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Articles

Diversity, distribution and host plants of armored scale insects (Hemiptera: Diaspididae) in Espírito Santo, Brazil

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Abstract: Armored scale insects (Hemiptera: Diaspididae), are phytophagous species that occur in major biogeographic regions of the world. Because of the importance of diaspidids as pests, there is widespread interest in countries that export and import unprocessed agricultural products in increased knowledge of this group which includes invasive and quarantine pests of great economic concern. The diversity, geographic distribution, and host of diaspidids were studied from November 2002 to December 2018 in 34 municipalities in the state of Espírito Santo, Brazil. Forty species of Diaspididae from 27 genera were collected and identified. The species *Acutaspis perseae* (Comstock), *A. umbonifera* (Newstead), *Aonidiella aurantii* (Maskell), *Comstockaspis perniciosa* (Comstock), *Lepidosaphes beckii* (Newman), *Lepidosaphes gloverii* (Packard), *Morganella longispina* (Morgan), *Mycetaspis apicata* (Newstead), and *Thysanofiorinia nephelii* (Maskel) were found for the first time in Espírito Santo. The plant families Myrtaceae, Moraceae, Arecaceae, Asparagaceae, and Rutaceae had the greatest number of host plant species of armored scale. Fifty-seven new host associations were observed for 25 species of diaspidids and 11 diaspidid species were recorded for the first time from nine families of plants. *Selenaspidus articulatus* (Morgan) was the most polyphagous species observed with 17 host plant species from 12 families, followed by *Pseudaonidia trilobitiformis* (Green), and *Parlatoria proteus* (Curtis). With these new records, 41 species and 28 genera of Diaspididae have been recorded in Espírito Santo.
Keywords: *Hemiptera; Diaspidids; Quarantine; Biodiversity; Biogeography.*

Diversidade, distribuição e plantas hospedeiras de cochonilhas escama (Hemiptera: Diaspididae) no Espírito Santo, Brasil

Resumo: As cochonilhas escama (Hemiptera: Diaspididae), são espécies fitófagas que ocorrem nas principais regiões biogeográficas do mundo. Devido à importância dos diaspidídeos como pragas, existe um amplo interesse nos países que exportam e importam produtos agrícolas não processados no aumento do conhecimento desse grupo, o que inclui pragas invasoras e quarentenárias de grande importância econômica. A diversidade, distribuição geográfica e hospedeiros de diaspidídeos foram estudadas de novembro de 2002 a dezembro de 2018 em 34 municípios do estado do Espírito Santo, Brasil. Quarenta espécies de Diaspididae de 27 gêneros foram coletadas e identificadas. As espécies *Acutaspis perseae* (Comstock), *A. umbonifera* (Newstead), *Aonidiella aurantii* (Maskell), *Comstockaspis perniciosa* (Comstock), *Lepidosaphes beckii* (Newman), *Lepidosaphes gloverii* (Packard), *Morganella longispina* (Morgan), *Mycetaspis apicata* (Newstead) e *Thysanofiorinia nephelii* (Maskel) foram encontradas pela primeira vez no Espírito Santo. Cinquenta e sete novas associações de hospedeiros foram observadas, em um total de 25 espécies de diaspidídeos; estes incluem 13 novos registros de famílias em um total de 11 espécies de diaspidídeos e nove famílias de plantas. Myrtaceae, Moraceae e Arecaceae foram as famílias botânicas com o maior número de espécies de diaspidídeos observadas. *Selenaspidus articulatus* (Morgan) foi a espécie mais polífaga, com 17 espécies de plantas hospedeiras de 12 famílias observadas, seguida por *Pseudaonidia trilobitiformis* (Green) e *Parlatoria proteus* (Curtis). Com esses novos registros, 41 espécies e 28 gêneros de Diaspididae foram registrados no Espírito Santo.
Palavras-chave: *Hemiptera; Diaspididae; Cochonilhas; Quarentena; Biodiversidade.*

Introduction

Among scale insects (Hemiptera: Coccoidea) the family Diaspididae has the greatest number of described species, with 2,643 species in 422 genera (García Morales et al. 2016). Members of the Diaspididae, commonly known as diaspidids or armored scale insects, occur in all major world biogeographic regions; however, the number of species of Diaspididae known in the Neotropical region is lower than in other major regions (García Morales et al. 2016). Diaspidids are phytophagous insects that feed on a great variety of plants grown for agricultural production including food, timber, and ornamentals, as well as wild, noncultivated species (Watson 2020). This group includes pests of great economic importance, including invasive and quarantine species that inhibit international commerce of agricultural products (Miller et al. 2005). Therefore, there is great interest in increased knowledge of diaspidids because of their potential impact on agriculture production and commerce worldwide as well as potential impacts on native species (Miller & Davidson 2005).

At least 163 species of Diaspididae from 53 genera have been recorded in Brazil, distributed in all regions of the country (García Morales et al. 2016). In the state of Espírito Santo, located in the Southeast region of Brazil, studies of the richness of species of diaspidids were almost nonexistent until the beginning of the decade of 2000 with only four species, *Comstockaspis perniciosa* (Comstock), *Costalimaspis eugeniae* Lepage, *Hemiberlesia lataniae* (Signoret) and *Ischnaspis longirostris* (Signoret), known to occur in the state (Silva et al. 1968, Claps et al. 1999). However, studies of insect pests, including diaspidids, and beneficial insects in agricultural crops in Espírito Santo beginning in 1999 contributed to a greater knowledge of the diversity and distribution of diaspidids and their natural enemies in the state with 29 additional diaspidid species identified in the state (Martins et al. 2004, Culik et al. 2008, 2009, 2011a, b). In addition, seven parasitoid species from the Aphelinidae and Encyrtidae (Hymenoptera: Chacidoidea) were found associated with the diaspidid species *Aonidiella comperei* McKenzie, *Diaspis boisduvalii* Signoret, *Hemiberlesia palmae* (Cockerell), *Melanaspis smilacis* (Comstock) and *Pseudaulacaspis pentagona* (Targioni Tozzetti) in crops of papaya, peach and pineapple (Culik et al. 2011a).

Studies of the species in regions provide the basic information necessary for knowledge of biological diversity and are indispensable for improved taxonomic and biogeographic understanding. In addition, knowledge of the pest species and their host plants and natural enemies in regions is of fundamental importance for the establishment of programs of integrated pest management (IPM) necessary for sustainable production and export of agricultural products. Therefore the objective of this study was to increase knowledge of the diversity and distribution of diaspidid species and their associated host plants in Espírito Santo state, Brazil.

Materials and Methods

Plant samples with associated diaspidids were collected in urban and rural areas of 34 of the 78 municipalities in Espírito Santo, Brazil, during the period from November 2002 to December 2018. Sampled municipalities were located throughout the state in the regions Central Litorânea (municipalities of Alfredo Chaves, Cariacica, Guarapari, Serra, Viana, Vila Velha, and Vitória); Centro Serrana (Domingos Martins, Santa Maria de Jetibá, Santa Teresa, Vargem Alta, and Venda Nova do Imigrante); Norte (Aracruz, Boa Esperança, Colatina, Conceição da Barra, Fundão, Jaguaré, João Neiva, Linhares, Marilândia, Montanha, Pancas, Pedro Canário, Pinheiros, Rio Bananal, São Mateus, and Sooretama); and Sul (Bom Jesus do Norte, Castelo, Guaçui, Jerônimo Monteiro, Marataízes, and Presidente Kennedy).

The diaspidid infested plant samples collected (leaves, stems, fruits, and pieces of trunk or branches) were placed in plastic bags and transported from the field to the Laboratory of Entomology of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), Vitória, Espírito Santo, where parts of the plant samples with attached diaspidids were stored and preserved in glass vials with 70% alcohol. For identification of species, the samples were cleared with potassium hydroxide (10%), dehydrated in 70% and 96% alcohol and mounted on glass slides in Canada balsam (Wolff et al. 2014). Identification of each species was based on morphological characteristics of the adult female, using an optical microscope and relevant identification keys (Ferris 1937, 1938, 1941, 1942, McKenzie 1937, Lepage & Gianotti 1944, Balachowsky 1954, Miller & Davidson 2005, Wolff 2008). The identifications of the species were made by second author of this publication.

A summary of collection records for each species of diaspidid that was collected in this study is provided, as well as the species previously known distribution in Brazil based on references noted, if any. New records of species of diaspidids in the state of Espírito Santo encountered in this study are noted with the expression ″new state record″, in parentheses, after the name of the state of Espírito Santo in the summary of the species distribution in Brazil.

New records of diaspidid host plants found in this study were determined based on previously known hosts noted by García Morales et al. (2016), and the names of the plant species follows the nomenclature of the Catalog of Life (2020). New host plant records for diaspidids species collected in this study are marked: new host plant family (*), new host plant species (**).

Voucher specimens of diaspidids identified in this study are deposited in the collections of arthropods of Incaper, Vitória, Espírito Santo and the Museu Ramiro Gomes da Costa (MRGC), Departamento de Diagnóstico e Pesquisa Agropecuária, Secretaria de Agricultura, Pecuária e Desenvolvimento Rural, Porto Alegre, Rio Grande do Sul, Brazil.

Results

A total of 302 samples of 80 species from 36 host plant families with armored scale insects were collected in this study. Forty species of Diaspididae from 27 genera were identified, demonstrating the diversity of species of this family in the state of Espírito Santo, Brazil.

Collection records for the species of Diaspididae collected in the present study are summarized as follow:

1. *Acutaspis perseae* (Comstock, 1881)

Samples examined: 2

Location (municipality) records: *Serra*: -20.21167° / -40.27153°, 1 sample, 07.ix.2016, B.C. Santos coll.; *Vitória*: -20.25811° / -40.26000°, 1 sample, 11.vi.2016, B.C. Santos coll.

Host plants examined: Malpighiaceae*: *Malpighia emarginata* [Moc. & Sesse] ex DC.**; Myrtaceae*: *Eugenia uniflora* L.**

Distribution in Brazil: Espírito Santo (new state record), Rio de Janeiro, Rio Grande do Sul.

Reference: Claps et al. (2001).

2. *Acutaspis umbonifera* (Newstead, 1920)

Samples examined: 1

Location (municipality) records: *Vitória*: -20.31444° / -40.28947°, 1 sample, 14.iii.2016, D.S. Martins coll.

Host plants examined: Asparagaceae*: *Yucca gigantea* Lem.**

Distribution in Brazil: Espírito Santo (new state record), Rio de Janeiro, São Paulo.

Reference: Claps et al. (2001), Imenes et al. (2002).

3. *Aonidiella aurantii* (Maskell, 1879)

Samples examined: 1

Location (municipality) records: *Vitória*: -20.29750° / -40.29114°, 1 sample, 27.i.2016, D.S. Martins coll.

Host plants examined: Apocynaceae: *Nerium oleander* L.

Distribution in Brazil: Alagoas, Ceará, Espírito Santo (new state record), Maranhão, Pará, Paraíba, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001).

4. *Aonidiella comperei* McKenzie, 1937

Samples examined: 72

Location (municipality) records: *Aracruz*: -19.57381° / -40.19150°, 4 samples, 12.i.2006, 16.i.2006, 18.v.2006, 18.v.2006, D.S. Martins coll.; -19.54164° / -40.90964°, 1 sample, 29.vi.2006, D.S. Martins coll.; 5 samples, 05.iv.2004, 01.ix.2004, 08.v.2006, 22.v.2006, 10.x.2006, D.S. Martins coll.; *Boa Esperança*: 3 samples, 12.iii.2004, D.S. Martins coll.; *Cariacica*: 1 sample, 19.iv.2007, M.P. Culik coll.; *Jaguaré*: -18.91900° / -40.17200°, 2 samples, 09.vi.2006, D.S. Martins coll.; *Linhares*: -19.14094° / -40.16975°, 3 samples, 10.vi.2003, 12.ii.2004, 15.iii.2006, D.S. Martins coll.; -19.17067° / -40.08558°, 2 samples, 12.ii.2004, D.S. Martins coll.; -19.23431° / -40.09439°, 1 sample, 28.vi.2006, D.S. Martins coll.; -19.25189° / -40.06806°, 3 samples, 16.xii.2005, 16.v.2006, 25.vii.2006, D.S. Martins coll.; -19.27183° / -39.98478°; 2 samples, 17.v.2006, 17.vii.2006, D.S. Martins coll.; -19.29511° / -40.14889°, 7 samples, 26.ii.2004, D.S. Martins coll.; 12 samples, 12.xi.2002, 12.xi.2002, 25.ii.2003, 25.ii.2003, 26.iii.2004, 07.iv.2004, 13.iv.2004, 13.i.2006, 28.vi.2006, 17.vii.2006, 13.ix.2006, 19.ix.2006, D.S. Martins coll.; 2 samples, 15.iii.2012, M.P. Culik coll.; *Montanha*: 3 samples, 20.i.2005, 19.iv.2006, 25.iv.2006, D.S. Martins coll.; *Pedro Canário*: 1 sample, 15.ix.2004, D.S. Martins coll.; 3 samples, 28.ix.2004, D.S. Martins coll.; 4 samples, 26.x.2004, D.S. Martins coll.; *Pinheiros*: -18.41258° / -40.28750°, 1 sample, 12.iii.2004, D.S. Martins coll.; *Rio Bananal*: 1 sample, 14.iii.2006, D.S. Martins coll.; 1 sample, 11.x.2006, D.S. Martins coll.; *São Mateus*: -18.65667° / -39.94269°, 1 sample, 27.xii.2005, D.S. Martins coll.; *Sooretama*: -19.11817° / -40.08011°, 1 sample, 11.x.2006, D.S. Martins coll.; -19.15286° / -40.13519°, 1 sample, 16.vi.2006, D.S. Martins coll.; -19.16575° / -40.10956°, 1 sample, 16.vi.2006, D.S. Martins coll.; -19.16708° / -40.08961°, 1 sample, 11.i.2006, D.S. Martins coll.; -19.19222° / -40.05697°, 1 sample, 15.viii.2006, D.S. Martins coll.; 4 samples, 16.xii.2005, 10.v.2006, 12.ix.2006, 12.ix.2006, D.S. Martins coll.

Host plants examined: Caricaceae: *Carica papaya* L.; Rubiaceae: *Morinda citrifolia* L.

Distribution in Brazil: Alagoas, Bahia, Ceará, Espírito Santo, Minas Gerais, Paraíba, Pernambuco, Rio de Janeiro, Rio Grande do Norte.

Reference: Claps et al. (2001), Martins et al. (2004, 2015), Culik et al. (2008), 2011a, b).

5. *Aspidiotus destructor* Signoret, 1869

Samples examined: 6

Location (municipality) records: *Guarapari*: -20.73136° / -40.53331°, 1 sample, 09.iv.2016, D.S. Martins coll.; *Serra*: 1 sample, 24.iv.2006, M.P. Culik coll.; *Vitória*: -20.29703° / -40.29181°, 1 sample, 21.xii.2015, D.S. Martins coll.; 3 samples, 15.xii.2005, 19.iv.2007, 07.viii.2007, M.P. Culik coll.

Host plants examined: Arecaceae: *Cocos nucifera* L., *Dypsis decaryi* (Jum.) Beentje & J. Dransf.**, species unidentified; Clusiaceae: *Clusia* sp.**.

Distribution in Brazil: Amazonas, Bahia, Ceará, Espírito Santo, Fernando de Noronha, Maranhão, Pará, Paraíba, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte, Santa Catarina, São Paulo, Sergipe.

Reference: Silva et al. (1968), Culik et al. (2008, 2011b).

6. *Aspidiotus nerii* Bouche, 1833

Samples examined: 3

Location (municipality) records: *Serra*: -20.17328° / -40.25758°, 1 sample, 12.ix.2016, B.C. Santos coll.; *Vitória*: -20.31689° / -40.32181°, 1 sample, 01.v.2004, D.S. Martins coll.; 1 sample, 29.ix.2016, B.C. Santos coll.

Host plants examined: Apocynaceae: *Nerium oleander* L.; Lauraceae: *Persea americana* Mill.; Rosaceae: *Rosa* sp.

Distribution in Brazil: Espírito Santo, Minas Gerais, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009).

7. *Aulacaspis tubercularis* Newstead, 1906

Samples examined: 6

Location (municipality) records: *Bom Jesus do Norte*: -21.13153° / -41.67581°, 1 sample, 21.vii.2016, B.C. Santos coll.; *Domingos Martins*: -20.32250° / -40.81689°, 1 sample, 23.v.2006, D.S. Martins coll.; -20.37256° / -41.06356°, 1 sample, 11.iii.2012, M.P. Culik coll.; *Serra*: -20.17058° / -40.25839°, 1 sample, 07.viii.2016, B.C. Santos coll.; 1 sample, 15.xii.2003, D.S. Martins coll.; *Vitória*: 1 sample, 31.viii.2004, D.S. Martins coll.

Host plants examined: Anacardiaceae: *Mangifera indica* L.

Distribution in Brazil: Espírito Santo, Goiás, Maranhão, Minas Gerais, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008), Ramos et al. (2018).

8. *Chrysomphalus aonidum* (Linnaeus, 1758)

Samples examined: 6

Location (municipality) records: *Jerônimo Monteiro*: -20.79453° / -41.37472°, 1 sample, 23.xi.2012, M.P. Culik coll.; *Serra*: -20.16889° / -40.25350°, 1 sample, 14.viii.2016, B.C. Santos coll.; *Sooretama*: -19.21700° / -40.05192°, 1 sample, 08.vii.2016, B.C. Santos coll.; *Vitória*: -20.29811° / -40.31650°, 1 sample, 26.xi.2015, D.S. Martins coll., -20.31344° / -40.30636°, 1 sample, 18.xii.2015, D.S. Martins coll.; 1 sample, 12.xii.2004, D.S. Martins coll.

Host plants examined: Asparagaceae: *Dracaena reflexa* var. *angustifolia* Baker (syn. *Dracaena marginata* Lam.); Rutaceae: *Citrus aurantium* L. (syn. *Citrus sinensis* (L.) Osbeck); Sapotaceae: *Manilkara subsericea* (Mart.) Dubard**; Vitaceae: *Leea rubra* Blume**.

Distribution in Brazil: Amapá, Amazonas, Bahia, Espírito Santo, Goiás, Maranhão, Mato Grosso, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001), Silva & Jordão (2005), Culik et al. (2008), Almeida et al. (2018).

9. *Chrysomphalus dictyospermi* (Morgan, 1889)

Samples examined: 9

Location (municipality) records: *Aracruz*: 1 sample, 17.viii.2006, D.S. Martins coll.; *Domingos Martins*: -20.37269° / -40.97608°, 1 sample, 28.xi.2015, D.S. Martins coll.; *Linhares*: -19.15189° / -40.07081°, 1 sample, 24.xi.2006, M.P. Culik coll.; *Santa Teresa*: 1 sample, 10.vii.2016, D.S. Martins coll.; *Vitória*: -20.29653° / -40.29275°, 1 sample, 24.vi.2006, D.S. Martins coll.; -20.29708° / -40.29272°, 2 samples, 16.xii.2005, D.S. Martins coll.; -20.30669° / -40.30239°, 1 sample, 20.i.2016, D.S. Martins coll.; -20.31892° / -40.30556°, 1 sample, 01.xii.2015, D.S. Martins coll.

Host plants examined: Araceae: *Zamioculcas zamiifolia* (G. Lodd.) Engl.**, Arecaceae: unidentified species; Asparagaceae: *Beaucarnea recurvata* Lem.; Moraceae: *Ficus benjamina* L.; Myrtaceae: *Syzygium jambos* (L.) Alston; Rosaceae: *Rosa* sp.; Vitaceae: *Leea guineenses* G.Don** (syn. *Leea coccinea* Planch), *Leea rubra* Blume**.

Distribution in Brazil: Bahia, Espírito Santo, Pará, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

10. *Comstockaspis perniciosa* (Comstock, 1881)

Samples examined: 1

Location (municipality) records: *Venda Nova do Imigrante*: 1 sample, 05.xii.2012, M.P. Culik coll.

Host plants examined: Rosaceae: *Prunus persica* (L.) Stokes.

Distribution in Brazil: Espírito Santo (new state record), Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001).

11. *Costalimaspis eugeniae* Lepage, 1937

Samples examined: 0

Location (municipality) records: Without specific locality.

Host plants examined: not collected in the present study.

Distribution in Brazil: Espírito Santo, Rio de Janeiro, São Paulo.

Reference: Silva et al. (1968), Claps et al. (1999), Culik et al. (2008).

12. *Diaspidiotus ancylus* (Putnam, 1878)

Samples examined: 2

Location (municipality) records: *Vitória*: -20.27625° / -40.29872°, 1 sample, 21.viii.2016, B.C. Santos coll.; -20.29653° / -40.29275°, 1 sample, 05.ii.2006, D.S. Martins coll.

Host plants examined: Myrtaceae*: *Psidium guajava* L.**.

Distribution in Brazil: Espírito Santo, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

13. *Diaspis boisduvalii* (Signoret, 1869)

Samples examined: 9

Location (municipality) records: *Domingos Martins*: -20.37256° / -41.06356°, 2 samples, 08.iii.2006, 16.i.2008, M.P. Culik coll.; *Serra*: -19.91650° / -40.12869°, 3 samples, 15.iii.2006, M.P. Culik coll.; *Sooretama*: -19.11817° / -40.08011°, 3 samples, 24.x.2006, M.P. Culik coll.; -19.11817° / -40.08011°, 1 sample, 11.ix.2007, M.P. Culik coll.

Host plants examined: Bromeliaceae: *Ananas comosus* (L.) Merr.

Distribution in Brazil: Espírito Santo, Minas Gerais, Pará, Paraná, Piauí, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009, 2011a).

14. *Diaspis bromeliae* Kerner, 1778

Samples examined: 9

Location (municipality) records: *Domingos Martins*: -20.36878° / -40.97467°, 1 sample, 26.iii.2016, D.S. Martins coll.; -20.37269° / -40.97608°, 1 sample, 03.ix.2006, D.S. Martins coll.; -20.37256° / -41.06356°, 1 sample, 22.ii.2005, M.P. Culik coll.; *Marataízes*: 1 sample, 18.viii.2005, M.P. Culik coll.; -21.05475° / -40.86397°, 5 samples, 20.x.2005, M.P. Culik coll.

Host plants examined: Bromeliaceae: *Ananas comosus* (L.) Merr.; Myrtaceae*: *Plinia cauliflora* (DC.) Kausel**; Orchidaceae: unidentified species.

Distribution in Brazil: Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009).

15. *Fiorinia fioriniae* (Targioni Tozzetti, 1867)

Samples examined: 9

Location (municipality) records: *Domingos Martins*: -20.37275° / -40.97600°, 1 sample, 28.xi.2015, D.S. Martins coll.; *Guarapari*: -20.73136° / -40.53331°, 1 sample, 24.vi.2006, D.S. Martins coll.; *Linhares*: -19.43511° / -40.08494°, 1 sample, 21.vi.2016, D.S. Martins coll.; *Serra*: -20.16889° / -40.25350°, 1 sample, 14.viii.2016, B.C. Santos coll.; -20.16994° / -40.25714°, 1 sample, 07.viii.2016, B.C. Santos coll.; -20.24503° / -40.26144°, 1 sample, 18.ix.2016, B.C. Santos coll.; *Vitória*: -20.29592° / -40.29572°, 1 sample, 01.i.2016, D.S. Martins coll.; -20.29653° / -40.29275°, 1 sample, 10.vi.2006, D.S. Martins coll.; -20.31175° / -40.30522°, 1 sample, 14.xii.2015, D.S. Martins coll.

Host plants examined: Iridaceae*: *Dietes bicolor* (Steud.) Sweet ex Klatt**; Lauraceae: *Laurus nobilis* L.; Moraceae: *Artocarpus heterophyllus* Lam; Myrtaceae: *Eugenia sprengelii* DC.**; Rutaceae: *Murraya paniculata* (L.) Jack.

Distribution in Brazil: Espírito Santo, Paraná, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Culik et al. (2008).

16. *Furcaspis biformis* (Cockerell, 1893)

Samples examined: 4

Location (municipality) records: *Vitória*: 3 samples, 29.ix.2006, 29.ix.2006, 28.iii.2007, M.P. Culik coll.; -20.31103° / -40.30236°, 1 sample, 26.viii.2013, J.A. Ventura coll.

Host plants examined: Asparagaceae: *Agave angustifolia* Haw.**; Orchidaceae: unidentified species.

Distribution in Brazil: Bahia, Espírito Santo, Rio de Janeiro, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

17. *Hemiberlesia cyanophylli* (Signoret, 1869)

Samples examined: 8

Location (municipality) records: *Conceição da Barra*: -18.56264° / -39.74250°, 1 sample, 25.xi.2015, A.F.S. Costa coll.; *Linhares*: 1 sample, 29.xi.2005, D.S. Martins coll.; *Santa Teresa*: 1 sample, 26.vii.2004, D.S. Martins coll.; *Vargem Alta*: 1 sample, 17.x.2005, M.J. Fornazier coll.; *Vitória*: -20.29653° / -40.29275°, 1 sample, 06.v.2006, D.S. Martins coll.; -20.30150° / -40.29886°, 1 sample, 01.viii.2006, D.S. Martins coll.; -20.30189° / -40.30117°, 1 sample, 19.i.2016, D.S. Martins coll.; -20.30961° / -40.28689°, 1 sample, 02.xii.2015, D.S. Martins coll.

Host plants examined: Anacardiaceae: *Anacardium occidentale* L.**; Arecaceae: *Cocos nucifera* L., *Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.; Cactaceae: *Cereus hildmannianus* K. Schum; Clusiaceae*: *Clusia fluminensis* Planch. & Triana**; Moraceae: *Artocarpus altilis* (Parkinson) Fosberg.; Myrtaceae: *Plinia cauliflora* (DC.) Kausel** (syn. *Myrciaria jaboticaba* (Vell.) O. Berg); Vitaceae*: *Vitis vinifera* L.**.

Distribution in Brazil: Bahia, Espírito Santo, Minas Gerais, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

18. *Hemiberlesia lataniae* (Signoret, 1869)

Samples examined: 3

Location (municipality) records: *São Mateus*: -18.73425° / -39.80231°, 1 sample, 12.v.2015, J.A. Ventura coll.; *Serra*: -20.19933° / -40.19528°, 1 sample, 06.x.2006, M.P. Culik coll.; *Venda Nova do Imigrante*: -20.41778° / -41.08486°, 1 sample, 22.xi.2011, M.P. Culik coll.

Host plants examined: Anacardiaceae: *Schinus terebinthifolia* Raddi**; Meliaceae: *Azadirachta indica* A. Juss.**; Vitaceae: *Vitis vinifera* L.

Distribution in Brazil: Amazonas, Espírito Santo, Minas Gerais, Pará, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Culik et al. (2008), Almeida et al. (2018).

19. *Hemiberlesia palmae* (Cockerell, 1893)

Samples examined: 3

Location (municipality) records: *Domingos Martins*: 1 sample, 03.ix.2007, M.P. Culik coll.; *Fundão*: 1 sample, 27.iv.2006, M.P. Culik coll.; *Serra*: -20.17311° / -40.25742°, 1 sample, 04.iv.2016, B.C. Santos coll.

Host plants examined: Arecaceae: *Dypsis lutescens* (H. wendl.) Beentje & J.Dransf., species unidentified; Rutaceae: *Citrus reticulata* Blanco.

Distribution in Brazil: Bahia, Espírito Santo, Paraná, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Culik et al. (2008, 2011a, b).

20. *Howardia biclavis* (Comstock, 1883)

Samples examined: 1

Location (municipality) records: *Vitória*: 1 sample, 26.xii.2007, M.P. Culik coll.

Host plants examined: unidentified ornamental species, leaf.

Distribution in Brazil: Bahia, Espírito Santo, Minas Gerais, Pará, Pernambuco, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2011b).

21. *Ischnaspis longirostris* (Signoret, 1882)

Samples examined: 13

Location (municipality) records: *Bom Jesus do Norte*: -21.13153° / -41.67581°, 1 sample, 21.vii.2016, B.C. Santos coll.; *Guarapari*: -20.67086° / -40.49914°, 1 sample, 23.i.2016, D.S. Martins coll.; *Linhares*: -19.15189° / -40.07081°, 2 samples, 24.xi.2006, 25.xi.2006, M.P. Culik coll.; *Venda Nova do Imigrante*: -20.34144° / -41.11547°, 1 sample, 24.vii.2016, D.S. Martins coll.; 1 sample, 15.iv.2005, M.P. Culik coll.; *Vitória*: -20.29708° / -40.29272°, 1 sample, 16.xii.2005, D.S. Martins coll.; -20.29914° / -40.29147°, 3 samples, 11.xii.2018, D.S. Martins coll.; -20.30592° / -40.29369°, 1 sample, 13.vi.2016, D.S. Martins coll.; -20.31175° / -40.30522°, 1 sample, 14.xii.2015, D.S. Martins coll.; 1 sample, 27.iv.2005, D.S. Martins coll.

Host plants examined: Anacardiaceae: *Mangifera indica* L.; Arecaceae: *Chamaedorea seifrizii* Burret**(syn. *Chamaedorea erumpens* H.E. Moore), *Phoenix sylvestris* (L.) Roxb.**; Davalliaceae*: *Davallia fejeensis* Hook.**; Iridaceae: *Dietes bicolor* (Steud.) Sweet ex Klatt; Moraceae: *Ficus variegata* Bl.**; Rubiaceae: *Ixora chinensis* Lam.**; Sapotaceae: *Mimusops thouarsii* M.M. Hartog ex Dubard**.

Distribution in Brazil: Bahia, Espírito Santo, Minas Gerais, Pará, Pernambuco, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

22. *Lepidosaphes beckii* (Newman, 1869)

Samples examined: 1

Location (municipality) records: *Domingos Martins*: -20.36878° / -40.97467°, 1 sample, 27.iii.2016, D.S. Martins coll.

Host plants examined: Rutaceae: *Citrus aurantiifolia* (Christm.) Swingle**.

Distribution in Brazil: Bahia, Espírito Santo (new state record), Goiás, Paraíba, Pernambuco, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Azevedo (1923), Carvalho & Carvalho (1939), Lepage & Giannotti (1942), Wolff & Corseuil (1994a), Almeida et al. (2018).

23. *Lepidosaphes gloverii* (Packard, 1869)

Samples examined: 1

Location (municipality) records: *Domingos Martins*: -20.36878° / -40.97467°, 1 sample, 27.iii.2016, D.S. Martins coll.

Host plants examined: Rutaceae: *Citrus aurantiifolia* (Christm.) Swingle.

Distribution in Brazil: Espírito Santo (new state record), Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Wolff & Corseuil (1994a).

24. Melanaspis smilacis (Comstock, 1883)

Samples examined: 7

Location (municipality) records: *Domingos Martins*: -20.37256° / -41.06356°, 1 sample, 25.ix.2007, M.P. Culik coll.; -20.37256° / -41.06356°, 1 sample, 16.i.2008, M.P. Culik coll.; *Sooretama*: -19.11817° / -40.08011°, 5 samples, 24.x.2006, M.P. Culik coll.

Host plants examined: Bromeliaceae: *Ananas comosus* (L.) Merr.

Distribution in Brazil: Espírito Santo, Rio de Janeiro, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009, 2011a, b), Almeida et al. (2018).

25. Morganella longispina (Morgan, 1889)

Samples examined: 1

Location (municipality) records: *Venda Nova do Imigrante*: 1 sample, 03.ii.2012, M.P. Culik coll.

Host plants examined: Moraceae: *Ficus carica* L.

Distribution in Brazil: Espírito Santo (new state record), Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001).

26. Mycetaspis apicata (Newstead, 1920)

Samples examined: 1

Location (municipality) records: *Vitória*: -20.29914° / -40.29147°, 1 sample, 11.xii.2018, D.S. Martins coll.

Host plants examined: Arecaceae*: *Phoenix sylvestris* (L.) Roxb.**.

Distribution in Brazil: Espírito Santo (new state record), Rio de Janeiro.

Reference: Claps et al. (2001).

27. Odonaspis ruthae Kotinsky, 1915

Samples examined: 1

Location (municipality) records: *Serra*: 1 sample, 25.xii.2005, M.P. Culik coll.

Host plants examined: Poaceae: *Cymbopogon winterianus* Jowitt ex Bor.**.

Distribution in Brazil: Bahia, Espírito Santo, Rio de Janeiro.

Reference: Silva et al. (1968), Ben-Dov (1988), Culik et al. (2008).

28. Parlatoria cinerea Hadden in Doane & Hadden, 1909

Samples examined: 4

Location (municipality) records: *Domingos Martins*: -20.38586° / -40.60733°, 1 sample, 29.iv.2016, D.S. Martins coll.; *Santa Maria de Jetibá*: 1 sample, 22.iv.2016, D.S. Martins coll.; *Santa Teresa*: -19.95956° / -40.51122°, 1 sample, 08.ii.2016, B.C. Santos coll.; *Viana*: 1 sample, 01.ix.2004, D.S. Martins coll.

Host plants examined: Rutaceae: *Citrus aurantium* L. (syn. *Citrus paradisi* Macfad, syn. *Citrus sinensis* (L.) Osbeck), *Citrus latifolia* (Tanaka ex Yu. Tanaka) Tanaka**, *Citrus reticulata* Blanco.

Distribution in Brazil: Amapá, Espírito Santo, Paraíba, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Fonseca (1965), Wolff & Corseuil (1994b), Silva & Jordão (2005), Culik et al. (2008).

29. Parlatoria pergandii Comstock, 1881

Samples examined: 4

Location (municipality) records: *Domingos Martins*: -20.36878° / -40.97467°, 2 samples, 27.iii.2016, D.S. Martins coll.; *Serra*: -20.19844° / -40.26053°, 1 sample, 03.ix.2016, B.C. Santos coll.; *Vitória*: -20.29653° / -40.29333°, 1 sample, 19.viii.2006, D.S. Martins coll.

Host plants examined: Moraceae: *Ficus recurva* Bl.**; Rutaceae: *Citrus latifolia* (Tanaka ex Yu. Tanaka) Tanaka**.

Distribution in Brazil: Bahia, Espírito Santo, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Wolff & Corseuil (1994b), Culik et al. (2008).

30. Parlatoria proteus (Curtis, 1843)

Samples examined: 15

Location (municipality) records: *Serra*: -20.19936° / -40.27108°, 1 sample, 22.v.2016, B.C. Santos coll.; *Vitória*: -20.29336° / -40.29336°, 1 sample, 29.xi.2015, D.S. Martins coll.; -20.29622° / -40.29578°, 1 sample, 03.i.2016, D.S. Martins coll.; -20.29642° / -40.29314°, 2 samples, 06.xii.2015, D.S. Martins coll.; -20.29644° / -40.29514°, 1 sample, 06.xii.2015, D.S. Martins coll.; -20.29653° / -40.29275°, 1 sample, 21.x.2006, D.S. Martins coll.; -20.29939° / -40.29214°, 1 sample, 03.i.2016, D.S. Martins coll.; -20.31444° / -40.28947°, 1 sample, 14.iii.2016, D.S. Martins coll.; -20.31867° / -40.30536°, 1 sample, 02.vi.2016, B.C. Santos coll.; -20.31872° / -40.30539°, 1 sample, 28.vii.2006, D.S. Martins coll.; -20.29694° / -40.29261°, 1 sample, 12.xii.2004, D.S. Martins coll.; 3 samples, 30.xii.2004, 09.xii.2005, 20.v.2013, M.P. Culik coll.

Host plants examined: Araceae: *Anthurium andraeanum* Linden ex André**; Araliaceae: *Schefflera arboricola* (Hayata) Merr.; Arecaceae: *Dypsis lutescens* (H. Wendl.) Beentje & J.Dransf., *Phoenix roebelenii* O'Brien**; Asparagaceae: *Beaucarnea recurvata* Lem.**, *Dracaena reflexa* Lam.**, *Yucca gigantea* Lem.**; Cycadaceae: *Cycas revoluta* Thunb.; Euphorbiaceae: *Euphorbia milii* Des Moul; Moraceae: *Ficus benjamina* L.; Rutaceae: *Murraya paniculata* (L.) Jack**; Vitaceae: *Leea rubra* Blume**.

Distribution in Brazil: Espírito Santo, Paraíba, Pernambuco, Rio Grande do Sul, São Paulo.

Reference: Silva et al. (1968), Corseuil & Silva (1971), Culik et al. (2008).

31. Pinnaspis aspidistrae (Signoret, 1869)

Samples examined: 12

Location (municipality) records: *Domingos Martins*: -20.37256° / -41.06356°, 2 samples, 10.iii.2012, 19.iv.2012, M.P. Culik coll.; -20.36861° / -40.97425°, 2 samples, 18.vi.2006, 28.xi.2015, D.S. Martins coll.; -20.37269° / -40.97608°, 1 sample, 10.ix.2006, D.S. Martins coll.; -20.38586° / -40.60733°, 1 sample, 29.iv.2016, D.S. Martins coll.; -20.37256° / -41.06356°, 1 sample, 19.iv.2012, M.P. Culik coll.; *Jerônimo Monteiro*: -20.79453° / -41.37472°, 2 samples, 10.i.2012, M.P. Culik coll.; *Santa Maria de Jetibá*: 1 sample, 22.iv.2016, D.S. Martins coll.; *Serra*: -20.16889° / -40.25350°, 1 sample, 14.viii.2016, B.C. Santos coll.; *Venda Nova do Imigrante*: -20.41433° / -41.16481°, 1 sample, 23.vii.2006, D.S. Martins coll.; *Vitória*: -20.31175° / -40.30522°, 1 sample, 14.xii.2015, D.S. Martins coll.

Host plants examined: Iridaceae*: *Dietes bicolor* (Steud.) Sweet ex Klatt**; Rutaceae: *Citrus aurantium* L. (syn. *Citrus sinensis* (L.) Osbeck), *Citrus reticulata* Blanco.

Distribution in Brazil: Amapá, Amazonas, Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Pará, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Claps et al. (2001), Silva & Jordão (2005), Culik et al. (2008), Almeida et al. (2018).

32. Pinnaspis buxi (Bouché, 1851)

Samples examined: 5

Location (municipality) records: *Marataízes*: 1 sample, 20.x.2005, M.P. Culik coll.; *Vitória*: -20.29708° / -40.29272°, 1 sample, 16.xii.2005, D.S. Martins coll.; -20.31825° / -40.32397°, 1 sample, 22.iii.2016, D.S. Martins coll.; 2 samples, 12.xii.2004, 27.iv.2005, D.S. Martins coll.

Host plants examined: Araceae: *Spathiphyllum wallisii* Regel**; Asparagaceae: *Cordyline fruticosa* (L.) A. Chev. (syn. *Cordyline terminalis* L. Kunth.); Iridaceae: *Dietes bicolor* (Steud.) Sweet ex Klatt; Malvaceae: *Sida* sp.

Distribution in Brazil: Bahia, Espírito Santo, Rio de Janeiro, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008).

33. Pinnaspis strachani (Cooley, 1899)

Samples examined: 8

Location (municipality) records: *Domingos Martins*: -20.36711° -40.97269°, 2 samples, 03.ix.2006, D.S. Martins coll.; *Linhares*: -19.15189° / -40.07081°, 1 sample, 25.xi.2006, M.P. Culik coll.; *Santa Teresa*: -19.95956° / -40.51122°, 1 sample, 07.ii.2016, B.C. Santos coll.; *Sooretama*: 1 sample, 16.v.2006, D.S. Martins coll.; *Vitória*: -20.29497° / -40.28650°, 1 sample, 30.iii.2015, D.S. Martins coll.; 2 samples, 15.iv.2005, 24.xii.2005, M.P. Culik coll.

Host plants examined: Anacardiaceae: *Mangifera indica* Bl.; Arecaceae: *Chamaedorea seifrizii* Burret (syn. *Chamaedorea erumpens* H.E. Moore); Malvaceae: *Hibiscus mutabilis* L., *Hibiscus rosa-sinensis* L.; Moraceae: *Artocarpus heterophyllus* Lam.; Poaceae: unidentified weed species; Rutaceae: *Citrus aurantium* L. (syn. *Citrus paradisi* Macfadyen, syn. *Citrus sinensis* (L.) Osbeck).

Distribution in Brazil: Amazonas, Bahia, Espírito Santo, Pernambuco, Rio Grande do Sul, São Paulo.

Reference: Carvalho & Carvalho (1939), Foldi (1988), Imenes et al. (2000), Wolff & Corseuil (1994a), Culik et al. (2008, 2009), Castro et al. (2020b).

34. Pseudaonidia trilobitiformis (Green, 1896)

Samples examined: 25

Location (municipality) records: *Castelo*: 1 sample, 09.x.2006, R.G. Ferrão coll.; *Colatina*: -19.50719° / -40.55392°, 1 sample, 07.i.2016, I. Monnerat coll.; *Conceição da Barra*: -18.56264° / -39.74250°, 1 sample, 25.xi.2015, A.F.S. Costa coll.; *Guarapari*: -20.67078° / -40.50161°, 1 sample, 12.i.2016, D.S. Martins coll.; -20.67083° / -40.49656°, 1 sample, 10.iv.2016, D.S. Martins coll.; -20.67292° / -40.49936°, 1 sample, 12.i.2016, D.S. Martins coll.; -20.73136° / -40.53331°, 1 sample, 24.vi.2006, D.S. Martins coll.; *Linhares*: -19.41708° / -40.07936°, 1 sample, 08.vii.2016, B.C. Santos coll.; -19.64586° / -39.82447°, 1 sample, 18.vi.2016, B.C. Santos coll.; 1 sample, 10.v.2006, D.S. Martins coll.; *Marilândia*: -19.43386° / -40.63300°, 1 sample, 07.i.2016, I. Monnerat coll.; *Pancas*: -19.22381° / -40.84339°, 1 sample, 23.xii.2015, M.J. Fornazier coll.; *Santa Teresa*: 1 sample, 07.vii.16, B.C. Santos coll.; *Sooretama*: -19.21700° / -40.05192°, 1 sample, 08.vii.2016, B.C. Santos coll.; *Vitória*: -20.27708° / -40.29886°, 1 sample, 21.viii.2016, B.C. Santos coll.; -20.28808° / -40.29358°, 1 sample, 10.vi.2006, D.S. Martins coll.; -20.29642° / -40.29314°, 1 sample, 06.xii.2015, D.S. Martins coll.; -20.29653° / -40.29275°, 1 sample, 10.vi.2006, D.S. Martins coll.; -20.29708° / -40.29272°, 1 sample, 24.xi.2015, D.S. Martins coll.; -20.29750° / -40.29114°, 1 sample, 27.i.2016, D.S. Martins coll.; -20.30711° / -40.32128°, 1 sample, 06.xii.2015, D.S. Martins coll.; -20.30711° / -40.32128°, 1 sample, 05.ii.2016, B.C. Santos coll.; -20.31175° / -40.30522°, 1 sample, 14.xii.2015, D.S. Martins coll.; -20.31867° / -40.30536°, 1 sample, 02.vi.2016, B.C. Santos coll.; 1 sample, 19.xii.2004, D.S. Martins coll.

Host plants examined: Anacardiaceae: *Anacardium occidentale* L.; Apocynaceae: *Nerium oleander* L.; Iridaceae*: *Dietes bicolor* (Steud.) Sweet ex Klatt**; Lauraceae: *Laurus nobilis* L.; Lythraceae: *Punica granatum* L.; Moraceae: *Ficus pumila* L.; Myrtaceae: *Psidium cattleianum* Afzel. ex Sabine, *Psidium guajava* L.; Oleaceae: *Olea europaea* L.; Rubiaceae: *Coffea canephora* Pierre ex A. Froehner**, *Ixora chinensis* Lam.**, *Ixora coccinea* L.; Rutaceae: *Murraya paniculata* (L.) Jacq.

Distribution in Brazil: Bahia, Ceará, Espírito Santo, Minas Gerais, Pará, Paraíba, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009).

35. Pseudaulacaspis pentagona (Targioni Tozzetti, 1886)

Samples examined: 7

Location (municipality) records: *Domingos Martins*: -20.37256° / -41.06356°, 3 samples, 16.i.2008, 14.xi.2011, 14.xi.2011, M.P. Culik coll.; -20.37269° / -40.97608°, 1 sample, 29.xi.2015, D.S. Martins coll.; *Linhares*: 1 sample, 19.v.2006, D.S. Martins coll.; *Sooretama*: -19.21700° / -40.05192°, 1 sample, 08.vii.2016, B.C. Santos coll.; 1 sample, 10.v.2006, D.S. Martins coll.

Host plants examined: Cannabaceae: *Trema micrantha* (L.) Bl.; Didiereaceae*: *Portulacaria afra* Jacq.**; Passifloraceae: *Passiflora edulis* Sims; Rosaceae: *Prunus persica* (L.) Stokes.

Distribution in Brazil: Bahia, Ceará, Distrito Federal (Brasília), Espírito Santo, Maranhão, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo.

Reference: Silva et al. (1968), Culik et al. (2008, 2011a, b), Castro et al. (2020a).

36. Pseudischnaspis bowreyi (Cockerell, 1893)

Samples examined: 2

Location (municipality) records: *Linhares*: -19.15189° / -40.07081°, 1 sample, 25.xi.2006, M.P. Culik coll.; *Serra*: -20.16889° / -40.25350°, 1 sample, 14.viii.2016, B.C. Santos coll.

Host plants examined: Myrtaceae: *Eugenia stipitata* Mc Vaugh**.

Distribution in Brazil: Espírito Santo, Paraiba, São Paulo.

Reference: Hempel (1900), Silva et al. (1968), Culik et al. (2008).

37. Pseudoparlatoria argentata Hempel, 1912

Samples examined: 1

Location (municipality) records: *Guarapari*: -20.73136° / -40.53331°, 1 sample, 24.vi.2006, D.S. Martins coll.

Host plants examined: unidentified parasitic species (Santalales)

Distribution in Brazil: Bahia, Distrito Federal (Brasília), Espírito Santo, Mato Grosso, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (1999), Wolff (2008), Culik et al. (2008), Castro et al. (2020c).

38. Pseudoparlatoria parlatorioides (Comstock, 1883)

Samples examined: 2

Location (municipality) records: *Alfredo Chaves*: 1 sample, 06.vii.2004, D.S. Martins coll.; *Vila Velha*: 1 sample, 28.v.2012, M.P. Culik coll.

Host plants examined: Myrtaceae: *Plinia cauliflora* (DC.) Kausel (syn. *Myrciaria jaboticaba* (Vell.) O. Berg, *Psidium guajava* L.

Distribution in Brazil: Espírito Santo, Minas Gerais, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Wolff (2008), Culik et al. (2008).

39. Selenaspidus articulatus (Morgan, 1889)

Samples examined: 25

Location (municipality) records: *Aracruz*: 1 sample, 18.xii.2005, D.S. Martins coll.; *Domingos Martins*: -20.36878° / -40.97467°, 1 sample, 24.iv.2005, D.S. Martins coll.; *Guaçui*: 2 samples, 12.i.2006, 07.iii.2006, H. Costa coll.; *João Neiva*: 1 sample, 01.vi.2004, D.S. Martins coll.; *Linhares*: -19.15189° / -40.07081°, 5 samples, 25.xi.2006, M.P. Culik coll.; -19.41708° / -40.07936°, 1 sample, 08.vii.2016, B.C. Santos coll.; *Presidente Kennedy*: -21.27861° / -40.96400°, 1 sample, 16.iv.2017, B.C. Santos coll.; -21.27861° / -40.96400°, 2 samples, 22.xii.2016, 14.iv.2017, B.C. Santos coll.; *Santa Teresa*: -19.95956° / -40.51122°, 2 samples, 08.ii.2016, 09.ii.2016, B.C. Santos coll.; 1 sample, 07.vii.2016, B.C. Santos coll.; *Serra*: -20.16889° / -40.25350°, 1 sample, 14.viii.2016, B.C. Santos coll.; -20.17311° / -40.25742°, 1 sample, 07.iv.2016, B.C. Santos coll.; -20,21047° / -40,27297°, 1 sample, 07.ix.2016, B.C. Santos coll.; -20.21167° / -40.27153°, 3 samples, 07.ix.2016, B.C. Santos coll.; -20.24503° / -40.26144°, 1 sample, 18.ix.2016, B.C. Santos coll.; *Vitória*: -20.25233° / -40.27319°, 1 sample, 11.vii.2016, B.C. Santos coll.

Host plants examined: Annonaceae: *Annona atemoya* Mabb.**; Apocynaceae: *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.** (syn. *Ervatamia coronaria* (Jacq.) Stapf); Arecaceae: *Cocos nucifera* L., *Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.; Malpighiaceae: *Malpighia emarginata* [Moc. & Sesse] ex DC.**; Malvaceae: *Theobroma cacao* L.**; Myrtaceae: *Eugenia uniflora* L.**; Oleaceae: *Ligustrum* sp.**, *Olea europaea* L.; Oxalidaceae: *Averrhoa carambola* L.; Passifloraceae: *Passiflora edulis* Sims; Rutaceae: *Citrus aurantiifolia* (Christm.) Swingle (syn. *Citrus limettioides* Tanaka), *Citrus aurantium* L. (syn. *Citrus sinensis* (L.) Osbeck), *Citrus latifolia* (Tanaka ex Yu. Tanaka) Tanaka**, *Citrus reticulata* Blanco; Sapotaceae: *Labramia bojeri* A. DC.**; Solanaceae: *Brunfelsia uniflora* (Pohl) D.Don**.

Distribution in Brazil: Amapá, Amazonas, Bahia, Espírito Santo, Goiás, Pará, Rio de Janeiro, Rio Grande do Norte, São Paulo.

Reference: Perruso & Cassino (1993), Claps et al. (2001), Martins et al. (2004, 2015), Silva & Jordão (2005), Culik et al. (2008), Silva et al. (2020).

40. Thysanofiorinia nephelii (Maskell, 1897)

Samples examined: 2

Location (municipality) records: *Domingos Martins*: -20.37256° / -41.06356°, 1 sample, 27.i.2012, M.P. Culik coll.; *Venda Nova do Imigrante*: -20.33964° / -41.11533°, 1 sample, 10.xi.2011, M.P. Culik coll.

Host plants examined: Sapindaceae: *Litchi chinensis* Sonner.

Distribution in Brazil: Espírito Santo (new state record), Rio de Janeiro.

Reference: Claps et al. (2001).

41. Unaspis citri (Comstock, 1883)

Samples examined: 10

Location (municipality) records: *Domingos Martins*: -20.36861° / -40.97425°, 3 samples, 27.iii.2016, 27.iii.2016, 28.xi.2015, D.S. Martins coll.; -20.37256° / -41.06356°, 1 sample, 11.iii.2012, M.P. Culik coll.; 1 sample, 23.viii.2007, M.P. Culik coll.; *Fundão*: 2 samples, 27.iv.2006, M.P. Culik coll.; *Linhares*: -19.15189° / -40.07081°, 1 sample, 25.xi.2006, M.P. Culik coll.; *Santa Maria de Jetibá*: 1 sample, 22.iv.2016, D.S. Martins coll.; *Santa Teresa*: -19.95956° / -40.51122°, 1 sample, 09.ii.2016, B.C. Santos coll.

Host plants examined: Rutaceae: *Citrus aurantium* L. (syn. *Citrus sinensis* (L.) Osbeck), *Citrus latifolia* (Tanaka ex Yu. Tanaka) Tanaka**, *Citrus reticulata* Blanco.

Distribution in Brazil: Alagoas, Espírito Santo, Mato Grosso, Rio de Janeiro, Rio Grande do Sul, São Paulo.

Reference: Claps et al. (2001), Culik et al. (2008, 2009, 2011b), Ferreira et al. (2013).

Discussion

Acutaspis perseae (Comstock), *Acutaspis umbonifera* (Newstead), *Aonidiella aurantii* (Maskell), *Comstockaspis perniciosa* (Comstock), *Lepidosaphes beckii* (Newman), *Lepidosaphes gloverii* (Packard), *Morganella longispina* (Morgan), *Mycetaspis apicata* (Newstead) and *Thysanofiorinia nephelii* (Maskel) were recorded for the first time in the state. *Costalimaspis eugeniae* (Silva et al. 1968, Claps et al. 1999) was the only species of diaspidid not observed in the present study that has previously been noted in Espírito Santo.

Fifty-seven new host associations were observed, across a total of 25 species of diaspidids; these include 13 new family records acros a total of 11 diaspidid species and 9 plant families: *A. perseae* on Malpighiaceae (*Malpighia emarginata* [Moc. & Sesse] ex DC.) and Myrtaceae (*Eugenia uniflora* L.); *A. umbonifera* on Asparagaceae (*Yucca gigantea* Lem.); *Diaspidiotus ancylus* (Putnam) on Myrtaceae (*Psidium guajava* L.); *Diaspis bromeliae* (Kerner) on Myrtaceae (*Plinia cauliflora* (DC.) Kausel); *Fiorinia fioriniae* (Targioni Tozzetti) on Iridaceae (*Dietes bicolor* (Steud.) Sweet ex Klatt); *Hemiberlesia cyanophylli* (Signoret) on Clusiaceae (*Clusia fluminensis* Planch. & Triana) and Vitaceae (*Vitis vinifera* L.); *I. longirostris* on Davalliaceae (*Davallia fejeensis* Hook.); *M. apicata* on Arecaceae (*Phoenix sylvestris* (L.) Roxb.); *Pinnaspis aspidistrae* (Signoret) and *Pseudaonidia trilobitiformis* (Green) on Iridaceae (*Dietes bicolor* (Steud.) Sweet ex Klatt); and *P. pentagona* on Didiereaceae (*Portulacaria afra* Jacq.).

The plant families Myrtaceae, Moraceae, Arecaceae, Asparagaceae, and Rutaceae had the greatest number of host plant species of diaspidids collected in the present study. However, worldwide the plant families with the greatest number of associated diapsid species are Fabaceae, Poaceae, Euphorbiaceae, Myrtaceae, Rosaceae, Moraceae, Oleaceae, Arecaceae, and Rutaceae (García Morales et al. 2016). The diaspidid *Selenaspidus articulatus* (Morgan) was observed to be the most polyphagous species and was associated 17 host plant species of 12 families in the preset study, followed by *P. trilobitiformis* (associated with 13 plant species of 9 families), and *Parlatoria proteus* (associated with 12 plant species of 9 families). In a study of diaspids in tropical forests (Rainforest) of Panama (Central America) *S. articulatus* was also the most polyphagous species. (Normark et al., 2014; Peterson et al., 2020).

Some species of diaspidids stand out in Espírito Santo because of the damage that they cause to agricultural crops of great socioeconomic importance in the state. *Aonidiella comperei* is the most important diaspidid pest in Espírito Santo because of the great damage it causes to papaya, the principal fruit of exportation of the state (Ibge 2020, Agrostar 2020). This species is widespread and common in the main papaya producing regions of Brazil, causing damage to the trunks and fruits of papaya, and because of its rapid multiplication and spread in fields, it has been the most important scale insect pest of papaya in Brazil (Martins et al. 2015); *D. boisduvalii*, *D. bromeliae*, and *M. smilacis* are important pests in the culture of pineapple (Culik et al. 2009); *P. trilobitiformis* is associated with conilon coffee (*Coffea canephora*) (Silva et al. 2019); *C. perniciosa* and *P. pentagona* infest the peach crop in the Serrana Region of Espírito Santo (Fornazier et al. 1987). In the present study diaspidids were also found in many other crops of economic importance in Espírito Santo state including acerola, cacao, citrus (lemon, orange, and tangerine), coconut, guava, lichee, mango, passionfruit, and grape.

With the nine new records of diaspidids observed in the present study, 41 species are now recorded in this Espírito Santo state, Brazil which is slightly more than 25% of the 163 species which have been recorded in this country (García Morales et al. 2016).

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Associate Editor

José Mermudes

Authors Contributions

David dos Santos Martins: contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision.

Vera Regina dos Santos Wolff: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision.

Mark Paul Culik: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision.

Beatriz Crisostomo dos Santos: contribution to data collection, contribution to data analysis and interpretation, and contribution to manuscript preparation.

Maurício José Fornazier: contribution to data collection, contribution to data analysis and interpretation, and contribution to manuscript preparation.

José Aires Ventura: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript, and the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Short Communication

Potential dispersal of aquatic snails by waterbird endozoochory in neotropical wetlands

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Abstract: Waterbird-mediated zoochory is one of the main ecological mechanisms by which non-flying freshwater invertebrates can disperse between isolated wetlands. Passive dispersal through gut passage inside waterbirds (endozoochory) may explain how many organisms spread in the landscape. Here, we evaluate the potential for dispersal of aquatic snails by three waterbird species in neotropical wetlands. A total of 77 faecal samples from *Coscoroba coscoroba* (n = 28), *Dendrocygna viduata* (n = 36) and *Anas flavirostris* (n = 13) were collected in the field and taken to the laboratory. There, the samples were examined under a stereomicroscope to check for the presence of gastropod shells. We found 496 intact gastropod shells, and *Heleobia piscium* was the most abundant species (n= 485). We also found two shells of *Drepanotrema* sp. and nine others distributed between two different morphotypes of Planorbidae. Snails were present in 20.8 % of all samples, and were more frequent in faeces of coscoroba swan (50%) than the other two bird species. Our data suggest that aquatic snails may disperse by avian endozoochory between neotropical wetlands, with vectors including migratory bird species.
Keywords: *Gastropods; waterfowl; wetlands; neotropics.*

Dispersão potencial de caramujos por endozoocoria de aves aquáticas em áreas úmidas neotropicais

Resumo: A zoocoria mediada por aves aquáticas é um dos principais processos ecológicos que explicam como invertebrados não-voadores habitantes de água doce se dispersam entre áreas úmidas isoladas. A dispersão passiva que ocorre através no interior dos intestinos de aves aquáticas (endozoocoria) pode explicar como estes invertebrados se distribuem na paisagem. Neste trabalho, avaliamos o potencial de dispersão de caramujos aquáticos por endozoocoria promovida por três espécies de aves aquáticas em áreas úmidas neotropicais. No total, 77 amostras fecais de capororoca (*Coscoroba coscoroba,* n = 28), irerê (*Dendrocygna viduata*, n = 36) e marreca-pardinha (*Anas flavirostris*, n = 13) foram coletadas em campo e levadas ao laboratório. As amostras foram examinadas em estereomicroscópio para verificar a presença de conchas de gastrópodes. Encontramos 496 conchas intactas, sendo *Heleobia piscium* a espécie mais abundante (n = 485). Também encontramos duas conchas de *Drepanotrema* sp. e nove de outros dois morfotipos de Planorbidae. Os caramujos estiveram presentes em 20,8% de todas as amostras, sendo mais frequentes nas fezes do capororoca (50%). Nossos dados sugerem que caramujos aquáticos podem se dispersar por endozoocoria de aves entre áreas úmidas neotropicais, com vetores incluindo espécies de aves migratórias e residentes.
Palavras-chave: *gastrópodes; aves aquáticas; áreas úmidas; região neotropical*

Introduction

How some aquatic invertebrates with low locomotion capacity became widely distributed is an issue that has long intrigued naturalists (Darwin 1859, Bohonak & Jenkins 2003, Van Leeuwen 2012 a, b). Waterbird-mediated zoochory is one of the main ecological mechanisms by which non-flying freshwater invertebrates disperse between isolated waterbodies such as lakes and temporary ponds (Figuerola & Green 2002; Silva et al., 2021, Martín-Vélez et al., 2022). Global distribution, high abundance and flight capacity make waterbirds vital vectors for the dispersal of aquatic invertebrates in the landscape (Figuerola et al. 2003, Brochet et al. 2010). Endozoochory, when whole invertebrates or their propagules are passively transported inside the animal vector, has been demonstrated for a wide spectrum of taxa, including organisms without any apparent adaptation to gut passage, such as rotifers, nematodes and dipteran larvae, and others with a resistant structure that may favour survival during stressful conditions, such as bryozoan statoblasts or whole snails (Brown 1933, Proctor 1964, Malone 1965a, 1965b, Green & Figuerola 2005, Brochet et al. 2010, Laux & Kolsch 2014, Simonová et al. 2016, Lovas-Kiss et al. 2018, Moreno et al. 2019, Silva et al., 2021). Even fish eggs and whole plants can be dispersed by waterfowl endozoochory (Silva et al. 2018, Silva et al. 2019).

Gastropod shells are adapted to survive hard environmental conditions and mechanical stress (Chapuis & Ferdy 2012, Havel et al. 2012). Peculiarities of the shell provide physical and chemical resistance that may allow some gastropod species to survive inside the anoxic and high temperature environment of the bird alimentary tract after being ingested, although many shells are excreted empty (or with dead bodies in them) by birds (Cadeé 2011, Wada et al. 2012, Van Leeuwen 2012 a). Avian endozoochory has been considered a plausible explanation for dispersal of some aquatic snails, such as *Physella acuta* (Physidae)*, Bithynia tentaculata* (Bithyniidae) and *Potamopyrgus antipodarum* (Tateidae) (Alonso & Castro-Diez 2008, Kappes & Haase 2012, Vinarski 2017, Martín-Vélez et al., 2022). Van Leeuwen et al. (2012 a, b) demonstrated that whole *Hydrobia ulvae* (Hydrobiidae) may survive after gut passage of mallards (*Anas platyrhynchos*), even remaining five hours inside the bird. Considering a waterbird can fly at speeds of 50-78 km/h (Welhun 1994, García-Alvarez *et al.* 2015, Lovas-Kiss et al. 2020), we can assume that snails may be dispersed at different spatial scales, including long-distance dispersal during waterbird migration. Avian vectors have often been proposed as an explanation for the genetic structure of snail metapopulations, or the phylogeography of closely related species (Miller et al. 2006, Holland et al. 2007, Zielske & Haase 2014). Here, we report the occurrence of aquatic snails found in faeces of three waterfowl species, and address the potential for dispersal by waterfowl endozoochory in the neotropic region.

Material and Methods

We analysed data collected in the Coastal Plain of Rio Grande do Sul, southern Brazil, one of the most important regions for waterbird conservation in South America (Silva et al. 2021; Figure 1). We obtained faecal samples of coscoroba swan (*Coscoroba coscoroba*, n= 28); white-faced whistling-duck (*Dendrocygna viduata*, n= 36) and yellow-billed teal (*Anas flavirostris*, n=13) from August 2017 to December 2019 in wetlands located in Tavares and Santa Vitória do Palmar municipalities. Field sample collection and laboratory procedures followed Silva et al. (2021). Briefly, we identified individuals or monospecific groups of three bird species resting or feeding around lake edges, and collected fresh droppings from the grass. We stored samples individually in plastic tubes and frozen (- 4 °C) to avoid fungal infestation. In the laboratory at UNISINOS University, the samples were defrosted, weighed and washed in tap water using a sieve (53 μm). The washed content was analyzed under a stereomicroscope (10x to 1.6x - 5 x of total magnification) to separate the visible snails from other material. We compared the frequency of occurrence of snails in waterfowl faeces through a Chi-square test.

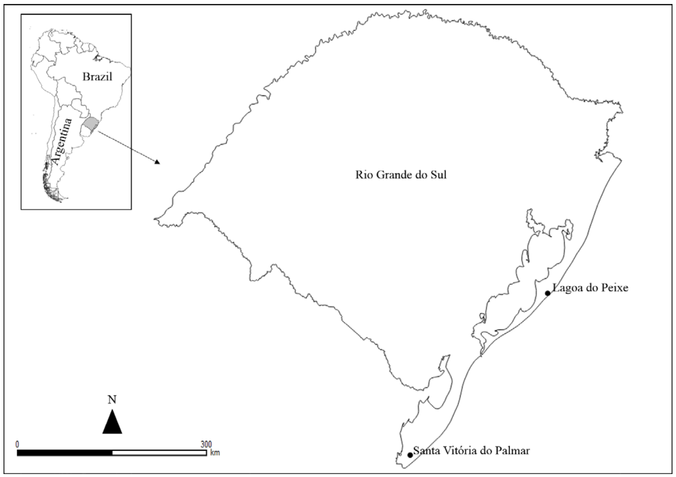

Figure 1. Study region of the Coastal Plain of Rio grande do Sul, southern brazil, where waterflow faecal samples were collected (black dots).

Results

We found 496 intact shells of four different gastropod taxa, *Heleobia piscium* (Hydrobiidae, n= 485; Figure 2), *Drepanotrema* sp. (Planorbidae, n= 2; Figure 3) and nine shells of two other unidentified genera of Planorbidae. Snails were present in 20.8 % of the total samples, and were more frequent in faecal samples of coscoroba swan (57.1%; n=16) than white-faced whistling-duck (2.8%; n=1) and yellow-billed teal (7.7%, n=1). Snails were also more abundant in coscoroba swan samples ($X^2 = 1388,2$; $df = 3$; $P < 0,001$) than the other two waterfowl species, and this result was influenced by the high abundance of *Heleobia piscium* shells (Table 1). We confirmed the presence of snail bodies inside 68 shells of *Heleobia piscium* (14%), by close inspection under the microscope. The dispersed shells of *Heleobia piscium* had 2.9 mm length (ranging from 2.4 to 3.5 mm) and 1.9 mm width (from 2.4 to 3.5 mm). *Drepanotrema* sp. shells had 4.9 mm length (4.7 mm to 5.5 mm) and 1.3 mm width (1.2 mm to 1.6 mm).

Discussion

Dispersal by avian endozoochory is an accepted explanation for dispersal of some aquatic snails, and the survival by gastropods of passage through avian guts has been repeatedly demonstrated (Cadeé 2011, Wada et al. 2012, Van Leeuwen *et al.* 2012 a, Simonova et al. 2016). Although our method necessarily involved freezing of the samples, making a survival test unfeasible, our study provides evidence that endozoochory may be a valid dispersal process for four different snail taxa in wetlands of southern Brazil. Further studies in which fresh

Figure 2. Helobia piscium shell with parts of animal body inside, found in a faecal sample from coscoroba swan.

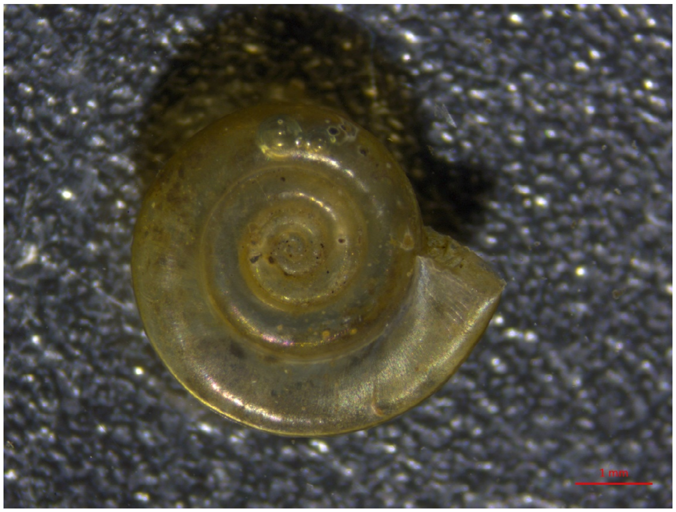

Figure 3. Drepanotrema sp. shell found in a faecal sample from coscoroba swan.

samples are analysed immediately after collection in the field are needed to assess whether these snails were indeed viable.

Some reports indicate that *Heleobia piscium*, the most abundant species observed in our study*,* is distributed in the Coastal Plain of Rio Grande do Sul and in the region of La Plata River estuary (Darrigran *et al.* 1998, Pfeifer & Pitoni 2003, Coimbra et al. 2013, Martin & Díaz 2016). *Drepanotrema* species are mostly endemic to the Neotropical region, occurring in Southern Brazil, Uruguay and Argentina (Rumi *et al*. 2006, Núñez et al. 2010, Martin *et al*. 2013, Palasio et al. 2019). Shells of *Heleobia piscium* were found in faeces of coscoroba swan and white-faced whistling-duck, and *Drepanotrema* sp. in coscoroba swan. Coscoroba swan is a migratory species and can move up to a thousand kilometres in their seasonal displacement between Argentina and Brazil (Silva et al. 2020). Similarly, white-faced whistling-duck covers hundreds of kilometres in their regular movements through the region, according to resource availability. The distributions of *Heleobia piscium* and *Drepanotrema* sp. overlap with those of coscoroba swan and white-faced whistling-duck, this being consistent with a role for these birds as vectors of snail dispersal.

Table 1. Intact gastropod shells found in faecal samples of three waterbird species in southern Brazil.

Waterbird	**Gastropods**	**Number of shells**	**Number of samples with shells**	**Percentage of samples with shells**
Coscoroba swan				
	Heleobia piscium	479	9	32.1%
	Drepanotrema sp.	2	1	3.6%
	Planorbidae - Morphotype I	7	5	17.8%
	Planorbidae - Morphotype II	1	1	3.6%
White-faced whistling-duck				
	Heleobia piscium	6	1	2.8%
Yellow-billed teal				
	Planorbidae - Morphotype I	1	1	7.7%

Two unidentified Planorbidae morphotypes (Morphotypes I and II) showed morphology characteristic of young individuals, and for that reason the identification to a lower taxonomic level was not possible. Morphotypes I and II were found in coscoroba swan samples, and Morphotype I was also found in faeces of yellow-billed teal, a resident waterfowl that remains in the region all year-round, making local movements between wetlands separated by several km.

Waterfowl body size may lead to variation in the access to different depths for feeding, and consequently to habitat segregation between species (Pöysä, 1983; Green, 1998; Guillemain et al., 2002; Ntiamoa-Baidu et al., 1998). Despite some overlapping, this general pattern was observed for waterfowl in our study, where extremes of body size (large and small) may affect the species composition of seeds dispersed by endozoochory (Silva et al. 2021). Coscoroba swan was the largest bird species (c.3.500 g), and had access to deepest water for feeding (c.1–1.5 m), where they often fed with the head or neck partially submerged (Silva et al., 2021). In contrast, yellow-billed teal (c. 500 g) fed by dabbling at the surface of shallower water (c. 0.5 cm) while white-faced whistling-duck (c. 800 g) fed by submerging their head in the same deep water (Silva et al., 2021). These differences in access to the bottom of the waterbody, combined with possible unknown differences in the preferred diet, may explain the variation in the abundance of shells among waterfowl species.

With the exception of killifish eggs that were found to be retained for at least 30 h inside the digestive tract of coscoroba swan (Silva et al., 2019), there is no information about gut retention times of any other taxa in the waterfowl species studied here. Furthermore, information about flight patterns of South American waterfowl is limited compared with North American or European species. However, considering flight speeds of 50-78 km/h (Welhun 1994) and that a snail may survive at least five hours inside a waterbird (Van Leeuwen et al. 2012 a), it is possible that dispersal of snails recorded in our study may occur over long distances, especially for taxa dispersed by coscoroba swan and white-faced whistling-duck. For example, satellite tracking data from white-faced whistling-duck in Argentina found birds moving up to >600 km away from the capture site, with individuals having daily average movements of 0.1 - 23 km, and a mean of 4 km (Don Pablo Research Team 2012). In this case, stopover sites used during bird displacement can also be important for snail dispersal in the region. Further studies should investigate the survival during gut passage of the snails identified in our study and the success of their dispersal by waterbird endozoochory in neotropical wetlands, as previously demonstrated in other regions.

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Author Contributions

Luiz C. Barboza: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Giliandro G. Silva: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Andy J. Green: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Leonardo Maltchik: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Cristina Stenert: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics

This work was authorized by the Brazilian agency SISBIO (n° 59225-1)

Data Availability

The information necessary to replicate this study is present in the manuscript.

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Article

Fish fauna from a fragmented river in the Atlantic Forest

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Abstract: This study sought to identify the ichthyofauna composition of the Muzambinho River, an upland tributary of the Paraná River. We also investigate whether waterfalls in the region can serve as barriers to the dispersal of fish species. For this purpose, collections were carried out at 34 points on the Muzambinho River using different techniques. In all, 37 species were recorded, some of which were endemic, and the majority were native. Among these species, some are predominant in degraded places and can be used as bioindicators. The results also demonstrate zonation in the ichthyofauna composition in Muzambinho that segregates the fauna into three sessions separated by waterfalls: Lower Muzambinho, Upper Muzambinho, and Sao Domingos. We conclude that, for the ideal preservation of the ichthyofauna of the Muzambinho River, it is necessary to preserve its sections independently, which would guarantee the maintenance of naturally isolated strains.
Keywords: *Upper Parana; Biodiversity; Ictiofauna; Mantiqueira.*

Fauna de peixes de um rio fragmentado na Mata Atlântica

Resumo: Este estudo buscou identificar a composição da ictiofauna do rio Muzambinho, um tributário de terras altas do Rio Paraná. Também buscamos investigar se as cachoeiras da região podem servir de barreiras para dispersão de espécies de peixes. Para isso, foram realizadas coletas em 34 pontos do Rio Muzambinho, com diferentes técnicas. Ao todo foram registradas 37 espécies de peixes, sendo algumas endêmicas e a maioria nativa. Dentre essas espécies algumas são predominantes em locais degradados e podem ser usadas como bioindicadores. Os resultados também demonstram que há uma zonação na composição ictiofaunística no Muzambinho que segrega a fauna em três sessões separadas por cachoeiras, Baixo Muzambinho, Alto Muzambinho e São Domingos. Concluímos que para a ideal preservação da ictiofauna do rio Muzambinho é necessária a preservação independente de suas sessões o que garantiria a manutenção de linhagens naturalmente isoladas.
Palavras-chave: *Alto Paraná; Biodiversidade; Ictiofauna; Mantiqueira.*

Introduction

The Neotropical freshwater ichthyofauna accounts for almost 30% of the world's freshwater fish species, with more than 6000 described species and potentially a further uncataloged 3000 species (Reis et al. 2016). Most of these species are distributed in the Amazon, Orinoco, and Paraná–Paraguay river basins, which are among the largest rivers worldwide. However, several researchers have pointed out that a significant percentage of this biodiversity is found in small headwater streams (Castro 1999, Langeani et al. 2007).

Headwater streams (perennial water bodies of the first and second order) (Fagan 2002, Meyer et al. 2007, Richardson 2019) are a prominent feature of the Neotropical region. The Headwater streams provide small areas of suitable habitat for residents species, resulting in relatively small isolated populations and ultimately leading to population segregation and speciation (Richardson 2019, Richardson & Danehy 2007). Furthermore, physical barriers, such as waterfalls and temperature gradients, can constitute impediments to the dispersal of stream species (Kurylyk et al. 2015, Torrente-Vilara et al. 2011), which can in turn contribute to the formation of refugia and eventually to the maintenance of relictual lineages (Buckup 2011), regardless of their taxonomic distinction. This segregation can also promote accelerated adaptive radiation, as small populations tend to be more susceptible to genetic drift-related factors, such as natural selection and local extinction (Frankham et al. 2004).

Located in the Brazilian Shield, the Muzambinho River is a typical Atlantic Forest river, which are commonly characterized by their high variation between lotic and lentic stretches. They are also home to a high diversity of endemic fish species whose evolutionary history is generally closely associated with the evolution of the rivers themselves (Castro 1999). The Muzambinho is a fourth-order river that can be divided into three sections separated by two waterfalls. 1) The Kita waterfall, locates at the mouth of the São Domingo stream, separates this tributary from the Muzambnho river. 2) The Usina waterfall separates the lower from the upper Muzambinho (Figure 1). This region, in which human activities are based on agriculture and livestock rearing, is characterized by an incredible diversity of plants and terrestrial animals (Domingos, 2014) typical of high-altitude Atlantic Forest areas and biodiversity hotspots. However, the fish fauna inhabiting the Muzambinho River in this region have yet to be systematically studied.

Given the growing anthropogenic pressure on natural areas and the lack of knowledge regarding the fish fauna of rivers and streams that occur in the Muzambinho basin, we conducted a detailed survey of the ichthyological components of the Muzambinho River (upper Paraná) to establish whether the waterfalls that separate the main stretches of this river can serve as barriers to the dispersal of species, and consequently provide havens for species inhabiting the upper reaches.

Material and methods

1. *Study area*

The Muzambinho river presents both lentic and lotic systems. The is entirely present in the Serra da Matiqueira region, in the south of Minas Gerais. The Muzambinho flows into the Muzambo river, an important tributary of the Grande river. Small agricultural companies and enterprises mostly occupy the surroundings of the Muzambinho River. The climate is typically tropical at altitude, and the predominant vegetation is Atlantic forest. The sampling points were divided into three groups, which are herein defined as sections: (1) the São Domingos stream (SD - P1 to P14), (2) the lower Muzambinho (LM - P15 to P22), and (3) the upper Muzambinho (UM - P23 to P34). The definition of sections is based on the location of two waterfalls mentioned above, the Kita (coordinate 21°17’37.94”S 46°29’6.33”W) and Usina (coordinate 21°21’0.79”S 46°31’2.87”W), and on the premise that these waterfalls represent physical barriers to the dispersal of populations (Figure 1).

2. *Sampling design*

Sampling was conducted at 34 collection points approximately 100 m from the main river channel based on sweeping at sampling points.

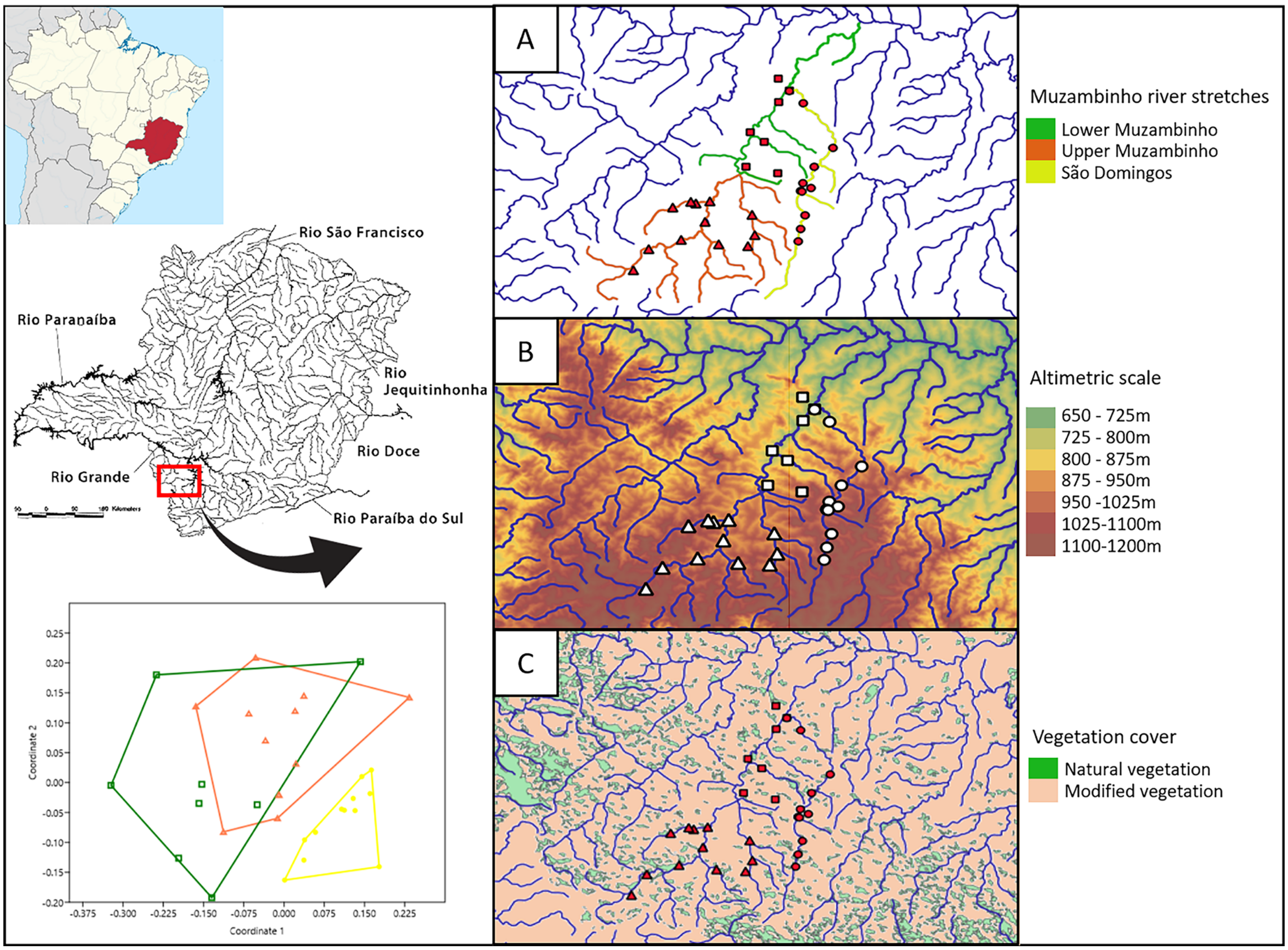

Figure 1. Study area map and Muzambinho river sections characteristics. A. A hydrographic map of the Muzambinho River basin. B. An altimetric map of the Muzambinho River basin. C. A map showing the vegetation cover of the Muzambinho River basin. Triangles, squares, and circles denote collection points in the Alto Muzambinho, Baixo Muzambinho, and São Domingos sections, respectively. The nMDS graphic shows the isolation between river section communities, mainly the São Domingos (yellow) from Upper Muzambinho (red) and Lower Muzambinho (green).

We applied a combination of active and passive capture methods to obtain samples that were as representative as possible of the total ichthyofauna present in each area sampled. Sampling was performed using manually deployed nets, trawls, and gillnets. Each sampling point was georeferenced, and the collection team assessed the corresponding environmental characteristics, such as water speed, type of bottom substrate, and state of preservation of the riparian forests by visual estimation. The sampling team consisted of four members, totaling approximately 384 hours of total sampling effort (about 2.5 hours of collection effort per sampled point). All three field trips were carried out in the rainy summer period.

Small and medium-sized fish (up to 15 cm in length) were fixed and preserved in 95% ethanol. Larger fish (over 15 cm) were fixed in 10% formaldehyde and transferred to 70% ethanol. Whenever feasible, collected specimens were identified to the lowest possible taxonomic level with the aid of identification keys and the assistance of specialists in the field. All specimens have been deposited in the fish collection of the Botucatu Fish Biology and Genetics Laboratory (LBP).

3. *Similarity analysis*

To visualize differences between river sections' faunal compositions, we performed an similarity analysis-"Muldimensional Scaling (nMDS)" in the PAST 3 software (Hammer *et al.* 2001). In this analysis, we used the Bray-Curtis similarity index, and for the other parameters, we used the Default.

Results

Different types of environments characterized each sampled section. The bottom substrates varied considerably from sandy to muddy, sandy-muddy, gravel, pebbles, rocks, and slabs. The preservation status of riparian vegetation ranged from well-preserved stretches to those that had been substantially degraded and converted to pastures, plantations, or urban areas. We collected 4,101 individual fish belonging to six orders, 24 genera, and 37 species (Table 1). The species were not homogeneously distributed along the sampled sections, with some showing a notably restricted distribution within the basin (Table 1). The similarity analysis of nMDS reinforces this hypothesis showing that the fish communities of the sections are distinct. Especially when we compare the São Domingos community to the other two studied, this result suggests that waterfalls, especially Kita, can act as a barrier to the dispersion of species. (Figure 1)

Section LM was found to be characterized by the highest species richness (27 species), among which only 11 species were also found in other sections. The lowest richness (15 species) was recorded in section UM, with four species being characterized as exclusive, whereas 17 species were collected from section SD, of which five were section specific (see Table 1).

Table 1. List of species collected in the demarcated sections of the Muzambinho River. (UM) Upper Muzambinho section; (LM) Lower Muzambinho section, (SD) São Domingos stream. The first number in each cell represents the total of individuals sampled in each section and the value in parentheses indicates the number of sample points at which the species was captured.

Order/Family	Species ID	UM	LM	SD
Characiformes				
Characidae				
	Astyanax altiparanae Garutti & Britski, 2000		1 (1)	
	Psalidodon fasciatus (Cuvier, 1819)		21 (5)	
	Psalidodon paranae (Eigenmann, 1914)	22(5)	25 (5)	46 (4)
	Bryconamericus stramineus Eigenmann, 1908		56 (2)	
	Piabina argentea Reinhardt, 1867		54(5)	
Anastomidae				
	Leporinus marcgravii Lütken, 1875		2(1)	
	Leporinus striatus Kner, 1858		5(1)	
Prochilodontidae				
	Prochilodus lineatus (Valenciennes, 1837)		1(1)	
Crenuchidae				
	Characidium aff. *zebra* Fowler, 1914		45 (5)	
	Characidium aff. *gomesi* Travassos, 1956	8(3)	36 (2)	96 (19)
	Characidium gomesi Travassos, 1956			12(1)
Erythrinidae				
	Hoplias malabaricus (Bloch, 1794)	13(3)	7(4)	19(6)
Gymnotiformes				
Gymnotidae				
	Gymnotus sp.	32(3)	1(1)	3(2)
Sternopygidae				
	Eigenmannia sp.		2(2)	

Discussion

The Muzambinho River is home of at least 37 species of fish, which corresponds to 11% of the total species inhabiting the Alto Paraná, within an area less than 0.04% of the total (Langeani et al. 2007). Compared with other rivers of similar size in this region, such as the Sapucai River inhabited by 24 species (Azevedo-santos *et al.* 2019), the Muzambinho River could be considered relatively rich in species. The ichthyofauna of the Muzambinho River brings together species such as *Neoplecostomus langeanii* and *Cetopsorhamdia* sp., which appear to be exclusively distributed in this basin, indicating that it may be a region of high endemism, a characteristic of brook rivers (Richardson 2019).

Some of our observations in the present study have raised concerns regarding the preservation of this biodiversity. For example, we recorded the occurrence of alien species introduced in the region, such as *Synbranchus marmoratus* and *Gymnotus* sp., whereas residents in this region have indicated that other nonnative species, such as *Oreochromis niloticus* and *Cyprinus carpio,* were not sampled in the study. In addition, at certain sampling points, we noted the disproportionate prevalence of bioindicator species such as *Poecilia* sp. and *Phalloceros harpagos*, which tend to proliferate in disturbed environments (Vieira et al. 2007). Collectively, these observations may serve to indicate that the degradation of habitats and the introduction of alien species in this region are contributing to a loss of diversity and local extinction (Reis et al. 2016).

This scenario is of particular concern if the populations in question are endemic and isolated, as thus, there may be little or no opportunity to restore populations in the event of local extinction (Bizerril 1998, Richardson 2019). Our findings showing that migratory and widely distributed species, such as *Leporinus* spp., *Iheringichthys labrosus*, *Hoplosternum littorale*, *Psalidodon fasciatus*, and *Astyanax altiparanae* (Langeani et al. 2007)*,* do not occur in the UM and SD regions tend to indicate that the two aforementioned waterfalls delimit these sections, effectively isolating these stretches from section LM (Figure 1), and acting as barriers to species dispersal. The nMDS analysis confirms segregation, at least the separation of SD from the other two sections (Figure 1). Further evidence of the efficacy of these waterfalls as physical barriers is the presence of several exclusive species (11 in total) in the stretches of river upstream of these barriers.

Neoplecostomus langeanii, a highly rheophilic species (Bressman et al. 2020; Menezes et al. 2007), reinforces the assumed segregational role played by the Kita and Usina waterfalls as barriers to dispersal. It is predicted that constant gene flow between populations would promote population homogeneity (Frankham et al. 2004). However, *N. langeanii* has undergone population segregation for thousands of years (Roxo et al. 2012). These data are even more evident when considering that this is a highly rheophilic species and might be one of the species most adept at negotiating barriers of this type.

Modern preservationist approaches tend to be based on the maintenance of the most significant possible number of strains, regardless of taxonomic rank (Frankham et al. 2004). Accordingly, given that the stretches of river surveyed in the present study appear to be characterized by a relatively distinctive ichthyofauna, they should ideally be preserved independently; local extinctions could represent the total extinction of distinct lineages.

Supplementary Material

The following online material is available for this article:

Table S1. Species per sampled point.

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Authors' Contributions

Paulo Roberto Monteiro de Brito: Contribution to data analysis and interpretation, contribution to manuscript preparation, and contribution to critical revision, adding intellectual content.

Luiz Henrique Garcia Pereira: Contribution to manuscript preparation, substantial contribution in the concept and design of the study contribution to data collection.

Fábio Fernandes Roxo: Contribution to manuscript preparation, substantial contribution in the concept and design of the study contribution to data collection.

Jefferson Henriques Monteiro: Contribution to manuscript preparation, substantial contribution in the concept and design of the study contribution to data collection.

Claudio Oliveira: Contribution to manuscript preparation, substantial contribution in the concept and design of the study contribution to data collection.

Guilherme José da Costa Silva: Contribution to data analysis and interpretation, contribution to manuscript preparation, substantial contribution in the concept and design of the study contribution to data collection, and contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

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Inventory

Living at the top of the forest line: medium and large mammals in a high-mountain ecotone in Peruvian Central Andes

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Abstract: Among the ecoregions that comprise the high-Andean zone of the Tropical Andes, the Puna and the Yungas stand out for covering a large part of the Peruvian Eastern Andes. Located in the ecotone of these two ecoregions, the Pampa Hermosa National Sanctuary (PHNS) houses one of the priority areas for conservation in Peru. However, the biodiversity of the high-Andean zone of the sanctuary and its surroundings remains poorly studied. Thus, through camera traps and transects, we sought to inventory for the first time the medium and large mammals from the high-Andean region of the PHNS and its buffer zone. We recorded 11 native and three domestic species of medium and large mammals. The richness of native mammals sampled reached 91.7% of the estimated richness (S est.= 11.99 ± 1.85). Among the native species *Odocoileus virginianus* had the highest relative frequency (56%). We recorded three endemic mammals from the Tropical Andes, *Cuniculus taczanowskii*, *Tremarctos ornatus*, and *Pudu mephistopheles*. The observed richness was higher than most surveys of medium and large mammals carried out in the Puna-forest ecotone in Peru, where the reported richness ranged from 4 to 13 species. In addition, our records of *Leopardus pardalis* and *Eira barbara* are the highest for all distribution of these two carnivores. Our results showed that more than 90% of the species recorded were found in it, demonstrating that the entire high-Andean region of the PHNS and its surroundings has an important value for the local mammal community.
Keywords: *Camera-trap; Eira barbara; Leopardus pardalis; Odocoileus virginianus; Puna; Tropical Andes.*

Vivendo no topo da linha florestal: mamíferos de médio e grande porte em um ecótono alto-montano nos Andes Centrais Peruanos

Resumo: Dentre as ecorregiões que compreendem a zona alto-andina dos Andes Tropicais, a Puna e as Yungas se destacam por abranger grande parte dos Andes Orientais peruanos. Localizado no ecótono entre estas duas ecorregiões, o Santuário Nacional Pampa Hermosa (SNPH) abriga uma das áreas prioritárias para conservação no Peru. No entanto, a biodiversidade da zona alto-andina do santuário e seu entorno permanece pouco estudada. Dessa forma, através de armadilhas fotográficas e transectos, buscamos inventariar pela primeira vez os mamíferos de médio e grande porte da região alto-andina do SNPH e de sua zona de amortecimento. Nós registramos 11 espécies nativas e três espécies domésticas de mamíferos de médio e grande porte. A riqueza observada dos mamíferos nativos atingiu 91,7% da riqueza estimada (S est.= 11,99 ± 1,85). Dentre as espécies nativas, *Odocoileus virginianus* foi a que apresentou a maior frequência relativa (56%). Registramos três mamíferos endêmicoss dos Andes Tropicais, *Cuniculus taczanowskii*, *Tremarctos ornatus*, e *Pudu mephistopheles*. A riqueza observada foi superior que a maioria dos levantamentos de mamíferos de médio e grande porte feitos em ecótono Puna-bosque no Peru, onde a riqueza reportada variou de 4 a 13 espécies. Além disso, os registros de *Leopardus pardalis* e *Eira barbara* são os mais elevados para toda distribuição destes dois carnívoros. Nossos resultados demonstraram que mais de 90% das espécies registradas foram encontradas nela, demonstrando que toda região alto-andina do SNPH e seu entorno tem um importante valor para a fauna de mamíferos local.
Palavras-chave: *Andes Tropicais; Armadilhas fotográficas; Eira barbara; Leopardus pardalis; Odocoileus virginianus; Puna.*

Introduction

Peru is considered as one of the most megadiverse countries in the world (Noss 1990, Shanee et al. 2017) and much of this biological diversity is due to the climatic and geographic influence created by the Andes Mountains and the Humboldt Maritime Current (Fajardo et al. 2014). The diversity of ecosystems and habitats created by these factors give rise to a scenario where the neotropical fauna finds countless opportunities for speciation and radiation (Pacheco et al. 2009).

Among the countries with the greatest diversity of mammals, Peru is in the fourth place, with 569 species (Pacheco et al. 2020), only behind Brazil within the neotropical region (SERFOR 2018). Of the 10 large ecoregions existing in Peru (Brack-Egg 1986), the Selva Baixa and the Yungas (or Selva Alta) have the highest rates of mammalian diversity and endemism, respectively (Pacheco et al. 2009). However, with a significant mammal diversity (63 spp.) (Pacheco et al. 2009), the Puna is the dominant ecoregion of the Peruvian high-Andean zone, rising from 3,000-3,500 m to 4,500-5,000 m (Rolando et al. 2017). The Puna is characterized by high-altitude grasslands, and it is subdivided into two ecosystems based on rainfall: (1) Dry Puna, which spans the high plateaus of southern Peru; and (2) Wet Puna, which is found from the center-south to the center-north of the Peruvian Andes (Josse et al. 2009). In the ecotone regions between the Wet Puna and the Cloud Forests of Yungas, a long transition zone is formed between grassland and forest environments, where there is a great sharing of the native fauna and flora of these two ecoregions (Simpson 1983, Buytaert et al. 2011).

Because they are regions with extremely rugged terrain, adverse climatic conditions, and difficult access (Jiménez et al. 2010), these high-Andean zones of the Peruvian Eastern Andes are poorly studied (Pacheco et al. 2009, Medina et al. 2012) and, consequently, the status of biological diversity remains with several local gaps in most of these Andean areas. In this sense, the Protected Areas are essential tools for the conservation of this Andean biodiversity to be explored and, therefore, have a fundamental role in the scientific and ecological development of the country (Shanee et al. 2017, Bax & Francesconi 2019).

In the Peruvian Andes only 36% of the biological diversity existing is protected by the Protected Areas system (Fajardo et al. 2014). This becomes even more worrying given the fact that Peru is the country with the largest extension of the Tropical Andes, one of the main hotspots of global biodiversity and holder of the highest rate of endemism in the world (Myers et al. 2000, Josse et al. 2011).

Located in the Peruvian Central Andes, the Pampa Hermosa National Sanctuary (PHNS) houses an important transitional area of the Puna and Yungas ecoregions of the department of Junín (SERNANP 2012). Despite being considered a priority area for the conservation of Andean-Amazonian biodiversity (SERNANP 2009, 2012, Arias et al. 2016), few expeditions sought to systematically inventory the local biological diversity, especially in the high-Andean zone (> 2,500 m) from the sanctuary, due to its difficult access. Historically, the fauna of the region was first studied in the mid-19th century by naturalist Johann Jakob von Tschudi in the Montaña de Vitoc (Tschudi, 1844a, 1844b). In this pioneering expedition, fish, amphibians, reptiles, birds and mammals were collected (Serrano-Villavicencio et al. 2020), mainly in the Yungas ecoregion, about 20km away from the PHNS. In the recent decades, among the groups already systematically inventoried in the PHNS and its surroundings, there are insects, birds (Silva et al. 2016), bats (Arias et al. 2016) and plants (La Torre-Cuadros et al. 2007, Silva et al. 2016). However, all these surveys were carried out in the lower part (< 2,000 m) of the sanctuary and its buffer zone.

Thus, the biodiversity of the high-Andean zone of the sanctuary and its surroundings remains poorly studied, especially with regard to the terrestrial mammals community. In the PHNS buffer zone , there are several reports of conflict between the communities in the San Pedro de Churco village and the Andean bear (*Tremarctos ornatus*) (Rojas-Vera Pinto & Butrón 2016, Rojas-Vera Pinto 2019), signaling the presence of important endangered species in the sanctuary area. For this reason, in this present study, we sought to inventory, for the first time, the medium and large mammals from the high-Andean zone of the PHNS and its buffer zone. In addition, we compared our observed richness with that of other surveys carried out in similar altitudinal ranges in the Peruvian Eastern Andes.

Material and Methods

1. *Study area*

The study was carried out in the PHNS (75º35’09” - 75º24’43”W; 10º58’53” - 11º06’30”S) and in its buffer zone, concentrating on the west side of sanctuary, where the S.P. Churco village (75º33’24”W; 11º02’13”S) is located (Figure 1). The site is situated on the eastern flanks of the Tropical Andes, in the department of Junín, central Peru (SERNANP 2012). The area of the PHNS is 11,543 ha and its buffer zone is 20,292 ha, totaling 31,835 ha of direct and indirect protection of the Andean-Amazonian Peruvian mountain ecosystems along a wide altitudinal range (1,130 to 4,080 m) (SERNANP 2012). The study area was concentrated in the high-Andean zone of the sanctuary and its surroundings, covering an area of approximately 2,500 ha, between 3,200 and 3,700 m.

The study area is located in an ecotone, where two ecosystems belonging to distinct ecoregions are found (Pulgar-Vidal 1987): (1) the Wet Puna of the Puna ecoregion, extending from 3,250 to 5,000 m, characterized by high-Andean grasslands with herbaceous-shrubby vegetation padded with mosses and grasses arranged in clumps (Brack 1986, López 2010) and; (2) the Cloud Forests (or High-Montane Forests) of the Yungas ecoregion, present from 2,500 to 3,300 m, with dense, steep forest vegetation and with a great abundance of epiphytes (Pulgar-Vidal 1987, SERNANP 2012).

The climate of the PHNS and its buffer zone presents a gradual variation along its altitudinal ranges (Junquas et al. 2018, Eghdami & Barros 2019). The highest part of the sanctuary where the Wet Puna and the S.P. Churco village are located corresponds to the climate of High-Montane Dry Tundra (ETH) according to Köppen (1936) (SERNANP 2012). The average annual precipitation varies from 2,000 to 3,000 mm, with greater concentration in the summer and with an average annual temperature of 6 to 10ºC (Yarupaitan & Giraldo 2007, SERNANP 2012). As the altitude decreases to 2,600 m, there is a zone that comprises, the Continental Humid Boreal (Dwb) climate (Köppen 1936). This range also presents low average annual temperatures, ranging from 10 to 12ºC and high average annual precipitation between 3,000 and 4,000 mm (SERNANP 2012, Eghdami & Barros 2019).

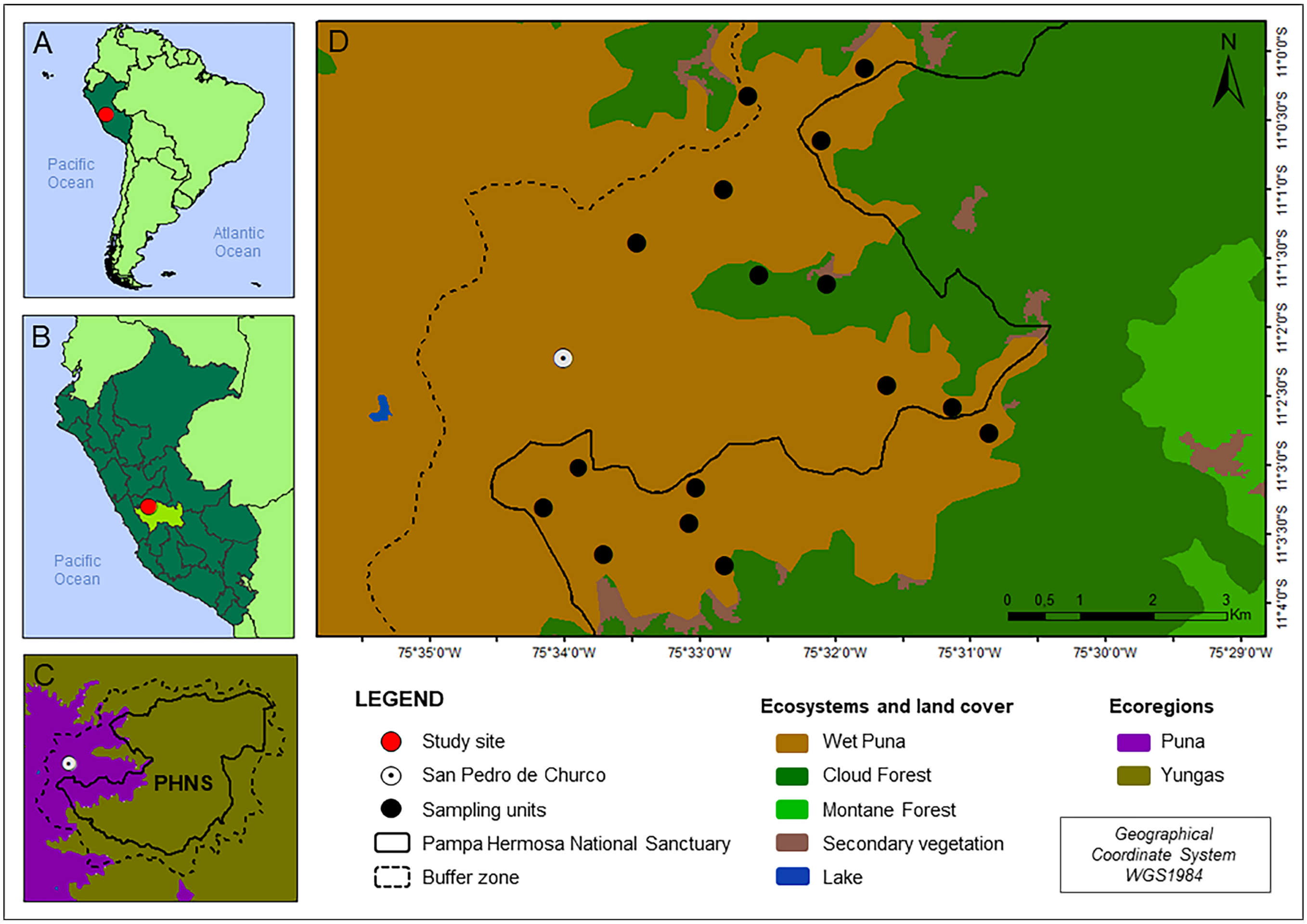

Figure 1. a) Location of Peru in South America and the study site; b) Location of department of Junín (light green) in Peru and the study site; c) Ecoregions covering the Pampa Hermosa National Sanctuary (PHNS) and its buffer zone and location of the S.P. Churco village; d) Ecosystems and land cover of the high-Andean region of the PHNS and its buffer zone and location of the 16 sampling units in the study area.

2. *Data collection*

We distributed 16 sampling points in the study site, eight inside the PHNS and eight in its buffer zone (Figure 1). We used two complementary sampling methods, camera traps and active search through transects, at a sampling unit scale of 1 km². Each sampling point was centered on a camera trap (Bushnell Trophy Cam HD 2018 ©), respecting a minimum distance of 1 km between them, with a maximum deviation of 20% being accepted, due to inaccessibility and difficulty of installation in some areas. We installed the camera traps in places commonly used by mammals through the recognition of signs and animal tracks and no bait was used to avoid the artificial attraction of animals (Srbek-Araújo & Chiarello 2013). We configured the equipment to capture three consecutive photos every 10 seconds, whenever the motion sensor was triggered. We carried out the maintenance of the camera traps every six months to change the batteries and collect the stored data. The sampling time was one year and six months (May/2019 – November/2020), a sampling effort of 4,402 traps/day.

For the methodology of active search for transects, we covered 1 km trails (of people and animals) in each of the 16 sample units, in search of mammal tracks (e.g., feces, tracks, food consumed, marks on trees) and direct visualization. The tracks found along each transect were registered and georeferenced. We carried out three visits to each sampling unit (1st: May/2019; 2nd: October/2019; 3rd: November/2020), totaling approximately 48 km of transects covered within the sample units.

3. *Data analysis*

For the independence of the records obtained by camera traps, we considered an interval of 1 h between the photographic records of all mammals with more than 1 kg (Srbek-Araújo & Chiarello 2005). In order to measure the sampling sufficiency of the survey, we constructed species rarefaction curves using the Estimate S 9.1.0 program (Colwell 2013) with the Chao 2 estimator. For this purpose, we used the independent records obtained through camera traps and transects, using one week as the sampling effort unit (n=76). We also calculated the relative frequency (RF) by camera traps for each taxon using the formula given in percentage: (nº records of the species/nº total records) x 100.

The scientific nomenclature used followed Pacheco et al. (2009, 2020) and Nascimento et al. (2021). For species' identifications we followed Eisenberg & Redford (1989), Pereira & Aprile (2012), and Duarte & González (2010). We also consulted specialists in cases of uncertainty in the identifications. For the identification of tracks, we used the guides of Torres (2011) and Becker & Dalponte (2013). The threat status of native species at the national level followed the Red List of Threatened Species of Peru (SERFOR 2018), and at the global level followed the International Union for Conservation of Nature's Red List of Threatened Species (IUCN 2021).

Results

We recorded 11 native and three domestic species of medium and large mammals distributed in five orders and nine families (Table 1 and Figures 2, 3). We obtained 525 independent records through camera traps of 10 native species and three domestic mammal species in the study area. By the transect method, we obtained 70 records from four species of native mammals and three domestic species, with *Puma concolor* being the only species recorded by feces alone.

The richness of native mammals sampled (S obs.= 11) reached 91.7% of the estimated richness (S est.= 11.99 ± 1.85), showing a stabilization of the rarefaction curve from the 40th week onwards (Figure 4). The order Carnivora was the most representative, accounting for 72.7% of the native species recorded, followed by Artiodactyla and Rodentia. The native species with the highest total RF was *Odocoileus virginianus* (25.5%), followed by *Conepatus chinga* (7.8%) and *Lycalopex culpaeus* (7.0%). In addition, 90.9% of native mammals were registered in the PHNS buffer zone, surpassing the proportion found within the sanctuary boundaries, which was 63.6% (Table 1).

The proportion of endemic species of Tropical Andes recorded in the area was 27.2% (*Tremarctos ornatus*, *Pudu mephistopheles* and *Cuniculus taczanowskii*). Only two species of mammals surveyed are categorized as Vulnerable*,* *T. ornatus* and *P. mephistopheles*; nonetheless, only the former species is also listed as Vulnerable globally. On the other hand, *P. mephistopheles* together with *Leopardus garleppi* present deficient data for a categorization of their conservation status globally. In addition, two species (*P. concolor* and *C. taczanowskii*) are listed as Near Threatened within Peruvian territory. Finally, 63.6% of recorded native mammals have a status of Least Concern as pertains their conservation at a global level, 72.7% of them show a trend towards population reduction according to the IUCN (2021).

The domestic species *Bos taurus* had the highest number of photographic records among all the surveyed mammals and, consequently, the highest RF (31.04%), in addition to being found both inside the PHNS and in its buffer zone. In total, native species obtained 45.24% of photographic records against 54.75% of records of domestic species (Table 1).

Table 1. Medium and large mammals recorded in the high-Andean region of the Pampa Hermosa National Sanctuary and its buffer zone, department of Junín, Peru and Tropical Andes endemic species, sampling methods, number of records, relative frequency, recording site and national (SERFOR, 2018) and global (IUCN, 2021) conservation status of recorded species. Legend: *Domestic species. Sampling methods: camera trap (Ct); feces (Fe); carcass (Ca); food consumed (Fc); tracks (Tr); visualization (Vi). N(Ct) = number of independent records through camera traps. N(Tr) = number of records through tracks and direct views. RF(Ct) = relative frequency by camera traps. Species record location: Pampa Hermosa National Sanctuary (NS), buffer zone (BZ). Conservation status: Least Concern (LC); Near threatened (NT); Vulnerable (VU), Data Deficient (DD). Population trend (Pt) at global level (IUCN, 2021): stable (s); reducing (r).

Taxon	Common name	Endemic	Methods	N(Ct)	N(Tr)	RF(Ct)	NS/BZ	Status conservation	
								Peru	IUCN (Pt)
ARTIODACTYLA									
Cervidae									
Odocoileus virginianus (Zimmermann, 1780)	White-tailed Deer	-	Ct, Fe, Tr, Vi	134	5	25.52	NS/BZ	LC	LC (s)
Pudu mephistopheles (de Winton, 1896)	Northern Pudu	En	Ct	3	0	0.57	NS/BZ	VU	DD (r)
Bovidae									
Bos taurus (Linnaeus, 1758)*	Cattle	-	Ct, Fe, Tr, Vi	163	33	31.04	NS/BZ	-	-
CARNIVORA									
Canidae									
Lycalopex culpaeus (Molina, 1782)	Andean Fox	-	Ct	37	0	7.04	NS/BZ	LC	LC (s)
Canis lupus familiaris (Linnaeus, 1758)*	Domestic dog	-	Ct, Tr	28	2	5.33	NS/BZ	-	-
Felidae									
Leopardus garleppi (Matschie, 1912)	Garlepp’s Pampas Cat	-	Ct	1	0	0.19	NS	DD	DD (r)
Leopardus pardalis (Linnaeus, 1758)	Ocelot	-	Ct	3	0	0.57	NS/BZ	LC	LC (r)
Puma concolor (Linnaeus, 1771)	Puma	-	Fe	-	1	-	BZ	NT	LC (r)
Mephitidae									
Conepatus chinga (Molina, 1782)	Molina's Hog-nosed Skunk	-	Ct	41	0	7.8	NS/BZ	LC	LC (r)
Mustelidae									
Eira barbara (Linnaeus, 1758)	Tayra	-	Ct	3	0	0.57	BZ	LC	LC (r)
Mustela frenata (Lichtenstein, 1831)	Long-tailed Weasel	-	Ct, Ca	4	1	0.76	BZ	LC	LC (s)
Ursidae									
Tremarctos ornatus (F. G. Cuvier, 1825)	Andean Bear	En	Ct, Fe, Fc, Tr	10	19	1.9	NS/BZ	VU	VU (r)
PERISSODACTYLA									
Equidae									
Equus caballus (Linnaeus, 1758)*	Horse	-	Ct, Fe, Vi	97	9	18.47	NS/BZ	-	-
RODENTIA									
Cuniculidae									
Cuniculus taczanowskii (Stolzmann, 1865)	Mountain Paca	En	Ct	2	0	0.38	BZ	NT	NT (r)

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Figure 2. Medium and large native mammals recorded by camera traps in the high-Andean region of the Pampa Hermosa National Sanctuary and its buffer zone, departament of Junín, Peru: a) *Lycalopex culpaeus*; b) *Leopardus garleppi*; c) *Leopardus pardalis*; d) *Tremarctos ornatus*; e) *Conepatus chinga*; f) *Pudu mephistopheles*; g) *Odocoileus virginianus*; h) *Cuniculus taczanowskii*.

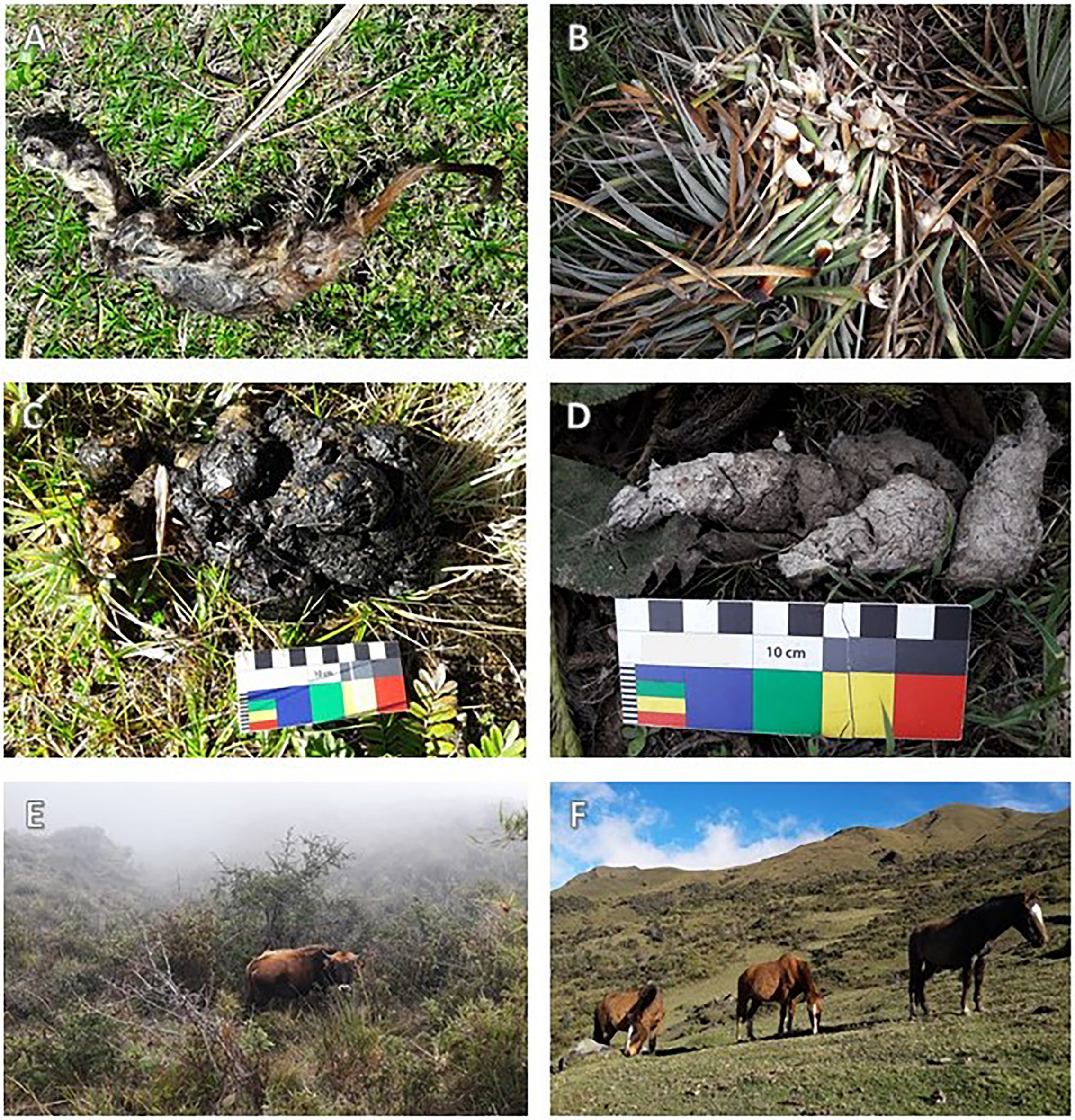

Figure 3. Medium and large sized mammals recorded through tracks and direct views in the high-Andean region of the Pampa Hermosa National Sanctuary and its buffer zone, department of Junín, Peru: a) *Mustela frenata* carcass; b) bromeliad of the genus *Puya* eaten by *Tremarctos ornatus*; c) *T. ornatus* feces; d) *Puma concolor* feces; e) Creole *Bos taurus* grazing native vegetation; f) *Equus caballus* grazing in the high-Andean grasslands.

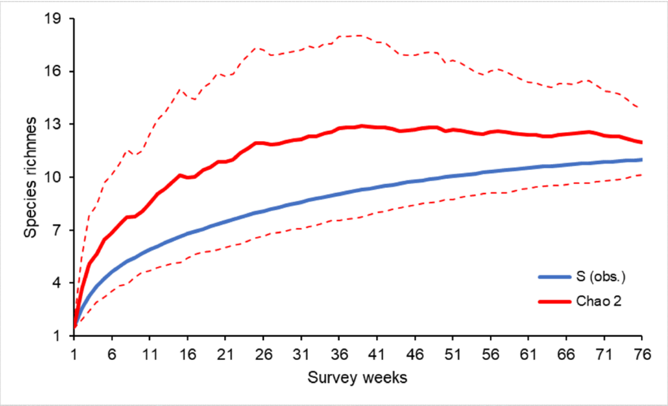

Figure 4. Rarefaction curve of medium and large mammals recorded in the high-Andean region of the Pampa Hermosa National Sanctuary and its buffer zone, department of Junín, Peru. Legend: Obs.: total observed richness; S est.: richness estimated by *Chao 2* (95% confidence interval – red dashed lines).

Discussion

The present study is the first and only systematic survey that sought to inventory the medium and large mammals present in the PHNS and its buffer zone. The richness of mammals was higher than most surveys of medium and large mammals carried out between the Wet Puna and Cloud Forests (2,000 – 4,000 m) in Peruvian Eastern Andes, where the reported richness ranged from 4 to 13 species (Shanee & Shanee 2018, López 2020) (Table 2). Taking into account the altitude range of the surveys, only studies carried out in regions with a difference greater than or equal to 1,000 m obtained a higher number of species than in our study (Medina et al. 2012, Shanee & Shanee 2018), which was performed at an altitudinal amplitude of only 500 m (Table 2). Furthermore, in most of aforementioned surveys the study areas are located mostly in the Yungas region, which has a known greater diversity of mammals (Pacheco et al. 2009). Unlike our study area, where the Puna ecoregion is dominant, there is a recognizably lower diversity of mammals compared to the Yungas (Pacheco et al. 2009, 2020). In addition, the present study is one of the few surveys that used only primary data in its methodology compared to other studies conducted in similar altitudinal ranges in the Peruvian Eastern Andes (Table 2).

The richness of medium and large mammals found in the high-Andean region of the PHNS and its buffer zone represents about 65% of the native mammal species of this group with known distribution for the study area (17 spp.) (Bernal 2016, Cassola 2016, Pacheco et al. 2009, 2020). Thus, six potential species were not recorded for the area: taruca (*Hippocamelus antisensis*), llama (*Lama glama*), Andean cat (*Leopardus jacobita*), southern mountain viscacha (*Lagidium viscacia*), hairy long-nosed armadillo (*Dasypus pilosus*), and Andean opossum (*Didelphis pernigra*). The first four species are typically found in higher rocky areas of the Andes (FAO 2005, Barrio 2010, Cossíos et al. 2012a, Bernal 2016), making it difficult to record these mammals in transition zones between Puna and Yungas (J. Barrio 2021, personal communication), especially taruca, llama and southern mountain viscacha that only occur in the Puna (Pacheco et al. 2009). The hairy long-nosed armadillo and the Andean opossum are found mainly in the Yungas (Pacheco et al. 2020), so possibly the presence of only 18% of our camera traps in the Cloud Forests of the Yungas may have rendering the recording of these two species in the area difficult.

Regarding the species richness of the two ecoregions in which the study area is located, we recorded 41.1% of the community of medium and large mammals known to occur in the Peruvian Puna ecoregion (17 spp.) (Pacheco et al. 2009, 2020). While, in relation to the Cloud Forests (>2,500 m) of the Peruvian Yungas, we found 40.7% of medium and large mammals known for this ecosystem (27 spp.) (Pacheco et al. 2009, 2020). Based on literature, *P. mephistopheles*, *L. pardalis* and *E. barbara* were only known to occur in the Yungas and other forest ecosystems in Peru (Sanborn 1953, Hurtado et al. 2016, Shanee & Shanee 2018, Pacheco et al. 2020). Nonetheless, we recorded theses three species in areas of Puna (Table 3). These are the first well-documented and georeferenced records within the Peruvian territory that confirm the presence of these three species in this Andean ecoregion. These records demonstrate the ecological plasticity of these typically forest mammals in the use of different types of environments for foraging and obtaining resources (Lyra-Jorge et al. 2008a, Escamilo et al. 2010, Lima et al. 2020, Pasa et al. 2021).

In addition, we recorded the species *P. mephistopheles*, *L. pardalis* and *E. barbara* at the highest altitudes known to the literature in Peruvian territory (Pacheco et al. 2020) (Table 3). For *P. mephistopheles* the increment was 56 m (3,506 m) in relation to the previous record of highest altitude of the species in Peru (3,450 m) (Shanee & Shanee 2018).

Table 2. Comparison of species richness of medium and large mammals found in the present study and in other surveys carried out in the Puna and Cloud Forest ecotones of the Peruvian Eastern Andes, in addition to the altitudinal range (in meters above sea level), altitude range amplitude (in meters) and sampling methods used in each study: camera trap (Ct); tracks and casual observations (Tr); interview (In); bibliographic review (Br).

Article	Location	Altitudinal range (amplitude)	Methods	Richness
Present study	Pampa Hermosa National Sanctuary	3,200-3,700 (500)	Ct, Tr	11
Pacheco et al. (2007)	Apurímac River Basin	2,751-3,500 (749)	Tr, In	10
Ramirez et al. (2007)	Tupala/Acjanaco	3,450-4,000 (550)	Tr	7
Jiménez et al. (2010)	Pagaibamba/ San Lorenzo Forests	2,500-3,700 (1,200)	Ct	8
Medina et al. (2012)	Kcosñipata Valley	2,550-3,600 (1,050)	Tr, In	12
Shanee & Shanee (2018)	Valley of Marañón and Huallaga Rivers	2,000-3,000 (1,000)	Tr, In	13
Pacheco & Noblecilla (2019)	Carpish Mountain Forest	2,700-3,000 (1,300)	Tr, In	6
Palomino & Ataucusi (2019)	Huáscaran National Reserve	2,300-4,000 (1,700)	Br	11
López (2020)	Yanachaga-Chemillén National Park	1,900-3,200 (1,300)	Ct	4

Table 3. Comparison between altitudinal ranges (in meters above sea level) and high-Andean ecoregions (Puna and Yungas) of occurrence of medium and large mammal species recorded in this study in relation to known records for the Peruvian territory according to Pacheco et al. (2020). Legend: [a]According to Medina et al. (2012). [b]According to Pacheco et al. (2009). [P]Highest altitude record for the species within its range of occurrence in Peru. [G]Highest altitude record for the species for its distribution range. *These species can also occur in other Peruvian ecoregions.

Species	Altitudinal range in Peru	Altitudinal range of records	Puna/ Yungas*	Record ecoregions
Odocoileus virginianus	3-4,400	3,326-3,699	P/Y	P/Y
Pudu mephistopheles	2,000-3,450	3,379-3,506[P]	Y	P
Lycalopex culpaeus	3-4,800	3,326-3,699	P/Y	P/Y
Leopardus garleppi	0-4,982	3,655	P/Y	P
Leopardus pardalis	150-3,379	3,210-3,623[G]	Y	P/Y
Puma concolor	3-5,800	3,523	P/Y	P
Conepatus chinga	0-4,530	3,326-3,649	P/Y	P/Y
Eira barbara	150-3,379	3,326-3,439[G]	Y	P/Y
Mustela frenata	1,514-4,000	3,326-3,573	P/Y	P/Y
Tremarctos ornatus	210-4,750	3,210-3,655	P/Y	P/Y
Cuniculus taczanowskii	1,920-3,530[a]	3,326-3,469	P/Y[b]	P/Y

However, throughout its distribution, this small deer has already been found at 4,500 m in the Ecuadorian Páramos (Escamilo et al. 2010). As for *L. pardalis*, the altitudinal increment was 244 m (3,623 m) whereas for *E. barbara* it was 60 m (3,439 m) compared to previous records of 3,379 m recorded by Jiménez et al. (2010) for both species in northern Peru (Table 3). These species are rarely reported for such high altitudes, and the altitudinal distribution of *E. barbara* hardly exceeds 1,200 m (Cuarón et al. 2016), while *L. pardalis* is uncommon to be found above 3,000 m (Paviolo et al. 2016). Thus, these records of *L. pardalis* and *E. barbara* are also the ones with the highest altitude for the entire area of occurrence of these two Neotropical carnivores.

The order Carnivora was the most representative in the study area, having 72.7% of the species belonging to this taxon, although most of them have a low relative frequency. Our results are similar to other studies with medium and large mammals conducted in transitional Puna-forest regions in the Peruvian Andes (Pacheco et al. 2007, Jiménez et al. 2010, Medina et al. 2012, Shanee & Shanee 2018). Despite occurring at low densities, species of this order have large home ranges and high mobility (Robinson & Redford 1986, Hodge & Arbogast 2016), increasing the probability of these mammals being detected at least once, certainly depending on the sampling effort. Furthermore, most of the registered carnivores have generalist habits, having the ability to explore resources in different environments (Lyra-Jorge et al. 2008b, Cossíos et al. 2012b), moving from more forested landscapes such as the Cloud Forests, to open areas like the Puna. The presence of large top-chain carnivores, such as *Puma concolor* and *Tremarctos ornatus*, also demonstrates that the high-Andean region of the PHNS and its buffer zone has healthy populations of prey and good environmental integrity (Cardillo et al. 2004).

Among the recorded native species, *Odocoileus virginianus* had the highest relative frequency (25.5%), and this rate is even higher when compared only among records of native mammals (56%). Such a high relative frequency of this deer species for the Eastern Andes has never been reported in the literature. The highest relative frequency recorded for *O. virginianus* reported for the Eastern Andes was 15.2% found by Jiménez et al. (2010) in a survey carried out in the Páramos and Cloud Forests of northern Peru. For other areas of Latin America with occurrence of the species, such as Protected Areas of Mexico, Honduras and Ecuador, this frequency rate varies from 1 to 9% only (Monroy-Vilchis et al. 2011, Gonthier & Castañeda 2013, Lizcano et al. 2016). Despite occurring in several ecosystems of the Neotropical region, *O. virginianus* presents greater abundances in dry tropical forests and regions of grassland-forest ecotone, such as our study area (Delfin 2002, Gallina et al. 2010). Furthermore, in the high-Andean zones there is a partitioning of habitat use between *O. virginianus* and *Hippocamelus antisensis*, with *O. virginianus* tending to avoid such high altitudes (> 3,700 m) and rocky environments (Barrios 2006), but it also does not have a great preference for tropical rainforests, such as the Yungas (Palomino & Ataucusi 2019). For these reasons, the high relative abundance of this deer is due to the altitudinal range and the puna-forest ecotone that the study area is located in, generating very favorable environmental conditions for the establishment of the species. Therefore, the high presence of *O. virginianus* in the area makes the high-Andean zone of PHNS a key location in Peru for potential ecological studies of the species, which still lacks information on its population and conservation status in the Peruvian territory (Gallina et al. 2010).

Among all recorded mammals, *Bos taurus* was the most frequent. The high presence of this domestic species in the area is due to the extensive livestock by the high-Andean rural communities (Paisley 2001, Goldstein 2002). The absence of more productive livestock management makes the rustic cattle move freely over extensive areas, enabling interaction with native species, generally in a negative way (Goldstein et al. 2006). A study by Barrio (2006) in the Peruvian Andes showed that the presence of cattle has a negative effect on the habitat use of native deer *O. virginianus* and *H. antisensis*, displacing these species to other areas. On the other hand, this extensive livestock makes the cattle very vulnerable to attacks from native predators such as pumas, foxes and Andean bears, generating retaliation by local communities and, consequently, persecution of these carnivores (Goldstein et al. 2006, Rojas-Vera Pinto et al. 2019). In addition, unmanaged cattle grazing in these high-Andean regions can impact negatively the soil, the water retention of the high-Andean mash, the biodiversity of plants, aquatic macro invertebrates and even the abundance of specialist birds in the upper Andean grasslands (Astudillo et al. 2018, Avellaneda-Torres et al. 2018, Machaca et al. 2018, Meza-Salazar et al. 2020).

The proportion of endemic species of Tropical Andes as *Cuniculus taczanowskii*, *T. ornatus*, and *P. mephistopheles* was relatively low for the area. This might be explained by the fact that the study was mostly carried out in the Puna ecorregion, which has an endemism rate of only 15.4% in Peruvian territory (Pacheco et al. 2009). In turn, the Yungas and the Low Jungle are the ecoregions with the largest number of endemic mammal species in the Tropical Andes (Myers et al. 2000, Pacheco et al. 2009, 2020).

According to the IUCN, about 73% of the species registered in the study show a trend towards population reduction, indicating the relevance of the area for mammal conservation. However, only two species (*T. ornatus* and *P. mephistopheles*) are listed as threatened in Peru (Velez-Liendo & García-Rangel 2018, SERFOR 2018), and globally, *P. mephistopheles* still lacks data to define its current conservation status (Barrio & Tirira 2019). Considered as smallest deer in the world, *P. mephistopheles* is still a poorly known species, especially the populations of central Peru, which are isolated from populations north of the Andes (Ecuador and Colombia), where there is more information about the species (Escamilo et al. 2010). Due to its low density, the record of three individuals of *P. mephistopheles* at the study site highlights the importance of the conservation of this area to maintain this species in the region.

Although the area belonging to the PHNS has a good conservation status of its Andean-Amazonian ecosystems, its surrounding areas are being impacted by human activities at different levels (SERNANP 2012). Extensive livestock and firewood extraction are the main threats to the ecosystems of the high-Andean region of the sanctuary's buffer zone, where the S.P. Churco village is located. Despite having a high environmental vulnerability, our results showed that more than 90% of the species recorded were found outside the sanctuary boundaries, showing that this high-altitude buffer zone has an important value for the high-Andean mammals. However, these anthropogenic disturbances added to climate change, could strongly impact the diversity, abundance, and distribution of these mammals in the long term, mainly if natural resources are not rationally used.

As the first systematic survey of medium and large mammals for the PHNS region, we demonstrate that the area has a good representation of this biological community in the high zone of the Peruvian Eastern Andes.

In addition, this study is one of the few surveys concentrated on the Wet Puna that only uses primary data and one of the first for the entire Peruvian department of Junín. Biological knowledge gaps in the high-Andean ecosystems are still huge in most Peru. The results we presented here offer new and important information on the local diversity of mammals, as well as updates on the altitudinal distribution of some species. However, further studies are needed on the biodiversity of the high-Andean zone of the PHNS and its surroundings, mainly because the high-Andean ecosystems are the most vulnerable and threatened to climate change throughout the Tropical Andes.

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Author Contributions

Mateus Melo-Dias: substantial contribution in the concept and design of the study, data collection, data analysis and manuscript preparation.

Jesenia Flora Aliaga Huatuco: substantial contribution to data collection and design of the study.

Marco Aurelio Arizapana-Almonacid: contribution in the concept and design of the study, data collection, data analysis and critical revision, adding intelectual content.

Marco Italo Castañeda-Tinco: contribution to data collection, data analysis and interpretation.

Fernán Chanamé: substantial contribution in the concept and design of the study and data collection.

Jesus Ulloa Ninahuamán: substantial contribution in the concept and design of the study and data collection.

Marcelo Passamani: substantial contribution in the concept and design of the study, data collection and critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that there are no conflicts of interests related to the publication of this manuscript.

Ethics

The authors declare compliance with all guidelines established by ethics committees' universities and government agencies involved. They also state that the manuscript has not been previously published.

Data Availability

The authors declare that the data present in this manuscript may have open access. The data resulting from this research has been archived in the public data repository Biota Neotropica Dataverse, that provides free access and guaranteed preservation. Access URL: https://data.scielo.org/dataset.xhtml?persistentId=doi:10.48331/scielodata.3KOVKP.

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Article

Hummingbird-plant networks in rupestrian fields and riparian forests in altitudinal areas of the Serra da Canastra National Park, Minas Gerais, Brazil

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Abstract: Hummingbirds are specialized in consumption of nectar and play an important role in Neotropical plant communities acting as pollinator organisms. Despite the importance of this mutualistic interaction, studies about hummingbird-plant relationships remain scarce regarding the Cerrado domain (Brazilian savanna). In this study, we aimed to describe the interaction network between hummingbirds and plants in rupestrian fields and riparian forests located in altitudinal areas of the Serra da Canastra National Park. We established two transects in each phytophysiognomy, that were sampled monthly for four days, from November 2018 to October 2019. Flowering plants in each transect were observed each survey, and all the visiting hummingbirds were recorded. Networks were constructed using the R bipartite package, considering each phytophysiognomy type, and grouping data of both environments. From these three network arrangements, we extracted complementary metrics at the community level (modularity, nestedness, and network specialization index), and at the species level (species specialization index and species strength in the network). We recorded 647 interactions between 10 hummingbird species and 23 flowering plant species. The hummingbird Colibri serrirostris was responsible for most of observed bird-plant interactions and the plant Qualea cordata was the most visited. The general network was significantly modular, comprising four modules, and showed considerable high specialization and low nestedness. The interaction network in the rupestrian field showed a higher specialization, nestedness, and modularity index when compared to riparian forests, while the metrics of this ecosystem did not differ from those of the general network. However, the metrics at hummingbird species level did not differ significantly between phytophysiognomies. This study corroborated some findings about hummingbird-plant networks in other areas of the Cerrado domain, but also pointed idiosyncrasies in networks of the investigated phytophysiognomies, especially the rupestrian fields.

Keywords: *Cerrado; gallery forest; nectivory; pollination; rocky grassland.*

Redes de beija-flor-planta em campos rupestres e florestas ripárias em áreas de altitude do Parque Nacional da Serra da Canastra, Minas Gerais, Brasil

Resumo: Beija-flores são especializados no consumo de néctar e desempenham um papel importante em comunidades de plantas Neotropicais, agindo como organismos polinizadores. Apesar da importância dessa relação mutualística, estudos sobre a relação de plantas e beija-flores ainda são escassos no Cerrado. Neste estudo, objetivamos descrever a rede de interações entre beija-flores e plantas em campos rupestres e matas ripárias localizadas em áreas de altitude do Parque Nacional da Serra da Canastra. Estabelecemos dois transectos em cada fitofisionomia, os quais foram amostrados mensalmente durante quatro dias, de novembro de 2018 a outubro de 2019. Plantas em floração em cada transecto foram observadas em cada amostragem, e todos os beija-flores visitantes foram registrados. As redes de interação foram construídas utilizando o pacote bipartite do R, considerando cada fitofisionomia e agrupando os dados de ambos os ambientes. Destes três arranjos de rede, extraímos métricas complementares no nível de comunidade (modularidade, aninhamento e índice de especialização de rede) e no nível de espécies (índice de especialização da espécie e força da espécie na rede). Registramos 647 interações entre 10 espécies de beija-flores e 23 espécies de plantas. O beija-flor Colibri serrirostris foi o responsável pela maior parte das interações ave-planta observadas e a planta Qualea cordata foi a mais visitada. A rede geral foi significativamente modular com quatro módulos e apresentou considerável especialização e baixo aninhamento. A rede de interações no campo rupestre apresentou maior especialização, aninhamento e índice de modularidade quando comparada à mata ripária, enquanto as métricas deste ecossistema não foram diferentes da rede geral. Entretanto, as métricas no nível de espécies de beija-flores não apresentaram diferenças significativas entre as fitofisionomias. Este estudo corrobora alguns resultados de redes de beija-flores-plantas em outras áreas do domínio do Cerrado, mas também aponta idiossincrasias nas redes das fitofisionomias investigadas, especialmente os campos rupestres.

Palavras-chave: *Campo rupestre; cerrado; mata de galeria; nectivoria; polinização.*

Introduction

Interactions between plants and animals are present in all terrestrial ecosystems, and biotic pollination is estimated to occur in 94% of tropical plants (Ollerton et al. 2011). These mutualistic interactions create complex networks and the comprehension of patterns and processes that structure them can be used to predict the dynamics and stability of biological communities (Bascompte 2009, Thompson et al. 2012, Schleuning et al. 2015, Bartomeus et al. 2016; Tinoco et al. 2017). Recently, an increasing number of studies have been elucidated pollination relationships patterns and its architecture in the light of the network approach (e.g., Vizentin-Bugoni et al. 2014, Souza et al. 2018, Maruyama et al. 2019). However, networks remain under investigated in the tropics with a particular gap in central Neotropical savanna areas (Vizentin-Bugoni et al. 2018), especially in attitudinal environments and open vegetation.

The Cerrado is the second largest morphoclimatic and phytogeographic domain of Brazil and the most biodiverse savanna of the world (Ratter et al. 1997, Ribeiro & Walter 1998). It harbors diverse types of phytophysiognomies, that encompasses grasslands, savanic and forest formations, considered within the Cerrado *sensu lato* classification, and other singular ecosystems such as rupestrian environments and riparian forests (see Ribeiro & Walter 1998). The rupestrian fields, regionally called *campos rupestres,* are a montane vegetation complex that occurs over rock outcrops (Alves et al. 2014, Silveira et al. 2016). It is predominantly dominated by herbaceous and shrubby vegetation that have xeromorfic features (Alves et al. 2014, Conceição et al. 2016, Morellato & Silveira 2018). The riparian forests occur alongside waterbodies and, unlike the adjacent grassland vegetation, are little affected by water restriction (Ribeiro & Walter 1998, Coelho et al. 2007). This feature may have implications for the dynamics of interactions between animals and plants since the phenology of plant species is often associated with the water regime (Conceição et al. 2007). Additionally, animals can move between different habitats according to the availability of resources, preferences for habitats and behavioral characteristics (Maruyama et al. 2014, 2019, Rodrigues & Rodrigues 2015). Therefore, differences between phytophysiognomies may play an important role in structuring the plant-pollinator interaction networks, which is particularly evident among hummingbirds (e.g., Maruyama et al. 2014, 2019, Rodrigues & Rodrigues 2015).

Hummingbirds are highly specialized in nectar consumption and act as important pollinator agents in the Neotropics, in such an extent that many plant species have evolved adaptations to attract then (Jordano 1987, Cronk & Ojeda 2008). Interactions between hummingbirds and plants provide good models to explore the structure of mutualistic networks, due to high dependence of hummingbirds on nectar, convenient sampling, and wide distribution of hummingbird species across nearly the entirety of the Americas (McGuire et al. 2014, Vizentin-Bugoni et al. 2018). In this sense, several studies have addressed structural patterns in hummingbird-plant networks, such as considerable specialization (e.g., Maglianesi et al. 2014), modular structure (e.g., Maruyama et al. 2014) and usually low nestedness (e.g., Vizentin-Bugoni et al. 2014). In other words, hummingbird-plant networks are mainly characterized by: (1) few species have many partners and/or interactions, while most have just a few links (Bascompte et al. 2006); (2) modules of species that interact strongly with each other (Olesen et al. 2007); and (3) species with few interactions do not always form subgroups with species with many interactions (Bascompte et al. 2003). Despite these general patterns, hummingbird-plant networks can show considerable variability through different habitats (e.g., Maruyama et al. 2019).

In altitudinal areas inside the Cerrado domain, the riparian forests and open vegetation in rupestrian fields can occur in mosaics at landscape levels (IBAMA 2005, Fieker et al. 2014), creating an interesting situation in which hummingbirds can use floral resources from both ecosystem types. Thus, using the ecological network approach, we aimed to characterize the interactions between hummingbirds and their floral resources in the main plateau of the Serra da Canastra National Park (SCNP), a federal Conservation Unit in the state of Minas Gerais, Brazil, where, as far as we know, there are no surveys on mutualistic interactions between bird pollinators and the visited flower community. We investigated whether the interactions between hummingbirds and their floral resources differ between vegetation types, reflected in network topologies and specialization metrics.

Material and Methods

1. *Study area*

This study was conducted in the SCNP, in the plateau known as *Chapadão da Canastra* (20°13’51.52”S, 46°29’11.39”W; Cunha et al. 2019) located in the southwestern portion of the Minas Gerais state, Brazil (IBAMA, 2005). The geographical limits comprise an area of 71,525 ha at 1,250 to 1,450 m of elevation (Cunha et al. 2019), harboring several phytophysiognomies of the Cerrado domain. The regional climate is characterized by a well-marked seasonality, with cold and dry winters (dry season) from April to September, and rainy summers (wet season) from October to March (IBAMA, 2005). Our fieldwork was carried out in two phytophysiognomies: the rupestrian fields (*campos rupestres*), characterized by open vegetation (grassland and open savanna) in gravelly/sandy soil with clumps of rocky outcrops in altitudinal mountaintops, and the riparian forests, composed by riverine forests alongside streams and rivers, and small patches of forests in hydromorphic soils, commonly associated to water sources and connected with gallery forests.

2. *Field methods*

From November 2018 to October 2019, we made 11 expeditions to the SCNP with an interval of 30 to 35 days. Each data collection comprised four days of observations in four different sites. Two transects were established in riparian forest (each of approximately 100 m) and two in the rupestrian fields (each of approximately 200 m) (see Silva 2021 for detailed information). The differences in the transect length between the two phytophysiognomies is due to the difficulty in going through the fragments of riparian forests. Each site was sampled monthly during morning and afternoon, from 8 am to 12 pm and later from 3 pm to 6 pm (approximately seven hours per site per month), totalizing 308 hours of sampling effort. Observations of hummingbird-plant interactions normally start at sunrise (Machado & Rocca 2010). However, we defined the observation time as mentioned above due to logistical difficulties to reach the study sites.

Flowering plants were observed for 30 minutes with direct visualization with the aid of binoculars (Nikon 8 x 42 mm). Whenever possible, more than one plant was observed simultaneously.

For each plant species, we recorded the number of visits by hummingbirds, considering as one visit the foraging performed within an uninterrupted flight regardless of the number of flowers visited (Las-Casas et al. 2012). A new visit was counted when the bird perched and returned to the floral resource or when it left the site and returned to forage on flowers again. Only legitimate visits were considered, that is, when the bird accesses the floral resource through the opening of the corolla and meets the reproductive structures of the flower.

Identification of the hummingbirds were confirmed with the help of a specialized guide when necessary (Grantsau 1989) and scientific nomenclature followed Piacentini et al. (2015). Samples from all plants visited were collected for identification to the highest possible taxonomic resolution and subsequently deposited at the herbarium of the Federal University of São Carlos (SPSC, acronym according to Thiers 2020).

3. *Data analysis*

To characterize and analyze hummingbird-plant network in the SCNP, we built interaction matrices weighted by the total number of visits observed between pairs of species. In these, three arrangements of interaction networks considering the entire period of study were considered: (1) general interaction network, in which all data collected in this study were grouped into a single matrix; (2) network of interactions in the rupestrian fields; (3) network of interactions in the riparian forests.

From these different network arrangements, we extracted three complementary metrics that describe different aspects of the network, namely: modularity, nestedness and network specialization index. The modularity in interaction networks allows the identification of subgroups (modules) of species that interact strongly with each other and with less intensity with species outside the module (Olesen et al. 2007). Here, we compute modularity through DIRTLPAwb+ algorithm (Beckett 2016). The modularity index ranges from 0 to 1, for the minimum modularity and maximum modularity, respectively. Nestedness was calculated using the weighted version of the metric NODF, the wNODF (Almeida-Neto & Ulrich 2011, Almeida-Neto et al. 2008). The wNODF index range is from 0 to 100, indicating, in this order, the minimum and maximum nestedness structure. The complementary specialization of the network was estimated using the metric H2’. This index is derived from Shannon entropy and describes the degree of specialization of a quantitative network (Blüthgen et al. 2006). Its variation is from 0 to 1, indicating the lowest and highest specialization, respectively.

The metrics of the observed networks described above were compared with the averages of the estimated metrics of 1.000 random null models, using Z tests. We consider the significance level of 0.05, so if test values ranged between -1.96 and 1.96, it is accepted the null hypothesis of equality of metrics observed at the expense of those expected at random. The null models were generated using the vaznull method (Vázquez et al. 2007). In this procedure, the observed network connectance is maintained and the totals of individual interactions are randomized. The analyzes were performed using the “bipartite” package (Dormann et al. 2008), with the metrics extracted by the function “networklevel” (Dormann et al. 2009).

Regarding the species level network metrics, we consider only the metrics related to hummingbirds, as the plants may have other pollinators that were not identified here. The estimated metrics were: (1) species specialization by the d’ index and (2) species strength in the network. The first metric calculates how much a species deviates from a random sample of available interaction partners (Blüthgen et al. 2006). Its variation occurs from 0 to 1, in a minimum and maximum specialization scale, respectively. The last one estimates the sum of the dependencies of each species (Bascompte et al. 2006). We obtained these metrics also through the “bipartite” package, using the function “specieslevel” (Dormann 2011). All analyzes were performed in the computational environment R Core Team (2020).

Results

We recorded 647 interactions from 10 hummingbird species (Table 1) on 23 flowering plant species distributed in 14 plant families (Table 2). Seven species of hummingbirds visited 12 plant species in rupestrian fields and nine hummingbird species visited 13 species in riparian vegetation (see Figure 1). Only two plant species were recorded in both phytophysiognomies: *Psittacanthus robustus* and *Hololepis pedunculata*. Six bird species were observed interacting with plants in both vegetation types, while *Calliphlox amethystina* used nectar resources only in rupestrian fields, and *Amazilia fimbriata*, *Anthracothorax nigricollis*, and *Heliomaster squammosus* interacted with flowering plants only in riparian forests.

Table 1. Hummingbird assemblage (Aves: Trochilidae) recorded visiting plant species in riparian forests and rupestrian fields in the Serra da Canastra National Park, MG, southeastern Brazil, from Nov/2018 to Oct/2019. NI = number of interactions recorded; ss = species strength in the network; d’ = species specialization.

Species	**Rupestrian fields**			**Riparian forests**			**General network**		
	NI	**ss**	**d'**	**NI**	**ss**	**d'**	**NI**	**ss**	**d'**
Phaethornis pretrei (Lesson & Delattre, 1839)	2	0.0329	---	29	1.5443	0.4948	31	1.4737	0.4756
Eupetomena macroura (Gmelin, 1788)	56	1.3011	0.7252	51	0.9211	0.2615	107	1.6659	0.4548
Colibri serrirostris (Vieillot, 1816)	152	9.2305	0.6234	207	4.8778	0.5947	359	13.2649	0.6
Anthracothorax nigricollis (Vieillot, 1817)	---	---	---	2	0.2857	0.7374	2	0.2857	0.7483
Chlorostilbon lucidus (Shaw, 1812)	18	0.8687	0.2917	84	3.1769	0.1335	102	3.6593	0.1729
Thalurania furcata (Gmelin, 1788)	2	0.0294	0.1427	23	0.7204	0.5017	25	0.7119	0.4759
Amazilia fimbriata (Gmelin, 1788)	---	---	---	4	0.0889	0.4368	4	0.0354	0.2839
Amazilia lactea (Lesson, 1832)	2	0.0941	0.2192	8	0.3627	0.1619	10	0.4569	0.2027
Heliomaster squamosus (Temminck, 1823)	---	---	---	3	1.0222	0.7155	3	1.0088	0.6705
Calliphlox amethystina (Boddaert, 1783)	4	0.4433	0.5613	---	---	---	4	0.4374	0.6105

Table 2. Plants visited by hummingbirds in riparian forests and rupestrian fields of the Serra da Canastra National Park, MG, southern Brazil, from Nov/2018 to Oct/2019. ● Plants with ornithophilous syndrome; N.V = Number of visits. D = Degree of visits by hummingbirds.

Family/Species	Habitat	N.V	D
Acanthaceae			
Justicia monticola (Ness) Profice ●	riparian forest	75	4
Amaryllidaceae			
Hippeastrum cipoanum (Ravenna) Meerow ●	rupestrian field	1	1
Asteraceae			
Asteraceae sp.1	riparian forest	2	1
Lessingianthus sp.	rupestrian field	5	2
Eremanthus sp.	rupestrian field	3	1
Hololepis pedunculata (DC. ex Pers.) DC. ●	riparian forest - rupestrian field	55-26	5-3
Lychnophora sp.	rupestrian field	7	2
Wunderlichia mirabilis Riedel ex Baker	rupestrian field	4	2
Bignoniaceae			
Pyrostegia venusta (Ker Gawl.) Miers ●	riparian forest	6	1
Bromeliaceae			
Aechmea bromeliifolia (Rudge) Baker ●	riparian forest	7	3
Dyckia minarum Mez ●	rupestrian field	14	2
Vriesea friburguensis Mez ●	riparian forest	6	3
Ericaceae			
Gaylussacia brasiliensis (Spreng.) Meisn. ●	riparian forest	38	3
Gaylussacia pseudogaultheria Cham. & Schltdl ●	rupestrian field	44	3
Gaylussacia reticulata Mart. ex Meisn. ●	rupestrian field	3	1
Lamiaceae			
Hypenia reticulata (Mart. ex Benth.) Harley	rupestrian field	2	1
Loganiaceae			
Spigelia sellowiana Cham. & Schltdl. ●	riparian forest	2	1
Loranthaceae			
Psittacanthus robustus (Mart.) Mart. ●	riparian forest -rupestrian field	68-45	5-6
Orobanchaceae			
Esterhazya splendida J.C.Mikan ●	riparian forest	6	3
Sapindaceae			
Serjania erecta Radlk	riparian forest	5	2
Theaceae			
Laplacea fruticosa (Schrad.) Kobuski	riparian forest	7	2
Velloziaceae			
Barbacenia lymansmithii Mello-Silva & N.L.Menezes	rupestrian field	30	1
Vochysiaceae			
Qualea cordata Spreng.	riparian forest	186	4

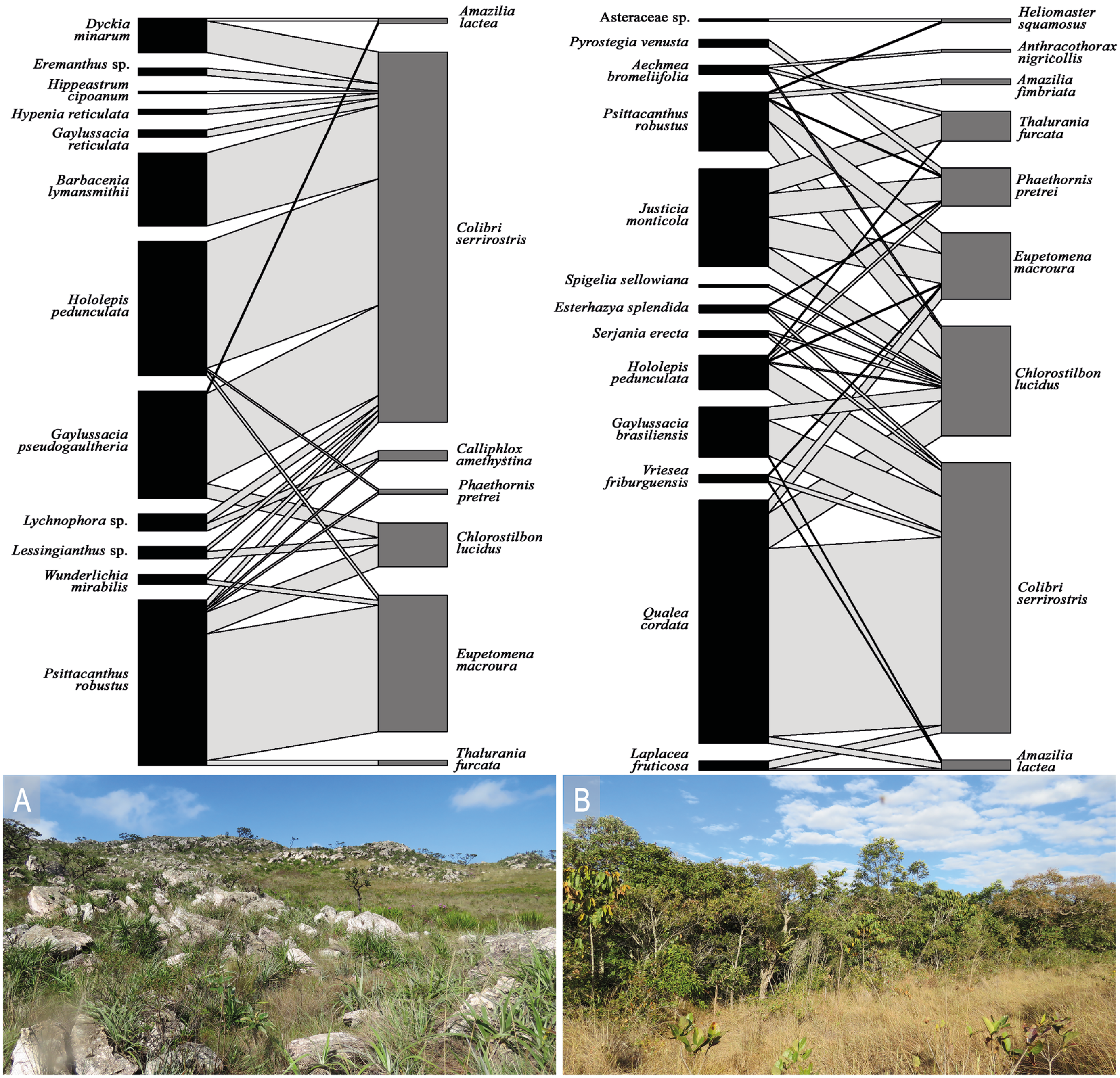

Figure 1. Plant-hummingbird network in (A) rupestrian fields and (B) riparian forests of the Serra da Canastra National Park, from Nov/2018 to Oct/2019. Plant species are represented by black and hummingbirds by gray polygons.

Colibri serrirostris was responsible for most of observed bird-plant interactions (55.49%), followed by *Eupetomena macroura* (16.54%) and *Chlorostilbon lucidus* (15.76%). The high number of interactions observed for *C. serrirostris* was mainly attributed to visits to the entomophilous species *Qualea cordata* during massive flowering events, representing 22.27% of all observed interactions, which made this plant the most visited in the study area. The ornithophilous species *Psittacanthus robustus*, *Hololepis pedunculata* and *Justicia monticola* also received a high number of visits, however, from more species of hummingbirds (Figure 1 and Table 2).

The overall hummingbird-plant network was significantly modular (Qobs = 0.36, Qd = 0.24, z-score = 17.58, p-value < 0.0005), comprising four modules of interacting species (Figure 2). Of those, two modules were composed of a single interacting pair, *Heliomaster squamosus* grouped with Asteraceae sp.1 and *Calliphlox amethystina* with *Lychnophora* sp. The hummingbirds *C. serrirostris* and *Amazilia lactea* were arranged in a single module with 14 plant species. The module with the highest number of hummingbird species, namely, *Amazilia fimbriata*, *Antrhacothorax nigricollis*, *Chlorostilbon lucidus*, *Eupetomena macroura*, *Thalurania furcata* and the only hermit species in the community, *Phaethornis pretrei*, has been associated with eight plant species, as shown in Figure 2. The network also indicated a considerable specialization (H2obs = 0.44, H2d = 0.32, z-score = 17.12, p-value < 0.0005) and low nestedness (wNODFobs = 33.49, wNODFd = -17.08, z-score = -3.72, p-value < 0.0005).

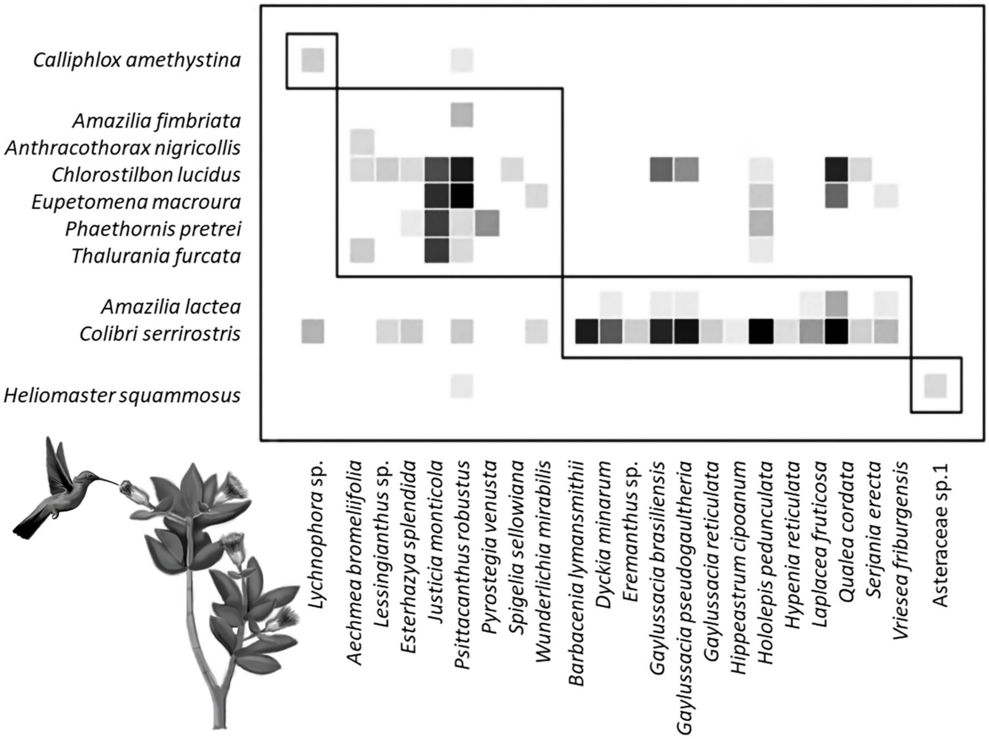

Figure 2. Plant-pollinator community in the main plateau of the Serra da Canastra National Park with indication of interaction abundance (darker tones represent a greater number of records) and the four identified modules of interactions. Data collected from Nov/2018 to Oct/2019.

Regarding to the species level network, the highest values of species strength index were registered to *C. serrirostris* (s.s = 13.2649) and *C. lucidus* (s.s = 3.6593). The lowest values of species strength were attributed to the hummingbird species *A. fimbriata* (s.s = 0.0354) and *A. nigricollis* (s.s = 0.2857). On the other hand, *C. lucidus* obtained the lowest values of d’ index (d’ = 0.1729), indicating to be a generalized pollinator, while *C. serrirostris* was moderately generalized to specialized according to the metric (d’ = 0.6). The species that had d’ indexes above that observed for *C. serrirostris* were *A. nigricollis*, *H. squamosus* and *C. amethystina* (Table 1). These hummingbirds visited one or two plant species, while *C. serrirostris* obtained the highest degree from the community, interacting with 18 plant species, of which it was the exclusive visitor of five plants, including *Barbacenia lymansmithii*, an endemic species only recorded in grasslands and rupestrian fields of the SCNP (Mello-Silva & Menezes 1999).

When considering networks for the two sampled physiognomies, we observed some idiosyncrasies between the environments and in relation to the completeness network. The network of the rupestrian fields showed higher specialization (H2obs = 0.55, H2d = 0.4, z-score = 9.29, p-value < 0.0005) and low nestedness, but obtained the highest wNODF of comparisons (wNODFobs = 44.64, wNODFd = -8.01, z-score = -1.23, p-value > 0.05) and a higher modularity index (Qobs = 0.38, Qd = 0.26, z-score = 9.85, p-value < 0.0005). The estimated network metrics for riparian forest were similar to those observed for the overall community network, therefore, also considerably specialized (H2obs = 0.41, H2d = 0.32, z-score = 15.01, p-value < 0.0005), but with the lowest estimated nestedness of the analyzes (wNODFobs= 24.02, wNODFd = -32.39, z-score = -5.12, p-value < 0.0005) and the same observed modularity index of the general network (Qobs = 0.36, Qd = 0.23, z-score = 15.86, p-value < 0.0005). The hummingbird species level metrics separated by phytophysiognomies did not differ significantly from each other (dfd’ = 11.54, p-value = 0.53; W species strength = 23, p-value = 0.41).

Discussion

Studies on hummingbird-plant networks have revealed structural patterns characterized by heterogeneity in the distribution of interactions between species (Rodríguez-Flores et al. 2019), considerable specialization (Maglianesi et al. 2014), modular structure (Maruyama et al. 2014), and low nestedness (Vizentin-Bugoni et al. 2014). These patterns were also detected in this study, considering the complete network and its arrangements for the two phytophysiognomic types sampled (rupestrian fields and riparian forests). However, when comparing the hummingbird-plant interactions network of the rupestrian field and riparian forest, the rupestrian field network was more specialized and nested than the riparian forest network. The structure of the hummingbird-plant interactions network can be influenced, among other factors, by the animals’ habitat preference for forest or open vegetation (Maruyama 2014, 2019). In this sense, the preference for open habitats of the hummingbird with the highest degree and number of interactions in SCNP seems to lead to differences in network topology between the two phytophysiognomies sampled in this study, as discussed below.

In view of the high topographic heterogeneity, a common feature in the rupestrian ecosystems (Alves et al. 2014), a high spatial turnover in interactions between pollinators and plants can be detected, which are more linked to variations in floristic composition than to differences in pollinator species between sites (see Carstensen et al. 2014).

Similarly, we observed noteworthy differences in the floristic composition, even between the same phytophysiognomy sampled in different areas, while the composition of hummingbird species was similar between the sampling sites. In fact, given the high mobility of hummingbirds and frequent generalized choice of floral resources (Stiles 1981; Wolf et al. 1976), it is expected a more homogeneous distribution of hummingbird species between different sites of the same region.Conversely, considering explicit variations in the vegetation landscape, such as forest patches immersed in open grasslands, the preference for habitat and/or the restriction on the occurrence of certain species before another hierarchically dominant species can emerge as a structuring pattern for interactions (Dupont et al. 2009, Morales & Vázquez, 2008). In this sense, the network topologies were consistently different between environments, with the greatest specialization and nestedness in the rupestrian fields.

The formation of subgroups of species that interact more strongly with each other than with species outside the group, that is, the modular arrangement of interactions (Olesen et al. 2007), supported the findings about the differences in network topologies between phytophysiognomies. The module with the highest number of hummingbird species was, above all, associated with the plant species observed in forest patches. Interestingly, this module also included the long-billed hummingbird, *Phaethornis pretrei*, the only recorded species of the hermit clade, which is capable of travelling long distances using the trapline foraging behavior (McGuire et al. 2014). This result contrasts with other studies also developed in the Cerrado domain, in which *P. pretrei* has been separated from the other hummingbirds in a single module (Maruyama et al. 2014, Queiroz 2018), what can be expected given the frequent association of hermit hummingbirds with morphologically specialized flowers of the ornithophilous plants (Feinsinger & Colwell 1978, Maglianesi et al. 2014, Maruyama et al. 2014). However, this particularity reported here for the interactions between hummingbirds and plants in forest patches does not seem to be associated with greater generalization in the choice of *P. pretrei*, but the greater frequency of visitation to ornithophilous plant species by hummingbirds belonging to other clades.

While the module formed by the largest number of hummingbird species was mostly composed of plant species from forest patches, another module with only two hummingbird species, *Colibri serrirostris* and *Amazilia lactea*, was associated with more than half of the plant species registered in the entire community, mainly with flowering species observed in the rupestrian fields. Similarly, the arrangement of *Colibri serrirostris* and *Amazilia lactea* in the same module closely related to plant species in open landscapes of the Cerrado domain was detected in another study (Maruyama et al. 2013). The hummingbird *C. serrirostris* interacted not only with all plant species in the module, but with all plants recorded in the rupestrian fields. The high degree of visitation performed by this hummingbird indicated its connecting role in the network of rupestrian fields, which is in line with his preference for open vegetation types (Sick 2001). In the forest patches, the role as connector species, according to the degree distribution to the plant species visited, was played by *Chlorostilbon lucidus*.

As pointed out by the modular structure of the network, we found that the interaction network in the rupestrian fields is more specialized and, still, nested than the network in forest patches. These variations in the network topology are probably related to the preference of habitats of the main interacting hummingbird species in each phytophysiognomy. The greater abundance in the rupestrian fields of a hummingbird known to be territorialist (Jacobi & Antonini 2008, Justino et al. 2012) seems to be associated with the greater exclusivity of the interactions in these environments. In this context, *C. serrirostris* was the exclusive visitor of five plant species in the rupestrian field, including the species *Barbacenia lymansmithii*, endemic to the SCNP (Mello-Silva & Menezes 1999). In other study, carried out in rupestrian ecosystems, the main visitor was *C. lucidus* (Rodrigues & Rodrigues 2014), but in this study, it showed the highest degree of visitation in forest patches. The larger body size and high abundance of *C. serrirostris*, coupled with its preference for open habitats, seems to lead *C. lucidus* to forage preferentially in forest environments in the SCNP.

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Associate Editor

Márcia Alexandra Rocca

Author Contributions

Monique Maianne and Carolline Zatta Fieker: contribution in the concept and design of the study; data collection in the field; data analysis and interpretation; and manuscript preparation.

Manoel Martins Dias: contribution in the concept and design of the study; and manuscript preparation.

Matheus Gonçalves dos Reis: contribution in the data analysis and interpretation; contribution to critical review adding intellectual content; and manuscript preparation.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Article

Entomological survey of phlebotominae sand flies (diptera: psychodidae) and vector species in the tegumentary leishmaniasis endemic area in eastern brazilian Amazon, Amapá state

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Abstract: American tegumentary leishmaniasis is an endemic that has increased considerably in recent decades in the Amazon region, sand flies are the vectors of the transmission of the protozoan that causes leishmaniasis, so the objective of this study was to carry out a survey of the diversity of species and the presence of *Leishmania* DNA in vectors circulating in three endemic counties for tegumentary leishmaniasis in the eastern Brazilian Amazon (Amapá state, Brazil). Using CDC light traps, a total of 10,773 specimens were collected between February 2019 and February 2020, representing 64 species in 15 genera. The vector specie *Nyssomyia umbratilis* Ward and Frahia, 1977 was the predominant species (13.20% of the total), being collected in all three counties, followed by *Trichopygomyia trichopyga* Floch & Abonnenc, 1945 (11.41%), *Trichophoromyia ubiquitalis* Mangabeira,1942 (9.47%) and *Nyssomyia anduzei* Rozeboom, 1942 (7.61%). For the identification of *Leishmania* DNA, 775 pools of unengorged females were used, of which 5 tested positive, 2 of *Nyssomya umbratilis* Ward & Fraiha,1977, 1 of *Nyssomyia anduzei* and 2 of *Psychodopygus davisi* Root,1934, demonstrating a natural total infection rate of 0.64%. This study increases the knowledge of vector diversity, as well as identifying *Leishmania* spp. in circulation in the eastern region of the Amazon.
Keywords: *Entomological surveillance; PCR; Molecular detection; Sand flies Diversity.*

Levantamento entomológico de flebotomíneos (Diptera: Psychodidae) e espécies vetoras na área endêmica de leishmaniose tegumentar na Amazônia oriental brasileira, Estado do Amapá

Resumo*:* A leishmaniose tegumentar americana é uma endemia que aumentou consideravelmente nas últimas décadas na região amazônica, os flebotomíneos são os vetores da transmissão do protozoário causador da leishmaniose, portanto o objetivo deste estudo foi realizar um levantamento da diversidade de espécies e a presença de DNA de *Leishmania* em vetores que circulam em três municípios endêmicos de leishmaniose tegumentar na Amazônia oriental brasileira (Amapá, Brasil). Usando armadilhas luminosas do tipo CDC, um total de 10.773 espécimes foram coletados entre fevereiro de 2019 e fevereiro de 2020, representando 64 espécies em 15 gêneros. As espécie vetoras - singular *Nyssomyia umbratilis* Ward e Frahia 1977 foram as espécies predominantes (13,20% do total), sendo coletadas nos três municípios, seguido por *Trichopygomyia trichopyga* Floch & Abonnenc, 1945 (11,41%), *Trichophoromyia ubiquitalis* Mangabeira, 1942 (9,47%) e *Nyssomyia anduzei* Rozeboom, 1942 (7,61%). Para a identificação do DNA de *Leishmania*, foram utilizados 775 pools de fêmeas não ingurgitadas, dos quais 5 foram positivos, 2 de *Nyssomya umbratilis* Ward & Fraiha, 1977, 1 de *Nyssomyia anduzei* e 2 de *Psychodopygus davisi* Root, 1934, demonstrando uma taxa de infecção total de 0,64%. Este estudo aumenta o conhecimento da diversidade de vetores, bem como a identificação das espécies de *Leishmania* spp. em circulação na região oriental da Amazônia.

Palavras-chave: *Vigilância Entomológica; PCR; Detecção Molecular; Diversidade de Flebotomíneos.*

Introduction

Sand flies (Diptera: Psychodidae) are small insects that play a fundamental role in the transmission of protozoa of the genus *Leishmania* Ross (Kinetoplastida: Trypanosomatidae), which are the etiological agents of American Tegumentary Leishmaniasis (ATL) and Visceral Leishmaniasis (VL) in humans (Ready 2013). Leishmaniasis infects approximately 12 million people around the world, with approximately 600,000 new cases being reported each year. It is estimated that 90% of ATL cases occur in Latin America (Bolivia, Peru, and Brazil) and Middle East (Iran, Saudi Arabia, Syria, Afghanistan) (WHO 2021).

In Brazil, ATL is a disease that requires attention due to the growing number of cases and the characteristics of the country that are conducive to the development of the disease, such as the great diversity of phlebotomine vectors of several *Leishmania* species that occur in the country (Brazil et al. 2014). Currently there are approximately 1,000 species of sand flies described in the world, 530 in the Americas and approximately 280 species have already been found in Brazil (Shimabukuro et al. 2017, Galati 2018), of these, at least 19 species are identified as proven or probable vectors of medical-veterinary importance (Aguiar & Medeiros 2003). According to the Notifiable Diseases Information System (SINAN), from the Ministry of Health, between 2003 and 2018, more than 300,000 cases of ATL were reported, with an average of 21,158 cases per year. ATL is largely neglected, and Brazil is responsible for the majority of human cases in the West (Alvar et al. 2012, Da Silva et al. 2020). The northern region of the country, where the state of Amapá is located, it is responsible for the largest number of cases in the period (Brasil 2019).

The state of Amapá, located in the Eastern Amazon, has environmental conditions that support one of the greatest biodiversity in the world, which provide opportunities for the development of vector-host relations and, consequently, of the leishmaniasis cycle (Ellwanger et al. 2020). To date there has been a record of five species of *Leishmania* coexisting in the region: *Leishmania (Viannia) guyanensis* Floch, 1954, *L. (V.) braziliensis* Vianna, 1911, *L. (V.) lainsoni* Silveira, Shaw, Braga & Ishikawa, 1987, *L. (V.) naiffi* Laison and Shaw, 1989, *L. (Leishmania) amazonensis* Lainson and Shaw, 1972. *L. (V.) guyanensis* being responsible for 80% of ATL cases in this area (Brasil 2017, de Souza et al. 2017). Despite the high incidence of ATL in the region, there are few studies that study the diversity of the sandfly and *Leishmania* fauna (Costa et al. 2021). The aim of this study is to characterize the sandfly fauna, as well as their natural infection by *Leishmania* spp. in an endemic region for ATL in the state of Amapá (AP), eastern Amazon.

Material and Methods

1. *Study area*

This study was conducted in the rural area of three counties in the state of Amapá (AP): **Mazagão – P1** (0° 6' 54" S, 51° 17' 20" W)**, Porto Grande – P2** (0° 53' 45" N, 52° 0' 7" W) and **Serra do Navio – P3** (0° 53' 45" N, 52° 0' 7" W) (Figure 1), located in the eastern Amazon, northern region of Brazil. According to Köeppen's classification criteria, the region's climate is in the humid tropical category, predominantly in the Am category, with the mildest month temperature above 18°C and monthly average rainfall below 60mm (Tavares 2014, Garcia et al. 2020). The collections were carried out in areas characterized as dry land with predominant vegetation of dense rainforest. The research was carried out in areas of proven ATL transmission in the three counties, in which an increase in cases was detected in recent years by the state agency for Health Surveillance (SVS 2019). Records have shown that human ATL cases have increased in rural areas in villages that are close to forested areas, and where villagers engage in agriculture and extractive activities of forest products.

2. *Sandfly collections and morphological identification*

The sand flies were captured in villages in rural areas of the three counties with Center for Disease Control (CDC) light traps placed in the peridomicile of homes that were selected for their proximity to the forest and the presence of chicken coops and pig breeding, characteristics that favor the presence of sand flies. The traps worked for three consecutive nights from 6:00 pm to 7:00 am, monthly for one year (February 2019 to February 2020), totalizing 468 hours of capture at each collection point. The 30 traps were distributed in locations with ATL human case records, in each of the three selected counties.

The collected insects were taken to the Arthropoda Laboratory (ArtroLab) at the Federal University of Amapá (UNIFAP) for screening by sex and dissection process. The final three segments of the abdomen and the head were removed for mounting on Berlese liquid glass slides. The rest of the phlebotomine bodies were conditioned in 94% ethanol at -20ºC for the subsequent extraction of genomic DNA. Species identification was based on the morphology of male genitalia and the spermatheca and by the characters present in female's head, , using the updated classification key developed by Galati in 2003 (Galati 2019), and following the abbreviation of the genera proposed by Marcondes (2007).

3. *Molecular detection of Leishmania*

The thorax of unengorged female sand flies were grouped according to date, species, and county of collection to form pools with 2 to 10 specimens of the 65 species collected. DNA extraction and Polymerase Chain Reaction (PCR) were performed to amplify the molecular targets of the region of kinetoplast DNA minicircles (*k*DNA) and *hsp*70, as described elsewhere (Pereira Júnior et al. 2015, Resadore et al. 2017). For the negative control, purified water was used and for the positive controls, DNA from *Le. (L.) amazonensis* Lainson & Shawn, 1972 and *Le. (V.) braziliensis* Vianna, 1911 strains.

4. *Data analysis*

The collection effort and the number of species in the study were measured using the non-parametric Jackknife1 estimator, generating a rarefaction curve for each of the collection points using the R software (R Core Team 2021) using the vegan package (Oksanen et al. 2019).

The sequences of the *hsp*70 molecular target were analyzed using Phred, Phrap and Consend software (Ewing & Green 1998), with the minimum value defined as Q=30. The identification of *Leishmania* species was performed by comparing the consensus sequences obtained in the study with reference sequences deposited in the GenBank database (http: www.ncbi.nlm.nih.gov/genbank), using the BLAST tool (Basic Local Alignment Search Tool) (Altschul et al. 1990).

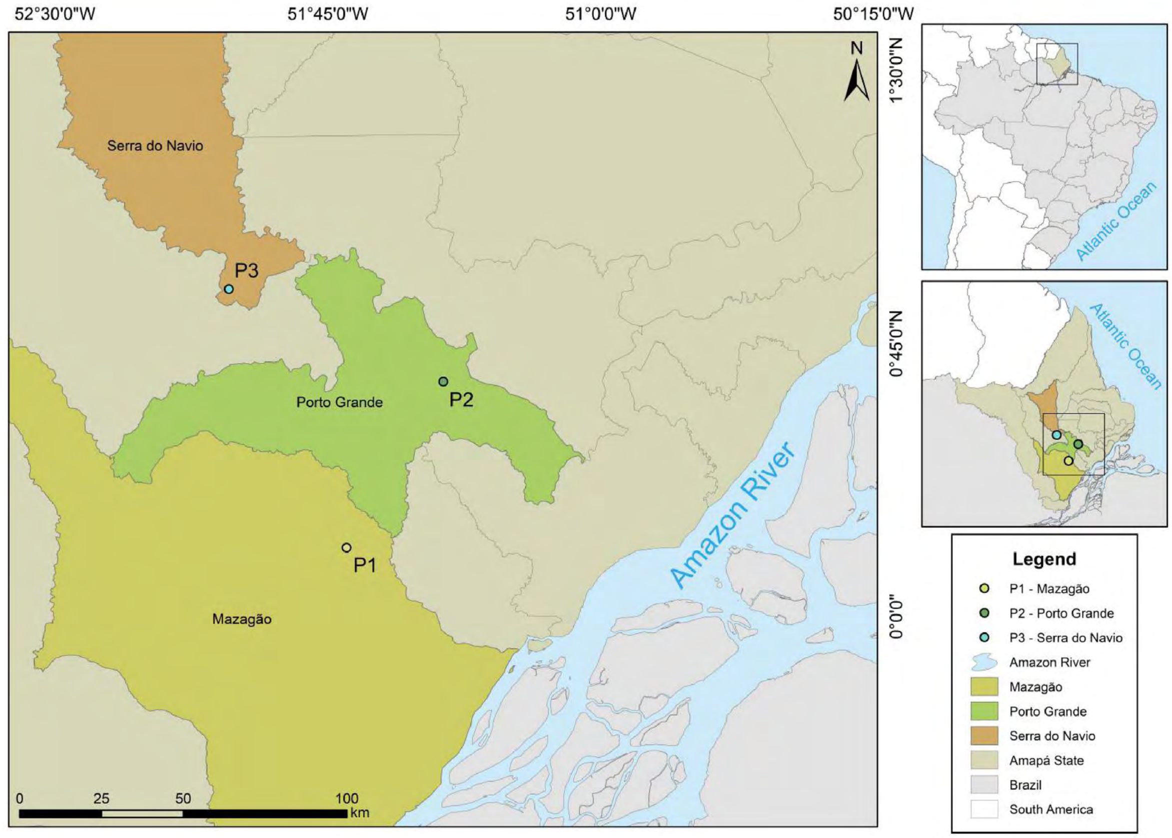

Figure 1. Map of the study area, location counties of Mazagão (P1), Porto Grande (P2) and Serra do Navio (P3), Amapá State, Brazil, where the Sand flies collections with the CDC light traps were carried out.

Results

The total number of sand flies collected in the three locations (P1, P2, P3) was 10,773 specimens, being: 4,512 males (41.89%) and 6,261 females (58.11%), totalizing 15 genera and 64 species (Table 1). The most abundant genera were *Nyssomyia* Barretto (3,559 individuals, 33.04%), *Trichophoromyia* Barreto (1,619, 15.02%), *Trichopygomyia* Barretto (1,278, 11.87%) and *Psychodopygus* Mangabeira (1,176, 10.92%). The genera collected in lesser abundance were *Pintomyia* Costa Lima (72 individuals, 0.67%), *Vianamyia* Mangabeira (65, 0.60%) and *Pressatia* Mangabeira (35, 0.32%). The most abundant species were *Ny. umbratilis* (n=1.422, 13.20%), *Ty. trichopyga* (n=1.229, 11.41%), *Th. ubiquitalis* (n=1020, 9.47%) and *Ny. anduzei* (n=820, 7.61%); these species accounted for 40.69% of the sand flies collections. The least abundant species were *Pa. bigeniculata* Floch & Abonnenc, 1941 (n=11,0.10%), *Br. beaupertuiy* Ortiz, 1954 (n=11,0.10%), *Pa. lutziana* Costa Lima, 1932 (n=8, 0.07%), *Lu. spatotrichia* Martins, Falcão & Silva, 1963 (n=6, 0.06%); among the less collected species is the vector *Ps. ayrozai* Barretto & Coutinho, 1940 (n=45, 0.42%).

Based on the data from the study, the rarefaction curves demonstrated a good result of the sampling efforts, with collection efficiency approaching 100% at the three collection points. The Jackknife 1 estimator showed that species richness corresponded to 96% in Mazagão (P1) and Porto Grande (P2), and 100% in Serra do Navio (P3) (Figure 2), demonstrating a tendency to stabilization in the three sampling points of the curve in an asymptote.

A total of 775 pools of unengorged females were formed for the detection of *Leishmania* DNA, of which PCR for molecular targets *k*DNA and *hsp*70 identified that a total of five pools were positive: two pools of *Ny. umbratilis* infected with *L. (V.) guyanensis* (query cover=100%, identity=100%, Genbank accession MW094227.1) collected in Porto Grande (P1) and Serra do Navio (P3), a pool of *Ny. anduzei* infected with *L. (V.) naiffi* (query cover=100%, identity=100%, Genbank accession MT469994.1) in Serra do Navio (P3), and two pools of *Ps. davisi* infected with *L. (V.) braziliensis* (query cover = 98%, identity = 98%, Genbank accession MT543301.1) collected in Mazagão (P2) and Serra do Navio (P3). The minimum infection rate (number of positive samples / total samples tested x 100) was calculated for each of the species that tested positive for *Leishmania* DNA: *Ny. umbratilis* (1.0%), *Ny. anduzei* (1,5%) and *Ps. davisi* (5.7%).

Discussion

Our sampling included 64 species of sand flies among the 77 recorded in the state of Amapá (Galati 2019), 50 in Mazagão (P1),49 in Porto Grande (P2) and 56 in Serra do Navio (P3). The highest number of species was found in Serra do Navio, which corroborates the 55 species already found in the county in a previous study (de Souza et al. 2017),

Table 1. Species of Sand flies from Amapá State, Eastern Amazon, Brazil, collected with CDC light traps from February 2019 to February 2020.

Species	P1	P2	P3	Total	% Total	MIR
Nyssomya umbratilis Ward & Fraiha, 1977	420	385	522	1327	13.20	1.0%
Trichopygomya trychopyga Floch & Abonnenc, 1945	450	255	524	1229	11.41	-
Trichophoromya ubiquitalis Mangabeira, 1942	357	204	459	1020	9.47	-
Nyssomya anduzei Rozeboom, 1942	227	280	313	820	7.61	1.5%
Nyssomya yuilli pajoti Abonnenc, Léger & Fauran, 1979	89	198	352	639	5.93	-
Nyssomya whitmani Antunes & Coutinho, 1939	159	200	256	615	5.71	-
Trichophoromya brachipyga Mangabeira, 1942	225	157	182	564	5.24	-
Psychodopygus squamiventris maripaensis Floch & Abonnenc, 1946	109	79	255	443	4.11	-
Evandromyia infraspinosa Mangabeira, 1941	78	117	180	375	3.48	-
Psychodopygus davisi Root, 1934	118	87	125	330	3.06	5.7%
Migonemyia migonei França, 1920	45	74	92	211	1.96	-
Evandromyia monstruosa Martins, Falcão & Silva, 1965	59	77	64	200	1.86	-
Migonemyia pilosa Damasceno & Causey, 1944	35	49	77	161	1.49	-
Migonemyia micropyga Mangabeira, 1942	21	40	78	139	1.29	-
Evandromyia brachyphalla Mangabeira, 1941	18	45	75	138	1.28	-
Bichromomyia flaviscutellata Mangabeira, 1942	45	33	57	135	1.25	-
Brumptomyia cunhai Mangabeira, 1942	54	-	77	131	1.22	-
Psychodopygus paraensis Costa Lima, 1941	42	12	74	128	1.19	-
Evandromyia bacula Martins, Falcão & Silva, 1965	15	46	65	126	1.17	-
Evandromyia sericea Floch & Abonnenc, 1944	-	78	72	120	1.11	-
Brumptomyia travassosi Mangabeira, 1942	7	-	94	101	0.94	-
Lutzomyia gomezi Nitzulescu, 1931	28	35	19	82	0.76	-
Psathyromyia inflata Floch & Abonnenc, 1944	14	22	45	81	0.75	-
Migonemyia oswaldoi Mangabeira, 1942	-	41	38	79	0.73	-
Evandromyia walkeri Newstead, 1914	78	-	-	78	0.72	-
Psychodopygus amazonensis Root, 1934	-	27	48	75	0.70	-
Sciopemyia fluviatilis Floch & Abonnenc, 1944	22	18	35	75	0.70	-
Evandromyia bourroli Barretto & Coutinho, 1941		25	49	74	0.69	-
Evandromyia pinottii Damasceno & Arouck, 1956	25	42	-	67	0.62	-
Psychodopygus claustrei Abonnenc, Léger & Fauran, 1979	22	18	27	67	0.62	-
Psathyromyia dreisbachi Causey & Damasceno, 1945	22	13	30	65	0.60	-
Migonemyia bursiformis Floch & Abonnenc, 1944	18	30	15	63	0.58	-
Micropygomyia longipennis Floch & Abonnenc, 1944	24	20	17	61	0.57	-
Micropygomyia rorotaensis Floch & Abonnenc, 1944	17	25	19	61	0.57	-
Nyssomya antunesi Coutinho, 1939	28	4	18	50	0.46	-
Trichopygomya depaquiti Floch & Abonnenc, 1944	25	-	24	49	0.45	-
Psychodopygus hirsutus Mangabeira, 1942	25	5	18	48	0.45	-
Evandromyia saulensis Floch & Abonnenc, 1943	45	-	-	45	0.42	-
Psychodopygus ayrozai Barretto &Coutinho, 1940	12	8	25	45	0.42	-
Pintomyia damascenoi Mangabeira, 1941		28	16	44	0.41	-
Micropygomyia chassigneti Floch & Abonnenc, 1944		15	27	42	0.39	-
Psychodopygus corossoniensis Le Pont & Pajot, 1978	18		22	40	0.37	-
Psathyromyia aragaoi Costa Lima, 1932		15	22	37	0.34	-
Trichophoromya ininii Floch & Abonnenc, 1943	12	5	18	35	0.32	-
Viannamyia tuberculata Mangabeira, 1941	15	19		34	0.32	-

Continua...

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Psathyromyia runoides Fairchild & Hertig, 1953		12	20	32	0.30	-
Viannamyia furcata Mangabeira, 1941	3		28	31	0.29	-
Psathyromyia pradobarrientosi Le Pont, Matias, Martinez & Dujardin, 2004	25			25	0.23	-
Evandromyia evandroi Costa Lima & Antunes, 1936	-	24	-	24	0.22	-
Sciopemyia sordelli Shannon & Del Ponte, 1927	12	-	9	21	0.19	-
Lutzomyia evangelistai Martins & Fraiha, 1971	-	19	-	19	0.18	-
Pressatia choti Floch & Abonnenc, 1941	-	2	17	19	0.18	-
Evandromyia inpai Young & Arias, 1977	1	-	16	17	0.16	-
Evandromyia williamsi Damasceno, Causey & Arouck, 1945	5	12	-	17	0.16	-
Pressatia trispinosa Mangabeira, 1942	7	-	9	16	0.15	-
Lutzomyia carvalhoi Damasceno, Causey & Arouck, 1945	-	14	2	16	0.15	-
Pintomyia pacae Floch & Abonnenc, 1943	5	-	10	15	0.14	-
Brumptomyia pintoi Costa Lima, 1932	-	7	8	15	0.14	-
Nyssomyia richardwardi Ready & Fraiha, 1981	8	-	5	13	0.12	-
Pintomyia serrana Damasceno & Arouck, 1949	4	-	9	13	0.12	-
Brumptomyia beaupertuiy Ortiz, 1954	5	-	6	11	0.10	-
Psathyromyia bigeniculata Floch & Abonnenc, 1941	5	2	4	11	0.10	-
Psathyromyia lutziana Costa Lima, 1932	1	3	4	8	0.07	-
Lutzomyia spathotrichia Martins, Falcão & Silva, 1963		1	5	6	0.06	-
Total of individuals	3157	2899	4717	10773	100%	-
Total species	50	49	56	64		

Collection points: P1: Mazagão; **P2:** Porto Grande; **P3:** Serra do Navio; **MIR:** Minimum Infection Rate; The abbreviation of genera is in agreement with Marcondes (2007).

the present study found the highest number (83.1%) of species among all species with occurrence for the state. Our survey demonstrated a great range of sandfly species in the three collection sites, demonstrating the great diversity of the sandfly fauna in the Eastern Amazon. The diversity found in the study generally corresponded to the pattern of studies carried out in forest areas that demonstrate the phlebotomine fauna usually composed of a few dominant species and many species with specimens (Rosário et al. 2016)

The genus *Nyssomia* is of great importance for surveillance studies and understanding of the ecopidemiology of ATL, occurring from North America (Mexico) to South America (Argentina), with a total of seven species proven or suspected of transmission of ATL (Marcondes et al. 1998). Three species of the genus showed great abundance: *Nyssomyia umbratilis* (13.2%), *Nyssomyia anduzei* (7.61%) and *Nyssomyia whitmani* Antunes & Coutinho, 1939 (5.71%), these species identified as vectors in Brazil (Brasil 2017) and already associated with ATL transmission in the state of Amapá in previous studies (De Souza et al. 2017). *Ny. umbratilis* is a constant presence in endemic areas for ATL (Pinheiro et al. 2008), and is considered one of the main vectors for *L. (V.) guyanensis* in most of Latin America, in countries such as Brazil, Bolivia, Colombia, Peru, Venezuela, Suriname and French Guiana (Brazil et al. 2014), as well as in the Amazon region (Brasil 2019). *Nyssomyia anduzei* is considered a secondary vector of *L. (V.) guyanensis* and appears as the fourth species with the largest sampling in the present study, which corroborates studies carried out in the Western Amazon region (Barbosa et al. 2008, Grimaldi et al. 1991a), *Ny. whitmani* is considered one of the vectors of great medical importance in the Amazon region as it can transmit *L (V.) braziliensis, L. (V.) shawi* and *L. (V.) guyanensis* (Lainson et al. 1994), this species is considered a wild species and in the northern region of Brazil it has a lower anthropophilic habit (Silveira et al. 1991), having been identified previously infected with *Leishmania* in the region (Rangel & Lainson 2009).

Other species with medical importance such as *Bichromomyia flaviscutellata* Mangabeira 1941*, Tricophoromyia ubiquitalis. Psychodopygus squamiventris maripaensis* Floch & Abonnenc, 1946 and *Migonemia migonei* França,1920 were found at the three collection points in peridomicile areas. This is a factor that should be carefully observed as finding females of these species in these environments may indicate that the species are looking for blood meal sources in the domestic environment or being attracted because of the lights in the houses; the presence of these vectors near the dwellings greatly increases the risk of contracting ATL in the home environment (Tanure et al. 2015).

For the incrimination of *Leishmania* vectors, one of the crucial points is to determine the occurrence of natural infection in sandfly populations, identifying possible vector species. The test considered that the gold standard for the natural detection of infection is the dissection of the digestive tract to indicate the presence of *Leishmania* promastigotes by light microscopy (Kato et al. 2005), but this method is extremely laborious and requires the dissection of a large number of specimens, isolation and culture of protozoa from dissected sand flies. Thus, molecular techniques such as PCR for the detection of *Leishmania* DNA have been increasingly used in studies with sand flies (Teles et al. 2016, Da Silva et al. 2020). The minimum infection rate of *Leishmania* DNA detected with molecular methods was 0.64%, a result compatible

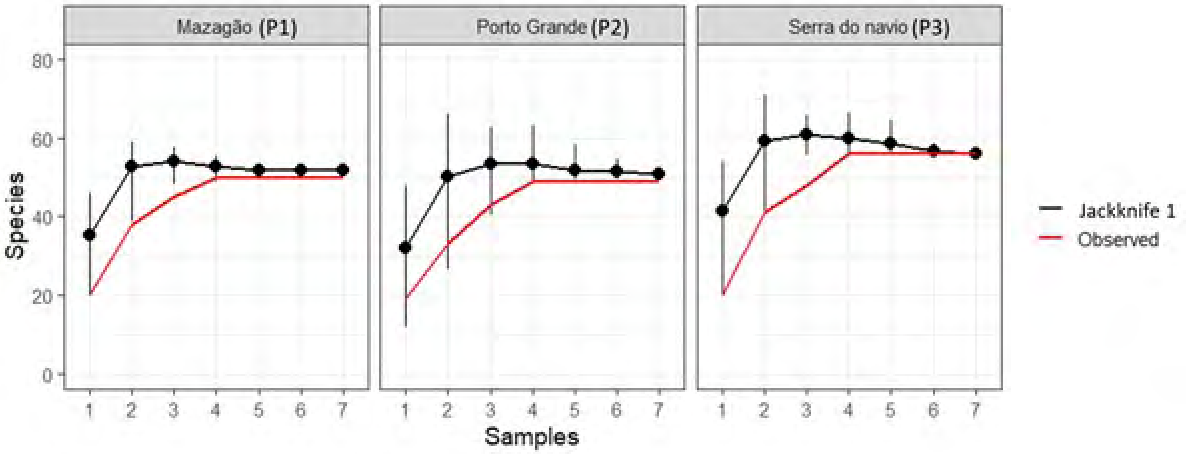

Figure 2. The rarefaction curves (observed and estimates by jackknife 1) of sand flies species collected in the Mazagão (P1), Porto Grande (P2) and Serra do Navio (P3), Amapá State, Brazil.

with a study conducted in the state of Amapá (0.78%) (Vasconcelos Dos Santos et al. 2019), as well as in other states in the Amazon region, such as Rondônia (0.28%) (Resadore et al. 2019), Amazonas (0.83%) and Acre (0.99%).

The DNA of the species *L. (V.) guyanensis* was detected in two samples of *Ny. umbratilis* in the collection locations (P1 and P3). This sandfly species is pointed out as the main vector of *L. (V.) guyanenis* in northern Brazil (Gil et al. 2009). Several other studies conducted in areas endemic to ATL found this sandfly species infected with *L. (V.) guyanensis* (Lainson et al. 1981, Pinheiro et al. 2008), including in studies previously conducted in the state of Serra do Navio (P3) (de Souza et al. 2017) and in Oiapoque, a region on the state border with French Guiana (Vasconcelos Dos Santos et al. 2019), where it is considered the main vector (Vasconcelos dos Santos et al. 2018). Infection by *L. (V.) guyanensis* represents a high risk of complications for human health, as the protozoan is resistant to the drug most used in clinical practice in Brazil, Glucantime (Brasil 2017).

Nyssomyia anduzei was found infected with the DNA of *L. (V.) naiffi* in P3 (Serra do Navio). This species is considered a secondary vector of *L. (V.) guyanensis* in the region (Rangel & Lainson 2009, Chagas et al. 2018), but has already been found infected with *L. (V.) naiffi* DNA in the state of Amapá (de Souza et al. 2017), demonstrating its likely participation in the cycle of leishmaniasis in the region. This species of *Leishmania* has been commonly associated with the *Ps. squamiventris maripaensis* vector in northern Brazil (Naiff et al. 1991), French Guiana (Fouque et al. 2007) and Suriname (Kent et al. 2013). In Brazil, *L.(V.) naiffi* has already been reported in other states of the Amazon region such as Pará (Lainson et al. 1981), Amazonas (Grimaldi et al. 1991a) and Acre (Tojal da Silva et al. 2006).

The *Psychodopygus davisi* species was found infected with *L. (V.) braziliensis*. The species is considered a potential vector for this species of *Leishmania*, having already been found in previous studies in forest environments infected by both *L. (V.) braziliensis* and *L. (V.) naiffi* (Grimaldi et al. 1991b, Gil et al. 2003). This species of sandfly is considered one of the main vectors of the etiological agent of Leishmaniasis, so it should be considered carefully in entomological surveys as it has relevant characteristics such as a high level of anthropophily. In Brazil, the transmission of *L. (V.) braziliensis* has as potential vectors 17 species of sand flies. In Amapá the DNA of *L. (V.) braziliensis* was found in other species of sand flies such as *Th. ininni* Floch & Abonnenc 1943, *Ny. umbratilis* and *Ev. infraspinosa* Mangabeira, 1941 (Vasconcelos Dos Santos et al. 2019).

The sandfly fauna of the eastern Amazon region is still poorly known. In this study we demonstrate the high level of species diversity of sand flies in the state of Amapá, the three collection counties demonstrate a diversity of proven or putative vectors of *Leishmania* in the region, as well as the detection of three different species of the *Leishmania Viannia* complex in sandfly species that already have a history of vectors in the literature. Thus, our studies suggest that the sand flies found are acting as vectors in the ATL transmission cycle, as well as indicating a high risk of transmission in the three collection counties of the three main *Leishmania* species. Thus, it is necessary that measures against ATL transmission be planned for the state of Amapá, based on entomological inventories to monitor the vector arthropod species as well as the species infected by *Leishmania spp.* that cause ATL in Eastern Brazilian Amazon.

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Author Contributions

Tiago Silva da Costa: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Ricardo Marcelo dos Anjos Ferreira: Contribution to data collection. Contribution to manuscript preparation.

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Camila Barbosa Pinto: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Erratum: Contribution of the Brazilian National Forest Inventory to the knowledge of Cerrado woody flora

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Article

Contribution of the Brazilian National Forest Inventory to the knowledge of Cerrado woody flora

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Abstract: The National Forest Inventory (Inventário Florestal Nacional-IFN) is a large initiative that uses standardised methods to survey Brazilian forestry resources. One target of the IFN is the Cerrado, which contains one of the richest floras in the world. The aim of this study was to assess the contribution of the IFN to the knowledge of Cerrado woody flora. We analysed data from field-collected vouchers sampled by the IFN Cerrado. We restricted our analyses to IFN collections of native trees and shrubs, including palms, which were identified at the species level. Habitat of each collection was obtained by overlaying specimens' geographic coordinates with land cover maps available in the Mapbiomas platform. Our final dataset comprised 28,602 specimens distributed in 2,779 sites (conglomerates) in Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo and Tocantins. Collections were located in the following habitats: savannas (40.5%), forests (30.2%), anthropic areas (25.6%), grasslands (3.5%), and water (0.2%). We recorded 1,822 species belonging to 543 genera and 105 families, representing 34% of Cerrado woody species recorded on Flora do Brasil 2020. Fabaceae had the largest number of species, while *Tapirira guianensis* and *Matayba guianensis* were the most collected species. We highlight 60 potentially new records of occurrence for several states and 64 new records for the Cerrado, primarily in riparian forests where species from other biomes occur. In addition, 232 recorded species are Cerrado endemics, while 36 are cited in the CNCFlora's red list as endangered. The systematic sampling carried out by the IFN enabled vegetation sampling in remote and poorly known areas, which expanded the geographic range of many woody species and contributed to the knowledge of plant diversity in the Cerrado.

Keywords: *conservation; endemism; plant diversity; sampling effort; survey; vegetation types.*

A contribuição do Inventário Florestal Nacional para o conhecimento da flora lenhosa do Cerrado

Resumo: O Inventário Florestal Nacional (IFN) é uma ampla iniciativa que emprega métodos padronizados para inventariar recursos florestais brasileiros. Um dos alvos do IFN é o Cerrado, o qual possui uma das floras mais ricas do mundo. O objetivo deste estudo foi avaliar a contribuição do IFN para o conhecimento da flora lenhosa do Cerrado. Nós analisamos dados de vouchers coletados em campo pelo IFN Cerrado. Nós restringimos nossas análises a coletas do IFN pertencentes a árvores e arbustos, incluindo palmeiras, identificadas ao nível de espécie. O habitat de cada coleta foi obtido pela intersecção entre as coordenadas geográficas dos espécimes com mapas de cobertura disponíveis na plataforma Mapbiomas. O conjunto final de dados foi composto por 28.602 coletas distribuídas em 2.779 sítios (conglomerados) localizados na Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo e Tocantins. As coletas foram realizadas nos seguintes habitats: savanas (40,5%), florestas (30,2%), áreas antrópicas (25,6%), campos (3,5%) e água (0,2%). Ao todo foram registradas 1.822 espécies pertencentes a 543 gêneros e 105 famílias, representando 34% das espécies lenhosas do Cerrado registradas na Flora do Brasil 2020. Fabaceae apresentou o maior número de espécies, enquanto que *Tapirira guianensis* e *Matayba guianensis* foram as espécies mais coletadas. Destacam-se possíveis novos registros de ocorrência de 60 espécies para diversos estados e de 64 espécies para o Cerrado, predominantemente nas florestas ripárias onde geralmente ocorrem espécies de outros biomas. Além disso, foram registradas 232 espécies endêmicas do Cerrado, bem como 36 espécies citadas na lista vermelha do CNCFlora como ameaçadas. A amostragem sistemática realizada pelo IFN permitiu o inventário da vegetação em áreas remotas e pouco coletadas, permitindo a expansão da distribuição geográfica de diversas espécies lenhosas, e contribuindo para o conhecimento da diversidade vegetal no Cerrado.

Palavras-chave: *conservação; endemismo; diversidade vegetal; esforço amostral; inventário; tipos de vegetação.*

Introduction

Brazil harbours one of the richest floras in the world, and it is home to a large number of endemics (BFG 2021). Recent advances took place after the compilation of an updated national checklist containing information on species descriptions and geographic distributions (Flora do Brasil 2020; http://floradobrasil.jbrj.gov.br). However, large gaps still exist in the documentation of plant diversity in Brazil. Such knowledge gaps are related to incomplete taxonomic knowledge ("Linnean shortfall"; Whittaker et al. 2005), as represented by a high number of new species being described every year in the country (BFG 2021). These gaps are also a product of unsatisfactory understanding of the geographic distribution of species ("Wallacean shortfall"), particularly in poorly sampled regions of the country (e.g., Sousa-Baena et al. 2014, Oliveira et al. 2016).

Since 2007, the Brazilian Forest Service (Serviço Florestal Brasileiro-SFB) has been coordinating a survey of Brazilian forest resources through the National Forest Inventory (Inventário Florestal Nacional-IFN). The IFN aims to provide data about forest structure, composition, vitality, biomass, wood and carbon stock (SFB 2020). This initiative seeks to support development policies and assist in the identification of strategies and opportunities for sustainable use, restoration and conservation of forest resources (SFB 2019, 2020). The IFN adopts a standardised sampling method applied to each Brazilian biome (Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal and Pampa; SFB 2017a). IFN systematic sampling generates a vast amount of scientific data, including thousands of plant occurrence records widely distributed across the country, mostly trees and shrubs. The compilation and analysis of such dataset would be a useful source of biodiversity information on Brazilian biomes that would help to increase floristic knowledge and support conservation planning. Although summary reports for some states have been published (https://www.florestal.gov.br/resultados), comprehensive analyses, including the large volume of plant diversity data sampled by the IFN surveys, are still missing for most states (but see Versieux et al. 2017 and Vibrans et al. 2020 for analyses of Rio Grande do Norte and Santa Catarina, respectively).

The Cerrado, which originally occupied around 23% of the Brazilian territory, is located in Central Brazil between two areas of wet forests, the Amazon and Atlantic Forest, and forms a dry corridor, together with the Caatinga, in the northeast and the Chaco in the southwest (Oliveira-Filho & Ratter 2002, Werneck et al. 2012). The Cerrado presents notable physiographic variation (e.g., Sano et al. 2019) and an associated number of vegetation types. These include grasslands, wetlands, savannas, and seasonally dry and wet forests (Ribeiro & Walter 2008), the occurrence of which depends on ecological factors at the local scale, such as soil fertility, water availability and fire regime (Bueno et al. 2018). Because of this complex mosaic of vegetation types, the Cerrado is a savanna-dominated biome with the richest flora in the world (Klink & Machado 2005). It has around 12,000 angiosperm species, including 40% endemics (Flora do Brasil 2020). However, the Cerrado has been highly threatened by deforestation, which has resulted in the loss of around 50% of its native vegetation (Alencar et al. 2020). Because of its species richness, high levels of endemism and anthropic pressure, the Cerrado is considered one of the 35 global biodiversity hotspots for conservation (Mittermeier et al. 2011). Its plant species extinctions are projected to increase as a consequence of habitat loss (Strassburg et al. 2017).

Considering the elevated rates of habitat loss and outstanding species richness, it is essential to increase knowledge about the Cerrado's rich and endangered flora through large-scale inventories. Such inventories are certain to result in new collections of herbarium specimens and occurrence data. This would, in turn, tackle both Linnean and Wallacean shortfalls (Whittaker et al. 2005) on plant diversity by increasing the number of specimens available for taxonomic work. It would also expand the geographic ranges of known species, particularly in poorly collected areas. Information provided by new inventories can help in the development of conservation strategies, identify priority areas for threatened species, and foster the discovery of new species.

Here, we carried out a data compilation of species occurrence records generated by the IFN Cerrado, seeking to assess the project's contribution to knowledge of the Cerrado's woody flora. It is expected that the systematic data survey and wide geographic breadth of the IFN will provide useful information on species geographical distribution in the Cerrado. Questions we intended to resolve herein are as follows: (1) How many species were recorded by the IFN? (2) What is the proportion of woody species surveyed by IFN compared to information available in the literature? (3) Were any species found and recorded for the first time for the Cerrado flora or for individual states? (4) How many endemic or endangered species were recorded?

Material and Methods

The Serviço Florestal Brasileiro provided the data collected by the IFN Cerrado, which followed a standardised methodology (SFB 2017b). Data were surveyed across most of the extension of the Cerrado biome (sensu Instituto Brasileiro de Geografia e Estatísticas; https://www.ibge.gov.br) in different habitats, except in indigenous lands, where plant collecting would demand special permissions. Surveys were carried out in sampling sites called conglomerates (0.4 ha) that were systematically distributed on a 20 km x 20 km grid that sampled different habitats, including various types of natural vegetation and also anthropic areas (e.g. pastures, agriculture). Each conglomerate was composed of four crosswise subunits formed by rectangles of 20 m x 50 m located 30 m from the conglomerate's centre. Sampling included all individuals with diameter at breast height (DBH) ≥ 10 cm, or diameter at 30 cm height from the soil (DSH) ≥ 10 cm in cases of adult individuals with height > 1.5 m, but DBH absent. Individuals with DBH ≥ 5 cm were measured in two smaller subplots (10 m x 10 m each). Individuals taller than 1.3 m, but with DBH/DSH < 5 cm, were sampled in two subplots (5 m x 5 m each). This sampling strategy was particularly designed for the IFN Cerrado in order to maximise sampling of savanna trees, which are normally stunted with twisted trunks. Individuals were assigned to morphotypes in the field and later received more accurate taxonomic identification. Detailed information on IFN Cerrado sampling methodology is provided in SFB (2017b).

The analyses presented here were based on the herbarium vouchers collected during field surveys carried out during the IFN Cerrado. Voucher specimens (sterile or fertile) representing species measured within conglomerates were collected in the field for identification in herbaria. Field teams were required to make a collection of any given species at least once every 15 conglomerates, including species readily identified in the field, as well as individuals with uncertain identification. Thus, specimens collected correspond to a subset of all individuals measured in IFN surveys, and are expected to comprise a representative sample of species surveyed. Conglomerates placed in areas harboring a richer flora (e.g., preserved forest) are expected to be more intensively collected than anthropic areas with fewer species (e.g., pastures). Extra collections not corresponding to sampled individuals within plots were also made. Appropriate information regarding collections, such as sampling unit, location, geographic coordinates, field name, habit and other observations, was annotated in a standardised form and later entered into a database. A total of 52,778 specimens collected were sent to the CEN, IBGE and UB herbaria for identification by generalist botanists and also by specialists in several families (Table S1, supplementary material). Specimens were databased, imaged, and finally incorporated into these collections. Duplicates were sent to other herbaria.

Our analysis included specimens collected in 2,779 conglomerates surveyed in ten states (Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo and Tocantins) from 2011 to 2020. Field surveys and botanical identifications for Minas Gerais and São Paulo were not completed by the finalization of our analyses, and data for these states are partial. We restricted our analyses to only herbarium specimens (sterile and fertile) since these records are more reliable than unvouchered occurrence data that were also available from the IFN Cerrado dataset. We included only woody species, the main focus of the IFN, by retaining records belonging to species with life form reported as "shrub" or "tree", according to the Flora do Brasil 2020, including shrubby and arborescent palms. From the initial dataset of 52,778 specimens, we excluded 3071 records corresponding to herbs, subshrubs, lianas and bamboos. We also excluded 20,710 records identified only at family, genus or not determined, as well as those records identified at the species level, but with an ambiguous identification (indicated by "cf." or "aff."). Also, 323 records corresponding to exotic or introduced species, according to the Flora do Brasil 2020, were excluded. Infraspecific categories were treated at the species level. After this filtering process, the final dataset totalled 28,602 records of native woody plants identified at the species level (Table S2, supplementary material).

Species names were checked in the RStudio program, version 3.6.3 (RStudio Team 2020), by using the flora package (Carvalho 2017), the underlying database of which is the Flora do Brasil 2020. By the end of name checking, species names identified as synonyms were replaced by their respective accepted names. The geographic distribution of each species was verified based on information provided by Flora do Brasil 2020 (accessed through flora package) in order to identify possible new occurrence records for the Cerrado or for individual states. Species that only occur within the Cerrado in Flora do Brasil 2020 were listed here as Cerrado endemics. To identify endangered species collected by the IFN Cerrado, we consulted the CNCFlora Red list (National Centre for Plant Conservation; CNCFlora 2021).

To characterise the environmental variation of IFN Cerrado sampling sites, we analysed the different habitats in which each specimen was collected. The habitat of each collection was obtained by overlaying specimens' geographic coordinates with land cover maps available in the Mapbiomas platform (collection 6.0; Souza et al. 2020), which have a spatial resolution of 30 x 30 m that is compatible with the size of our sampling units (conglomerates). Original land use classes from Mapbiomes (see terms listed in parentheses) were merged into five major categories: grasslands ("formação campestre"), savannas ("formação savânica"), forests ("formação florestal"), anthropic areas ("pastagem", "agricultura", "mosaico de agricultura e pastagem", "silvicultura"), and water ("água"). A land use map for 2017 was downloaded from Mapbiomes as a shapefile, and habitat classes for each of the 28,602 occurrence records were retrieved using ArcGIS 10.3 (Environmental Systems Resource Institute).

We assessed the completeness of species richness recorded in the IFN Cerrado based on the sampling effort (Martins & Santos 1999). We verified sampling efficiency by using the rarefaction/extrapolation curve based on sample size (number of specimens) according to the Hill number (q=0) using the iNext package (Chao et al. 2014) in the RStudio program. We also compared the number of species in our dataset with the data available in Flora do Brasil 2020 to assess the representativeness of the woody flora sampled by the IFN Cerrado.

Results

Our clean dataset, composed of 28,602 specimens distributed in IFN's 2,779 conglomerates, covered most of the Cerrado (Figure 1). The number of specimens collected varied from one to 127 per conglomerate (average 10.3) with 68% of conglomerates presenting less than ten collections (Figure 2). The number of specimens was not equally distributed among states, with a higher incidence of collections in the central region of the Cerrado, especially in Goiás, and also in northern Maranhão (Figure 1). Collections were recorded in the following land use classes: savannas (40.5%), forests (30.2%), anthropic areas (25.6%), grasslands (3.5%), and water (0.2%).

We recorded a total of 1,822 woody species sampled by the IFN Cerrado. However, the rarefaction curve did not reach stability (Figure 3), suggesting that increasing sampling would result in recording additional species. Species numbers varied among states (Table 1) with the highest numbers in Goiás (735), followed by Mato Grosso (683), Maranhão (647), Tocantins (563), Bahia (513), Mato Grosso do Sul (382), São Paulo (314), Piauí (291), Distrito Federal (190), and Minas Gerais (132). Most species were found in savannas (1,204 species) and forests (1,200), followed by anthropic areas (891), grasslands (373), and water (130).

Species recorded belong to 543 genera and 105 families, including two *Podocarpus* gymnosperm species (see Table S3 for full species list, authorship, habit, number of collections and distribution). Among families collected, the top ten presenting the highest number of species were Fabaceae (336 species), Rubiaceae (85), Myrtaceae (78), Malvaceae (72), Melastomataceae (65), Euphorbiaceae (63), Asteraceae (60), Sapotaceae (49), Malpighiaceae (47) and Annonaceae (46). These families represented 47.2% of specimens and contributed to 49.6% of species.

The number of specimens collected per species varied widely with 29% of species (528) represented by a single collection (Figure 4). The most collected species were *Tapirira guianensis* (374 specimens), *Matayba guianensis* (305), *Vatairea macrocarpa* (290), *Myrcia splendens* (269), *Diospyros lasiocalyx* (249), *Machaerium acutifolium* (220), *Qualea parviflora* (215), *Terminalia argentea* (207), *Pouteria ramiflora* (206) and *Callisthene fasciculata* (204). All these species are typically found in savannas and forests within the Cerrado, including the widespread riparian forest species *Tapirira guianensis*. The IFN Cerrado contributed

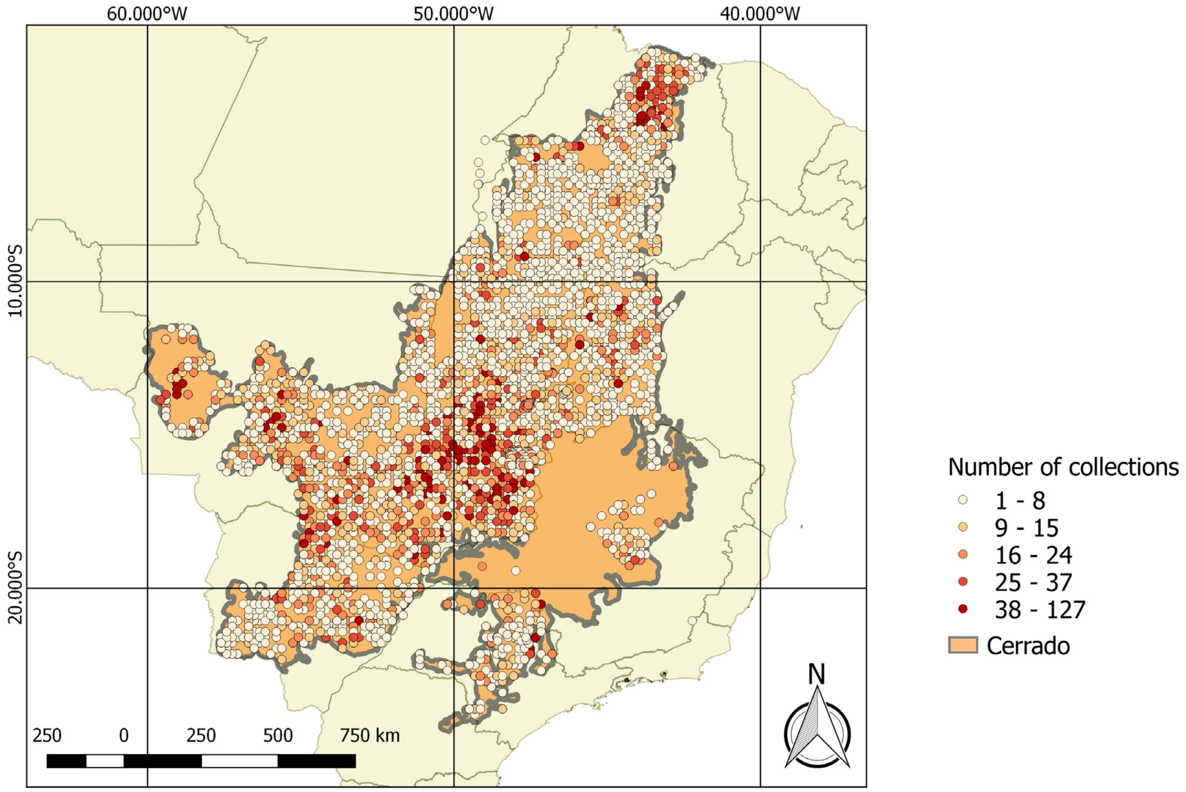

Figure 1. Geographic distribution of 2779 conglomerates (sampling sites) within the Cerrado showing the number of collections of woody plants identified at species level per conglomerate. The state of Minas Gerais was only partially sampled. Indigenous lands were not sampled, which correspond to large areas in Mato Grosso and Tocantins.

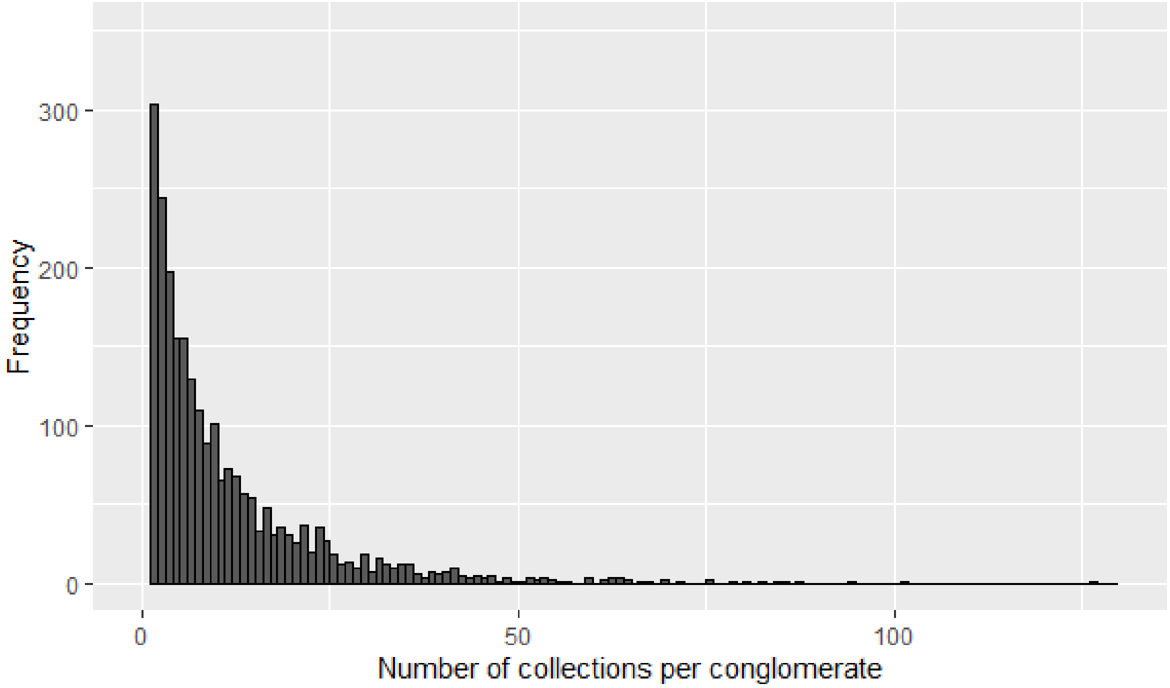

Figure 2. Histogram showing the number of collections of woody plants per sampling unit (conglomerate; 0.4 ha) in the IFN Cerrado. Among the 2779 conglomerates sampled, most were represented by less than ten collections.

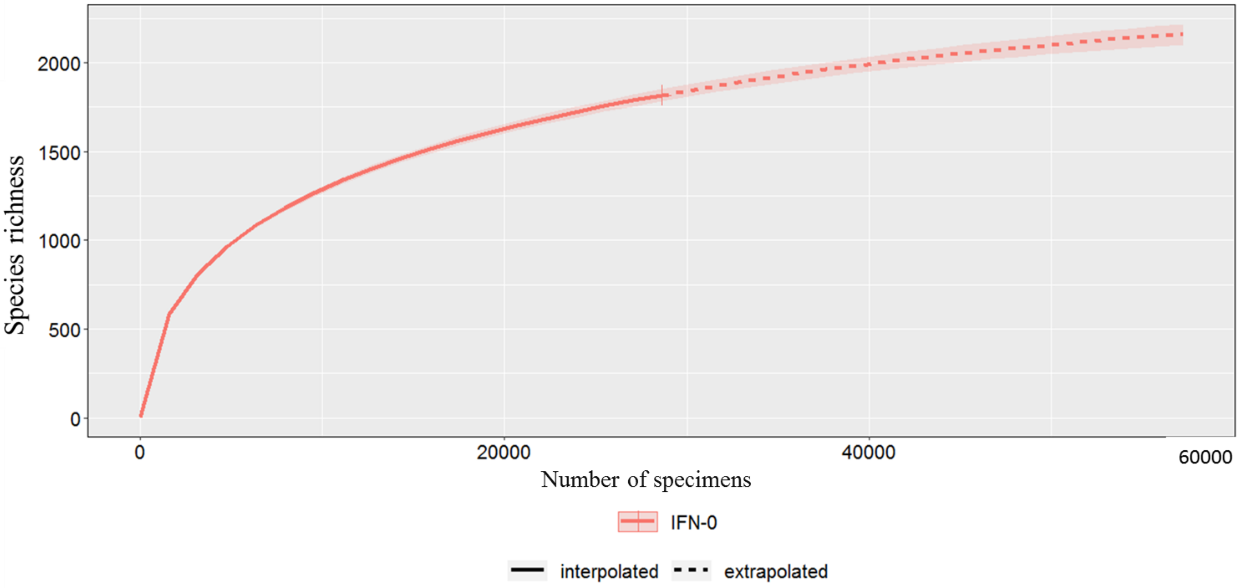

Figure 3. Rarefaction/extrapolation curve of richness of woody species based on the number of specimens (28,602) collected by the IFN Cerrado. Extrapolation of species richness is represented, considering a doubling of sampling effort.

Table 1. IFN Cerrado summary statistics by state. The total sampled area is the sum of sampled conglomerates (sampling units), each one with 0.4 ha. Figures are based on herbarium specimens belonging to woody species identified at the species level. The average of specimens is presented followed by its respective standard deviation. Habitat: G = grasslands, S = savannas, F = forests, A = anthropic areas, W = water.

State	Number of conglomerates	Total sampled area (ha)	Specimens collected	Average of specimens per conglomerate	Total number of species	Number of collected specimens per habitat (G/S/F/A/W)
Bahia	249	99.6	1,964	7.9 ± 8.2	513	122/1,551/137/100/54
Distrito Federal	34	13.6	275	8.1 ± 7.2	190	3/154/83/34/1
Goiás	641	256.4	10.618	16.6 ± 17.2	735	263/3,755/2,719/3,870/11
Maranhão	403	161.2	3,891	9.7 ± 11.2	647	80/1,276/2,157/336/42
Mato Grosso	446	178.4	4,625	10.4 ± 10.7	683	113/2,050/1,544/888/30
Mato Grosso do Sul	290	116.0	2,418	8.3 ± 9.5	382	57/519/993/841/8
Minas Gerais	56	22.4	419	7.5 ± 7.3	132	39/95/29/256/0
Piauí	148	59.2	751	5.1 ± 4.4	291	52/574/100/16/9
São Paulo	110	44.0	949	8.6 ± 9.4	314	14/49/393/465/28
Tocantins	402	160.8	2,692	6.7 ± 7.3	563	245/1,566/484/396/1
Total	2,779	1,111.6	28,602	10.3 ± 12.2	1,822	988/11,589/8,639/7,202/184

potentially new occurrence records for the Cerrado (64 species) and individual states (60), such as Tocantins (18), Mato Grosso do Sul (16), Goiás (11), and Maranhão (ten). Furthermore, 233 Cerrado endemics were recorded, as well as 36 endangered species (Table 2).

Discussion

Botanical collections made during IFN Cerrado surveys covered a broad geographic range that included poorly collected regions. However, the number of specimens collected varied greatly among sampling units (conglomerates) and states. The unbalanced number of collections per conglomerate could have been influenced by the number of species present within each conglomerate. For example, a conglomerate placed in a diverse, well-preserved forest is likely to produce more collections than a conglomerate situated in pastureland wherein only one or a few tree species occur. In addition, we observed that sampling effort varied among the field teams that conducted surveys in different regions within the Cerrado. Although all field teams are supposed to follow the same sampling protocol, we found that some teams were more likely to produce more collections per conglomerate than others. For example, sampling in Piauí averaged only 5.1 specimens per conglomerate, while in Goiás, this number was more than three times higher (16.6). Therefore, it is likely that differential sampling effort among regions may have biased our results. We recommend for future IFN surveys that field teams should increase the number of collections, assuring that a representative sample of the flora within each conglomerate is

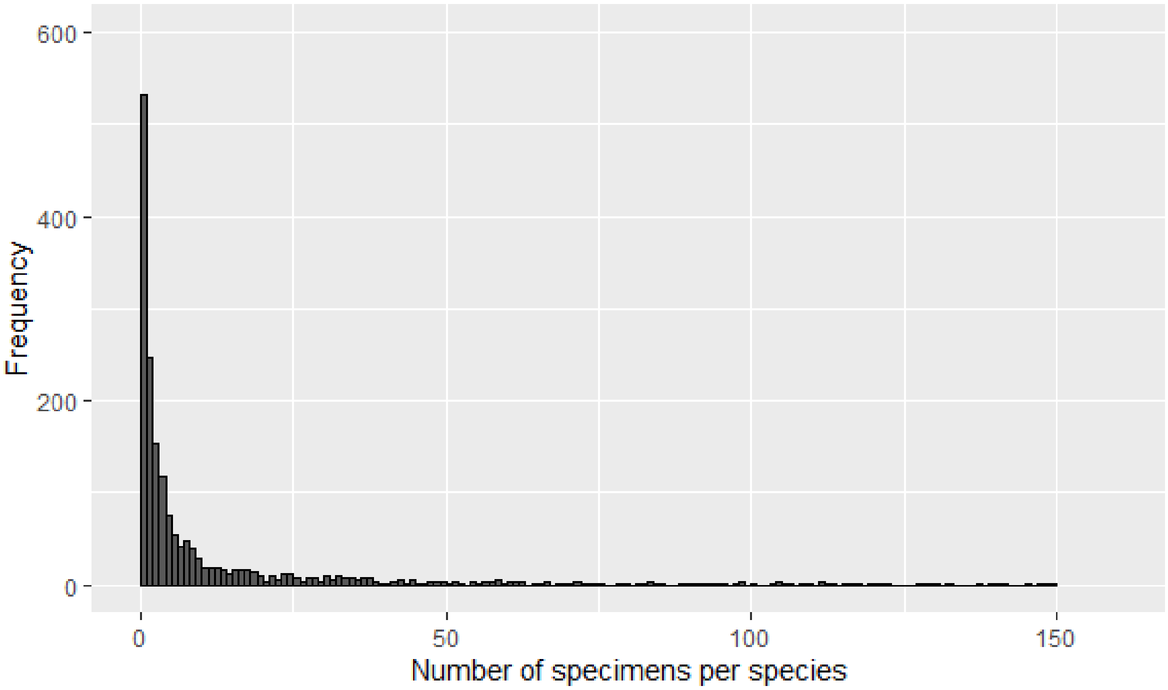

Figure 4. Histogram showing species frequency per range of number of collections made by the IFN Cerrado. Among the 1,822 species recorded, most were collected only once or twice, while 67 species are represented by more than 100 specimens.

represented by a set of herbarium specimens. This could be achieved by demanding field teams to collect a minimum number of vouchers per conglomerate, considering the peculiarities of each vegetation type. Such measures would reduce discrepancies in the number of specimens between conglomerates, resulting in a more even collecting effort across different regions. Increasing collecting effort would be particularly important for highly diverse sites where species identification tends to be more problematic (e.g., wet forests).

We listed a total of 1,822 woody species collected by the IFN Cerrado, which corresponds to 34% of the 5,373 woody species native to the Cerrado in the Flora do Brasil 2020 repository. These statistics indicate that the survey was not able to sample the rich Cerrado plant diversity in its entirety. It is likely that unsampled species are rare, i.e., having restricted geographic range and/or low abundance, since the most frequent species in the various habitats were sampled in the IFN Cerrado. The rarefaction curve clearly showed that additional collections would result in higher species richness. Doubling sampling effort would result in an estimated 2,380 species. However, we must recognise that a considerable number of collected specimens (20,806 or 39.4%) were not identified at species level. Improving the identification of these specimens would certainly result in an increased number of species sampled by the IFN Cerrado. Likewise, the completion of the survey in Minas Gerais would have also contributed to an increase in IFN Cerrado species numbers.

Accurate identification of specimens at the species level is clearly a challenge for many large and taxonomically complex plant families (e.g., Fabaceae, Lauraceae and Myrtaceae), and reliable identifications often require samples of fruits or flowers. The challenge of naming species is particularly difficult when identifications are based on sterile specimens, which make up 86.4% of the IFN Cerrado specimens analysed here. In many cases, we recognise that an accurate determination of sterile material at the species level is not possible, even for experienced taxonomists. Uncertainties underlying plant identifications pose a limitation to forest inventories in species-rich countries, and improving this situation demands innovative approaches (Drapper et al. 2020) that could be adopted by the IFN. Compared to our results, sampling efficiency of floristic diversity was apparently higher in the IFN survey carried out in Santa Catarina where 831 species of trees and shrubs were found (Vibrans et al. 2020). However, despite all collecting efforts, a considerable number (150 species) of trees and shrubs reported for Santa Catarina in previous studies remained unsampled by that survey. In general, the most frequently collected species in the IFN Cerrado are among the most common species cited in the literature for the different vegetation types of the Cerrado. Therefore, we can assume that an elevated number of collections for a given species reflects its high natural abundance. For example, among the 38 species considered the most frequent (oligarchic) in the *cerrado sensu lato* (Ratter et al. 2003), all were recorded by the IFN Cerrado, including some highly collected (>100 records) species, such as *Agonandra brasiliensis, Bowdichia virgilioides, Byrsonima coccolobifolia, Connarus suberosus, Machaerium acutifolium, Myrcia splendens, Plathymenia reticulata, Pouteria ramiflora, Qualea grandiflora, Q. parviflora, Terminalia argentea* and *Vatairea macrocarpa*. Typical species of "*cerradão*", a forest formation composed of mostly savanna tree species, such as *Callisthene fasciculata, Emmotum nitens, Lafoensia pacari* and *Magonia pubescens* (Ribeiro & Walter 2008), were also highly collected in the IFN Cerrado.

Wet forests in the Cerrado are often associated with water courses. Among the species frequently found in Cerrado's riparian forests, several were highly collected in the IFN Cerrado, such as *Tapirira guianensis*, which was the most collected species in the whole survey, as well as *Calophylllum brasiliense, Chysophyllum marginatum,*

Table 2. Selected species collected by the IFN Cerrado highlighting those of high conservation value (threatened and endemics), as well as potential new occurrence records for states. Cerrado endemic species and new records of occurrence are based on information available in Flora do Brasil 2020. BA = Bahia, DF = Distrito Federal, GO = Goiás, MA = Maranhão, MG = Minas Gerais, MS = Mato Grosso do Sul, MT = Mato Grosso, PI = Piauí, SP = São Paulo, TO = Tocantins. Endangered species listed by the CNCFlora according to the categories of the International Union for Conservation of Nature (IUCN): CR = critically endangered, EN = endangered, VU = vulnerable.

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Acanthaceae				
Justicia nodicaulis (Nees) Leonard	X			
Annonaceae				
Duguetia calycina Benoist		X		
Guatteria rigida R.E.Fr.			MS	
Oxandra reticulata Maas			MS	
Trigynaea duckei (R.E.Fr.) R.E.Fr.		X		
Xylopia discreta (L.f.) Sprague		X		
Apocynaceae				
Aspidosperma dispermum Müll.Arg.	X			
Aspidosperma melanocalyx Müll.Arg.			MS	
Aspidosperma spruceanum Benth. ex Müll.Arg.		X		
Aspidosperma rizzoanum Scudeler & A.C.D. Castello	X			
Aspidosperma verbascifolium Müll.Arg.	X			
Rauvolfia weddelliana Müll.Arg.	X			
Araliaceae				
Dendropanax denticulatus Fiaschi		X		
Didymopanax macrocarpus (Cham. & Schltdl.) Seem.			MA, TO	
Didymopanax vinosus (Cham. & Schltdl.) Marchal			TO	
Arecaceae				
Euterpe edulis Mart.				VU
Asteraceae				
Acilepidopsis echitifolia (Mart. ex DC.) H.Rob.	X			
Chromolaena chaseae (B.L.Rob.) R.M.King & H.Rob.	X			
Chromolaena myriocephala (Gardner) R.M.King & H.Rob.	X			
Chromolaena pungens (Gardner) R.M.King & H.Rob.	X			
Eremanthus brasiliensis (Gardner) MacLeish	X			
Eremanthus glomerulatus Less.	X			
Eremanthus goyazensis (Gardner) Sch.Bip.	X			
Eremanthus mollis Sch.Bip.	X			
Eremanthus uniflorus MacLeish & H.Schumach.	X			
Lepidaploa muricata (DC.) H.Rob	X			
Lepidaploa remotiflora (Rich.) H.Rob.	X			
Lepidaploa rufogrisea (A.St.-Hil.) H.Rob.	X			
Lessingianthus brevipetiolatus (Sch.Bip. ex Baker) H.Rob.	X			
Lessingianthus floccosus (Gardner) H.Rob.	X			
Lessingianthus ligulifolius (Mart. ex DC.) H.Rob.	X			
Lessingianthus myrsinites H.Rob.	X			
Lessingianthus obscurus (Less.) H.Rob.	X			
Lessingianthus obtusatus (Less.) H.Rob.	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Lessingianthus zuccarinianus (Mart. ex DC.) H.Rob.	X			VU
Moquiniastrum barrosoae (Cabrera) G.Sancho	X			
Moquiniastrum blanchetianum (DC.) G.Sancho	X			
Moquiniastrum floribundum (Cabrera) G.Sancho	X			
Moquiniastrum paniculatum (Less.) G.Sancho	X			
Piptocarpha oblonga (Gardner) Baker	X		MS	
Piptocarpha rotundifolia (Less.) Baker	X			
Strophopappus glomeratus (Gardner) R.Esteves	X			
Vernonanthura ferruginea (Less.) H.Rob.	X			
Vernonanthura membranacea (Gardner) H.Rob.	X			
Wunderlichia crulsiana Taub.				EN
Bignoniaceae				
Anemopaegma arvense (Vell.) Stellfeld ex de Souza				EN
Fridericia cinerea (Bureau ex K.Schum.) L.G.Lohmann	X			
Handroanthus spongiosus (Rizzini) S.Grose				EN
Jacaranda copaia (Aubl.) D.Don		X		
Jacaranda grandifoliolata A.H.Gentry	X			EN
Jacaranda ulei Bureau & K.Schum.	X			
Xylophragma heterocalyx (Bureau & K.Schum.) A.H.Gentry	X			
Zeyheria tuberculosa (Vell.) Bureau ex Verl.				VU
Burseraceae				
Protium rhoifolium (Benth.) Byng & Christenh.		X	MA	
Calophyllaceae				
Kielmeyera grandiflora (Wawra) Saddi	X			
Kielmeyera lathrophyton Saddi			MA	
Kielmeyera neriifolia Cambess.	X			
Kielmeyera petiolaris Mart. & Zucc.	X			
Kielmeyera rubriflora Cambess.	X		PI	
Kielmeyera speciosa A.St.-Hil.	X			
Kielmeyera tomentosa Cambess.	X			
Cannabaceae				
Celtis fluminensis Carauta		X		
Caricaceae				
Jacaratia corumbensis Kuntze	X			
Caryocaraceae				
Caryocar brasiliense Cambess.	X			
Caryocar cuneatum Wittm.	X			
Celastraceae				
Monteverdia acanthophylla (Reissek) Biral				VU
Monteverdia gonoclada (Mart.) Biral			PI	
Monteverdia guyanensis (Klotzsch ex Reissek) Biral		X		
Salacia crassifolia (Mart. ex Schult.) G.Don	X			
Chrysobalanaceae				
Couepia ovalifolia (Schott) Benth. ex Hook.f.		X		
Exellodendron gardneri (Hook.f.) Prance	X			

Continued...

Family/ species	Cerrado Endemic	New records (Cerrado)	New records (states)	IUCN category
Hirtella hoehnei Pilg.	X		TO	
Leptobalanus parvifolius (Huber) Sothers & Prance		X		
Licania canescens Benoist		X		
Licania coriacea Benth.		X		
Licania gracilipes Taub.		X		
Licania nitida Hook.f.			MS, TO	
Clusiaceae				
Clusia criuva Cambess.	X			
Clusia renggerioides Planch. & Triana		X		
Symphonia globulifera L.f.			GO	
Combretaceae				
Combretum pyramidatum Ham.			GO	
Terminalia phaeocarpa Eichler	X			
Connaraceae				
Connarus suberosus Planch.	X			
Rourea induta Planch.	X			
Cunoniaceae				
Lamanonia brasiliensis Zickel & Leitão	X		GO	EN
Dilleniaceae				
Davilla elliptica A.St.-Hil.	X			
Davilla grandiflora A.St.-Hil. & Tul.	X			
Davilla lacunosa Mart.	X			
Davilla villosa Eichler	X			
Ebenaceae				
Diospyros coccolobifolia Mart. ex Miq.	X			
Diospyros tetrandra Hiern		X		
Elaeocarpaceae				
Sloanea subsessilis D.Sampaio e V.C.Souza	X			
Erythroxylaceae				
Erythroxylum argentinum O.E.Schulz		X		
Erythroxylum ayrtonianum Loiola & M.F.Sales			GO	
Erythroxylum betulaceum Mart.			MS	
Erythroxylum tianguanum Plowman				CR
Erythroxylum tortuosum Mart.	X			
Erythroxylum umbu Costa-Lima		X		
Euphorbiaceae				
Bernardia gardneri Müll.Arg.	X			
Croton micans Sw.	X			
Manihot anomala Pohl	X			
Manihot caerulescens Pohl	X			
Manihot cecropiifolia Pohl	X			
Manihot tripartita (Spreng.) Müll.Arg.	X			
Manihot triphylla Pohl	X			
Sapium laurifolium (A.Rich.) Griseb.		X		
Fabaceae				

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Abarema cochliacarpos (Gomes) Barneby & J.W.Grimes			GO	
Andira cordata Arroyo ex R.T.Penn. & H.C.Lima	X		MS	
Andira inermis (W.Wright) DC.			TO	
Apuleia leiocarpa (Vogel) J.F.Macbr.				VU
Bauhinia dumosa Benth.	X			
Bauhinia holophylla (Bong.) Steud.	X			
Bauhinia membranacea Benth.	X			
Bauhinia rufa (Bong.) Steud.	X			
Calliandra dysantha Benth.	X			
Calliandra silvicola Taub.	X			
Cassia fastuosa Willd. ex Benth.		X		
Cenostigma bracteosum (Tul.) Gagnon & G.P.Lewis			MS	
Chamaecrista acosmifolia (Mart. ex Benth.) H.S.Irwin & Barneby	X			
Chamaecrista ciliolata (Benth.) H.S.Irwin & Barneby	X		BA	
Chamaecrista claussenii (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista conferta (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista coradinii H.S.Irwin & Barneby	X			VU
Chamaecrista crenulata (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista desvauxii (Collad.) Killip	X			
Chamaecrista geminata (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista isidorea (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista ramosa (Vogel) H.S.Irwin & Barneby	X			
Copaifera depilis Dwyer	X			
Copaifera luetzelburgii Harms	X			
Copaifera magnifolia Dwyer	X			
Copaifera malmei Harms	X			
Copaifera oblongifolia Mart. ex Hayne	X			
Copaifera sabulicola J.Costa & L.P.Queiroz	X			
Cratylia mollis Mart. ex Benth.		X		
Dalbergia cuiabensis Benth.	X			
Dalbergia elegans A.M.Carvalho				VU
Dalbergia glandulosa Benth.	X			
Dalbergia miscolobium Benth.	X			
Dalbergia nigra (Vell.) Allemão ex Benth.				VU
Dimorphandra gardneriana Tul.			MS	
Dipteryx alata Vogel	X			
Diptychandra aurantiaca Tul.	X			
Enterolobium timbouva Mart.			TO	
Guibourtia chodatiana Hassl.	X			
Harpalyce brasiliana Benth.	X			
Harpalyce magnibracteata São -Mateus, D.B.O.S.Cardoso & L.P.Queiroz	X			
Harpalyce minor Benth.	X			
Hymenaea maranhensis Lee & Lang.	X			
Hymenaea parvifolia Huber				VU
Hymenolobium heringerianum Rizzini	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Leptolobium elegans Vogel	X		DF	
Luetzelburgia praecox (Harms) Harms	X			
Machaerium nigrum Vogel		X		
Machaerium opacum Vogel			MS	
Machaerium scleroxylon Tul.			TO	
Machaerium ternatum Kuhlm. & Hoehne		X		
Melanoxylon brauna Schott				VU
Mimosa claussenii Benth.	X			
Mimosa decorticans Barneby	X			
Mimosa densa Benth.	X			
Mimosa dichroa Barneby ex G.P.Lewis	X		MS	
Mimosa gardneri Benth.	X			
Mimosa gemmulata Barneby			MS	
Mimosa hapaloclada Malme	X			
Mimosa hebecarpa Benth.	X			
Mimosa hypoglauca Mart.	X			
Mimosa insignis (Hassl.) Barneby	X			
Mimosa interrupta Benth.	X			
Mimosa kalunga M.F.Simon & C.E.Hughes	X			
Mimosa laniceps Barneby	X			
Mimosa laticifera Rizzini & A.Mattos	X			
Mimosa melanocarpa Benth.	X			
Mimosa nitens Benth.	X			
Mimosa nothopteris Barneby		X		
Mimosa oedoclada Barneby	X			
Mimosa oligosperma Barneby	X			EN
Mimosa somnians Humb. & Bonpl. ex Willd.	X			
Mimosa xanthocentra Mart.	X			
Muellera montana (MJ.Silva & AMG.Azevedo) MJ.Silva & AMG. Azevedo	X			
Myrocarpus frondosus Allemão		X		
Ormosia coarctata Jacq.		X		
Peltogyne maranhensis Huber ex Ducke				VU
Schizolobium parahyba (Vell.) Blake		X		
Stryphnodendron fissuratum E.M.O.Martins	X			
Stryphnodendron polyphyllum Mart.	X			
Swartzia laurifolia Benth.		X		
Tachigali aurea Tul.	X			
Tachigali rubiginosa (Mart. ex Tul.) Oliveira-Filho	X			
Tachigali subvelutina (Benth.) Oliveira-Filho	X			
Zapoteca scutellifera (Benth.) H.M.Hern.			GO, TO	
Hypericaceae				
Vismia macrophylla Kunth		X		
Lacistemataceae				
Lacistema hasslerianum Chodat	X			

Continued...

Family/ species	Cerrado Endemic	New records (Cerrado)	New records (states)	IUCN category
Lamiaceae				
Hypenia calycina (Pohl ex Benth.) Harley	X			
Hypenia macrosiphon (Briq.) Harley	X			
Hyptidendron arbusculum (Epling) Harley	X			
Hyptidendron canum (Pohl ex Benth.) Harley	X			
Hyptidendron caudatum (Epling & Játiva) Harley	X			
Hyptidendron conspersum (Benth.) Harley	X			EN
Hyptidendron leucophyllum (Pohl ex Benth.) Harley	X			
Hyptis lutescens Pohl ex Benth.	X			
Hyptis pachyphylla Epling	X			VU
Hyptis rubiginosa Benth.	X			
Hyptis saxatilis A.St.-Hil. ex Benth.	X			
Medusantha multiflora (Pohl ex Benth.) Harley & J.F.B.Pastore			MA	
Mesosphaerum pectinatum (L.) Kuntze			MA	
Vitex flavens Kunth	X			
Lauraceae				
Aiouea macedoana Vattimo-Gil	X			
Aniba hostmanniana (Nees) Mez		X		
Aniba williamsii O.C.Schmidt		X		
Dicypellium caryophyllaceum (Mart.) Nees,				CR
Endlicheria lhotzkyi (Nees) Mez	X			
Nectandra warmingii Meisn.	X			
Ocotea leucoxylon (Sw.) Laness.		X		
Persea splendens Meisn.	X			
Lecythidaceae				
Cariniana legalis (Mart.) Kuntze				EN
Couroupita guianensis Aubl.		X		
Eschweilera grandiflora (Aubl.) Sandwith		X		
Eschweilera parviflora (Aubl.) Miers		X		
Loganiaceae				
Antonia ovata Pohl			MS	
Lythraceae				
Diplusodon virgatus Pohl	X			
Lafoensia pacari A.St.-Hil.	X			
Malpighiaceae				
Banisteriopsis argyrophylla (A.Juss.) B.Gates	X			
Banisteriopsis latifolia (A.Juss.) B.Gates	X			
Banisteriopsis malifolia (Nees & Mart.) B.Gates	X			
Banisteriopsis megaphylla (A.Juss.) B.Gates	X			
Banisteriopsis variabilis B.Gates	X			
Byrsonima affinis W.R.Anderson	X			
Byrsonima basiloba A.Juss.	X			
Byrsonima clausseniana A.Juss.	X			
Byrsonima guilleminiana A.Juss.	X			
Byrsonima variabilis A.Juss.			DF	

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Heteropterys byrsonimifolia A.Juss.	X			
Heteropterys dumetorum (Griseb.) Nied.	X			
Heteropterys procoriacea Nied.	X			
Heteropterys rhopalifolia A.Juss.	X			
Peixotoa glabra A.Juss.	X		PI	
Peixotoa magnifica C.E.Anderson	X			
Malvaceae				
Byttneria glazioui Hochr.	X			
Ceiba samauma (Mart.) K.Schum.			GO	
Eriotheca pubescens (Mart. & Zucc.) Schott & Endl.	X			
Hibiscus capitalensis Krapov. & Fryxell	X			
Luehea crispa Krapov.			TO	
Mollia lepidota Spruce ex Benth.			GO	
Matayba peruviana Radlk.			MT	
Pavonia immitis Fryxell	X			
Pavonia pohlii Gürke	X			
Pseudobombax longiflorum (Mart.) A.Robyns	X			
Pseudobombax tomentosum (Mart.) A.Robyns	X			
Theobroma speciosum Willd. ex Spreng.		X		
Marcgraviaceae				
Schwartzia adamantium (Cambess.) Bedell ex Gir.-Cañas	X			
Melastomataceae				
Cambessedesia hilariana (Kunth) DC.	X			
Lavoisiera pohliana O.Berg ex Triana	X			
Leandra chaetodon (DC.) Cogn.		X		
Leandra deflexa (Triana) Cogn.	X			
Miconia abbreviata Markgr.		X		
Miconia affinis DC.			TO	
Miconia burchellii Triana	X			
Miconia eugenioides Triana		X		
Miconia herpetica DC.	X			
Miconia pepericarpa DC.	X			
Miconia sclerophylla Triana	X		SP	
Microlicia euphorbioides Mart.	X			
Mouriri elliptica Mart.	X			
Mouriri gardneri Triana	X		MA	
Ossaea congestiflora (Naudin) Cogn.	X			
Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	X			
Meliaceae				
Cedrela fissilis Vell.				VU
Cedrela odorata L.				VU
Trichilia elegans A.Juss.			TO	
Trichilia stellato-tomentosa Kuntze				VU
Moraceae				
Ficus maxima Mill.		X		

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Sorocea hilarii Gaudich.			PI	
Myristicaceae				
Virola subsessilis (Benth.) Warb.	X			
Virola surinamensis (Rol. ex Rottb.) Warb.				VU
Virola urbaniana Warb.	X		MS	
Myrtaceae				
Eugenia cupulata Amshoff		X		
Eugenia matogrossensis Sobral	X			
Eugenia megaflora Govaerts	X			
Eugenia pyrifera Faria & Proença	X			
Eugenia stipitata McVaugh		X		
Myrcia camapuanensis N.Silveira	X			
Myrcia myrtillifolia DC.	X			
Myrcia neorubella A.R.Lourenço & E.Lucas		X		
Myrcia tortuosa (O.Berg) N.Silveira	X			
Psidium oligospermum Mart. ex DC.			MS, TO	
Psidium salutare (Kunth) O.Berg	X			
Psidium sessiliflorum (Landrum) Proença & Tuler	X			
Siphoneugena densiflora O.Berg			TO	
Nyctaginaceae				
Guapira campestris (Netto) Lundell	X			
Guapira noxia (Netto) Lundell	X			
Neea floribunda Poepp. & Endl.		X		
Ochnaceae				
Elvasia calophyllea DC.		X		
Elvasia canescens (Tiegh.) Gilg		X		
Ouratea acicularis R.G.Chacon & K.Yamam.				EN
Ouratea cauliflora Fraga & Saavedra		X		
Ouratea paraensis Huber		X		
Olacaceae				
Dulacia egleri (Bastos) Sleumer			GO	
Oxalidaceae				
Oxalis goyazensis Turcz.	X			
Peraceae				
Pera anisotricha Müll.Arg.			MA	
Polygalaceae				
Moutabea excoriata Mart. ex Miq.	X			
Polygonaceae				
Coccoloba brasiliensis Nees & Mart.	X			
Primulaceae				
Cybianthus cuneifolius Mart.		X		
Putranjivaceae				
Drypetes amazonica Steyerm.		X		
Rhabdodendraceae				
Rhabdodendron gardneranum (Benth.) Sandwith	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Rhamnaceae				
Rhamnidium glabrum Reissek				VU
Rubiaceae				
Borreria crispata (K.Schum.) E.L.Cabral & Bacigalupo	X			
Calycophyllum spruceanum (Benth.) K.Schum.		X		
Cordiera myrciifolia (K.Schum.) C.H.Perss. & Delprete			PI	
Guettarda pohliana Müll.Arg.	X			
Palicourea guianensis Aubl.			TO	
Palicourea justiciifolia (Rudge) Delprete & J.H.Kirkbr.		X		
Psychotria guianensis (Aubl.) Rusby		X		
Rutaceae				
Pilocarpus trachylophus Holmes				EN
Salicaceae				
Casearia altiplanensis Sleumer	X			
Casearia rufescens Cambess.	X			
Casearia rupestris Eichler	X			
Xylosma benthamii (Tul.) Triana & Planch.			TO	
Xylosma venosa N.E.Br.			MA	
Sapindaceae				
Cupania castaneaefolia Mart.			MA, TO	
Dilodendron bipinnatum Radlk.			PI	
Matayba peruviana Radlk.		X		
Talisia subalbens (Mart.) Radlk.				VU
Sapotaceae				
Chrysophyllum lucentifolium Cronquist		X		
Ecclinusa ramiflora Mart.		X		
Elaeoluma schomburgkiana (Miq.) Baill.		X		
Micropholis egensis (A.DC.) Pierre		X		
Micropholis emarginata T.D.Penn.			GO, PI	EN
Pouteria anomala (Pires) T.D.Penn.		X		
Pouteria bangii (Rusby) T.D.Penn.		X		
Pouteria cladantha Sandwith		X		
Pouteria furcata T.D.Penn.			TO	EN
Pouteria macrocarpa (Mart.) D.Dietr.		X		VU
Pouteria procera (Mart.) K.Hammer		X		
Pouteria subcaerulea Pierre ex Dubard	X			
Pouteria torta (Mart.) Radlk.	X			
Pradosia granulosa Pires & T.D.Penn.				VU
Schoepfiaceae				
Schoepfia lucida Pulle	X		MA	
Schoepfia velutina Sandwith	X		MA	
Simaroubaceae				
Homalolepis ferruginea (A.St.-Hil.) Devecchi & Pirani	X			
Homalolepis warmingiana (Engl.) Devecchi & Pirani			BA	EN

Continued...

Family/ species	Cerrado Endemic	New records (Cerrado)	New records (states)	IUCN category
Solanaceae				
Solanum falciforme Farruggia	X			
Styracaceae				
Styrax ferrugineus Nees & Mart.	X			
Symplocaceae				
Symplocos nitens (Pohl) Benth.	X			
Symplocos rhamnifolia A.DC.				EN
Turneraceae				
Piriqueta breviseminata Arbo	X			
Turnera lamiifolia Cambess.	X			
Turnera melochioides Cambess.	X			
Verbenaceae				
Citharexylum poeppigii Walp.		X		
Lippia eupatorium Schauer	X			
Vochysiaceae				
Callisthene major Mart.	X		SP	
Callisthene mollissima Warm.	X			
Qualea cordata Spreng.	X			
Qualea dichotoma (Mart.) Warm.	X			
Qualea hannekesaskiarum Marc.-Berti	X			
Qualea selloi Warm.	X		GO	
Vochysia cinnamomea Pohl	X			
Vochysia discolor Warm.	X			
Vochysia gardneri Warm.	X			
Vochysia herbacea Pohl	X			
Vochysia palmirana F.França & Proença	X			
Vochysia pruinosa Pohl	X			
Vochysia pumila Pohl	X			
Vochysia rufa Mart.	X			
Vochysia sessilifolia Warm.	X			
Vochysia thyrsoidea Pohl	X			

Copaifera langsdorffii, Coussarea hydrangeifolia, Dendropanax cuneatus, Eugenia florida, Hirtella glandulosa, Protium heptaphyllum, P. spruceanum, Tapura amazonica, Vochysia haenkeana and *Xylopia emarginata*. These species are widely distributed in Brazilian wet forests, and their shared occurrence between central Brazil riparian forests, Amazon and Atlantic Forest reinforces the floristic links among these domains (Oliveira-Filho & Ratter 1995, Oliveira-Filho & Fontes 2000, Miranda et al. 2018).

Seasonally dry forests occur as scattered patches within the Cerrado, often associated with limestone outcrops and high-fertility soils, which stand in contrast to the acidic and nutrient-poor soils that predominate in the Cerrado region. As a consequence, central Brazil dry forests, which are mostly deciduous during the dry season, differ markedly in species composition compared to adjacent savannas and wet forests (Pennington et al. 2000, Bueno et al. 2018). The characteristic dry forest species *Aspidosperma subincanum* and *Tabebuia roseoalba* were among those highly collected species in the IFN Cerrado, which also recorded other typical dry forest representatives mentioned in the literature, such as *Aspidosperma pyrifolium, Commiphora leptophloeos, Machaerium scleroxylon* and *Schinopsis brasiliensis* (Scariot & Sevilha 2005, Pereira et al. 2011). Although occurring in discontinuous patches, Cerrado dry forests share several species in common with the Caatinga, highlighting the floristic link between these seasonally dry vegetation nuclei (Prado & Gibbs 1993, Neves et al. 2015). In addition, a large sample of sites located at the Cerrado/Caatinga boundary, particularly in Bahia, contributed to increased numbers of typical dry forests and shrublands in our list.

It is worth highlighting that the systematic sampling methodology of the IFN generated new plant occurrence records in areas little sampled in the Cerrado (Sousa-Baena et al. 2014), such as Piauí, Maranhão (the second state in number of IFN specimens), Tocantins, Mato Grosso and southwestern Goiás, thereby filling some important gaps in species distributions. Another positive aspect of the IFN Cerrado methodology was the sampling in different physiognomies, which included habitats that are not frequently surveyed by botanists, such as riparian forests, swamps and seasonally dry forests, including remote areas difficult to access. Botanical exploration of these areas contributed to a number of new species occurrence records for the Cerrado and also for individual states, contributing floristic knowledge, particularly to some states, such as Maranhão, Piauí and Tocantins, for which floristic information is limited (BFG 2015). The contribution of IFN collections towards new state occurrence records has also been highlighted elsewhere for Rio Grande do Norte where 71 new angiosperm records were reported (Versieux et al. 2017).

A large number of new occurrence records for the Cerrado, such as *Aniba hostmaniana, Calycophyllum spruceanum, Elvasia calophyllea, Eugenia cupulata, Jacaranda copaia, Matayba peruviana, Miconia eugenioides, Pouteria anomala* and *Theobroma speciosum*, were mostly collected in wet forests located in northern Mato Grosso and Tocantins along the Cerrado/Amazonia boundary. This ecotone between the Cerrado and Amazonia, a complex transition zone, is composed of interdigitating patches of savannas and various forest types (Marques et al. 2020), and it has certainly incremented the number of species sampled by the IFN Cerrado. Coinciding with an area that lacks botanical records overall (Souza-Baena et al. 2014), this ecotone also helps to explain the large number of new occurrences in the Cerrado for species previously reported only to Amazonia. New occurrences for the Cerrado also comprised typical elements from the Caatinga (e.g., *Cratylia mollis, Pouteria furcata*) and Atlantic Forest (e.g., *Dendropanax denticulatus, Myrocarpus frondosus*), albeit to a lesser extent compared to Amazonia. These results highlight the contribution of marginal/ecotonal areas between the Cerrado and other biomes to the species richness of the Cerrado's tree flora, as reported in previous studies (Oliveira-Filho & Ratter 1995, Françoso et al. 2016, Miranda et al. 2018).

It is important to mention that new occurrence records should be viewed with caution since most were based on sterile specimens identified by non-specialists. Consequently, they may not represent accurate records. This means that potentially new occurrence records should be further confirmed, preferably based on fertile specimens determined by experts. Among the 214 species collected by the IFN Cerrado cited in the CNCFlora's red list, 36 are classified to some degree of threat, including 2 critically endangered (CR), 14 endangered (EN) and 20 vulnerable (VU). Another 179 species are classified as least concern (LC) or near threatened (NT). A total of 232 Cerrado endemic species were collected, representing 12.4% of 1,858 endemic woody species registered in the Flora do Brasil 2020 for this biome. New occurrence records for threatened, as well as rare and endemic species provide crucial data expanding knowledge of geographic ranges and, hence, enabling updates of species threat status and furthering efforts to subsidise conservation initiatives. In addition to new distribution records, specimens collected by the IFN Cerrado supported the description of a new *Harpalyce* (Fabaceae) species from western Bahia (São-Mateus et al. 2019). It is likely that ongoing taxonomic work by specialists based on IFN Cerrado collections deposited in herbaria will reveal more new species to science. Also, with progress in specimen identification in herbaria, new species occurrences for the Cerrado and states, as well as records for endemic and threatened species, are expected.

Conclusion

Although initially developed to assess land coverage, forest structure and wood production, national forest inventories have also played a role as a major source of data for monitoring forest biodiversity (e.g., Chirici et al. 2012). In the present study, focusing specifically on the biodiversity dimension, we showed that the IFN Cerrado has provided a useful source of occurrence data for woody species, spanning wide geographic and habitat coverage. The large number of woody species reported here reflects the floristic variation found in diverse vegetation types found in the Cerrado region, which includes savannas, seasonally dry forests, and wet forests. Our analyses, based on the latest dataset available, showed that about a third of the floristic diversity of the Cerrado woody plants was sampled in the IFN. Our results also show that the typical floristic composition of the different vegetation types of the Cerrado was captured by that survey, and that species cited in the literature as common appear in high numbers among IFN collections. However, differential sampling efforts between field teams and difficulties in naming specimens are expected to influence the floristic diversity reported here. The fact that 35% of conglomerates sampled were located in anthropic areas, which are likely to be less diverse than well-preserved areas, may have also influenced the great variation found in sampling units in terms of species richness. The floristic richness compiled by the IFN Cerrado, which included a number of potential new occurrence records, was greatly influenced by typical woody elements from surrounding biomes, such as Amazonia, Caatinga and Atlantic Forest. Species collected in these marginal/ecotonal zones greatly contributed to the overall number of species recorded here.

Although considered a biodiversity hotspot, it is estimated that the Cerrado will lose 31-34% of its remaining native vegetation by 2050, mostly from agricultural expansion and limited protected areas (Strassburg et al. 2017). Therefore, increasing Cerrado floristic knowledge is critical to support conservation planning in this threatened biome. We expect that the occurrence records derived from IFN collections, particularly those from rare, endemic and endangered species, will contribute to the identification of priority areas for further surveys and conservation of the rich Cerrado flora.

Supplementary Material

The following online material is available for this article:

Table S1 - Specialists that contributed with the identification of selected families, and generalist botanists that identified miscellaneous families (bottom of the table).

Table S2 - Herbarium specimens of woody species collected by the IFN Cerrado. Only records belonging to native species identified at the species level are listed.

Table S3 - List of woody species collected by the IFN Cerrado, including information on states of occurrence, number of specimens collected, habit, vegetation type, and selected voucher specimen. A voucher specimen, either sterile or fertile, was chosen to represent each species.

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Author Contributions

Fernanda K. Kiataqui: Conceptualization, Formal analysis, Writing – original draft-, Writing – review & editing. Sérgio Eustáquio de Noronha: Formal analysis, Writing – review & editing. Marcelo F. Simon: Data curation, Formal analysis, Writing – review & editing.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Inventory

Insect (Hexapoda) Diversity in the Oceanic Archipelago of Fernando de Noronha, Brazil: Scenopinidae (Diptera)

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Abstract: This paper covers the first record of window flies, Scenopinidae (Diptera), in the archipelago of Fernando de Noronha, Brazil: *Scenopinus schulzi* Enderlein, which is briefly characterized. This species was recorded in low numbers: three males and 13 female specimens. The occurrence of scenopinids in Fernando de Noronha is regarded as an extension of the Brazilian mainland continental fauna. The colonization of the archipelago is discussed.
Keywords: *Fernando de Noronha archipelago, window flies, neotropics, oceanic island fauna, Scenopinus.*

Diversidade de insetos (Hexapoda) no Arquipélago Oceânico de Fernando de Noronha, Brasil: Scenopinidae (Diptera)

Resumo: Este trabalho faz o primeiro registro de uma espécie de Scenopinidae no Arquipélago de Fernando de Noronha, Brasil: *Scenopinus schulzi* Enderlein, brevemente caracterizada. Esta espécie foi registrada em baixa densidade: três machos e 13 fêmeas. A ocorrência de scenopinideos em Fernando de Noronha é atribuída à extensão territorial da fauna continental e sua colonização no arquipélago é discutida.
Palavras-chave: *Arquipélago de Fernando de Noronha, fauna insular oceânica, mosca de janela, neotrópicos, Scenopinus.*

Introduction

The current list of hexapods of the Fernando de Noronha archipelago (FN) was presented by Rafael et al. (2020), which included 453 terrestrial species and morphospecies. The list was based on recently collected specimens and on several scientific and technical published works. It still is a preliminary list, and certainly incomplete since lots of specimens are not yet identified. The list is a dynamic process that requires continuous updating since the hexapod fauna from FN is still being studied by many different researchers based on recently collected specimens. Two papers were published based on new material from FN: on Pipunculidae (Diptera) (Rafael et al. 2021a) and on Tabanidae (Diptera) (Rafael et al. 2021b).

Here we present the results for Scenopinidae (Diptera), also known as window flies. Adults are 1.5–9 mm long (Winterton & Gaimari 2017), and feed on nectar and honeydew (Kelsey, 1975). Larvae are predators of arthropods in sandy, friable soils and leaf litter (Winterton & Gaimari 2017), they have also been reared from mammal, bird, and termite nests, and associated with dermestid and wood-boring beetle larvae (Kelsey, 1969, Yeates & Grimaldi 1993).

Two cosmopolitan species, *Scenopinus fenestralis* (Linnaeus, 1758) and *S. glabrifrons* Meigen, 1824, are both associated with human dwellings, being predators of carpet beetles (Dermestidae) (Winterton & Gaimari 2017). However, little else is known of their biology in the Neotropical Region. In the Amazon Basin, adults of *Metatrichia brunneipennis* Ale-Rocha & Limeira-de-Oliveira, 2021, previously treated as *Metatrichia robusta* Kröber, 1913, are known to be more active during the dry season (Rafael & Ale 1983).

The fauna of Scenopinidae has been previously recorded in other oceanic islands, always with low species numbers; 24 species of Scenopinidae are known for the Neotropical Region, and three species of *Scenopinus* have been recorded for Brazil (Lamas 2021; Ale-Rocha & Limeira-de-Oliveira 2021). Currently, the only Neotropical oceanic island records for Scenopinidae are of one species in the Galápagos Archipelago, *Scenopinus galapagosensis* Kelsey, 1970, and four records in the Caribbean islands, namely: *Scenopinus bermudaensis* Kelsey, 1971 (Bermuda); *Scenopinus pygmaeus* Loew, 1857 (Kelsey 1969) (Cuba, Jamaica), and *S. velutinus* Kröber, 1913 and *S. buscki* Kelsey, 1969 (Kelsey 1969) (Jamaica). There is also one fossil species record (*Metatrichia pria* Yeates & Grimaldi, 1993 (Perez-Gelabert 2020) in Dominican amber from Hispaniola. In the South Atlantic Ocean scenopinid was recorded in FN as two unidentified morphospecies of *Scenopinus* (Rafael et al. 2020), both analyzed again and the identification corrected to one species.

Material and Methods

The Brazilian oceanic archipelago of Fernando de Noronha is located in the equatorial South Atlantic region (latitude 3°45'S to 3°57'S; longitude 32°19'W to 32°41'W). It is located c. 360 km from the nearest continental port (in Natal, State of Rio Grande do Norte). The archipelago is entirely volcanic in origin and has never been connected to the mainland. The total land area is 18.4 km^2, of which 16.9 km^2 is the main island named Fernando de Noronha (Teixeira et al. 2003), the only human-inhabited area. All islands and islets are so close, no more than 300 meters apart from each other, that, in regard to insects, they can be treated as a single unit.

The FN archipelago has a tropical oceanic climate (Awi - Köppen classification). The temperature ranges from 23.5ºC to 31.5ºC, with an annual mean of 28ºC (IBAMA 2006) and annual precipitation of 1,400 mm, but with large inter-annual variability. It is characterized by a less rainy season, with a mean precipitation of 27.2 mm/month (August–January), and a rainy season, with a mean precipitation of 211.7 mm/month (March–July). The archipelago has a harsh environment, lacking a permanent source of freshwater, with a low vegetation diversity, and a shallow soil with little water retention (Freitas et al. 2013, Rafael et al. 2020).

The authorization to collect in FN was granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under the collecting license number 62.821. Voucher specimens are deposited at INPA, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, at CZMA, Coleção Zoológica da Universidade Estadual do Maranhão, Caxias, Maranhão and at MNRJ, Museu Nacional do Rio de Janeiro, Rio de Janeiro.

Three sites on the main island of FN were selected for continuous collecting, using interception traps, based on the following criteria: accessibility, diversity of vegetation, low degree of exposure to human activities, and geographical position inside the National Park area. The three sites that were continuously sampled during nine months (June/2019 to February/2020) were: 1) Sancho-Dolphins bay trail, near the information and control desk, using two interception traps, model Townes (Townes 1972), placed around 100 meters of distance from each other; 2) next to the lookout at dolphins bay, using one interception trap, model Gressitt and Gressitt (1962); and 3) on the Capim-Açu trail, also using one interception trap, model Gressitt and Gressitt (1962). Collections were interrupted on March 15, after sanitary restrictions were placed due to the covid-19 pandemic. Additionally, seven-day collecting, using both interception trap models, was conducted at Sueste Bay, on a mangrove ('mangue' on labels), in June/2019 (2-9) and February/2020 (20-27). Specimens were preserved in small containers with commercial ethanol (94°), posteriorly most of the specimens being dried, pinned and labeled.

Results

Fernando de Noronha is the only volcanic south Atlantic oceanic island where *Scenopinus* has been recorded (Rafael et al. 2020).

In nine months (from June 2019 to February 2020) using interception traps, only 14 adult specimens, three males and 13 female specimens, of *Scenopinus schulzi* Enderlein were collected.

Scenopinus schulzi Enderlein (Figures 1A -1D)
Scenopinus schulzei Enderlein, 1934: 429.
Scenopinus schulzi; Kelsey, 1969: 150, fig. 101 (revision); Lamas, 2021 (Brazilian on-line catalogue).
Omphrale caenofrons Kröber, 1937: 229.
Scenopinus sp. 1; Rafael et al. 2020: 15.
Scenopinus sp. 2; Rafael et al. 2020: 15.

The specimens from FN agrees very well with the redescription presented by Kelsey (1969). Males (Figures 1A -1C) and females (Figure 1D) run to *S. schulzi* in the key presented by that author based on the hyaline wing, brown to reddish brown halter knob, and the male abdomen (Figures 1A-1B) with three white bands (first two bands broad, third narrow). Additionally, it is the only neotropical species in the *S. velutinus* group with vein R_4 branching from vein R_5 at the middle of cell r_5.

This species was considered as two morphospecies by Rafael et al. (2020) based on the white bands across the male tergites. The male collected in the mangrove, Sueste Bay, was treated as *Scenopinus* sp. 1 due to its larger size and the wider white bands on the posterior margin of the tergites (Figures 1A-1B). The male collected in the Sancho trail was treated as *Scenopinus* sp. 2 due to its smaller size and the narrower white bands on the posterior margin of the tergites. Male specimens from both morphospecies have now been dissected and, after comparing the terminalia, we concluded that both are conspecific because of the identical terminalia that also fits the figures of *S. schulzi* (Kelsey (1969)). The narrower white bands on the abdominal tergites of one male specimen are here considered to be an artifact of preservation resulted from the dehydration of the specimen. The female specimens are all identical, only differing slightly in size.

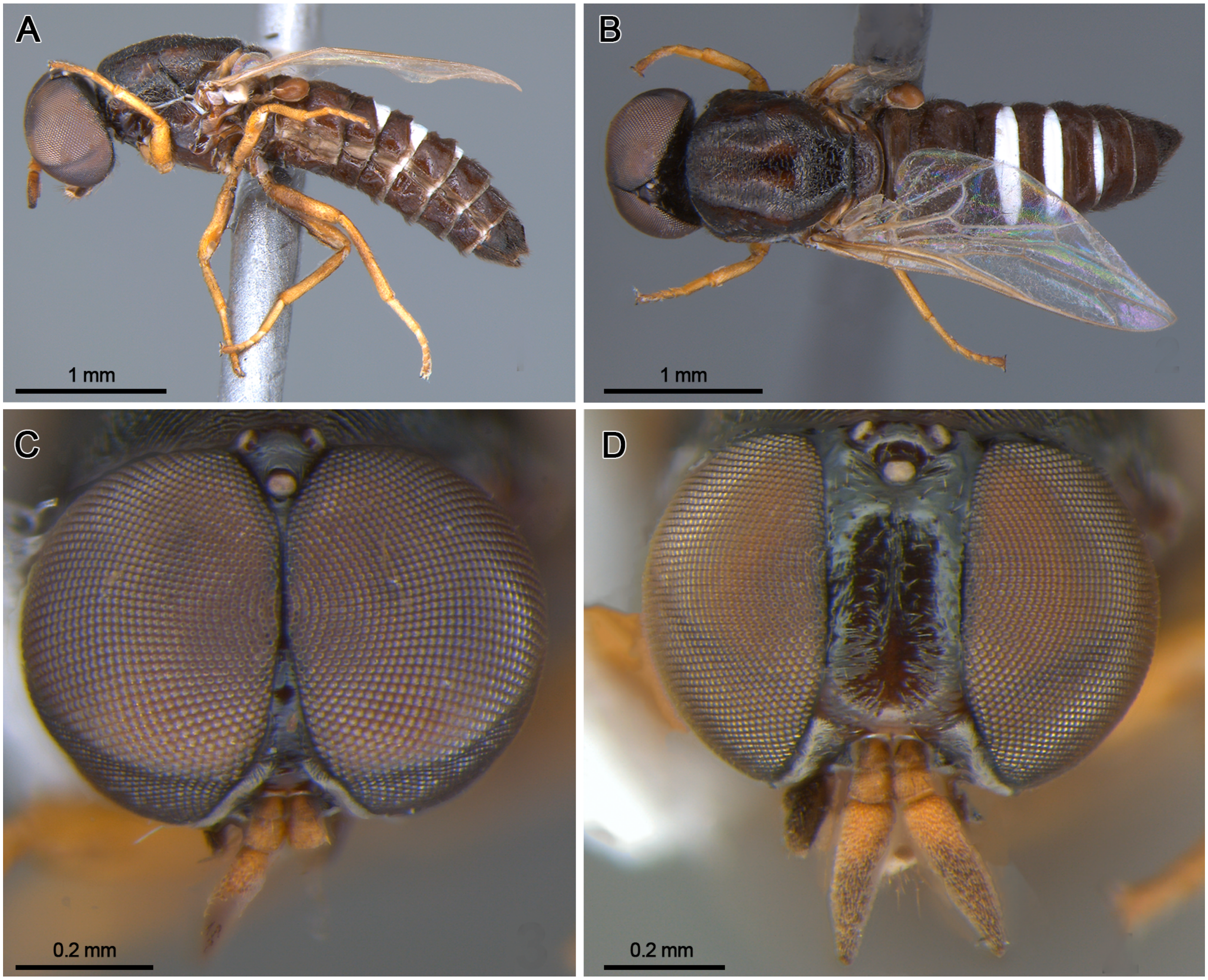
Distribution. Brazil: Pará, Mato Grosso and Santa Catarina (Kelsey 1969), Pernambuco (Rafael et al. 2020) and Cape Green archipelago (Baéz & Oroni 2005).

Examined Material. BRASIL, PE, Fernando de Noronha, 3°51'17"S-32°26'26"W, Tr. Golfinhos, 24.x–9.xi.2019, Malaise Gd, J.A. Rafael, F. Limeira-de-Oliveira, L.C. Castro cols. (1 male, 1 female, CZMA); 26.xi–8.xii, 2019, J.A. Rafael, F. Limeira-de-Oliveira, L.C. Castro cols.(1 female, CZMA); Sancho, 21.viii–8.ix.2019, Malaise peq., J.A. Rafael, F.Limeira-de-Oliveira, L.C. Castro cols. (1 male, 1 female, INPA); 8–27.x.2019 (1 female, INPA); 27.x–11-xi.2019 (2 females, MNRJ); 9–27.xii.2019 (4 females, INPA); Sueste mangue, 20–27.ii.2020, Malaise, J.A. Rafael, P.C. Grossi, F. Limeira-de-Oliveira cols. (1 male, MNRJ; 3 females, CZMA).

Bionomy. The specimens were collected from August/2019 to February/2020, indicating a continuous occurrence in the archipelago. No collections were made in the rainy season, from March to May. No effort was made to find the larvae in FN.

Discussion

Fernando de Noronha is one of the smaller oceanic islands around the world but the largest one belonging to Brazil. Its insect fauna is still poorly known, and it has been neglected for a long time.



Figures 1. *Scenopinus schulzi*: Male, A) *habitus*, lateral view; B) *habitus*, dorsal view; C) head, frontal view; female, D) head, frontal view.

It is uncertain whether *S. schulzi* has been brought to Fernando de Noronha by man or whether it was a natural dispersal. Although natural dispersion seems less likely, it should not be discarded at this moment. There has been active commerce between the continent and FN over the years, which most likely has facilitated the transport of this species as immatures (eggs, larvae or pupae) and/or as adults. About 300 plant species have been introduced to FN (Teixeira et al. 2003), and cargo ships are not inspected to control the introduction of exotic species in the archipelago.

Interception traps are effective to collect scenopinids (Rafael & Ale 1983) and in nine months using Malaise traps only 16 specimens were collected, indicating a low density in FN as in the mainland (authors, pers. obs.). As in other group of insects, Scenopinidae has been neglected, especially in South America, and much work remains to be done especially studies on their biology and behavior.

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Gustavo Graciolli

Authors Contribution

José Albertino Rafael: Contribution in the concept of the study; data collection; identification of the species; data analysis and interpretation; manuscript preparation.

Rosaly Ale-Rocha: Contribution in the concept and design of the study; contribution to critical revision; manuscript preparation; preparation of the figures.

Francisco Limeira-de-Oliveira: Contribution in the concept of the study; data collection; manuscript preparation; contribution to critical revision.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Checklist of Damselflies and Dragonflies (Odonata) from Acre state, and the first record of *Drepanoneura loutoni* von Ellenrieder & Garrison, 2008 for Brazil

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Abstract: Here we present the first Odonata (Insecta) species list for the state of Acre, Northern Brazil, adding ecological aspects and notes on its taxonomy and conservation status. Regarding Odonata samplings, Acre is one of the least explored states in the northern region of Brazil and an area of geographic importance, as it is a transition between the Andean and Amazon regions. Collections were carried out in 35 streams, distributed in nine municipalities. We also supplemented our database from the review of secondary literature and data from biological collections. We recorded 140 species, distributed in 55 genera, of which 16 species are new records for the state, making Acre state the second in the number of recorded species in northern Brazil. Of the recorded species, 113 are classified within some threat category of the IUCN red list and 110 in the ICMBio national list. Analyzing the taxonomic information on each recorded species, knowledge of females and larvae is still very limited and, most of the time, only available to adult males. For the first time, the occurrence of *Drepanoneura loutoni* von Ellenrieder & Garrison (2008) is reported for Brazil, and we also present photos of its main morphological characters, with comments on its biology. Our study shows the importance of conducting biodiversity research in poorly studied areas; such as the state of Acre, and serves as a basis for future expeditions in the region.
Keywords: *List of distribution; Aquatic insects; Inventory; Anisoptera and Zygoptera.*

Checklist das Libélulas (Odonata) do estado do Acre, e o primeiro registro de *Drepanoneura loutoni* von Ellenrieder & Garrison, 2008 para o Brasil

Resumo: Apresentamos a primeira lista de espécies de Odonata (Insecta) para o estado do Acre no Brasil, adicionando aspectos ecológicos e notas sobre o seu estado de conservação. O Acre é um dos estados com o menor esforço de coleta da região Norte do Brasil e uma área de importância geográfica, pois é uma transição entre os Andes e a Amazônia. Foram realizadas coletas em 35 riachos, distribuídos em nove municípios e também complementados com informações de dados secundários de revisão na literatura e bases de dados de coleções biológicas. Registramos 140 espécies, distribuídas em 55 gêneros, das quais 16 espécies são novos registros para o estado, tornando-o o segundo em número de espécies na região Norte do Brasil. Das espécies registradas, 113 estão classificadas dentro de alguma categoria de ameaça da lista vermelha da IUCN e 110 na lista nacional do ICMBio. Analisando as informações do conhecimento das espécies, o conhecimento das fêmeas e larvas ainda muito limitado e na maioria das vezes disponíveis apenas para os machos adultos. Pela primeira vez é registrada a ocorrência da Drepanoneura loutoni von Ellenrieder & Garrison (2008) no Brasil, também apresentamos fotos das principais estruturas, com comentários sobre sua biologia. Nosso trabalho mostra a importância da realização de estudos de biodiversidade em áreas ainda pouco estudadas como a do estado do Acre e serve como base para futuras expedições na região.
Palavras-chave: *Lista de distribuição; Insetos aquáticos; Inventário; Anisoptera e Zygoptera.*

Introduction

Dragonflies and damselflies (order Odonata) constitute a group of charismatic insects, with vibrant colors and great flight capacity (Souza et al. 2007). Worldwide, there are more than 6,300 Odonata valid species, however, considering the great diversity that has yet to be described, estimates indicate that the real number could exceed 7,000 (Suhling et al. 2015, Bybee et al. 2021), due to the preservation of environmental conservation areas focused on these organisms (Bede 2015). The Neotropical region alone contributes approximately 1,700 of the described species (Olaya 2019). In this region, South America stands out for its high species richness, althought much of its territorial extension has not yet been satisfactorily sampled (Kalkman 2008, Araújo et al. 2020). This is an even more evident problem in countries with a large territorial extension such as Peru, Colombia or Brazil (Tognelli et al. 2016).

In Brazil, 901 species of dragonflies are recorded (Pinto 2021), a number that reflects the growing number of studies with this target group in the last decade (Miguel et al. 2017). Despite this increase, only 29% of the Brazilian territory was surveyed so far, being a large part of these collections carried out in regions with more human and financial resources, such as the Southern, Southeastern and Midwestern regions (De Marco & Vianna 2005, Calvão et al. 2016, Rodrigues & Roque 2017). In this sense, these studies, in addition to being insufficient, have a heterogeneous spatial distribution with a recent and slow increase in poorly sampled areas and which access is difficult, such as the Northern, (Koroiva et al. 2020a, Garcia Junior et al. 2021) and Northeastern regions (Santos et al. 2021, Koroiva et al. 2021).

Acre is one of the nine states that compose the Brazilian Legal Amazon (Padrão et al. 2016), a region with large knowledge gaps, especially those related to which species exist and where they are distributed (Linnean and Wallacean gaps) (Hortal et al. 2015). So far, there are only studies by Raimundo et al. (2003), Oliveira (2017) and Garcia Junior et al. (2022) dealing with Odonata species for the state, none of which are specifically directed to the state of Acre in order to list records. However, research carried out in the vicinities of Acre (e.g., state of Amazonas) provides an idea of the diversity potential existing in the region. For example, Paulson (1985) cites the Manu National Park (Perú) as one of the most biodiverse regions for dragonflies on the planet, with 838 species recorded, corresponding to approximately 13% of the global Odonatofauna (reinforced by Venable 1996). Additionally, in Northern Brazil, 334 species have recorded the state of Amazonas alone, which neighbors the state of Acre (Koroiva et al. 2020b). Furthermore, carrying out studies in border regions such as Acre-Pando-Madre de Dios (Brazil, Bolivia and Peru) are of great importance for taking conservation actions for the forest fragments and transboundary water resources (Souza et al. 2013, Acre 2010).

Regarding this scenario, the objectives of our study were: i) to provide the first Odonata species list for the state of Acre; and ii) to provide information on the type of environment in which the species were collected and their level of degradation. Additionally, we recorded for the first time to Brazil the specie *Dreopanoneura loutoni* von Ellenrieder & Garrison, 2008, providing some taxonomic notes and images for the terminalia of both male and female, as well habitat characteristics. We believe that these results can mitigate some of the Linnean gap still present in this region, and consequently provide basic biological information that can be used in further studies for the Amazon forest.

Material and Methods

1. *Study Area*

The Acre state is located in the extreme west of the Northern region of Brazil, in the Western Amazon (Brasil 1968). The territorial extension of the state comprises an area of 164,173,431 km², representing 4% of the Brazilian Amazon and 1.9% of the total territory of Brazil (Figure 1) (Acre 2010, IBGE 2021). The state of Acre has international borders with Peru and Bolivia, and national borders with the states of Amazonas and Rondônia (Acre 2010).

The prevailing climate is the “Am” tropical type (according to Köppen’s classification), described as hot and humid, with high temperatures, high levels of rainfall, and high relative humidity (Peel et al. 2007). The Acre soils, of sedimentary origin, have a predominantly dense rainforest cover (SEMA 2021), characterized by floristic heterogeneity, which constitutes great economic value for the state (Acre 2010). Regarding the relief, the state of Acre has a stable platform that descends smoothly at 300 m on international borders to just over 110 m on the limits with the state of Amazonas. At the western end is the highest point in the state, where the relief changes with the Serra do Divisor, a branch of the Serra Peruana de Contamana, with maximum altitude of 734 m. The hydrography is quite complex, being formed by the hydrographic basins of the rivers Juruá and Purus, tributaries on the right bank of the Solimões river (Acre 2010).

The Acre state falls under the Amazon deforestation arc, where approximately 11% of its territory was already deforested due to the advance of agricultural frontiers (Aguiar et al. 2016). Furthermore, that region is characterized by extractive activities such as rubber and Brazil nut extraction (Ângelo et al. 2013, Martins 2020). However, activities such as the reforestation of Teak (*Tectona grandis* L. F.), fire control programs in the region (large contributors to the ecological imbalance of forests), and monitoring activities (Raimundo et al. 2003, Terra 2017) are preserving native species in the state.

1.1. Places Sampled on Excursions

The sampling was made in the municipalities of Assis Brasil, Brasiléia, Porto Acre, Rio Branco, Sena Madureira, and Senador Guiomard (TABLE 1). The sampled municipalities correspond to approximately 22% of the total area of the state (IBGE 2021). We selected streams that represent a wide gradient of forest cover at the landscape level, and different land uses, from areas altered by agriculture and pasture, to preserved areas located within conservation units. There are two extractive reserves among the sampled areas: RESEX Chico Mendes and RESEX Cazumbá-Iracema, both conservation units meant for sustainable use of natural resources. Data from the RESEX Alto Juruá, taken from the secondary database (Raimundo et al. 2003), were also used. The territorial extension of RESEX Chico Mendes is 970,570 acres, while in Cazumbá-Iracema the territory is 750,795 acres (Acre 2010). Even located within conservation units, these areas are under strong anthropogenic pressure, especially from activities with high environmental impact, such as the conversion of forest into pasture for cattle raising (Fantini & Crisóstomo 2009, Mascarenhas et al. 2018). All collections were made with permission from the Biodiversity Authorization and Information System – SISBio (License number: 11841-4).

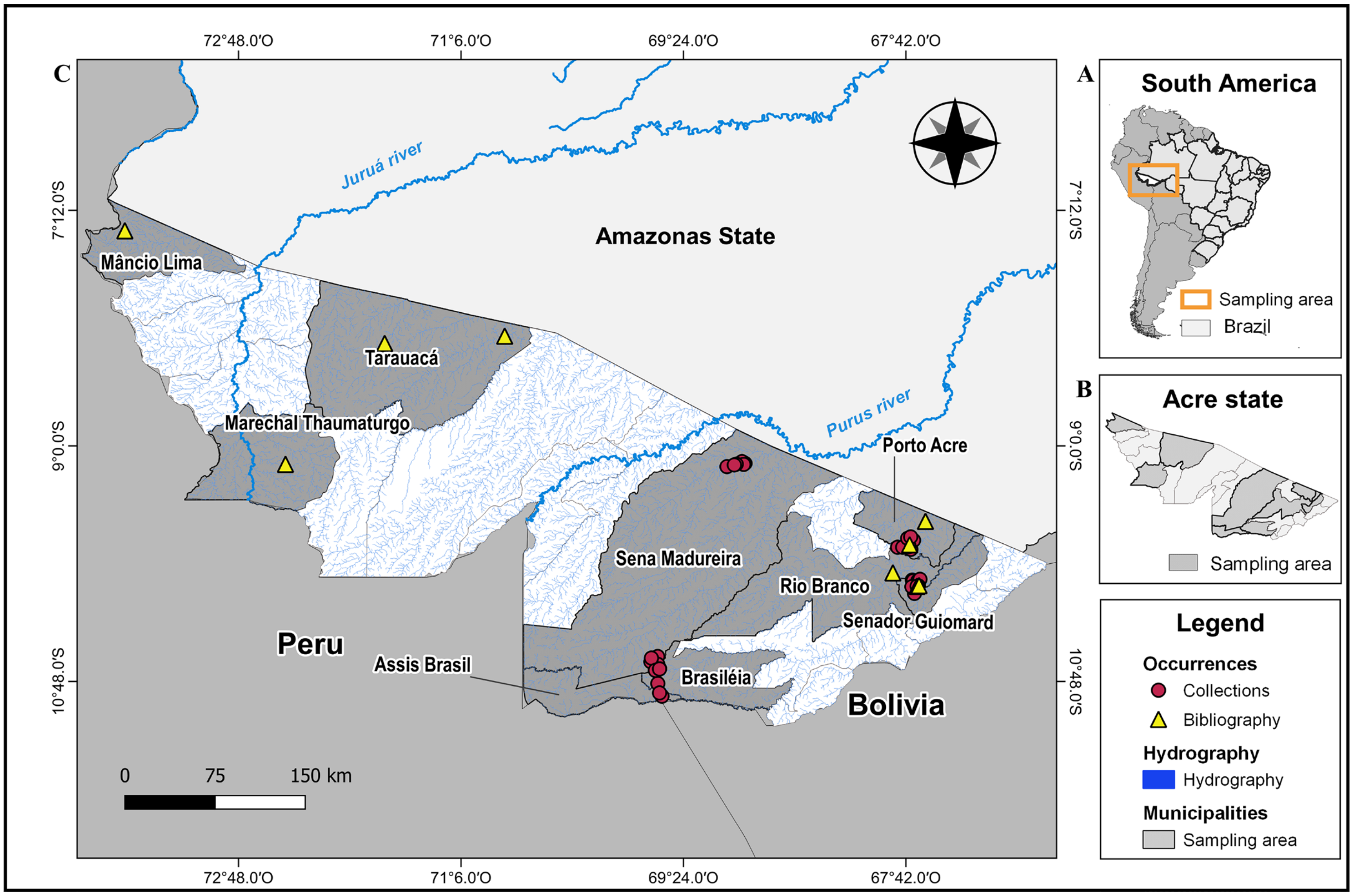

Figure 1. A: South America, with emphasis on the political division of Brazil and territorial extension of the Acre state; B: Map of Acre state; C: Close-up of Acre state, highlighting municipalities with Odonata records.

The Habitat Integrity Index (HII) (Nessimian et al. 2008) was used to relate the integrity of sampled sites to the presence of species. This index has been shown to be an effective metric to explain the distribution of aquatic insect communities, mainly Odonata (Brasil et al. 2021). The index consists of 12 items, which assess characteristics of the banks and the water body. The result varies between 0 (degraded) and 1 (preserved). To categorize the streams, we adopted the criterion proposed by Oliveira-Junior (2015), where environments are considered degraded when values are between $0.15 < HII < 0.49$; intermediate if $0.5 < HII < 0.74$; and preserved if $HII > 0.75$. This classification criterion has been used successfully in other research (Monteiro-Junior et al. 2015, Oliveira-Junior 2015, Oliveira-Junior & Juen 2019) (Table 1).

2. *Preparation of Species List*

The preparation of the species list was made using information from primary data (expeditions and field data collection in 35 streams) and secondary data (from literature and databases). To prepare the list, this information was gathered together with information from the Odonata collection of the Laboratory of Ecology and Conservation (LABECO) of the Federal University of Pará (UFPA), Belém (for more details on the database, access Brasil et al. 2021).

2.1. Collection of Specimens

Adult odonates were collected from 35 small streams (1st to 3rd order according to the Strahler's classification (1957)). A transect of 150 meters was established in each stream, and then subdivided into ten longitudinal sections of 15 m each, named "A" to "K" in the upstream direction. In addition, specimens were collected more specifically on the banks of the stream, with the aid of an entomological net and with a sampling effort of one hour along each transect, with an average of six minutes on each longitudinal section (Cezário et al. 2021). Sampling was always carried out on sunny days between 11:00 and 14:00, which is the ideal weather conditions for the activity of most Odonata species (Monteiro-Júnior et al. 2015, Oliveira-Junior & Juen 2019). The collected specimens were packaged and preserved according to the protocol by Lencioni (2006). For the taxonomic identification of the collected specimens, specific keys such as those by Lencioni (2005, 2006, 2013), and Garrison et al. (2006) were used. In addition, comparisons were made with specimens already identified and deposited in the collection of LABECO - UFPA, and, when necessary, specialists were consulted, such as Frederico Lencioni e Diogo Vilela.

2.2. Search in Literature and Databases

Additional secondary data were obtained from the following databases: SpeciesLink (http://splink.Cria.org.br/), and Brazilian Taxonomic Catalog of Fauna (http://fauna.jbrj.gov.br;Brazilian FaunaTaxonomic Catalog), making a filter for information on species in the state of Acre. Data was also gathered from specimen description studies, mainly seeking information from biology or natural history and where they occur in Brazil (Garrison et al. 2006, Lencioni 2005, 2006).

Table 1. Primary data with sampling points in the hydrographic basins of the Acre, Iguiri and Caeté rivers, Acre state, Brazil.

Point (SU)	Point Code	Municipality	Coordinates	Elevation (masl)	HII	Condition
1	AC01	Senador Guiomard	10º7'37.2"S 67º38'6"W	170	0.417	Degraded
2	AC02	Senador Guiomard	10º1'30"S 67º39'7.2"W	187	0.428	Degraded
3	AC03	Senador Guiomard	10º1'48"S 67º38'38.4"W	188	0.352	Degraded
4	AC04	Senador Guiomard	10º4'19.2"S 67º36'54"W	200	0.970	Preserved
5	AC05	Senador Guiomard	10º4'15.6"S 67º37'22.8"W	201	0.920	Preserved
6	AC06	Rio Branco	10º1'51.6"S 67º36'32.4"W	195	0.954	Preserved
7	AC07	Rio Branco	10º1'8.4"S 67º35'34.8"W	196	0.954	Preserved
8	AC08	Senador Guiomard	10º4'33.6"S 67º39'18"W	204	0.609	Intermediate
9	AC09	Senador Guiomard	10º4'8.4"S 67º36'18"W	202	0.870	Preserved
10	AC10	Porto Acre	9º47'24"S 67º39'46.8"W	182	0.664	Intermediate
11	AC11	Porto Acre	9º43'15.6"S 67º38'31.2"W	171	0.496	Intermediate
12	AC13	Porto Acre	9º46'8.4"S 67º40'33.6"W	166	0.596	Intermediate
13	AC14	Rio Branco	9º46'26.4"S 67º45'50.4"W	197	0.440	Degraded
14	AC15	Rio Branco	9º46'26.4"S 67º43'30"W	195	0.822	Preserved
15	AC16	Porto Acre	9º42'21.6"S 67º41'13.2"W	180	0.739	Intermediate
16	CZ01	Sena Madureira	9º7'57.972"S 68º55'49.548"W	173	0.819	Preserved
17	CZ02	Sena Madureira	9º7'49.584"S 68º56'15.612"W	169	0.764	Preserved
18	CZ03	Sena Madureira	9º7'51.996"S 68º56'27.096"W	165	0.764	Preserved
19	CZ04	Sena Madureira	9º7'11.964"S 68º57'9.936"W	147	0.613	Intermediate
20	CZ05	Sena Madureira	9º9'28.987"S 69º4'4.66"W	160	0.590	Intermediate
21	CZ06	Sena Madureira	9º8'5.496"S 68º56'57.876"W	146	0.494	Intermediate
22	CZ07	Sena Madureira	9º7'11.964"S 68º57'9.936"W	144	0.619	Intermediate
23	CZ08	Sena Madureira	9º8'28.248"S 68º56'23.028"W	189	0.875	Preserved
24	CZ09	Sena Madureira	9º8'20.033"S 68º59'48.934"W	185	0.671	Intermediate
25	CZ10	Sena Madureira	9º8'42.292"S 69º0'44.968"W	170	0.688	Intermediate
26	CM01	Brasiléia	10º48'54.472"S 69º35'43.897"W	288	0.585	Intermediate
27	CM02	Assis Brasil	10º54'41.936"S 69º33'52.088"W	273	0.606	Intermediate
28	CM03	Assis Brasil	10º53'13.546"S 69º35'0.172"W	268	0.336	Degraded
29	CM04	Brasiléia	10º41'59.741"S 69º34'59.596"W	273	0.543	Intermediate
30	CM05	Sena Madureira	9°09'29.0"S 69°04'04.7"W	266	0.460	Degraded
31	CM06	Brasiléia	10º40'7.622"S 69º35'46.32"W	302	0.425	Degraded
32	CM07	Sena Madureira	9°07'12.0"S 68°57'09.9"W	266	0.467	Degraded
33	CM08	Sena Madureira	9°08'28.3"S 68°56'23.0"W	307	0.664	Intermediate
34	CM09	Brasiléia	10º42'55.033"S 69º36'52.722"W	286	0.293	Degraded
35	CM10	Brasiléia	10º42'14.4"S 69º34'58.8"W	282	0.194	Degraded

Captions. SU=Sampling Unit, AC=Acre. CM=Chico Mendes. CZ=Cazumbá-Iracema. Non-sequential numbers were extracted from the literature (Table 2).

With the incorporation of secondary data, we added 12 more collection points in six municipalities, taken from the literature describing the corresponding species, three exclusively for literature data and another three already added from LABECO collection tours, data from two recently published articles were also incorporated, Oliveira (2017) as point L14 and Garcia Junior et al. (2022) as point L13 (TABLE 2). The literature search was based on the Web of Science and Scielo databases, using data from previously published studies that record odonates in the region of the Acre state. The published data are also search results on Google Scholar (http://scholar.google.com), we use the terms: "Odonata and Acre and Brasil" for more general literature, and we add specific terms to confirm information missing (e.g. "larva and female" or "taxonomy and description"). There have been species description works since the end of the 18th century and the beginning of the 19th century, with specimens of odonatas collected in the region of the state. Searches were carried out in May 2021. Additional searches were completed in February 2022. Collection information was considered: species name, occurrence, collection date, collector name and location (city and state), in addition to additional information about the altitude level of each point, habitat (municipality and additional information such as vegetation type or name of stream or lake, when available), number of individuals collected and number of males, females and larvae described.

Table 2. Information obtained from secondary data. L= Secondary data based on literature.

Point Code	Point (SU)	Municipality	Coodinates	Elevation (masl)
L1	36	Porto Acre	9º 41' 42"S 67º 39' 50.4"W	206
L2	37	Senador Guiomard	10º 4' 19.56"S 67º 36' 53.64"W	200
L3	38	Brasiléia	10º42'14.328"S 69º34'59.592"W	208
L4	39	Marechal Tramaturgo	9° 08' 23.0"S 72° 26' 28"W	295
L5	40	Tarauacá	8º 13' 0" S 71° 41' 0" W	240
L6	41	Porto Acre	9º 34′ 35″ S 67° 33′ 3″ W	164
L7	42	Mâncio Lima	7º 21' 23" S 73° 40' 4" W	197
L8	43	Senador Guiomard	10° 4' 20"S 67° 36' 53"W	200
L9	44	Tarauacá	8° 09' 39" S 70° 45' 57" W	172
L10	45	Porto Acre	09° 45' 19"S 67° 40' 18"W	189
L11	46	Rio Branco	09° 58' 13"S 67° 48' 00"W	135
L12	47	Senador Guiomard	10° 03' 60"S 67° 35' 59"W	191
L13	48	--	--	--
L14	49	--	--	--

Caption. L= Literature.

3. *Statistical Analysis*

To assess the efficiency of the sampling effort, we generated collector curves with rarefaction (interpolation) using the first-order Jackknife estimator, which allows us to evaluate collection efficiency and produce the collector curve. Using this method, we estimated the number of species per sampled transect. As a result, the observed richness with the average of Mao Tau Sobs was obtained and Jackknife (Burnham & Chazdon 1978; Burnham & Overton 1979), the most accurate and least biased estimator compared to other extrapolation methods (Palmer, 1990). All analyzes were performed in the R software (RCoreTeam 2019) using the "vegan" (Oksanen et al. 2005), and "BiodiversityR" (Kindt & Coe 2005) packages (Supplementary Material, Table S1).

4. *Taxonomic Notes*

Drepanoneura loutoni von Ellenrieder & Garrison, 2008 was recorded for the first time in Brazil. *D. loutoni* were collected in the two extractive reserves (RESEX). The specimens were photographed using a Leica M205 a stereomicroscope equipped with a Leica DFC 450 camera. Subsequently, we processed the image in the in a free image editor. Morphological terminology for *D. loutoni* follows von Ellenrieder & Garrison (2008). All the measurements are in millimeters (mm). Abbreviations: Ce, cercu; Pa, paraproct; Ep, epiprocto; Tru, truncated.

Results

Altogether, 140 species were recorded (Table 3; Figure 2, 3 and 4), being 57 from primary data and 108 from secondary records, with 19 of these records already in our collection data. The number of estimated species was 38 ± 0.652 (mean ± SD). When we analyzed the efficiency of the collection effort (average observed richness/average estimated richness), we obtained a value of 73%. These results show that the efficiency collection for the study was enough to sample the existing biodiversity in the region. A similar result was observed in the collector curve, where there was a tendency to stabilization in its final part (Figure 5; Supplementary material, Table S1).

The total number of Odonata genera thus far recorded for the state is 55, distributed in nine families. The suborder Zygoptera was the most representative, with six families, namely: Calopterygidae (two genera, six species), Coenagrionidae (17 genera, 54 species), Heteragrionidae (one genus, three species), Polythoridae (two genera, four species), Dicteriadidae (one genus, one species), Perilestidae (two genera, two species). In its turn, the suborder Anisoptera comprises three families: Libellulidae (23 genera, 62 species), Gomphidae (two genera, two species), and Aeshnidae (five genera, five species).

Of the 140 recorded species, 98 have described females. When considering the larval stages, only 56 species have their larvae described. In our study, the material identified to genus level was not considered, as it could represent an underestimation of the diversity presented here. However, we would like to record the existence of specimens of the following genera: *Dythemis* Hagen, 1861, *Elasmothemis* Westfall, 1988, *Oligoclada* Karsch, 1890, and *Heteragrion* Selys, 1862 that present different structures. More detailed analyzes are being carried out to determine if these taxa represent species new to science or new records for Brazil.

Knowledge concerning the conservation status of dragonflies in Acre state is still incipient. However, based on the Red List of Endangered Species of the Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (ICMBio 2018) and the International Union for Conservation of Nature (IUCN, https://www.iucnredlist.org), of the 140 listed species, 113 were evaluated, but we emphasize that none is threatened with extinction (EX), in critical danger (CR) or any threat category (TABLE 3). In the IUCN list, most species (103) are in the Least Concern (LC) category, 10 species have insufficient data (DD), while 27 species have not yet been evaluated (NE); on the ICMBio list, 105 species are listed as least concerning (LC), five have insufficient data (DD) and 30 have not yet been evaluated.

The registration data for Brazil come from the taxonomic keys of Lencioni (2005, 2006) and Garrison (2006), as well as for described females and larvae, being complemented, when necessary, with data from the "Brazilian Taxonomic Catalog of Fauna" and articles describing the species, that contains their collection points as well as geographic coordinates data.

Table 3. List of recorded species in the primary and secondary databases.

Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Zygoptera					**Yes**	**No**	**Yes**	**No**		
Coenagrionidae										
Hetaerina laesa Hagen in Selys, 1853	4, 7 e 10.	36, 48.	NE	LC	X			X	PA. RO. MT. AC	Garcia Junior et al. 2022
Hetaerina rosea Selys, 1853*	1 e 3.	--	LC	LC	X		X		RO. MG. RJ. SP. RS. MT. BH.	Lencioni 2005
Hetaerina sanguinea Selys, 1853	--	39, 48.	LC	LC	X			X	AM. AC	Garcia Junior et al. 2022
Mnesarete aenea (Selys, 1853)	5	48	NE	LC	X			X	PA. RO. AC.	Garcia Junior et al. 2022
Mnesarete cupraea (Selys, 1853)	1, 4, 5, 7, 9, 10, 16, 19, 25, 26, 27, 28, 29, 30 e 34.	39, 48.	NE	LC		X		X	AC. RO. PA. MA. MT.	Garcia Junior et al. 2022
Mnesarete loutoni Garrison, 2006*	17 e 21.	--	NE	LC	X			X	AM.	Lencioni 2005
Coenagrionidae										
Acanthagrion apicale Selys, 1876	5	36, 48.	LC	NE	X		X		PA. RO. AC.	Garcia Junior et al. 2022
Acanthagrion ascendens Calvert, 1909*	22, 32 e 33.	--	LC	NE	X		X		MT. SP.	Lencioni 2006
Acanthagrion floridense Fraser, 1946	2	--	LC	NE	X		X		RO. AC.	Lozano et al. 2017
Acanthagrion gracile (Rambur, 1842)	26	37, 48.	LC	NE	X			X	BH. MT. RJ. SP. RS. AC.	Garcia Junior et al. 2022
Acanthagrion obsoletum (Foster, 1914)	1, 2, 3, 8, 26, 29 e 33.	37	LC	NE	X			X	AC.	Lencioni 2006
Acanthagrion peruvianum Leonard, 1977	2	--	LC	DD	X			X	RO. AC.	Lozano et al. 2017
Acanthagrion temporale Selys, 1876*	--	36	LC	LC	X			X	RO. BH. MT. MG. SP.	Lencioni 2006
Amazoneura juruaensis Machado, 2004	--	42, 48.	DD	NE		X		X	AC.	Garcia Junior et al. 2022, Machado 2004.
Argia cf. Adamsi Calvert, 1902	18, 20 e 24.	--	NE	NE	X		X		AC.	De Marmels 2007
Argia collata Selys, 1865	1, 2, 6, 7, 9, 16, 17, 18, 21 e 22.	--	LC	NE	X			X	PA. AC. RO.	Garrison & Ellenrieder 2018
Argia dives Förster, 1914*	29, 31 e 35	--	LC	NE	X			X	MT.	Lencioni 2006
Argia euphorbia Fraser, 1946	--	39, 48.	LC	LC		X		X	AM. RO. AC.	Garcia Junior et al. 2022
Argia fumigata Hagen in Selys, 1865*	27, 28, 31 e 33.	--	LC	LC		X		X	AM. RO. MT.	Garrison & Ellenrieder 2015
Argia indicatrix Calvert, 1902*	2	--	NE	LC	X			X	AM.	Lencioni 2006
Argia infumata Selys, 1865	5 e 6.	48	NE	LC	X			X	PA. AM. RO. AC.	Garcia Junior et al. 2022

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Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Zygoptera					**Yes**	**No**	**Yes**	**No**		
Coenagrionidae										
Argia loutoni Garrison & von Ellenrieder, 2015	--	41	LC	NE	X			X	AM. AC.	Garrison & Ellenrieder 2015
Argia oculata Hagen in Selys, 1865*	2, 31, 32 e 33	--	NE	LC	X		X		AM. MT.	TORRES-PACHÓN et al. 2017, Garrison & Ellenrieder 2015
Argia tennesseni Garrison & von Ellenrieder, 2018	--	40	LC	NE	X			X	AC.	Garrison & Ellenrieder 2018
Argia thespis Hagen in Selys, 1865*	10, 12 e 15	36	NE	NE	X			X	AM. BH.	Lencioni 2006
Drepanoneura janirae von Ellenrieder & Garrison, 2008*	16, 17, 18, 21, 22, 23 e 24.	--	NE	DD		X		X	RO.	Von Ellenrieder & Garrison 2008
Drepanoneura loutoni von Ellenrieder & Garrison, 2015**	19, 21, 25, 27, 31, 33, 34 e 35	--	LC	NE	X			X	AC.	Von Ellenrieder & Garrison 2008
Epipleoneura tariana Machado, 1985	--	48	LC	LC		X		X	AM. AC.	Pessacq 2014, Garcia Junior et al. 2022
Epipleoneura venezuelensis Rácenis, 1955*	1, 2, 4, 5, 6, 9, 10, 14, 22, 27, 28, 29, 30 e 31.	--	LC	LC	X			X	DF. GO. MG. MT. PA. RJ. SP. ES.	Pessacq 2014
Ischnura capreolus (Hagen, 1861)	--	48	LC	LC	X		X		PA. PE. BA. MT. ES. RJ. SP. RS. AC.	Garcia Junior et al. 2022
Mecistogaster amalia (Burmeister, 1839)	--	48	LC	LC	X		X		RJ. SP. AC	Garcia Junior et al. 2022
Mecistogaster buckleyi McLachlan, 1881*	--	39	LC	NE		X		X	AM.	Lencioni 2006
Mecistogaster jocaste Hagen, 1869	--	39	LC	DD	X			X	AC.	Lencioni 2006
Mecistogaster linearis (Fabricius, 1777)*	--	39	LC	LC	X		X		AM. RO. PA. MS. AM. SP.	Lencioni 2006
Metaleptobasis falcifera von Ellenrieder, 2013	--	43, 48.	LC	NE	X			X	AC.	Von Ellenrieder 2013
Metaleptobasis inermis von Ellenrieder, 2013	--	48	DD	NE		X		X	PA. AC.	Von Ellenrieder 2013
Metaleptobasis minteri Daigle, 2003	--	44, 48.	DD	NE	X			X	AC.	Von Ellenrieder 2013
Microstigma anomalum Rambur, 1842	--	48	LC	LC	X			X	AM. PA. AC.	Garcia Junior et al. 2022
Microstigma rotundatum Selys, 1860	--	39, 48.	NE	LC	X			X	AC.	Garcia Junior et al. 2022
Neoneura bilinearis Selys, 1860	--	39, 48.	LC	LC	X			X	PA. ES. SP	Garcia Junior et al. 2022
Neoneura denticulata Williamson, 1917	--	48	LC	LC	X			X	AM. RO. RR. PA. AC.	Garcia Junior et al. 2022

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Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Zygoptera Coenagrionidaea					**Yes**	**No**	**Yes**	**No**		
Neoneura rubriventris Selys, 1860*	2	--	LC	LC	X			X	RR. PA. RO. RS.	Lencioni 2006
Neoneura rufithorax Selys, 1886	--	48	LC	LC	X			X	AM. AC.	Garcia Junior et al. 2022
Philogenia marinasilva Machado, 2010	--	42	LC	NE		X		X	AC.	Machado 2010a
Phoenicagrion flammeum (Selys, 1876)	--	36, 48.	LC	LC	X			X	PA. AM. AC. TO. AM. RO. PE. MT.	Garcia Junior et al. 2022
Platystigma humaita Machado & Soldati, 2017	--	45	DD	NE		X		X	AC.	Machado & Lacerda 2017
Platystigma jocaste (Hagen, 1869)	--	48	LC	NE	X			X	AC.	Garcia Junior et al. 2022
Platystigma minimum Machado & Soldati, 2017	--	46	DD	NE		X		X	AC.	Machado & Lacerda 2017
Platystigma quadratum Machado & Soldati, 2017	--	47	DD	NE		X		X	AC.	Machado & Lacerda 2017
Protoneura scintilla Gloyd, 1939*	2, 21, 22, 27 e 32.	--	LC	LC	X			X	RO.	Lencioni 2006
Protoneura tenuis Selys, 1860	1, 4, 5, 6, 9, 16 e 17.	36 e 39	LC	LC	X			X	PA. RO. AC.	Garcia Junior et al. 2022
Protoneura woytkowskii Gloyd, 1939	16, 18, 22, 26 e 31.	--	NE	NE	X			X	AC.	Lencioni 2006
Psaironeura bifurcata (Sjöstedt, 1918)	--	48	LC	LC	X			X	AM. PA. AC.	Garcia Junior et al. 2022
Psaironeura tenuissima (Selys, 1886)*	8, 17, 18, 19, 22 e 24.	--	NE	LC	X			X	AM. PA. RO.	Lencioni 2006
Telebasis carmesina Calvert, 1909	--	48	LC	LC	X			X	MT. MG. SP. AC.	Garrison 2009, Garcia Junior et al. 2022
Telebasis corbeti Garrison, 2009	--	43	NE	NE		X		X	AC.	Garrison 2009, Machado 2010b, Garcia Junior et al. 2022
Telebasis griffinii (Martin, 1896)	16, 17, 22, 26 e 31.	48	LC	LC	X		X		PA. AM. AC. MS. SP. RJ. MS.	Garrison 2009, Guilhermo-Ferreira 2013, Garcia Junior et al. 2022
Telebasis obsoleta (Selys, 1876)	--	44, 48.	LC	LC		X	X		PA. AM. AC. PR. MT. MS.	Garrison 2009, Lozano et al. 2017, Garcia Junior et al. 2022
Telebasis rubricauda Bick & Bick, 1995	--	48	LC	DD	X			X	RO. AC.	Garrison 2009, Garcia Junior et al. 2022

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Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Zygoptera					**Yes**	**No**	**Yes**	**No**		
Coenagrionidae										
Tigriagrion aurantinigrum Calvert, 1909	1 e 3.	36, 48.	LC	LC	X			X	MT. MG. SP. AC.	Garcia Junior et al. 2022
Dicteriadidae										
Heliocharis amazona Selys, 1853	16 e 35	39, 48.	NE	LC	X		X		GO. MT. MG. SP. AC.	Garcia Junior et al. 2022
Heteragrionidae										
Heteragrion bariai De Marmels, 1989	4, 6, 7, 9, 10, 13, 16, 17, 18, 21, 23, 26, 27, 33 e 34.	36, 48.	NE	LC	X			X	RO. AC.	Garcia Junior et al. 2022
Heteragrion bickorum Daigle, 2005	16, 18, 19, 21, 22, 23, 24 e 25.	--	LC	NE	X			X	AC.	Lencioni 2005
Heteragrion cf. Majus Selys, 1886	17 e 21.	--	LC	NE		X	X		AC.	Lencioni 2005
Perilestidae										
Perilestes kahli Williamson & Williamson, 1924*	21, 22 e 29, 31 e 32	--	LC	LC	X			X	RO. PA.	Kennedy 1937
Perissolestes paprzyckii Kennedy, 1941	--	48	DD	DD	X			X	AC. AM.	Garcia Junior et al. 2022
Polythoridae										
Chalcopteryx rutilans (Rambur, 1842)	7	48	LC	LC	X		X		AM. PA. RO. GO. MT. AC.	Garcia Junior et al. 2022
Polythore manua Bick and Bick 1990	--	39, 48.	LC	NE	X			X	AC. AM.	Garcia Junior et al. 2022
Polythore picta (Rambur, 1842)	--	48	LC	LC	X			X	AM. AC	Garcia Junior et al. 2022
Polythore vittata (Selys, 1869)	--	39, 48.	NE	LC	X			X	AC.	Garcia Junior et al. 2022
Anisoptera										
Aeshnidae										
Coryphaeschna adnexa (Hagen 1861)	--	39, 48.	LC	LC	X		X		AC.	Garcia Junior et al. 2022
Gynacantha interioris Williamson, 1923	--	48	LC	LC	X			X	AC.	Williamson 1932, Garcia Junior et al. 2022
Neuraeschna calverti Kimmins, 1951	--	39, 48.	LC	LC		X		X	AC.	Garcia Junior et al. 2022
Remartinia luteipennis (Burmeister, 1839)	--	48	LC	LC	X		X		AC. RJ.	Carvalho 1992, Garcia Junior et al. 2022
Staurophlebia reticulata (Burmeister, 1839)	--	39, 48.	LC	LC	X		X		AC.	Garcia Junior et al. 2022
Gomphidae										
Agriogomphus cf. Sylvicola Selys, 1869*	19	--	NE	LC	X		X		AM.	Garrison et al. 2006
Zonophora calippus Selys, 1869	--	48	LC	LC	X		X		AC	Belle 1966, Garcia Junior et al. 2022

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Species	**Primary data**	**Secundary data**	**Classification IUCN**	**Classification ICMBio**	**Female described**		**Larva described**		**Ocurrence in Brazil**	**Reference**
Anisoptera					**Yes**	**No**	**Yes**	**No**		
Libellulidae										
Argyrothemis argentea Ris, 1911*	5 e 17.	--	LC	LC	X		X		AM. PA. RO. PA. AM. MT.	Garrison et al. 2006
Brachymesia furcata (Hagen, 1861)	--	48	LC	LC		X	X		AC.	Garcia Junior et al. 2022
Brachymesia herbida (Gundlach, 1889)	--	36, 48.	LC	LC		X	X		AM. PA. MT. MS. SP. RJ. AC.	Garcia Junior et al. 2022
Dasythemis esmeralda Ris, 1910*	--	36	LC	LC	X			X	MT.	Garrison et al. 2006
Diastatops emilia Montgomery, 1940*	1 e 3.	--	DD	LC		X		X	PA.	Garrison et al. 2006
Diastatops obscura (Fabricius, 1775)	--	36, 48.	LC	LC	X		X		AC. AM. AP. BA. ES. GO. MA. MG. MS. MT. PA. PB. PE. PR. RJ. RO. RR. SP. TO.	Garcia Junior et al. 2022
Dythemis sterilis Hagen, 1861	11	48	NE	LC	X		X		AC.	Garcia Junior et al. 2022
Elasmothemis cannacrioides (Calvert, 1906)*	--	36	NE	LC	X		X		MG.	Garrison et al. 2006
Erythemis atalla (Selys in Sagra, 1857)	--	49	LC	LC	X		X		MG. AC.	Oliveira 2017, Bhukal 2017
Erythemis credula (Hagen, 1861)	--	36, 48.	NE	LC		X	X		AC.	Garcia Junior et al. 2022
Erythemis haematogastra (Burmeister, 1839)	--	36, 48.	LC	LC	X			X	AC. AM. AP. BA. ES. GO. MA. MG. MS. MT. PA. PB. PE. SP	Garcia Junior et al. 2022
Erythemis mithroides (Brauer, 1900)	--	36, 48.	LC	LC	X		X		AC.	Garcia Junior et al. 2022
Erythemis peruviana (Rambur, 1842)	--	46	LC	LC	X		X		PA. AM. AC. AP. RO. TO. CE. BA. AL. PI. SE. PE. PB. RN. MA. ES. MG. SP. RJ. RS. PR. SC.	Garrison et al. 2006
Erythemis vesiculosa (Fabricius, 1775)	--	36, 48.	LC	LC	X		X		AM. RJ. AC.	Garcia Junior et al. 2022
Erythrodiplax amazonica Sjöstedt, 1918*	--	39	LC	LC		X	X		AM.	Garrison et al. 2006
Erythrodiplax anatoidea Borror, 1942	--	49	LC	LC		X		X	AC.	Oliveira, 2017
Erythrodiplax attenuata (Kirby, 1889)	--	48	LC	LC	X			X	RO. AC.	Garcia Junior et al. 2022
Erythrodiplax basalis (Kirby, 1897)	12, 15 e 33.	48	LC	LC	X		X		AC. AM. GO. MA. MS. MT. PA. PE.	Kirby 1897, Garcia Junior et al. 2022
Erythrodiplax basalis avittata Borror, 1942*	--	36	NE	NE	X			X	MS. RJ. SP.	Garrison et al. 2006

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Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Anisoptera					**Yes**	**No**	**Yes**	**No**		
Libellulidae										
Erythrodiplax branconensis Sjöstedt, 1929	--	48	DD	LC		X		X	AC.	Garcia Junior et al. 2022
Erythrodiplax clitella Borror, 1942	--	36, 48.	LC	LC	X			X	RS. AC.	Garcia Junior et al. 2022
Erythrodiplax fusca (Rambur, 1842)	26	39, 48.	LC	LC	X		X		AC. AM. BA. ES. PA. PE. RJ. SP. GO. MA. MG. MS. MT. PR. RO. RR.	Garcia Junior et al. 2022
Erythrodiplax latimaculata Ris, 1911	--	48	LC	LC	X		X		AC.	Garcia Junior et al. 2022
Erythrodiplax paraguayensis (Förster, 1905)	--	48	DD	LC		X	X		AC.	Muzón & Garré 2005, Garcia Junior et al. 2022
Erythrodiplax umbrata (Linnaeus, 1758)	26	36, 48.	LC	LC	X		X		AC. AM. AP. BA. ES. GO. PA. PE.	Carvalho 1991, Garcia Junior et al. 2022
Erythrodiplax unimaculata (De Geer, 1773)*	--	36	LC	LC		X		X	AP. MT	Garrison et al. 2006
Fylgia amazonica Kirby, 1889	12 e 13	39, 48.	LC	LC	X			X	PA. AC.	Garcia Junior et al. 2022
Idiataphe cubensis (Scudder, 1866)*	--	36	LC	LC		X	X		AM.	Garrison et al. 2006
Miathyria marcella (Selys *in* Sagra, 1857)*	15 e 16.	36	LC	LC	X		X		RJ. PA. RS. SP.	Garrison et al. 2006
Miathyria simplex (Rambur, 1842)	--	36, 48.	NE	LC		X	X		AC.	Garcia Junior et al. 2022
Micrathyria artemis Ris, 1911	--	39, 48.	LC	LC	X		X		AM. AP. BA. ES. GO. MG. MS. MT. PA. RJ. RO. SP. AC.	Garcia Junior et al. 2022
Micrathyria atra (Martin, 1897)	--	39	LC	LC		X	X		AC.	Garrison et al. 2006
Micrathyria ocellata (Martin, 1897)	--	39, 48.	LC	LC		X	X		AC. RS. ES.	Garcia Junior et al. 2022
Micrathyria pseudeximia Westfall, 1992*	--	46	NE	LC		X		X	AM, GO, ES, MA, PA, PR, MG, MS , MT, RJ. RO..	Garrison et al. 2006
Nephepeltia flavifrons (Karsch, 1889)	--	48	LC	LC		X		X	AC.	Garcia Junior et al. 2022
Nephepeltia phryne (Perty, 1834)*	26	--	LC	LC	X		X		BA. SC. RO. PI	Garrison et al. 2006
Oligoclada monosticha Borror, 1981	--	48	LC	LC	X			X	PA. AC.	Garcia Junior et al. 2022
Oligoclada walkeri Geijskes, 1931*	16 e 22	--	LC	LC	X			X	RO. PA. MT.	Borror 1931
Orthemis biolleyi Calvert, 1906	--	36, 48.	LC	LC		X		X	RO. AC.	Garcia Junior et al. 2022
Orthemis cultriformis Calvert, 1899	5	36, 48	NE	LC	X		X		GO. MS. AC.	Garcia Junior et al. 2022
Orthemis discolor (Burmeister, 1839)*	29	--	LC	LC		X		X	RO.	Garrison et al. 2006

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Species	**Primary data**	**Secundary data**	**Classification IUCN**	**Classification ICMBio**	**Female described**		**Larva described**		**Ocurrence in Brazil**	**Reference**
Anisoptera					**Yes**	**No**	**Yes**	**No**		
Libellulidae										
Orthemis schmidti Buchholz, 1950	2 e 5	--	NE	LC		X	X		PA. AM. AC. AP. RO. TO. CE. BA. AL. PI. SE. PE. PB. RN. MA. ES. MG. SP. RJ. RS. PR. SC.	Costa & Santos 2009
Pantala flavescens (Fabricius, 1798)	--	36, 48.	LC	LC	X		X		PA. AM. BA. PE. MT. ES. MG. SP. RJ. RS. AC.	Garcia Junior et al. 2022
Perithemis cf. icteroptera (Selys *in* Sagra, 1857)	19, 20, 22, 25 e 32	--	LC	LC	X		X		AC. MG. MS. RS.	Ris 1930
Perithemis cornelia Ris, 1910	--	39, 48.	LC	LC	X			X	PA. AM. RR. RO. AP TO. CE. BA. AL. PI. SE. PE. PB. RN. MA. MT. GO. MS. DF. EP. MG. SP. RJ. RS. PR. SC.	Ris 1930, Garcia Junior et al. 2022
Perithemis electra Ris, 1930	--	39, 48.	LC	LC	X		X		AC. MS.	Ris 1930, Garcia Junior et al. 2022
Perithemis lais (Perty, 1834)*	19	36	LC	LC	X		X		PA. AM. RO. RR. PE. MA. MT. MS. ES. MG. RJ SP.	Ris 1930, Costa 2005
Perithemis tenera (Say, 1840)	19	--	LC	LC	X		X		MT. MG. RJ. SC.	Ris 1930, Santos 1973
Perithemis parzefalli Hoffmann, 1991	--	39	LC	NE		X		X	AC.	Garrison et al. 2006
Perithemis rubita Dunkle, 1982	--	49	LC	NE	X		X		AC.	Dunkle 1982, Oliveira 2017.
Perithemis thais Kirby, 1889	28, 31 e 33.	39, 48.	LC	LC	X		X		AM. AP. ES. PA. MS. MT. RJ. RO. SP. AC.	Ris 1930, Garcia Junior et al. 2022
Rhodopygia cardinalis (Erichson *in* Schomburgk, 1848)	14	36, 48.	LC	LC	X			X	AM. MT. MG. PA. AC.	Garcia Junior et al. 2022
Tauriphila argo (Hagen, 1869)	--	48	LC	LC		X	X		AC.	Costa 1994, Garcia Junior et al. 2022
Tramea binotata (Rambur, 1842)	--	48	LC	LC	X		X		AC. RO.	De Marmels 1994, Tennessen 2017, Garcia Junior et al. 2022

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Species	Primary data	Secundary data	Classification IUCN	Classification ICMBio	Female described		Larva described		Ocurrence in Brazil	Reference
Anisoptera					**Yes**	**No**	**Yes**	**No**		
Libellulidae										
Tramea cophysa Hagen, 1867	--	36	LC	LC	X		X		PB. MT. MS. ES. MG. SP. RJ. RS. PR. SC. AC.	De Marmels 1982, Costa 2000, Garcia Junior et al. 2022
Tramea rustica De Marmels & Rácenis, 1982	--	48	LC	LC	X			X	AM. RO. AC. MT.	De Marmels 1982, Garcia Junior et al. 2022
Uracis fastigiata (Burmeister, 1839)	--	39, 48	NE	LC		X		X	AM. AC. AP. RR. RO. TO PA. MA. PB. BA. MT.	Garcia Junior et al. 2022
Uracis imbuta (Burmeister, 1839)	5	39, 48.	LC	LC		X		X	AC.	Garcia Junior et al. 2022
Uracis infumata (Rambur, 1842)	--	39, 48.	LC	LC		X		X	AC. RO, PA, MT. AM.	Garcia Junior et al. 2022
Uracis siemensi Kirby, 1897	--	48	LC	LC	X			X	AC. PA.	Garcia Junior et al. 2022
Zenithoptera fasciata (Linnaeus, 1758)	--	36, 48.	LC	LC		X		X	PA. AM. AC. AM. RO. MA. MT. GO	Garcia Junior et al. 2022
Zenithoptera lanei Santos, 1941	1	48	LC	LC		X	X		PA. AM. AC. AP. RO. TO. CE. BA. AL. PI. SE. PE. PB. RN. MA. ES. MG. SP. RJ. RS. PR. SC. SC.	Garcia Junior et al. 2022

Caption. *New records for Acre state; **New records for Brazil; -- No data. Acre - AC; Alagoas - AL; Amapá - AP; Amazonas - AM; Bahia - BA; Ceará - CE; Distrito Federal - DF; Espírito Santo - ES; Goiás - GO; Maranhão - MA; Mato Grosso - MT; Mato Grosso do Sul - MS; Minas Gerais - MG; Pará - PA; Paraíba - PB; Paraná - PR; Pernambuco - PE; Piauí - PI; Roraima - RR; Rondônia - RO; Rio de Janeiro - RJ; Rio Grande do Norte - RN; Rio Grande do Sul - RS; Santa Catarina - SC; São Paulo - SP; Sergipe - SE; Tocantins - TO.

The habitat integrity of the sampled streams ranged from 0.194 to 0.970, suggesting a high environmental heterogeneity along with the sampled points. From the scores generated by the HII, ten points were classified as preserved, 15 had intermediate integrity and another ten were categorized as degraded (TABLE 1). The municipality of Sena Madureira was the one with the highest preservation index and the greatest collection effort due to its vast territorial extension (23,759,518 ha), which corresponds to 14% of the state of Acre. On the other hand, Brasiléia, with only 2% of the territory of Acre (3,928,174 ha), presents high degradation rates. In this location, five points were sampled, two of them being classified as intermediate and three as degraded (Table 1).

1. *Taxonomic Notes*

The specimens examined correspond to the morphological characteristics mentioned by the authors, which are: truncated paraprocts, cercus in lateral and laterodorsal views are truncated, with a wider base that tapers towards its distal apex, ending in a blunt tip (Figure 6). In addition, the characteristic yellow band can be seen below the ventral margin of the antehumeral band (Figure 7). According to von Ellenrieder & Garisson (2008), specimens of *D. loutoni* are associated with lotic environments, being generally found perched in riparian vegetation or performing active flights close to the water surface, making the individuals difficult to be captured. In our collections, these characteristics were evidenced. Our sampling localities for *D. loutoni* correspond to first-order streams with a well-established band of riparian vegetation, light entering at various points along the bed, with HII score of 0.497. When there was no direct sunlight, individuals were observed perched on the tips of branches and leaves at a maximum height of 1.5 m. Under strong light conditions, specimens were observed flying close to the water surface on the stream bank.

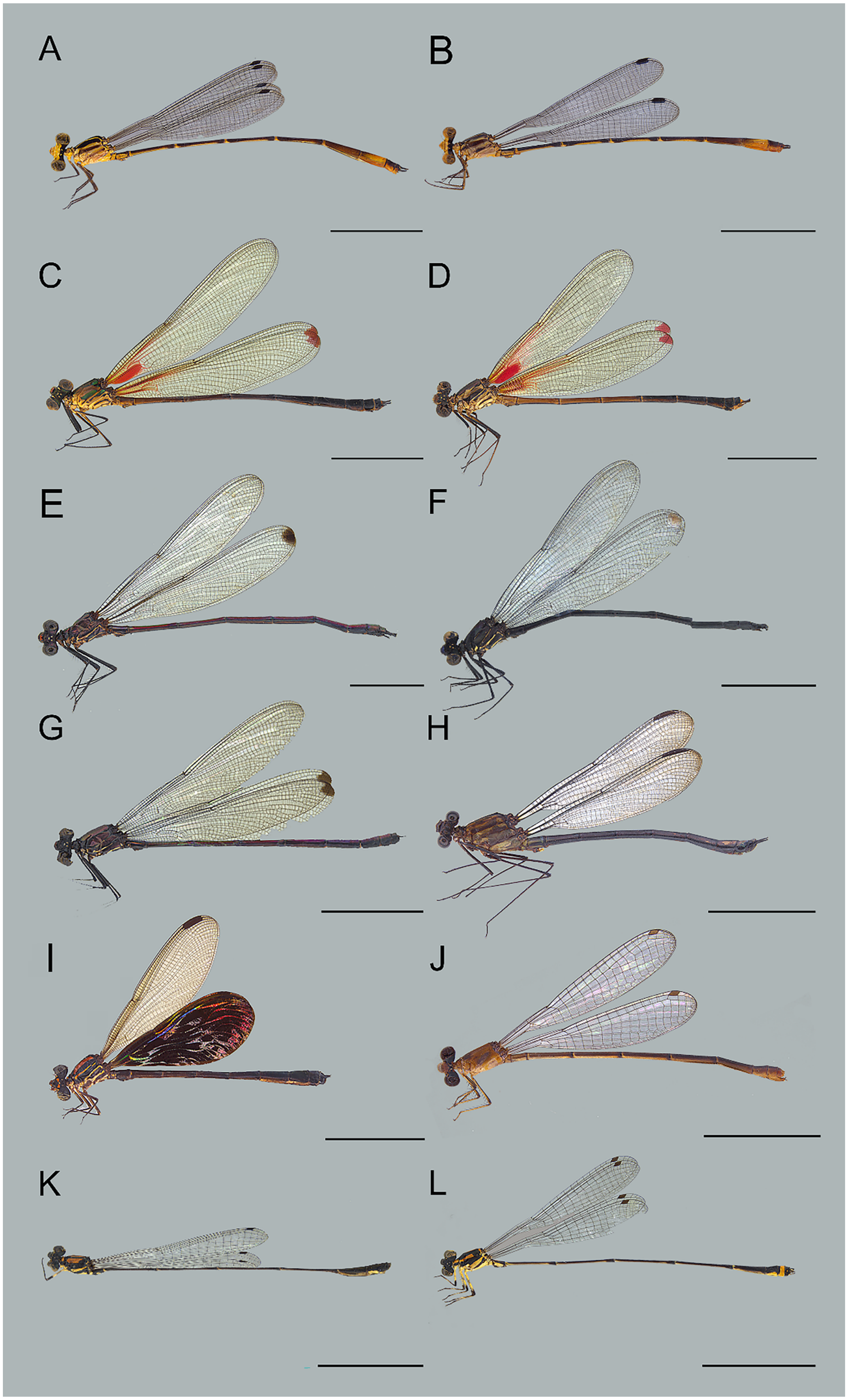

Figure 2. Some of the species presented in this study. Family Heteragrionidae: A) *Heteragrion bariai* De Marmels, 1989, B) *H. bickorum* Daigle, 2005; Family Calopterygidae: C) *Hetaerina laesa* Hagen *in* Selys, 1853, D) *H. rosea* Selys, 1853, E) *Mnesarete cupraea* (Selys, 1853), F) *M. loutoni* Garrison, 2006, G) *M. aenea* (Selys, 1853); Family Dicteriadidae: H) *Heliocharis amazona* Selys, 1853; Family Polythoridae: I) *Chalcopteryx rutilans* Ris, 1914; Family Coenagrionidae: J) *Phoenicagrion* sp., K) *Protoneuera tenuis* Selys, 1860, L) *P. woytkowskii* Gloyd, 1939.

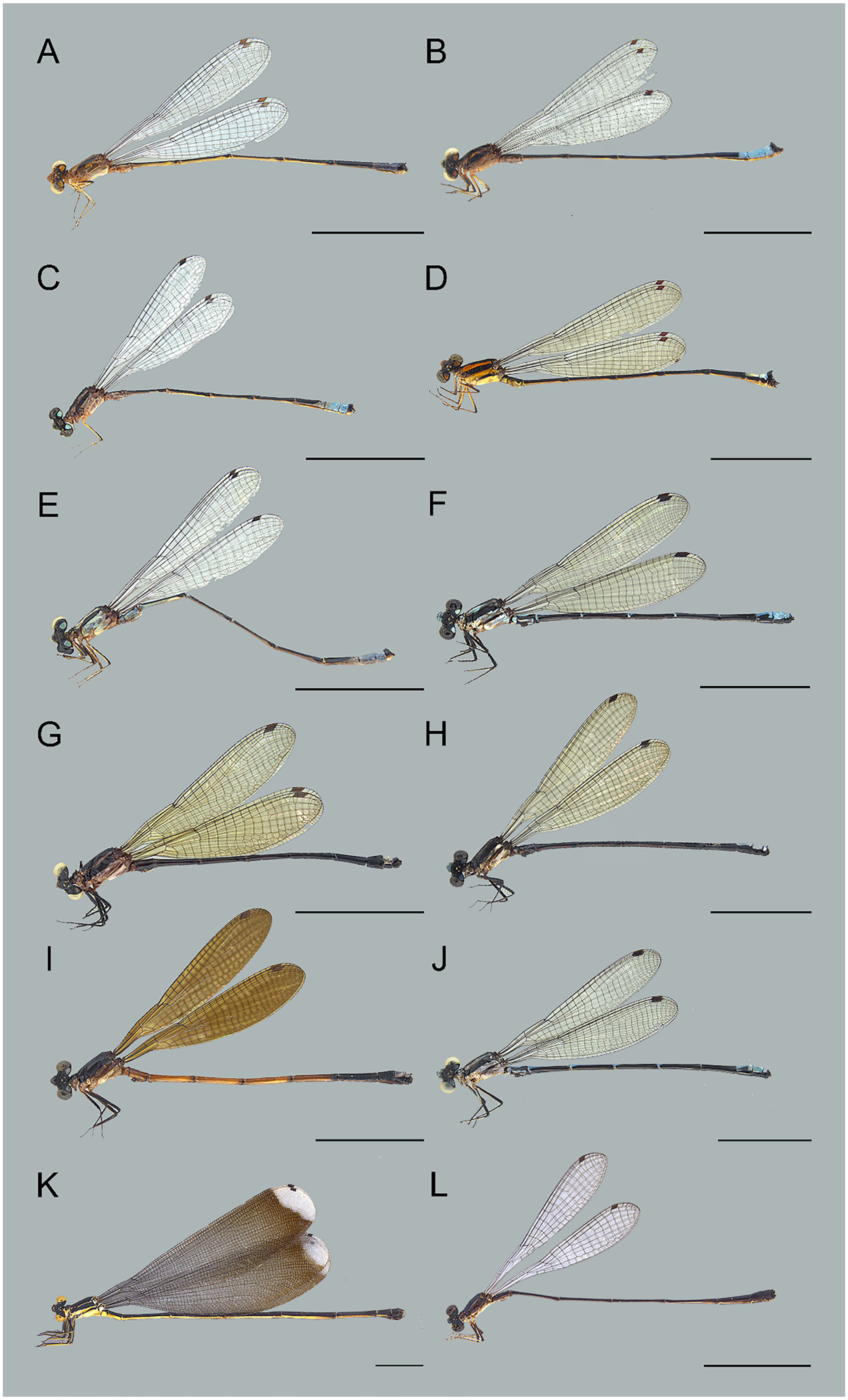

Figure 3. Some of the species presented in this study. Family Coenagrionidae: A) *Acanthagrion obsoletum* (Förster, 1914), B) *A. ascendens* Calvert, 1909, C) *A. gracile* (Rambur, 1842), D) *A. apicale* Selys, 1876, E) *A. floridense* Fraser, 1946, F) *Argia fumigata* Hagen in Selys, 1865, G) *A. indicatrix* Calvert, 1902, H) *A. collata* Selys, 1865, I) *A. infumata* Selys, 1865, J) *A. oculata* Hagen in Selys, 1865, K) *Microstigma sp.*, L) *Epipleoneura venezuelensis* Rácenis, 1955. All images in 10 mm scale.

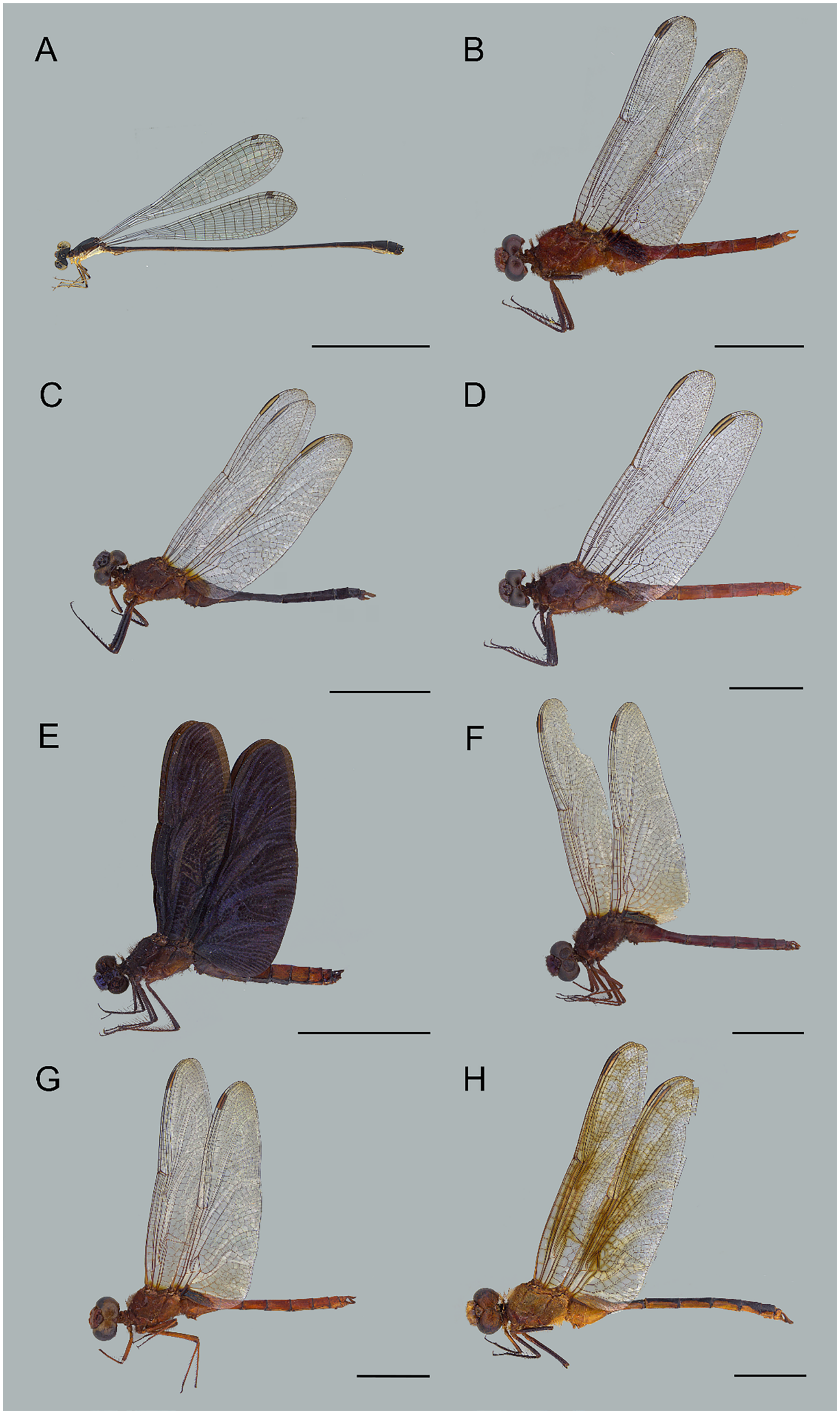

Figure 4. Some of the species presented in this study. Family Coenagrionidae: A) *Drepanoneura janirae* von Ellenrieder & Garrison, 2008; Family Libellulidae: B) *Erythemis credula* (Hagen, 1861), C) *E. mithroides* (Brauer, 1900), D) *Dasythemis esmeralda* Ris, 1910, E) *Diastatops obscura* (Fabricius, 1775), F) *Dythemis sterilis* Hagen, 1861; G) *Elasmothemis cannacrioides* (Calvert, 1906); H) *Brachymesia herbida* (Gundlach, 1889). All images in 1 cm scale.

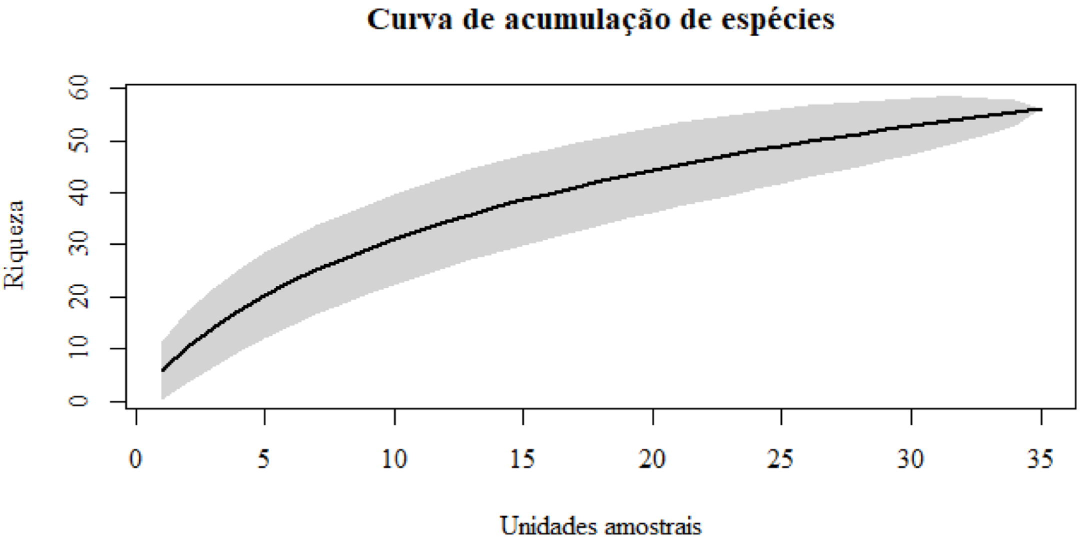

Figure 5. Species accumulation curve, representing the relation between species and sampled areas. The line represents the average calculated value of the collected species at the 35 sampling points in Acre state, Brazil. The lighter margins indicate their respective confidence intervals (95%).

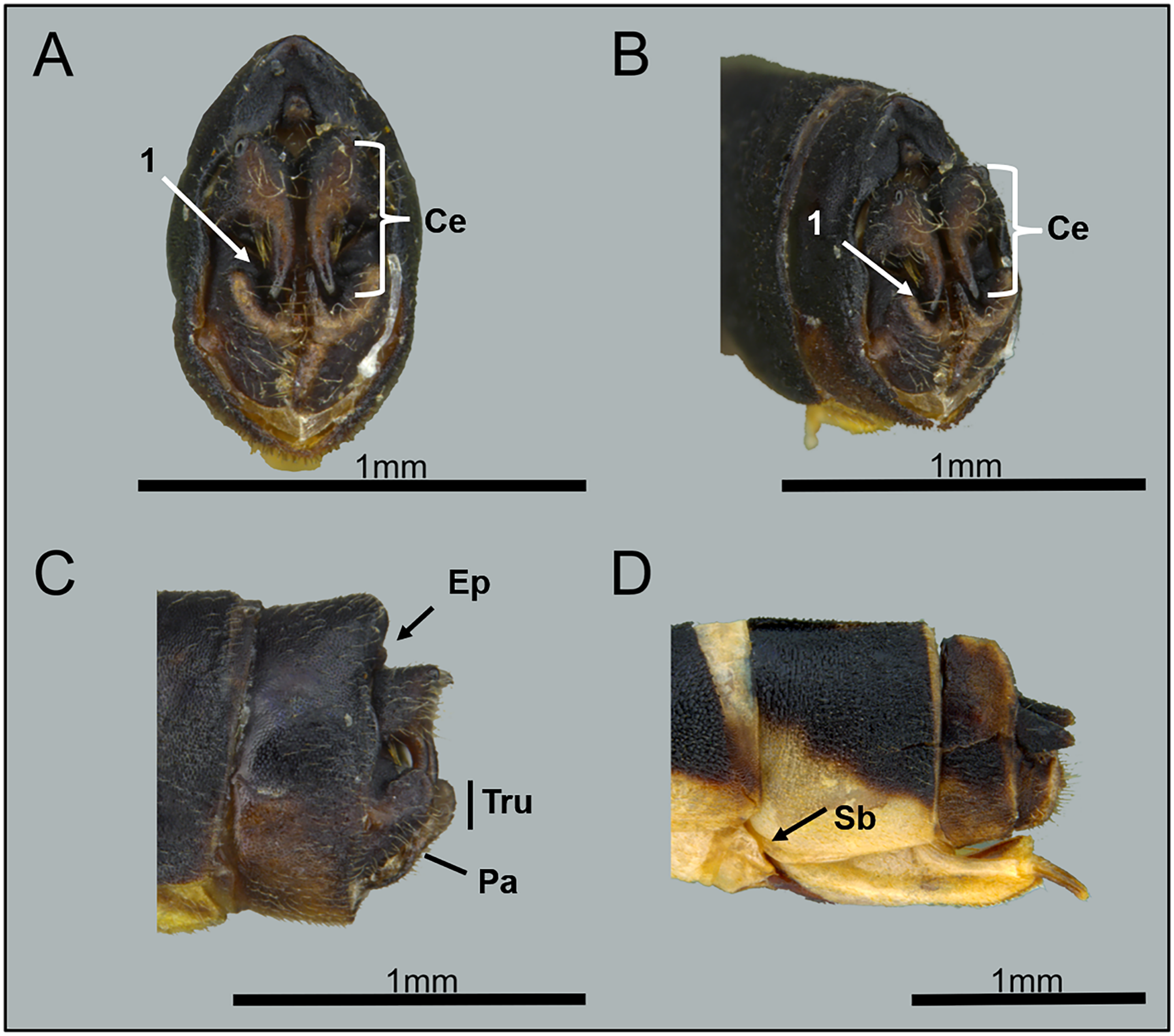

Figure 6. Male diagnostic structures of *Drepanoneura loutoni* von Ellenrieder & Garrison, 2008 (A, B and C) where A) cercus in frontal view; B) dorsolateral view; C) lateral view; D) Female ovipositor in lateral view; 1= Tapered tip of cercus; 2= ratio of ventro-apical curvature to base of cercus; Ep= epiproct; tru= truncated; pa= paraproct; sb= sub basal plate.

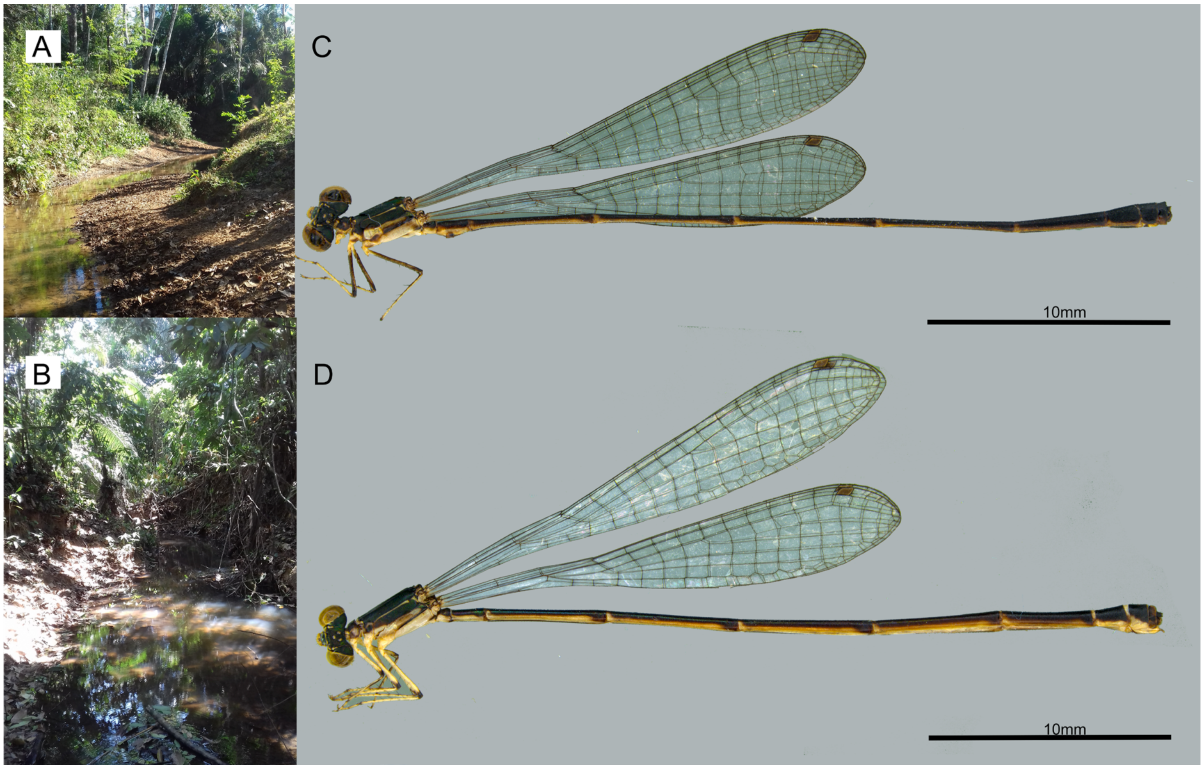

Figure 7. A) and B) habitat/specimens collection site of *Drepanoneura loutoni* von Ellenrieder & Garrison, 2008; C) male *D. loutoni*; D) female *D. loutoni*.

Discussion

In total, 140 species were recorded for the Acre state, becoming the fifth second the greatest diversity of Odonata in the Northern region, only behind Amazonas (n = 364), Pará (n = 310), Mato Grosso (n = 285) and Rondonia (n = 206) (García-Júnior et al. 2022). Following, we have the states of Amapá with 119 (Garcia Junior et al. 2022) and Roraima with 82 species (Garcia-Junior et al. 2022). However, considering that the information gathered in our study represents only a portion of the Acre state, we cannot rule out the possibility that this number could be even higher. Therefore, the need to carry out complementary samplings in other places in Acre is evident, as well as in more marginal points on the border with Amazonas and Perú. This is more evident in the center and northern portions of the state, where there are only a few records from the literature for the municipalities of Marechal Thaumaturgo (code number 39), Taraucá (code number 40) and Mâncio Lima (code number 42).

Our sampling effort was efficient but still shows a large gap for the Odonata knowledge in Acre. All information and occurrence records presented in this study come from nine municipalities in Acre, which correspond to approximately 22% of the total extension of the state, where the environmental protection units are the most representative in the study. As reported by Koroiva et al. (2020b), little information is found in the literature and in the databases, being Acre one of the most poorly explored states of Brazil. In this context, this study is the first compendium on the Odonatofauna for the state. Thus, this checklist represented a great effort and the first step towards trying to synthesize the state of knowledge of dragonflies for this region.

Recently, Garcia-Junior et al. (2022) reported the total number of species (n=82) for the state of Acre. However, they recorded from bibliographic research and did not inform the coordinates of the locations where the species were recorded, which significantly limits the use of this information. In our study, we increase the total number of Odonata species for the Acre state, based on information from field and literature, increasing the number to 140 species. In addition, we provide additional information on the localities where each species was collected and information related to the category of threat and knowledge about females and larvae. This type of information is essential as it allows more accurate assessments of the threats that species face, ensuring better strategies for their conservation (IUCN 2022). Our sampling effort was efficient but still shows a large gap for the Odonata knowledge in Acre.

As a result, the species *Drepanoneura loutoni* is reported for the first time in Brazil, being collected in the municipalities of Sena Madureira and Assis Brasil in eight streams. The occurrence of this species was expected because it is a species that has a distribution in the department of Madre de Dios, in Peru, which borders Brazil (Ellenrieder & Garrison 2008). This species has a strong connection with first and second order streams, in environments with dense vegetation cover. The genus *Drepanoneura* is known to occur along rivers and streams within forests, occupying large allopatric distributions from southern Panama through the foothills of the Andes Mountain range in Colombia, Ecuador, and Peru (Ellenrieder & Garrison 2008), and we found *D. loutoni* in a similar environment in Acre state. However, except for this basic occurrence information, *D. loutoni* has still large gap in its knowledge, as the larval stages are still undescribed, and little is known about its autoecological aspects.

Our results also show that many species still do not have descriptions of larvae and/or females, which hampers the advance of the taxonomic knowledge of the group. Therefore, in addition to taxonomic improvement, information regarding basic biology is of great importance. For instance, the distribution or status of populations is essential for us to advance and further integrate studies with Odonata, and effectively contribute to the assessment of endangered species such as the List of Threatened Species of ICMBio (2018), and IUCN (https://www.iucnredlist.org).

Our results show a high environmental heterogeneity (HII 0.194 - 0.970) within the sampled points, most of which are located within conservation units. However, Acre is within the deforestation arc, where a wide range of anthropogenic activities that generate changes in land use exists (Aguiar et al. 2016). Furthermore, in the Amazonian context, there are considerable evidence showing how odonates are affected by the loss of environmental integrity caused by land use modifications (Calvão et al., 2016, Oliveira-Junior & Juen 2019, Brasil et al. 2020). Thus, these results can indicate that Odonata communities are suffering strong pressures in the region. Therefore, we consider it necessary to expand the sampling efforts within a gradient of ecological conditions (from heavily impacted to pristine environments) likewise, the implementation of biomonitoring programs.

The information derived from our samplings will be of great importance in assessing the status of Odonata diversity in the Acre state, helping to identify threats and conservation strategies to be developed in the future. This study was carried out almost predominantly in the eastern zone of Acre, where the heaviest anthropogenic pressure in that state is located. The western region of the state still contain large areas of vegetation cover with many different phytophysiognomies such as campinaranas, indigenous lands (geographically more rugged), and with three types of water (clear, white and black). This entire arrangement of geophysical characteristics has a high potential for endemism and establishment of species with greater environmental demand.

Finally, this study highlights the importance of sampling efforts in poorly explored regions such as Acre state, making a significant contribution to the knowledge of the Neotropical odonatofauna and to one of the most biodiverse regions such as the Amazon biome.

Supplementary Material

The following online material is available for this article:

Table S1 - Richness estimate performed for each of the sampled points

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Cristian Camilo Mendoza Penagos: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual contente.

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Leandro Juen: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that there is no conflict of interest related to the publication of this manuscript.

Ethics

The authors agree with the guidelines established by the ethics committees and their respective research institutions and being aware of scientific ethical commitments.

Data availability

The data are already included in the SISBIO platform (link https://www.icmbio.gov.br/ran/o-que-fazemos/sisbio.html) and are already being used for the reassessment of endangered Odonata species of Brazil.

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Short Communication

Two in one: the little bat that pollinates and disperses plants at an urban site in Southeastern Brazil

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Abstract: The glossophagine Pallas's long-tongued bat (*Glossophaga soricina*) fares well in urban environments across its range. In addition to roost sites, there are nectar and fruit sources available in diverse situations across the urban gradient. Phyllostomid bats that thrive in urbanized situations are behaviorally plastic generalists and rely on patches of ornamental or feral plants as food sources. Herein we report on *G. soricina* and its food sources at an urbanized site in Southeastern Brazil. This small phyllostomid bat consumes nectar from landscaping ornamental plants, besides consuming the soft pulp along with the tiny seeds of pioneer trees and shrubs. In addition to these natural sources, the bat exploits hummingbird feeders to consume the sugared water. Ingested small seeds are defecated in flight, the bat acting as a disperser of pioneer plants that favor cleared areas. *Glossophaga soricina* role as flower-pollinator and seed-disperser at Neotropical urban areas merits further attention due both to the maintenance of urban biodiversity and delivery of ecosystem services.

Keywords: *Ecosystem services; Flowers; Foraging behavior; Fruits; Phyllostomidae.*

Dois em um: o pequeno morcego que poliniza e dispersa plantas em local urbano no Sudeste do Brasil

Resumo: O morcego beija-flor (*Glossophaga soricina*) adapta-se a ambientes urbanos na sua área de distribuição. Além de abrigos diurnos, há fontes de néctar e frutos ao longo do gradiente urbano. Morcegos filostomídeos que se adaptam a situações urbanas são generalistas comportamentalmente flexíveis e dependem de trechos com plantas ornamentais ou ferais como fonte alimentar. Relatamos aqui informações sobre o morcego beija-flor e suas fontes alimentares em um local urbanizado no sudeste do Brasil. Este pequeno morcego glossofagíneo busca néctar em plantas usadas em paisagismo, além de consumir a polpa macia, juntamente com as sementes minúsculas, de plantas pioneiras. Além destas fontes naturais, o morcego explora água açucarada dos bebedouros de beija-flores. Sementes pequenas são defecadas em voo e o morcego age como dispersor de plantas poineiras em áreas sem vegetação. A função de *G. soricina* como polinizador de flores e dispersor de sementes em áreas urbanas nos Neotrópicos merece atenção adicional devido à manutenção da biodiversidade urbana e da prestação de serviços ecossistêmicos.

Palavras-chave: *Comportamento alimentar; Flores; Frutos; Phyllostomidae; Serviços ecossistêmicos.*

Introduction

The Pallas's long-tongued bat (*Glossophaga soricina*) is a phyllostomid widespread in South America east of the Andes (Alvarez 1991, Dias et al. 2017, Calahorra-Oliart et al. 2021), and fares well in urbanized environments across its distribution (e.g., Lemke 1985, Ballesteros et al 2012, Nunes et al. 2017, Turcios-Casco et al. 2021). In addition to roost sites, there are nectar and fruit sources available for phyllostomid bats in diverse situations across the urban gradient (Bredt et al. 2002, Silva et al. 2005, Kruszynski et al. 2016, Vilar et al. 2016, Nunes et al. 2017). Phyllostomid bats that thrive in urbanized situations are behaviorally plastic and rely on patches of ornamental or feral plants as food sources (Bredt et al. 2002, Kruszynski et al. 2016, Garcia et al. 2000, Pellón et al. 2021, Turcios-Casco et al. 2021).

Notwithstanding its widespread occurrence in South America and commonness in urban areas, Pallas's long-tongued bat remains understudied from the perspective of food resources at a given urban area (but see Pellón et al. 2021). We had the opportunity to sporadically observe and record this phyllostomid bat at a very small urbanized site in Southeastern Brazil for a period spanning about 10 years. Herein, we present a snapshot report on the food sources of *G. soricina* and the behavior displayed on these resources at the site.

Material and Methods

The study area is a block of about 60.000 m^2, including streets, sidewalks, gardens, and backyards at an urban area (22°49'36"S, 47°04'15"W, 621 m.a.s.l.) in the vicinity of the Universidade Estadual de Campinas, São Paulo, South-eastern Brazil. We sporadically observed Pallas's bats feeding activity on trees and shrubs used in landscaping and gardening, besides some feral ones for a period spanning 10 years (2009-2019). We observed the bats with bare eyes and documented its behavior with a 70-300 mm telephoto lens mounted on a SLR camera from a distance of about 2-4 m. Streetlight and lamps in gardens and backyards allowed an adequate view of the bats' activity on most food sources. During the observational sessions we used "*ad libitum*" and "sequence" samplings (Altmann 1974), which are choice methods to record temporary or unpredictable events. One bat individual was recognized due to a natural mark on the forearm. We examined fecal samples scattered on vegetation after the bat's visits to a given food source. Images of the bats feeding on some of the food sources are on file in the Coleção de Imagens (ZUEC-PIC 448-453) at the Museu de Diversidade Biológica, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Results

At our study site, *Glossophaga soricina* exploited three night-blooming flower species in two families, and four fruit species in four families (Table 1). The flowers lasted one night only and the fruits lasted until consumed entirely by bats at night and birds during the day, which could last for weeks as new infructescences matured.

Glossophaga soricina used three different types of food sources at the studied small urban site (Figure 1). A regularly visited food source were night-blooming flowers, such as those of *Lafoensia pacari* and *Luehea alternifolia* trees (Figure 1a-b). Another regularly visited source were fruit-bearing trees and shrubs such as those of *Cecropia pachystachya* and *Piper aduncum* when the pulp was ripe and soft (Figure 1c). Sugared water in a hummingbird feeder (Figure 1d) was used sporadically, mostly when flower or fruit bearing plants grew near the feeder, which had a stable position for several years.

The *Lafoensia pacari* tree had numerous (up to 40) open flowers per night, whereas the *Luehea alternifolia* tree had smaller number (up to 8-10) of open flowers each night. The *Callianthe fluviatilis* shrub opened up to five flowers per night (but 2-3 was the usual number). *Lafoensia pacari* flowers were often visited by 2-3 bat individuals at the same time, which chased one another around the tree. A given bat made a flight pass over the tree and appeared to assess the flowers for

Table 1. Food sources used by the phyllostomid bat *Glossophaga soricina* at a small urban site in Campinas, São Paulo, Southeastern Brazil. Plant families, genera, and species in alphabetical order. E= exotic. Last line is provisioned food. Color flower/fruit is color visible to humans.

Plants	Habit	Food type	Color flower/fruit
Lythraceae			
Lafoensia pacari	Tree	Nectar	White
Malvaceae			
Callianthe fluviatilis	Shrub	Nectar	Light yellow
Luehea alternifolia	Tree	Nectar	White
Moraceae			
Morus nigra^E	Tree	Infructescence pulp	Purple
Muntingiaceae			
Muntingia calabura	Tree	Fruit	Yellowish green
Piperaceae			
Piper aduncum	Shrub	Infructescence pulp	Light green
Urticaceae			
Cecropia pachystachya	Tree	Infructescence pulp	Greyish yellow
Provisioned food source			
Hummingbird feeder	NA	Sugared water	Yellow base

Figure 1. The Pallas's long-tongued bat (*Glossophaga soricina*) exploits three food source types at an urbanized site in Campinas, São Paulo, Southeastern Brazil: (a) the bat laps the copious nectar from the flowers of a *Lafoensia pacari* tree, (b) the bat laps nectar from a flower of a *Luehea alternifolia* tree, (c) the bat chews a portion of the soft pulp of *Piper aduncum*, swallowing the tiny seeds along - note pulp already chewed out, (d) the bat laps sugared water from a hummingbird feeder left in place overnight. *Glossophaga soricina* visits each food source hovering fleetingly.

nectar content before the actual visit. After this exploratory flight, the bat visited up to 10-15 flowers in succession, making rounds over the tree during up to 5 min. It visited the flowers hovering fleetingly, tenths of a second. Visits to a given *L. pacari* tree were at intervals of up to 30 min, but sometimes the intermissions were shorter (about 10-15 min), possibly due to the bats being different individuals. The bat visited the *Luehea alternifolia* tree at intervals of 50-60 min, exploiting all the available flowers at each visit (we recognized the bat due to a natural marking). Its visits were similar to those described on *L. pacari* flowers. The flowers of *C. fluviatilis* were visited by the bat at irregular intervals of up to 60 min, and even more fleetingly than the visits to the *L. pacari* and *L. alternifolia* flowers. During visits to flowers of these three plant species, the bats touched the reproductive parts, which would result in pollination.

When visiting the *Cecropia pachystachya* tree and the *Piper aduncum* shrub, the bat chewed out a portion of the Infructescences while hovering and flew away with a mouthful. It visited these two food sources at irregular intervals that lasted about 5-40 min. We often observed bats defecating along their pathway, spraying small seeds on the ground or house walls. We also found seeds of both *C. pachystachya* and *P. aduncum* in the feces scattered on vegetation after the bat's visits to a given food source.

Pallas's long-tongued bat took out a portion of the *Morus nigra* tree in a way similar to those described above, also at irregular intervals that lasted about 5-30 min. Due to poor illumination of the single *Muntingia calabura* tree, we were unable to observe whether the bat grabbed a fruit while hovering or had to cling to be able to tear the fruit from its stalk and fly away with the fruit in its mouth.

The bat visited hummingbird feeders at irregular intervals that lasted 5-15 min, lapping the sugared water while hovering fleetingly. It combined its visits to the feeder with those on a few *L. pacari* flowers available at the time, and the *P. aduncum* shrub that was close to the sugared water source.

Discussion

Our observations centered on Pallas's long-tongued bat constitute the second study about food sources used by this bat species at an urban site. Plants used as food by *Glossophaga soricina* were recently studied at and urban site in Lima, Peru (Pellón et al. 2021). However, judging from the recent review of the genus by Calahorra-Aliart et al. (2021), the species that occurs in Peru is *Glossophaga valens* (distribution in Handley et al. 1991 as *G. soricina valens*), which renders our snapshot study as the first that address diverse food sources of *G. soricina* at an urban site.

The visits of *Glossophaga soricina* to nectar-offering flowers did not differ from available sudies on flower-visiting bats to night-blooming plants, including *Lafoensia pacari* and *Luehea alternifolia* (Silva & Peracchi 1999, Sazima et al. 1982). However, visits of this bat to flowers of *Callianthe fluviatilis* are not available in the scientific literature, besides a brief mention to its one night-lasting flowers in Buzato et al. (1994) as *Abutilon peltatum*. We were surprised by the exceedingly fleeting visits, which precluded photographic records with the equipment we had. Pollination of the three plant species would be expected, as the flowers fit within the known types usually pollinated by bats (Buzato et al. 1999).

Visits to fruits by *G. soricina* apparently remain undescribed in the scientific literature to date. This small bat secures the pulp of *Cecropia pachystachya*, *Piper aduncum*, and *Morus nigra* infructescences with a hovering flight similar to that it displays when visiting flowers for nectar. We expected that it would cling on the infructescence to chew a mouthful as displayed by some phyllostomid bats such as Seba's short-tailed bat *Carollia perspicillata*, which is able to hover while feeding on flowers but also cling to some fruits (Sazima & Sazima 1978, Sazima et al. 2003). We were unable to observe how *G. soricina* secures the *Muntingia calabura* fruits, but conceive it would cling on a branch.

Visits of Pallas's long-tongued bat to hummingbird feeders left unattended at night were observed since the nineteen-nineties in Southeastern Brazil. In Vitória, a seaside town in Espírito Santo, visits of this bat to feeders are known since about 1995 (J.L. Gasparini, pers. comm.), and we photographed the visits there in 1997. This behavior spread through *G. soricina* populations and now is a common view at several urban and suburban regions in Brazil (Esbérard et al. 1999, Santos & Uidea 2002). However, visits to hummingbird feeders are not restricted to urban sites. We recorded this bat species exploiting bird feeders on the veranda of a hotel within the Atlantic forest at the Itatiaia National Park, Rio de Janeiro state, at about 1.200 m a.s.l.

Despite its use of sugared-water feeders, Pallas's long-tongued bat still relies on flowers and fruits for its nutritional and energy intakes. This is likely due to its low energy reserves and failure to maintain an adequate level of blood glucose after a short-fasting period, contrary to which happens with essentially fruit-feeding phyllostomid species (Pinheiro et al. 2006, Amaral et al. 2019). There is some evidence that the use of hummingbird feeders interfere with pollination of plants in a given area covered by flower-visiting birds (Arizmendi et al. 2007, Maruyama et al. 1999). Even if the breeding success of a given plant is lower in the close presence of the feeder (Arizmendi et al. 2007), or the hummingbird assemblage may change with provision of feeders (Maruyama et al. 1999), the plants still are visited and pollinated. A similar situation is likely to occur with *G. soricina*.

In conclusion, Pallas's long-tongued bat exploited a variety of food sources available at our very small urbanized study site, including nectar, fruit pulp, and sugared water. Even in an urban settings, the bat retained its ecological functions as a flower-pollinator and seed-disperser. Some of these two ecosystem services are recorded in other urbanized areas across the range of this small bat (Bredt et al. 2002, Silva et al. 2005, Kruszynski et al. 2016, Vilar et al. 2016, Nunes et al. 2017), and contributes to maintain and even expand the local biodiversity, as it occurred with the "feral" *Piper aduncum* in our study. This plant was probably transported to the study site via defecated seeds, as *P. aduncum* was not present at the site until after *Glossophaga soricina* began visiting *Cecropia pachystachya* trees. The seed dispersal role of this bat was already commented upon by Augusto & Hayashi (2004), which lends support to our assumption on the important role of *G. soricina* in maintaining ecosystem services at urbanized areas.

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Ivan Sazima: Data collection; data analysis and interpretation; manuscript preparation; critical revision.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Inventory

A “hotspot” within a hotspot: the reptiles of the Estação Ecológica and Área de Proteção Ambiental de Murici, Atlantic Forest of northeastern Brazil

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Abstract: Currently the Atlantic Forest hotspot has less than 11% of its original coverage. However approximately 300 species of reptiles are known to inhabit this ecoregion, of which 34% are endemic. The creation of protected areas represents a strategy for preserving ecosystems and managing land use, and for attaining the proper management of these protected areas, information on local biodiversity is essential. Herein we provide the first list of reptile species for the Estação Ecológica and Área de Proteção Ambiental de Murici, two overlapping protected areas located in the state of Alagoas, one of the most important sets of forest remnants for the conservation of the Atlantic Forest in the northeast of Brazil. The species list was constructed based on expeditions and occasional encounters in the area between 1994 and 2022. A total of 89 reptile species were obtained during the 28 years of collection, being two species of Crocodylia, three species of Testudines and 84 species of Squamata. This richness is by far one of the greatest ever documented for the Atlantic Forest. Additionally, two species registered in the area are considered threatened according to the national list (*Amerotyphlops paucisquamus* and *Bothrops muriciensis*) and six are defined as data deficient for the assessment of their conservation status. Three species are recorded for the first time in the Atlantic Forest north of the São Francisco River: *Dipsas indica*, *Trilepida salgueiroi* and *Cercophis auratus*. We claim that the species list provided here will serve as a starting point for further studies in this rich reptile “hotspot” within the Atlantic Forest.
Keywords: *Conservation; Crocodylia; Checklist; Squamata; Testudines.*

Um “*hotspot*” dentro de um *hotspot*: os répteis da Estação Ecológica e Área de Proteção Ambiental de Murici, Mata Atlântica do nordeste do Brasil

Resumo: Atualmente o *hotspot* da Mata Atlântica tem menos de 11% de sua cobertura original. Porém aproximadamente 300 espécies de répteis são conhecidas por habitar esta ecorregião, das quais 34% são endêmicas. A criação de unidades de conservação representa uma estratégia de preservação de ecossistemas e gestão do uso do solo, e para o manejo adequado dessas áreas protegidas é fundamental a informação sobre a biodiversidade local. Apresentamos aqui a primeira lista de espécies de répteis para a Estação Ecológica e Área de Proteção Ambiental de Murici, duas unidades de conservação parcialmente sobrepostas localizadas no estado de Alagoas, um dos conjuntos de remanescentes florestais mais importantes para a conservação da Mata Atlântica no nordeste do Brasil. A lista de espécies foi construída com base em expedições e encontros ocasionais na área entre 1994 e 2022. Um total de 89 espécies de répteis foram registradas durante os 28 anos de coleta, sendo duas espécies de Crocodylia, três espécies de Testudines e 84 espécies de Squamata. Essa riqueza é de longe uma das maiores já documentadas para a Mata Atlântica. Além disso, duas espécies registradas na área são consideradas ameaçadas segundo a lista nacional (*Amerotyphlops paucisquamus* e *Bothrops muriciensis*) e seis são definidas como dados insuficientes para a avaliação de seu status de conservação.

Três espécies são registradas pela primeira vez na Mata Atlântica ao norte do rio São Francisco: *Dipsas indica*, *Trilepida salgueiroi* e *Cercophis auratus*. Afirmamos que a lista de espécies aqui fornecida servirá como ponto de partida para novos estudos neste rico "*hotspot*" de répteis dentro da Mata Atlântica.

Palavras-chave: *Conservação; Crocodylia; Lista de espécies; Squamata; Testudines.*

Introduction

The origin of reptiles dates back 300 million years ago, and countless morphological, physiological and behavioral adaptations have enabled this group to diversify across environments worldwide (Reisz 1997, Pough et al. 2008, Roll et al. 2017). Currently, over 11,500 living species are described (Uetz et al. 2022), although recent studies have emphasized that this richness is still underestimated (Mora et al. 2011, Moura & Jetz 2021). Certainly, many reptile species will become extinct before they are discovered because, in addition to current threats, such as habitat loss and degradation, pollution, species trafficking, pathogens and the introduction of invasive species (Gibbons et al. 2000), their metabolic rate is dependent on external sources of heat (ectothermy), resulting in their classification as the terrestrial vertebrates that are most threatened by climate change (e.g., Araujo et al. 2006, Closel & Kohlsdorf 2012, IUCN 2022). Furthermore, it represents the taxonomic group of which more species will be described in future years (Moura & Jetz 2021), mostly in the Neotropical region. Reptile diversity is not evenly distributed globally (Roll et al. 2017) and the greatest richness is concentrated among the 36 areas considered as global biodiversity hotspots (sensu Myers et al. 2000, Conservation International 2022).

Among these areas, the Atlantic Forest, a Neotropical ecoregion that originally occupied most of the east coast of South America (3° – 31° South and 35° – 60° West), has been highlighted as a hotspot for reptiles (Tozetti et al. 2017, Costa & Bérnils 2018, Uetz et al. 2022). Its original cover is estimated to have occupied about 150 million ha, extending as a coastal arc at a high latitudinal range that encompassed tropical and sub-tropical regions (Ribeiro et al. 2009). However, since European colonization, this ecoregion has been greatly threatened, mainly due to deforestation and the conversion of its natural areas for the expansion of the agricultural industry, exploitation of resources and advance of urban frontiers (Ribeiro et al. 2009). Currently, it is estimated that the remaining Atlantic Forest represents less than 11% of its original coverage, and these remnants are distributed in small and isolated fragments immersed in large matrices of monocultures and pastures (Ribeiro et al. 2009). Despite the high level of degradation, approximately 300 species of reptiles are currently known to inhabit this ecoregion, of which more than 34% are endemic (Tozetti et al. 2017).

The climatic and elevational variations in the Atlantic Forest throughout its latitudinal amplitude (about 29º and 0–2,700 meters; Ribeiro et al. 2009), together with the precipitation gradient from the coast to the interior (Ribeiro et al. 2009, Haddad et al. 2013), resulted in the formation of areas with distinct vegetation (Pinto & Brito 2003), allowing evolutionary processes in to occur at large and small scales (Vasconcelos et al. 2014; Moura et al. 2017a, b). The heterogeneity of this ecoregion, the geomorphological processes and climatic changes that have occurred since the Tertiary have consolidated areas of endemism (Ribeiro et al. 2009, Freire et al. 2018). Based on these areas, the Atlantic Forest was subdivided into eight Biogeographic Sub-Regions (BSR). Among these, one of the northernmost sub-regions is known as the Pernambuco Biogeographic Sub-Region (sensu Ribeiro et al. 2009), extending from the northern margin of the São Francisco River, in the state of Alagoas, to the remnants located in the state of Rio Grande do Norte. This BSR was historically the most deforested (only 12% of its original coverage remains) and still houses the least known biota of the entire Atlantic Forest (Ribeiro et al. 2009).

The delimitation of protected areas represents a strategy for preserving ecosystems and managing land use. In Brazil, for example, a country where more than 90% of the Atlantic Forest is located, there are over 2,400 protected areas in this hotspot (CNUC/MMA 2022). Despite the expressive number, the total area of these protected areas corresponds to less than 0.12% of the total coverage of the Atlantic Forest and when only considering the current remnants, these numbers are much smaller. Additionally, many of these protected areas are quite permissible in terms of use (protection category with similar objectives as the " VI: Protected area with sustainable use of natural resources" classified by the IUCN, sensu Dudley 2008), few being fully protected, and the growing pressure to search for resources and space means that even these areas considered as "protected" continue to suffer from a plethora of threats (Jones et al. 2018).

In order to properly manage these protected areas, biodiversity information is essential. As such, species lists become an essential tool and source for the development of management plans and strategies and the definition of priority areas for conservation (Ribeiro et al. 2009, Jones et al. 2018). Despite the importance of understanding the local biodiversity, the flora and fauna of many of these protected areas in the Atlantic Forest remain poorly studied (Pinto et al. 2006, Tabarelli et al. 2006, Ribeiro et al. 2009). Herein we provide the first list of reptile species for the Estação Ecológica and Área de Proteção Ambiental de Murici, two overlapping protected areas located in the state of Alagoas, one of the most important sets of forest remnants for the conservation of the Atlantic Forest from the northeast of Brazil.

Material and Methods

1. *Study area*

The Área de Proteção Ambiental (Environmental Protection Area/ APA) de Murici (APA de Murici; Figure 1; protection category with similar objectives as the " VI: Protected area with sustainable use of natural resources" classified by the IUCN, sensu Dudley 2008) is in the northeast of the state of Alagoas and partially covers the municipalities of Murici, União dos Palmares, São José da Laje, Ibateguara, Colônia Leopoldina, Novo Lino, Joaquim Gomes, Messias, Branquinha and Flexeiras. The total area is 133,100 ha, and within this protected area sustainable use is permitted (Law Decree No. 5.907/199; Alagoas 1997). Created in 1993, this APA is considered the largest terrestrial protected area in the state of Alagoas with its main objective being to protect the raised areas of the relief wrinkling for the Borborema Plateau and its water resources.

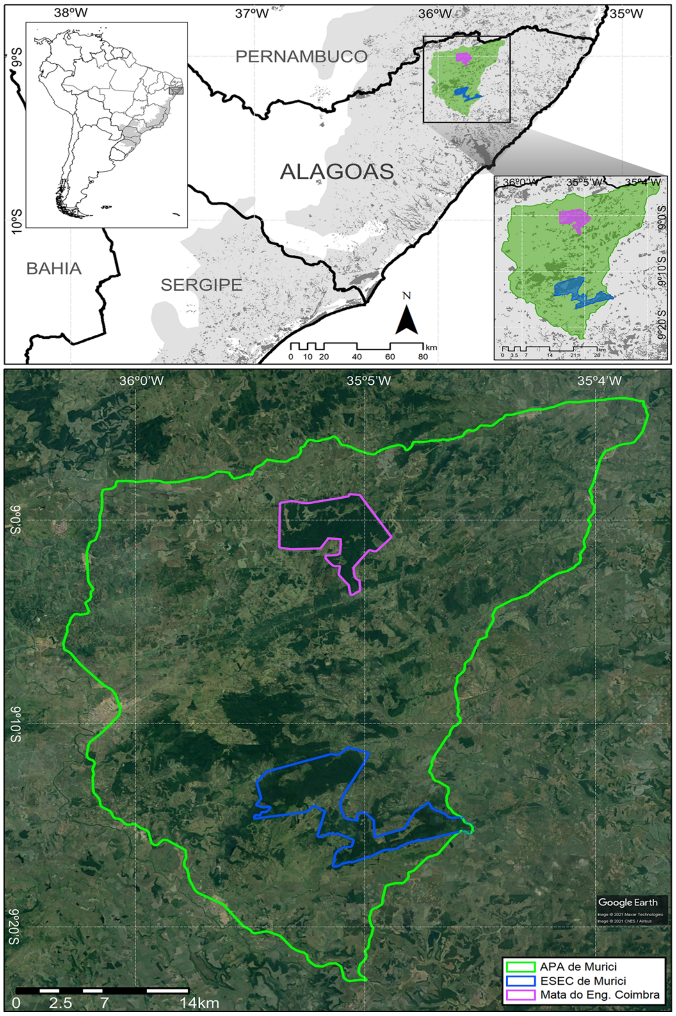

Figure 1. Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. A = Location and limits of APA (green) and ESEC (blue) de Murici and *Mata do Engenho Coimbra* (purple) and original cover (ligth green; adapted from IBGE 2022) and remaining cover (dark-grey; adapted from SOS MATA ATLÂNTICA 2022) of Atlantic Forest. B = Satellite image highlighting the limits of APA (green) and ESEC (blue) de Murici and *Mata do Engenho Coimbra* (purple). Inset map: South America.

Inserted within the limits of the APA de Murici, the Estação Ecológica (Ecological Station/ ESEC) de Murici (ESEC de Murici; Figures 1–2; protection category with similar objectives as the "Ia: Strict Nature Reserve" classified by the IUCN, sensu Dudley 2008) comprises a federal protected area of restrictive use and comprises one of the largest and continuous remnants of the original Atlantic Forest to the north of the São Francisco River. This ESEC was recently created by the decree of law s/No. of May 28, 2001 (Brasil 2001) and has an area of approximately 6,130 ha, partly covering the municipalities of Murici, Flexeiras and Messias.

In general, the forest remnants located in both protected areas have different phytophysiognomies, varying from Ombrophilous Forest, with dense and tall tree phytophysiognomies towards Seasonal Forest and wide rocky outcrops (Assis 2000). Elevation varies from 150 to 640 meters. The climate is tropical humid and sub-humid, with a dry period from October to March, and a rainy period from April to September. The annual precipitation and temperature range from 800 to 1,800 mm and 20 to 25 °C, respectively (Alvarez et al. 2013, SEMARH 2022).

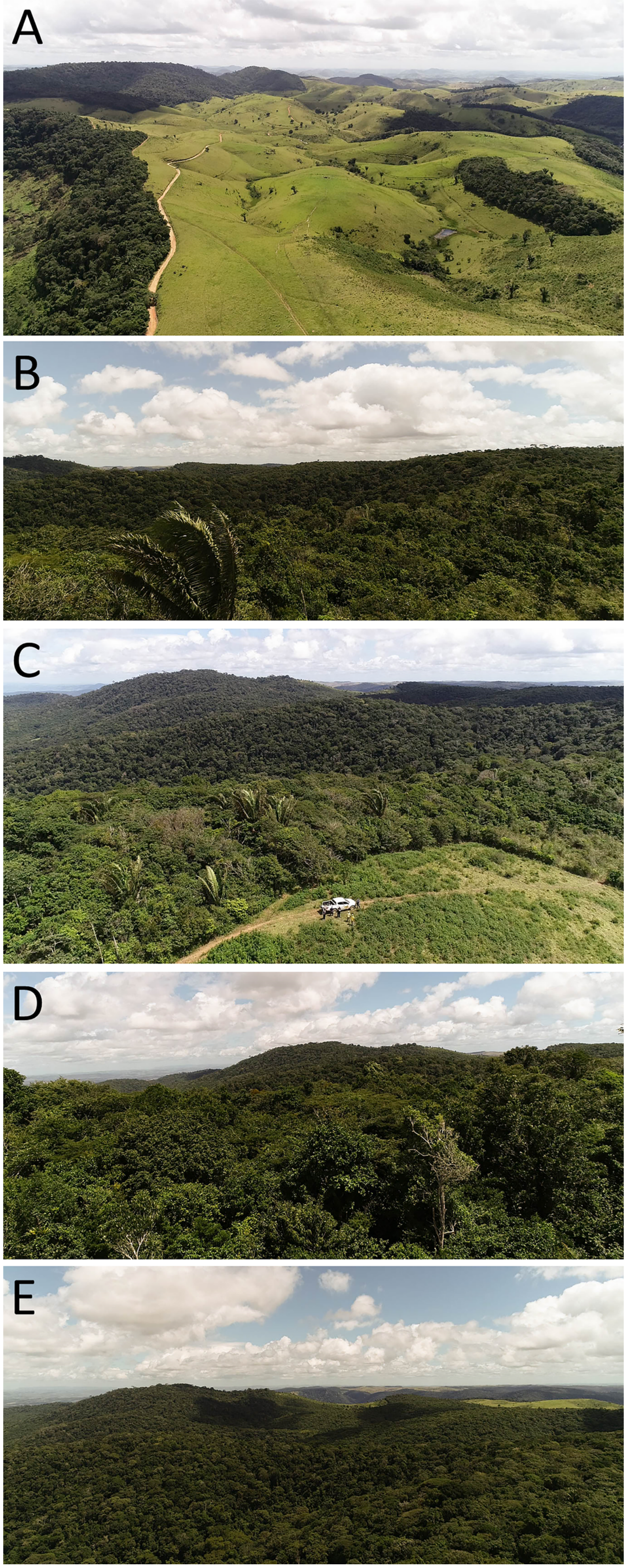

Figure 2. Aerial view of Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. A = Highlight for the small fragments surrounded by the pasture; B – E = Vegetation of the largest forest remnant (*Mata da Bananeira*). Photos: Rafael Cordeiro.

2. *Data collection*

The species list was constructed based on expeditions and occasional encounters in the APA and ESEC de Murici between 1994 and 2022 by different researchers. Specifically, in the ESEC de Murici and *Mata do Engenho Coimbra* (inserted in the limits of the APA de Murici, located 19 km north of the ESEC de Murici) intensive and standardized sampling efforts using different collection methods, were employed.

For the ESEC de Murici, expeditions were carried out every two months of 3 to 4 consecutive days by EMXF, ST and collaborators between the years 1994 to 1996. The sampling effort was entirely employed using the active search method, totaling 1,450 hours/person (Foster 2012). Expeditions were then performed between 2012 and 2015 by JVAN, ICST, BSL and collaborators. A total of 18 campaigns (totaling 114 field days) were carried out, with monthly visits lasting seven days between December 2012 and December 2013 and visits every two months lasting five days between March 2014 and March 2015. During the first year of sampling (December 2012 to December 2013), 24 sets of pitfall traps were installed at different points in the main fragment of the ESEC de Murici, called *Mata da Bananeira* (Figure 2B–E). Each station consisted of four 60-liter buckets, arranged in a "Y" and interconnected by a guide fence measuring four meters in length and 70 cm in height (adapted from Cechin & Martins 2000, Foster 2012). The traps were opened for five days during each expedition, totaling 1,440 hours/bucket. Additionally, three glue traps (dimensions 20 x 15 centimeters) were installed within a radius of six meters from each of the pitfall stations (totaling 72 traps), one in a fallen log and two in vertical logs (0.3–1.5 meters above from soil). Like the pitfalls, the glue traps were kept for five days during each expedition, totaling 1,440 hours/trap.

For *Mata do Engenho Coimbra*, four expeditions lasting 20 days each were carried out by Ubiratan Gonçalves and collaborators during 2006 and 2007, of which two were performed during the dry period (November to December 2006 and April 2007) and two during the rainy season (August to September 2007 and September to October 2007), totaling 80 field days. The sampling effort was directed towards the registration and collection of lizards, resulting in 524 hours/person of active and visual searches. During the period of field expeditions, three sets of pitfall traps were installed at different points in the fragment. Each station consisted of 32 buckets of 25–37 liters (96 buckets in total), arranged in a "Y" and interconnected by a guide fence measuring four meters in length and 70 cm in height. The traps were opened 15 days before each expedition and remained open during the 20 days in the field, totaling 3,360 hours/bucket.

All specimens collected (Collection and Transport License ICMBio/SisBio 33507) were euthanized using 10% lidocaine, fixed in 10% formalin (Beaver 2001) and incorporated into the Coleção Herpetológica do Museu de História Natural da Universidade Federal de Alagoas (MHN-UFAL), Coleção Herpetológica da Universidade Federal do Rio Grande do Norte (UFRN-CH), Coleção Herpetológica da Universidade Federal Rural de Pernambuco (CHP-UFRPE), and Coleção Herpetológica do Museu de Zoologia da Universidade de São Paulo (MZUSP). Material identification was carried out using the available literature and by consulting specialists in this area. The taxonomic nomenclature followed Uetz et al. (2022) (except Dipsadidae which follows Zaher et al. [2019]).

3. *Conservation status*

The conservation status of each taxon was determined following the Redlist of the International Union for the Conservation of Nature (IUCN 2022) and the Brazilian Redlist, the *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção* of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio 2018).

Results

A total of 89 reptile species were obtained during the 28 years of collection. Seventy-six species were recorded within the limits of the ESEC de Murici (85% of the total) of which 17 were recorded only in this protected area. As for the APA de Murici, 72 species (81% of the total) were recorded, of which 12 were only recorded in this protected area. Within Crocodylia, two species of Alligatoridae were recorded. Within Testudines, three species were recorded, two of Chelidae and one of Kinosternidae. Within Squamata, two species of amphisbaenians were recorded, both belonging to Amphibaenidae family. Twenty-seven species of lizards were recorded, where Gymnophthalmidae was the most diverse family with four species, followed by Dactyloidae, Dipoglossidae, Scincidae, Teiidae and Tropiduridae (3 spp. each), Phyllodactylidae, Polychrotidae and Sphaerodactylidae (2 spp. each), Gekkonidae, Iguanidae and Leiosauridae (1 spp. each). Fifty-four species of snakes were recorded, of which Dipsadidae was the most diverse family with 30 species, followed by Colubridae (8 spp.), Viperidae (5 spp.), Boidae (4 spp.), Elapidae and Typhlopidae (2 spp. each), Anomalepididae and Leptotyphlopidae (1 spp. each). Of these, two are currently considered endangered: *Amerotyphlops paucisquamus* and *Bothrops muriciensis* (Vulnerable [VU] and Endangered [EN], respectively, according to the national list; ICMBio 2018). The complete species list including information about voucher, the protected area of the record and global and national conservation status is provided in Table 1 (Figures 3 - 9).

Table 1. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. Collection acronym: **MHN-UFAL**= Coleção Herpetológica do Museu de História Natural da Universidade Federal de Alagoas; **UFRN-CH**= Coleção Herpetológica da Universidade Federal do Rio Grande do Norte; **CHP-UFRPE**= Coleção Herpetológica da Universidade Federal Rural de Pernambuco; **MZUSP**= Museu de Zoologia da Universidade de São Paulo. Area of record: **ESEC**= Estação Ecológica de Murici; **APA**= Área de Proteção Ambiental de Murici. Conservation status: **EN**= Endangered; **VU**= Vulnerable; **LC**= Least Concern; **DD**= Data Deficient; **NE**= Not Evaluated. For specimens that do not have a voucher, there is photographic record (Figures 3 – 9) or personal observation (P.O.).

SPECIES	VOUCHER	Recorded in		Conservation status	
		ESEC	APA	ICMBIO	IUCN
CROCODYLIA					
Alligatoridae					
Caiman latirostris (Daudin, 1801)	MHN-UFAL 16463		●	LC	LC
Paleosuchus palpebrosus (Cuvier, 1807)	Figure 3B	●	●	LC	LC

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SPECIES	VOUCHER	Recorded in		Conservation status	
		ESEC	APA	ICMBIO	IUCN
TESTUDINATA					
Chelidae					
Mesoclemmys tuberculata (Luederwaldt, 1926)	P.O.		●	LC	NE
Phrynops geoffroanus (Schweigger, 1812)	Figure 3C		●	LC	NE
Kinosternidae					
Kinosternon s. scorpioides (Linnaeus, 1766)	MHN-UFAL 12175	●	●	LC	NE
SQUAMATA					
AMPHISBAENIAS					
Amphisbaenidae					
Amphisbaena alba Linnaeus, 1758	MHN-UFAL 1883	●	●	LC	LC
Amphisbaena pretrei Duméril & Bibron, 1839	MHN-UFAL 1411	●	●	LC	LC
"LIZARDS"					
Dactyloidae					
Dactyloa punctata (Daudin, 1802)	MHN-UFAL 10827	●	●	LC	LC
Norops fuscoauratus (D'Orbigny, 1837)	MHN-UFAL 10877	●	●	LC	LC
Norops ortonii (Cope, 1868)	MHN-UFAL 11555	●	●	LC	NE
Dipoglossidae					
Diploglossus fasciatus (Gray, 1831)	MHN-UFAL 1643	●	●	LC	LC
Diploglossus lessonae Peracca, 1890	MHN-UFAL 1403	●	●	LC	LC
Ophiodes striatus (Spix, 1824)	MHN-UFAL 12269	●	●	DD	LC
Gekkonidae					
Hemidactylus mabouia (Moreau De Jonnès, 1818)	MHN-UFAL 1562	●	●	NE	LC
Gymnophthalmidae					
Acratosaura mentalis (Amaral, 1933)	UFRN-CH 2323		●	LC	LC
Cercosaura olivacea (Gray, 1845)	P.O.		●	NE	NE
Dryadosaura nordestina Rodrigues, Freire, Pellegrino & Sites, 2005	MHN-UFAL 10875	●	●	LC	LC
Stenolepis ridleyi Boulenger, 1887	MHN-UFAL 11686	●	●	LC	LC
Iguanidae					
Iguana i. iguana (Linnaeus, 1758)	MHN-UFAL 16135		●	LC	LC
Leiosauridae					
Enyalius aff. *catenatus* (Wied, 1821)	MHN-UFAL 10728	●	●	-	-
Phyllodactylidae					
Gymnodactylus darwinii (Gray, 1845)	MHN-UFAL 2123	●		LC	LC
Phyllopezus lutzae (Loveridge, 1941)	MHN-UFAL 10872	●	●	LC	LC
Polychrotidae					
Polychrus acutirostris Spix, 1825	MHN-UFAL 1594	●		LC	LC
Polychrus marmoratus (Linnaeus, 1758)	MHN-UFAL 10943	●	●	LC	LC
Scincidae					
Copeoglossum nigropunctatum (Spix, 1825)	MHN-UFAL 10845	●		LC	LC
Psychosaura agmosticha (Rodrigues, 2000)	P.O.	●	●	LC	LC
Psychosaura macrorhyncha (Hoge, 1946)	MHN-UFAL 1587	●		LC	LC
Sphaerodactylidae					
Coleodactylus elizae Gonçalves, Torquato, Skuk & Sena, 2012	MHN-UFAL 11417	●		DD	NE
Coleodactylus meridionalis (Boulenger, 1888)	MHN-UFAL 10838	●	●	LC	LC
Teiidae					
Ameiva a. ameiva (Linnaeus, 1758)	MHN-UFAL 10996	●	●	LC	LC
Kentropyx calcarata Spix, 1825	MHN-UFAL 10921	●	●	LC	LC
Salvator merianae Duméril & Bibron, 1839	Figure 5D	●	●	LC	LC
Tropiduridae					
Strobilurus torquatus Wiegmann, 1834	MHN-UFAL 10834	●	●	LC	LC
Tropidurus hispidus (Spix, 1825)	MHN-UFAL 10945	●	●	LC	LC
Tropidurus semitaeniatus (Spix, 1825)	MHN-UFAL 10944	●	●	LC	LC
SNAKES					
Anomalepididae					
Liotyphlops trefauti Freire, Caramaschi & Argolo, 2007	MZUSP 12178		●	DD	DD
Boidae					
Boa c. constrictor Linnaeus, 1758	MHN-UFAL 12020	●	●	LC	LC
Corallus hortulana (Linnaeus, 1758)	MHN-UFAL 10879	●	●	LC	NE
Epicrates assisi Machado, 1945	CHP-UFRPE 6019		●	LC	LC
Epicrates cenchria (Linnaeus, 1758)	MHN-UFAL 12241	●	●	LC	NE

continue...

...continue

SPECIES	VOUCHER	Recorded in		Conservation status	
		ESEC	APA	ICMBIO	IUCN
Colubridae					
Chironius carinatus (Linnaeus, 1758)	CHP-UFRPE 6010	●	●	LC	NE
Chironius flavolineatus Jan, 1863	MHN-UFAL 10888	●	●	LC	LC
Dendrophidion atlantica Freire, Caramaschi & Gonçalves, 2010	MHN-UFAL 12021	●	●	DD	NE
Drymoluber dichrous (Peters, 1863)	MHN-UFAL 10957	●	●	LC	LC
Oxybelis aeneus (Wagler, 1824)	MHN-UFAL 10891	●	●	LC	LC
Spilotes s. sulphureus (Wagler, 1824)	MHN-UFAL 11036	●	●	LC	LC
Spilotes p. pullatus (Linnaeus, 1758)	MHN-UFAL 11060	●		LC	LC
Tantilla melanocephala (Linnaeus, 1758)	MHN-UFAL 10956	●	●	LC	LC
Dipsadidae					
Atractus maculatus (Günther, 1858)	MHN-UFAL 12342	●		DD	LC
Cercophis auratus (Schlegel, 1837)	MHN-UFAL 16636	●		LC	DD
Dipsas i. indica Laurenti, 1768	MHN-UFAL 11037	●		LC	LC
Dipsas m. mikanii (Schlegel, 1837)	MHN-UFAL 1886	●	●	LC	LC
Dipsas neuwiedi (Ihering, 1911)	MHN-UFAL 12888	●	●	LC	LC
Dipsas sazimai Fernandes, Marques & Argôlo, 2010	MHN-UFAL 12890	●		LC	LC
Dipsas variegata (Duméril, Bibron & Duméril, 1854)	MHN-UFAL 13005	●	●	LC	LC
Echinanthera cephalostriata Di Bernardo, 1996	MHN-UFAL 12345	●		-	-
Erythrolamprus aesculapii venustissimus (Wied, 1821)	MHN-UFAL 12793	●	●	LC	LC
Erythrolamprus miliaris merremii (Wied, 1821)	Figure 7C–D			LC	LC
Erythrolamprus p. poecilogyrus (Wied, 1824)	MHN-UFAL 13922		●	LC	LC
Erythrolamprus reginae (Linnaeus, 1758)	MHN-UFAL 11033	●		LC	LC
Erythrolamprus taeniogaster (Jan, 1863)	MHN-UFAL 10426	●	●	LC	LC
Erythrolamprus v. viridis (Günther, 1862)	MHN-UFAL 12800	●	●	LC	LC
Helicops angulatus (Linnaeus, 1758)	MHN-UFAL 10752	●	●	LC	LC
Imantodes cenchoa (Linnaeus, 1758)	MHN-UFAL 10835	●	●	LC	LC
Leptodeira a. annulata (Linnaeus, 1758)	MHN-UFAL 10835	●	●	LC	LC
Oxyrhopus guibei Hoge & Romano, 1977	MHN-UFAL 12358	●	●	LC	LC
Oxyrhopus petolarius digitalis (Reuss, 1834)	MHN-UFAL 12350	●	●	LC	LC
Oxyrhopus trigeminus Duméril, Bibron & Duméril, 1854	MHN-UFAL 10427	●		LC	LC
Philodryas nattereri (Steindachner, 1870)	Figure 8D		●	LC	LC
Philodryas olfersii (Lichtenstein, 1823)	CHP-UFRPE 6014	●	●	LC	LC
Pseudablabes patagoniensis (Girard, 1858)	MHN-UFAL 16637		●	LC	LC
Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	MHN-UFAL 12352	●	●	LC	LC
Siphlophis compressus (Daudin, 1803)	MHN-UFAL 12214	●	●	LC	LC
Taeniophallus affinis (Günther, 1858)	MHN-UFAL 10723	●		LC	LC
Taeniophallus occipitalis (Jan, 1863)	MHN-UFAL 10724	●	●	LC	LC
Thamnodynastes pallidus (Linnaeus, 1758)	MHN-UFAL 10611	●	●	LC	LC
Xenodon merremii (Wagler, 1824)	Figure 8J	●	●	LC	NE
Xenodon r. rabdocephalus (Wied, 1824)	MHN-UFAL 1655	●	●	LC	LC
Xenopholis scalaris (Wucherer, 1861)	MHN-UFAL 12354	●	●	LC	LC
Elapidae					
Micrurus ibiboboca (Merrem, 1820)	MHN-UFAL 10750	●	●	DD	NE
Micrurus carvalhoi Roze, 1967	MHN-UFAL 12349	●		LC	LC
Leptotyphlopidae					
Trilepida salgueiroi (Amaral, 1955)	CHP-UFRPE 4957	●		LC	LC
Typhlopidae					
Amerotyphlops arenensis Graboski, Filho, Silva, Prudente & Zaher, 2015	MHN-UFAL 10874	●	●	LC	LC
Amerotyphlops paucisquamus (Dixon & Hendricks, 1979)	MHN-UFAL 2058		●	VU	LC
Viperidae					
Bothrops b. bilineatus (Wied, 1821)	MHN-UFAL 10753	●	●	LC	NE
Bothrops leucurus Wagler, 1824	MHN-UFAL 1551	●	●	LC	NE
Bothrops muriciensis Ferrarezzi & Freire, 2001	MHN-UFAL 10751	●	●	EN	NE
Crotalus durissus cascavella Wagler in Spix, 1824	MHN-UFAL 12343	●	●	LC	LC
Lachesis muta (Linnaeus, 1766)	MHN-UFAL 13784	●	●	LC	LC

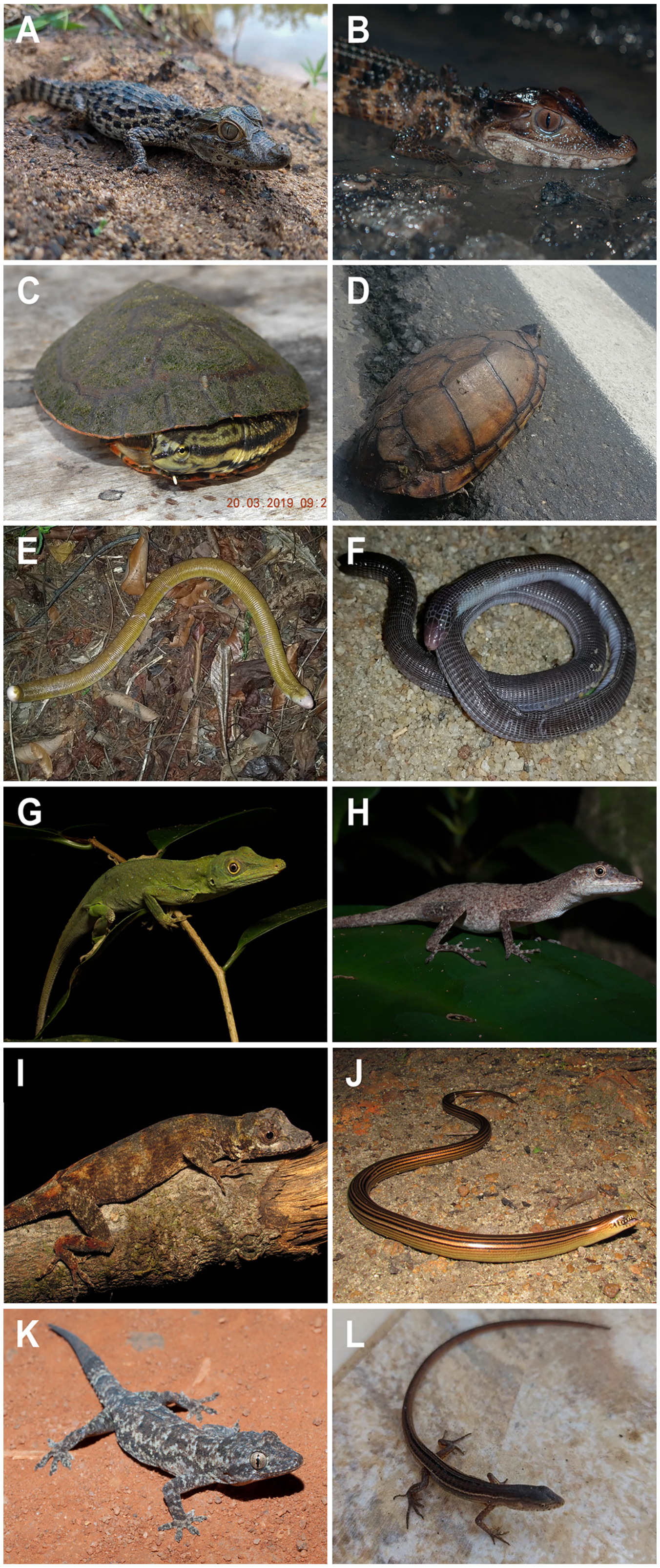

Figure 3. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Caiman latirostris*; **B** - *Paleosuchus palpebrosus*; **C** - *Phrynops geoffroanus*; **D** - *Kinosternon s. scorpioides*; **E** - *Amphisbaena alba*; **F** - *A. pretrei*; **G** - *Dactyloa punctata*; **H** - *Norops fuscoauratus*; **I** - *N. ortonii*; **J** - *Ophiodes striatus*; **K** - *Hemidactylus mabouia*; **L** - *Cercosaura olivacea*. All photos were taken from individuals found in the study area. Photos: A, C, D, E, F (Marco de Freitas); B, G, H, K (Marcos Dubeux); I, J (José Neto); L (Ubiratan Gonçalves).

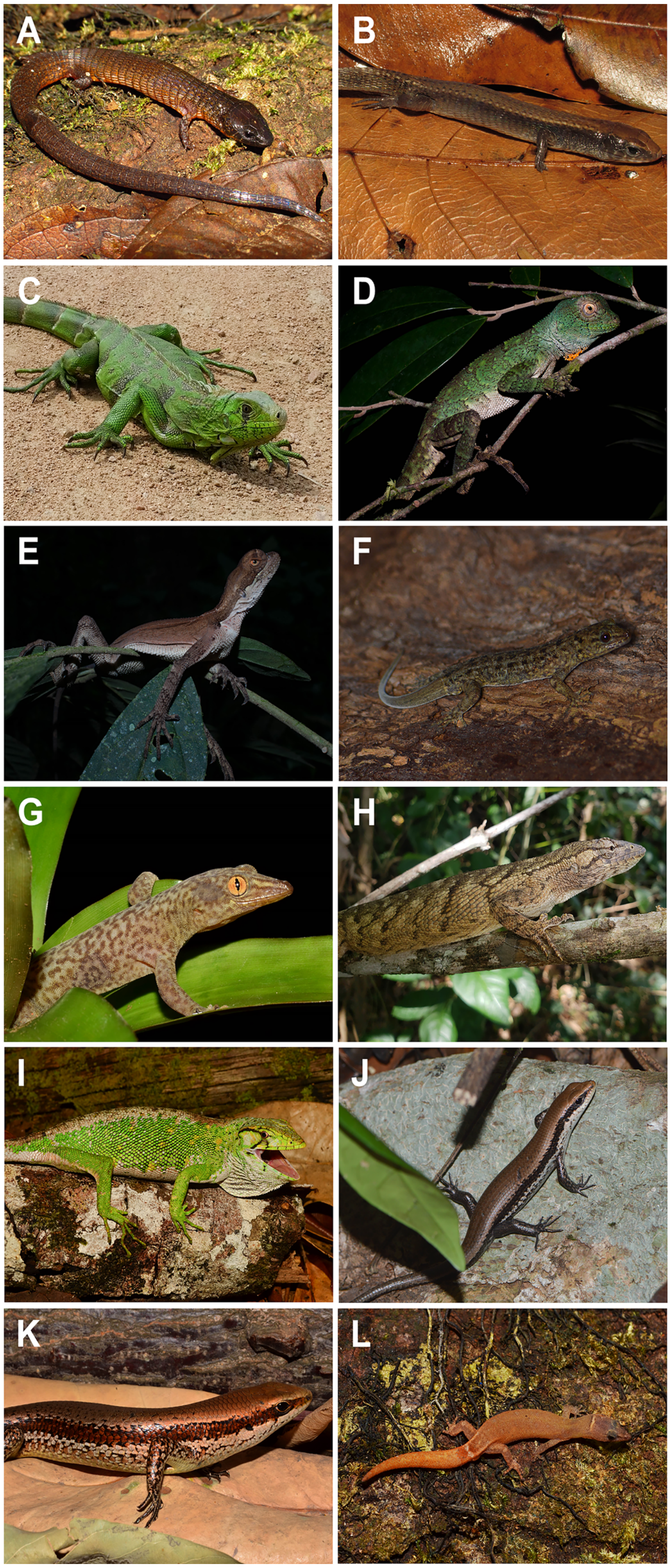

Figure 4. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Dryadosaura nordestina*; **B** - *Stenolepis ridleyi*; **C** - *Iguana i. iguana*; **D** - *Enyalius* aff. *catenatus* (male); **E** - *E.* aff. *catenatus* (female); **F** - *Gymnodactylus darwinii*; **G** - *Phyllopezus lutzae*; **H** - *Polychrus acutirostris*; **I** - *P. marmoratus*; **J** - *Copeoglossum nigropunctatum*; **K** – *C. nigropunctatum*; **L** - *Coleodactylus elizae*. All photos were taken from individuals found in the study area. Photos: A, B (José Neto); C, H, J (Marco de Freitas); D, E, M (Marcos Dubeux); F (Márcio Campelo); G, I, K, L (Barnagleison Lisboa).

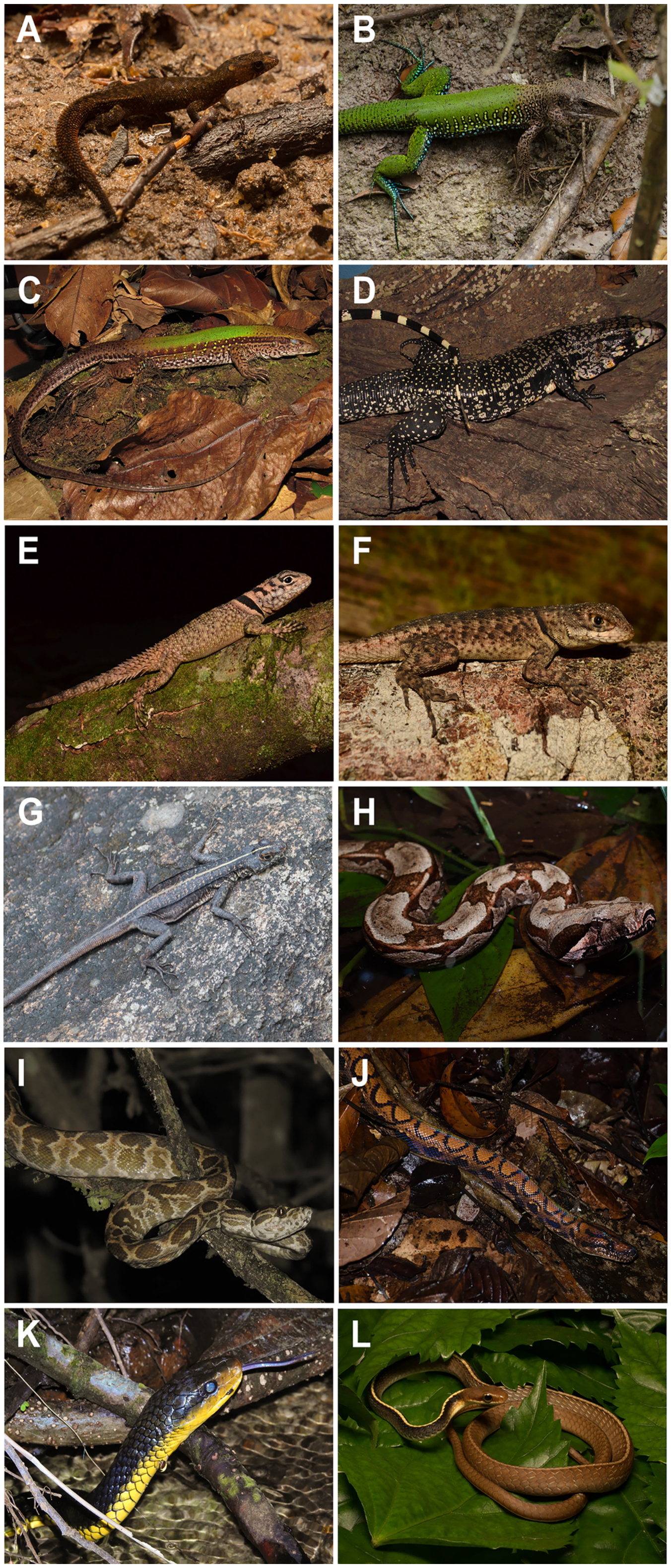

Figure 5. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Coleodactylus meridionalis*; **B** - *Ameiva* a. *ameiva*; **C** - *Kentropyx calcarata*; **D** - *Salvator merianae*; **E** - *Strobilurus torquatus*; **F** - *Tropidurus hispidus*; **G** - *T. semitaeniatus*; **H** - *Boa c. constrictor*; **I** - *Corallus hortulana*; **J** - *Epicrates cenchria*; **K** - *Chironius carinatus*; **L** - *C. flavolineatus*. All photos were taken from individuals found in the study area. Photos: A, H, L (Marcos Dubeux); B, I, J (Hermínio Vilela); C, D (José Neto); E, F (Barnagleison Lisboa); G (Márcio Campelo); K (Marco de Freitas).

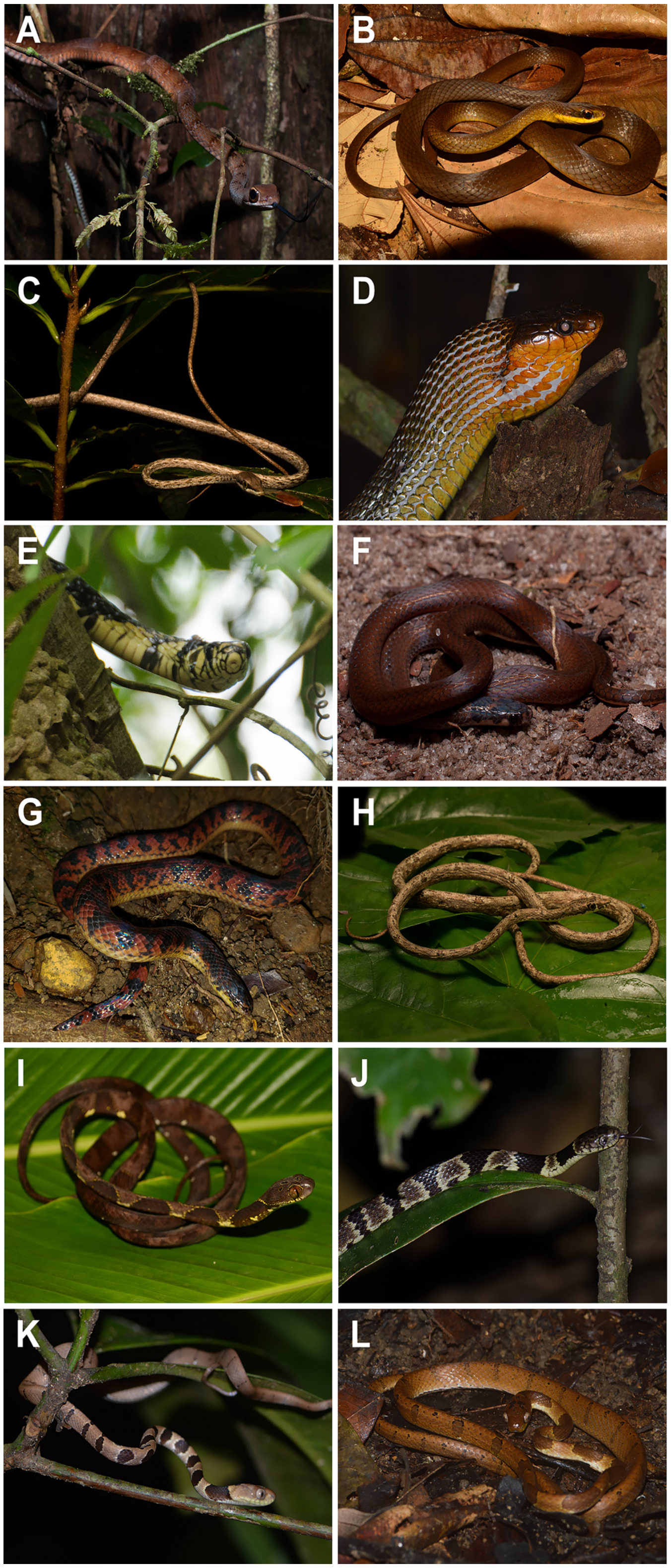

Figure 6. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Dendrophidion atlantica*; **B** - *Drymoluber dichrous*; **C** - *Oxybelis aeneus*; **D** - *Spilotes s. sulphureus*; **E** - *S. p. pullatus*; **F** - *Tantilla melanocephala*; **G** - *Atractus maculatus*; **H** - *Cercophis auratus*; **I** - *Dipsas i. indica*; **J** - *D. neuwiedi*; **K** - *D. sazimai* (juvenile); **L** - *D. sazimai* (adult). All photos were taken from individuals found in the study area. Photos: A, C, F, H (Marcos Dubeux); B, I (Barnagleison Lisboa); D, K, L (Márcio Campelo); E, J (Hermínio Vilela); G (Marco de Freitas).

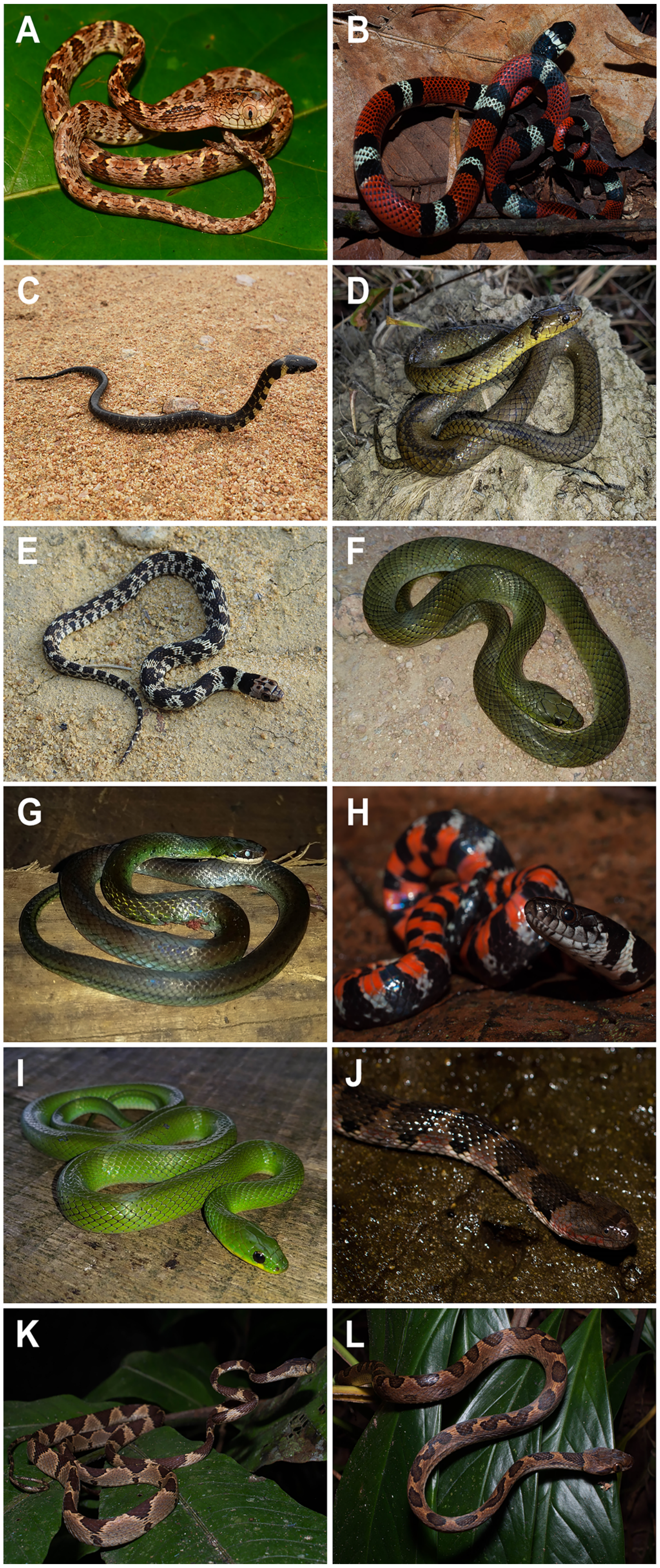

Figure 7. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Dipsas variegata*; **B** - *Erythrolamprus aesculapii venustissimus*; **C** - *Erythrolamprus miliaris merremii* (juvenile); **D** - *E. miliaris merremii* (adult); **E** - *E. p. poecilogyrus* (juvenile); **F** - *E. poecilogyrus* (adult); **G** - *E. reginae*; **H** - *E. taeniogaster*; **I** - *E. v. viridis*; **J** - *Helicops angulatus*; **K** - *Imantodes cenchoa*; **L** - *Leptodeira a. annulata*. All photos were taken from individuals found in the study area. Photos: A (Barnagleison Lisboa); B, H, J, K, L (Marcos Dubeux); C, D, E, F, G, I (Marco de Freitas).

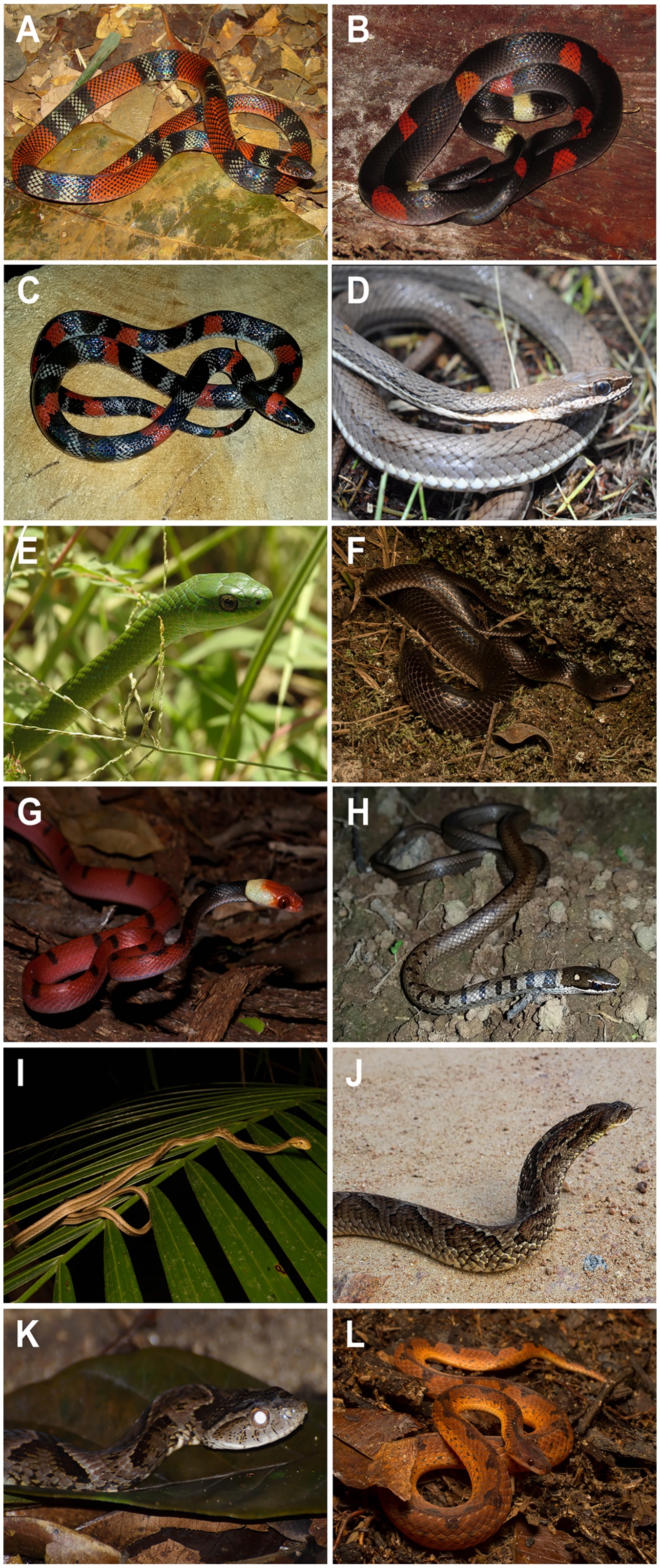

Figure 8. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Oxyrhopus guibei*; **B** - *O. petolarius digitalis*; **C** - *O. trigeminus*; **D** - *Philodryas nattereri*; **E** - *P. olfersii*; **F** - *Pseudoboa nigra*; **G** - *Siphlophis compressus*; **H** - *Taeniophallus occipitalis*; **I** - *Thamnodynastes pallidus*; **J** - *Xenodon merremii*; **K** - *X. r. rabdocephalus*; **L** - *Xenopholis scalaris*. All photos were taken from individuals found in the study area. Photos: A, B, C, E, H, J (Marco de Freitas); D, F, G, I, L (Marcos Dubeux); K (Hermínio Vilela).

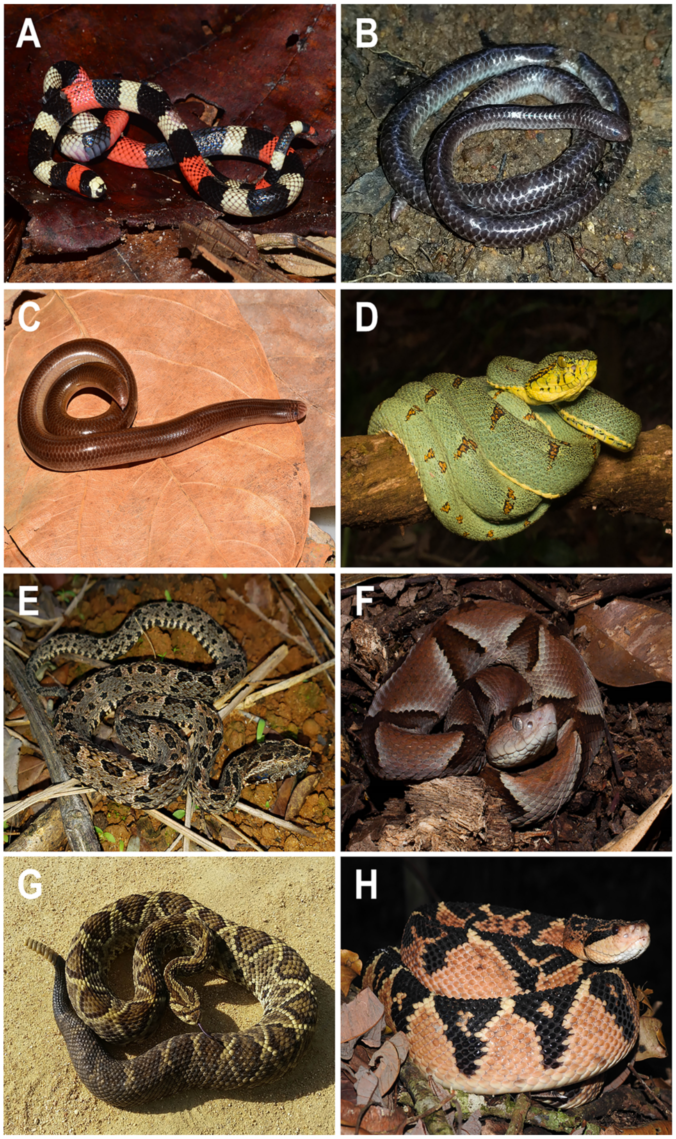

Figure 9. Reptiles recorded in the Estação Ecológica and Área de Proteção Ambiental de Murici, Alagoas state, northeastern Brazil. **A** - *Micrurus ibiboboca*; **B** - *Trilepida salgueiroi*; **C** - *Amerotyphlops arenensis*; **D** - *Bothrops b. bilineatus*; **E** - *B. leucurus*; **F** - *B. muriciensis*; **G** - *Crotalus durissus cascavella*; **H** - *Lachesis muta*. All photos were taken from individuals found in the study area. Photos: A, C (Barnagleison Lisboa); B, E, G, H (Marco de Freitas); D, F (Marcos Dubeux).

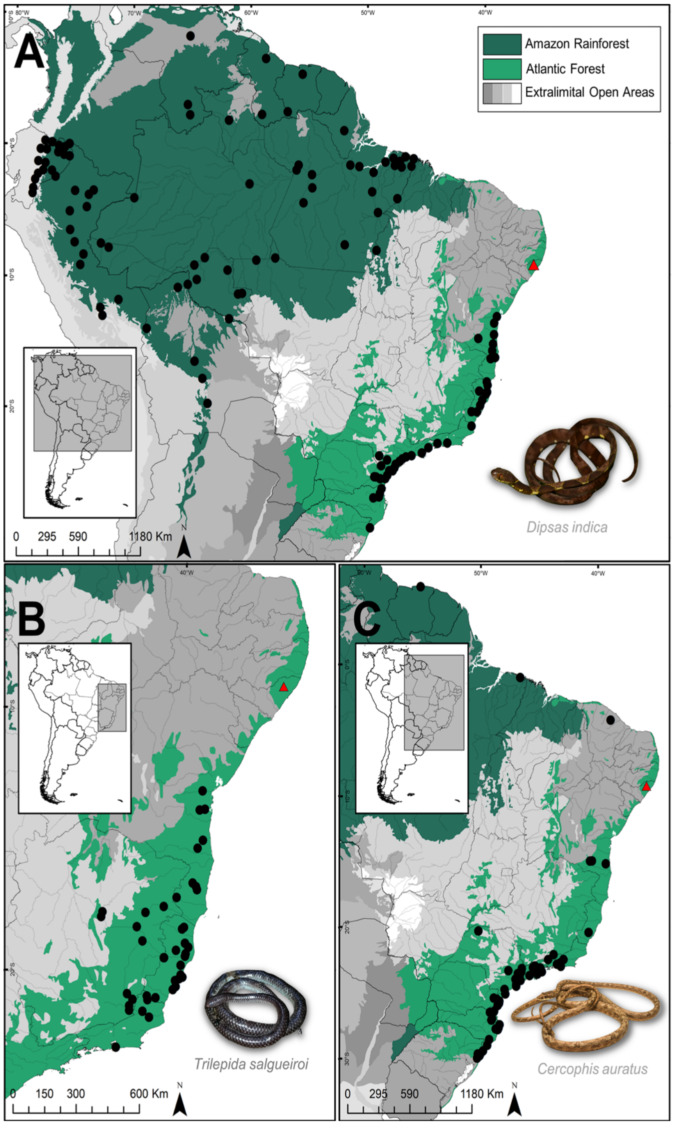

Figure 10. Geographical distribution and new records for **(A)** *Dipsas indica*, **(B)** *Trilepida salgueiroi* and **(C)** *Cercophis auratus* in the Atlantic Forest of Northeastern Brazil. Black circles = literature records (Nogueira et al. 2019); Red triangle = new records. Inset map = South America.

Discussion

The reptile richness recorded for the APA and ESEC de Murici is by far one of the greatest ever documented for the entire Atlantic Forest (e.g., Santana et al. 2008, Roberto et al. 2015, Roberto et al. 2017, Mesquita et al. 2018, Melo et al. 2018, Barbosa et al. 2019, Lima et al. 2021, Oliveira et al. 2021). This expressive richness is also the result of the high sampling effort and collection time applied in the study area, which is one of the more well sampled areas in the entire state of Alagoas. With 89 registered species, these protected areas surpass, in terms of number of species, the areas that previously housed the greatest reptile richness of the Atlantic Forest north of the São Francisco River - the Reserva Biológica (Biological Reserve) Guaribas, Paraíba state, and the Reserva Biológica de Pedra Talhada, Alagoas and Pernambuco states, both with 72 recorded reptile species (Roberto et al. 2015, Mesquita et al. 2018).

It is worth noting that although the ESEC de Murici area is comprised of only 6,130 ha, it has the most conserved forest fragments in the region and is home to more than 96% of the species recorded here. When considering its small geographic coverage, the ESEC de Murici can be considered the richest area, in terms of reptile fauna, in the entire Brazilian territory. This ESEC represents one of the last forest fragments of the Atlantic Forest north of the São Francisco River, and undoubtedly has an important conservation value (Filho et al. 2021).

Although there was a considerable sampling effort and the use of complementary sampling methodologies (active and passive), this effort was not evenly distributed temporally and geographically in the study area. This fact makes it difficult to present statistics on species richness and sampling effort or methodological comparisons covering the entire set of data obtained. In fact, these data will be better explored in future studies with a specific focus on the evaluation of techniques for collecting amphibians and reptiles in the northern Atlantic Forest (MJMD unpublished data).

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Two species registered in the area are considered threatened. *Amerotyphlops paucisquamus* is considered a Vulnerable [VU] species according to the national list (ICMBio 2018; not evaluated in the international list, IUCN 2022). This species of fossorial snake has a restricted distribution in the Atlantic Forest north of the São Francisco River and in some forest areas in the state of Maranhão (Dixon & Hendricks 1979, Rodrigues et al. 1988, Graboski et al. 2019, Nogueira et al. 2019). Since it is restricted to forested areas, deforestation and the consequent loss of habitat are considered the main threats to this snake (ICMBio 2018). *Bothrops muriciensis* (Figure 9F) is classified as Endangered [EN] on the national list (ICMBio 2018; not evaluated in the international list, IUCN 2022). This species is endemic to ESEC de Murici and known to occur in only a single forest remnant called *Mata da Bananeira*. Knowledge about basic aspects of its biology is still scarce and up until 2012 the species was known by only nine individuals, all found close to its original area of description (Ferrarezzi & Freire 2001, Freitas et al. 2012). Recent studies have been refining this knowledge and although still restricted to the ESEC de Murici, new records of the species have been described in recent years (MJMD unpublished data).

Additionally, six recorded species are defined as Data Deficient [DD] for the assessment of their conservation status (*Coleodactylus elizae*, *Ophiodes striatus*, *Liotyphlops trefauti*, *Atractus maculatus*, *Cercophis auratus* and *Micrurus ibiboboca*). These species are lonely and elusive, making an accurate assessment difficult due to the incomplete knowledge of their geographic distribution and population sizes, as well as their ecological and environmental requirements (ICMBio 2018). The evaluation of these taxa is necessary, as some are currently only known to occur in a few localities, such as *Coleodactylus elizae* (Figure 4L) which presents a disjointed distribution and is only known to occur in the ESEC de Murici and for its type locality, 37 km away in the municipality of Maceió, state of Alagoas (Gonçalves et al. 2012). While others, although widely distributed, are considered complexes of cryptic species, for example, *Ophiodes striatus* and *Micrurus ibiboboca* (Figure 3J and 9A; ICMBio 2018, Schools & Hedges 2021), where the current taxonomic context can give the false impression of widespread species rather than restricted distribution under different threats. Additionally, some species are known to be distinct evolutionary lineages and potential candidates for new species (e.g., *Enyalius* aff. *catenatus*; Rodrigues et al. 2014).

Three of the snakes found at the ESEC de Murici correspond to the first record of the species for the Atlantic Forest north of the São Francisco River (França et al. 2020). *Dipsas indica* (Figure 6I) is an arboreal Dipsadidae found in the interior of forests. The species has a widely disjointed distribution, occurring throughout practically the entire Amazon region and in the Atlantic Forest, from the states of Bahia to the extreme south of the state of Santa Catarina (Freitas 2015, Costa & Bérnils 2018, Arteaga et al. 2018, Nogueira et al. 2019; Figure 10A). The new record corresponds to the northernmost occurrence of this species, expanding its distribution 560 km north of its closest location (municipality of Jaguaripe, state of Bahia; Nogueira et al. 2019). The species was registered by a single individual (MHN-UFAL 11037), found in the interior of *Mata da Bananeira*.

The second species recorded was *Trilepida salgueiroi* (Figure 9B), a tiny snake belonging to the family Leptotyphlopidae with fossorial and semi-fossorial habits (Passos et al. 2005). The species is known to occur in the Atlantic Forest in the states of Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro (Costa et al. 2009, Figueiredo-de-Andrade et al. 2011, Nogueira et al. 2019; Figure 10B). The new record corresponds to the northernmost occurrence of the species, expanding its distribution 585 km north of its closest location (municipality of Laje, state of Bahia; Nogueira et al. 2019). The species was described by a single individual (CHP-UFRPE 4957), found dead near the edge of the forest.

The third record was of *Cercophis auratus* (Dipsadidae; Figure 6D), an arboreal snake with diurnal habits found within forests (Morato & Bernils 1989, Marques 2000). This species is the only representative of the genus *Cercophis* and has a disjointed distribution in the Amazon Forest (where its type locality is located), in the southern portion of the Atlantic Forest and in the *Brejo de Altitude* in the state of Ceará (Hoogmoed et al. 2019, Nogueira et al. 2019, Bezerra et al. 2020, Figure 10C). The new record expands the known distribution of the species 814 km north of its closest location (municipality of Barra do Choça, state of Bahia; Bezerra et al. 2020). The species was described by a single individual (MHN-UFAL 16636), found under vegetation at a height of approximately 1.5 m high on the banks of a stream in the interior of *Mata da Bananeira*.

Although the APA and ESEC de Murici have been receiving attention from researchers in recent decades, little information has been made available so far on the herpetofauna in the region, especially regarding reptile fauna. The information available so far is limited to the description of some species (Ferrarezzi & Freire 2001, Fernandes et al. 2010), distribution extensions (Andrade-Lima et al. 2020) and some specific information on natural history (Santos et al. 2018, Dubeux et al. 2019, Dubeux et al. 2020, Dubeux & Gonçalves 2021) and conservation (Freitas et al. 2012). We hope that the species list provided here will serve as a starting point for paving the way for further studies in this "hotspot" of reptile diversity.

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Author Contribution

Marcos Jorge Matias Dubeux: Contribution to the conception and design of the work, data acquisition, data analysis and interpretation; writing and critical review of the manuscript, adding intellectual content.

José Vieira de Araújo Neto: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Ingrid Carolline Soares Tiburcio: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Barnagleison Silva Lisboa: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Selma Torquato: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Marco Antônio de Freitas: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Eliza Maria Xavier Freire: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Míriam Camargo Guarnieri: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Tamí Mott: Contribution to the conception and design of the work and data acquisition; writing and critical review of the manuscript, adding intellectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this work

Data Availability

Supporting data are available at https://doi.org/10.48331/scielodata.1KX6SA

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Inventory

Floristic composition, pollination and seed-dispersal systems in a target cerrado conservation area

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BORGIANI, R., GROMBONE-GUARATINI, M.T., VARGAS, B.C., MARTINS, A.E., CAMARGO, M.G.G., MORELLATO, L.P.C. **Floristic composition, pollination and seed-dispersal systems in a target cerrado conservation area**. Biota Neotropica 22(2): e20211318. https://doi.org/10.1590/1676-0611-BN-2021-1318

Abstract: Cerrado remnants can hold an important diversity of plant species of environmental and ecological relevance. We presented a checklist of vascular plants based on 12 years of inventory carried out in 36 plots (10 m x 2 m; 0.18 ha in total) and during unsystematic walks in a remnant area of *cerrado sensu stricto* located at Itirapina municipality, state of São Paulo, southeastern Brazil. The list comprised 195 plant species, corresponding to 54 families and 131 genera. The richest families were Fabaceae (25 species), Asteraceae (16), Myrtaceae (16), Rubiaceae (11), Bignoniaceae and Malpighiaceae (10 each), Melastomataceae (9), and Erythroxylaceae, Sapindaceae and Annonaceae (6). Predominant life forms included shrubs and trees, with 68% of the species, followed by lianas with 12%, sub-shrub and herbs with 10% each. Bees were the dominant pollinators (67,5%) and the majority of species had seeds dispersed by animals (56.8%), mostly by birds, followed by wind (33.3%) and self-dispersed (11.2%). More than 60% of the total species were classified as "typical" Cerrado species. *Bowdichia virgilioides* was the only species classified as Near Threatened (NT) and 157 were regarded as Data Deficient (DD). Our dataset provides floristic, structural, and ecological information for one of the targeted areas for Cerrado survey at São Paulo state, contributing to the understanding of diversity patterns and future conservation and restoration actions in this threatened hotspot.
Keywords: *Brazilian savanna; hotspot; life form; functional traits.*

Composição florística, modos de polinização e dispersão de sementes de uma área de cerrado relevante para conservação

Resumo: Apresentamos uma lista de verificação de plantas vasculares baseada em 12 anos de inventário realizado em 36 parcelas (10 m x 2 m; 0,18 ha no total) e caminhadas assistemáticas em uma área remanescente de cerrado *sensu stricto* localizada em Itirapina, município do estado de São Paulo, sudeste do Brasil. A lista é composta por 195 espécies de plantas, correspondendo a 54 famílias e 131 gêneros. As famílias mais ricas foram Fabaceae (25 espécies), Asteraceae (16), Myrtaceae (16), Rubiaceae (11), Bignoniaceae e Malpighiaceae (10 cada), Melastomataceae (9) e Erythroxylaceae, Sapindaceae e Annonaceae (6). As formas de vida predominantes incluíram arbustos e árvores (33,7% das espécies), seguidas por lianas (12%), arbustos e ervas (10%). As abelhas foram os polinizadores dominantes (67,5%) e o principal modo de dispersão foi a zoocoria (56,8%), representada principalmente por pássaros, seguida por vento (33.3 %) e auto (11.2 %). Mais de 60% das espécies encontradas foram classificadas como espécies "típicas" de Cerrado. *Bowdichia virgilioides* foi a única espécie pertencente a uma categoria de ameaça "Quase Ameaçada (NT)", sendo 157 delas classificadas na categoria "Deficiente de Dados (DD)". Nosso conjunto de dados fornece informações florísticas, estruturais e ecológicas para uma das áreas-alvo do levantamento do Cerrado no estado de São Paulo, sudeste do Brasil, contribuindo para a compreensão dos padrões de diversidade e futuras ações de conservação neste *hotspot* ameaçado.
Palavras-chave: *Savana brasileira; hotspot; formas de vida; características funcionais.*

Introduction

The Cerrado – the Brazilian savanna - is the second most extensive biome in South America. It is the source of many water springs encompassing the main hydrographic basins and the largest reservoirs of freshwater in this continent, the Guarani Aquifer (Pereira et al. 2021). Cerrado is one of the 25 global *hotspots* (Myers et al. 2000) for biodiversity conservation (Mittermeier et al. 2005), and the most diverse neotropical savanna (Klink & Machado 2005), providing diverse and important ecosystem services essential to sustain agricultural systems (Lambers et al. 2020). Due to the extensive land conversion to agriculture and the high susceptibility to climatic change (Strassburg et al. 2017), the Cerrado is also the most severely threatened biome in Brazil (Lopes et al. 2021).

The Cerrado is a heterogeneous ecosystem regarding biodiversity and phytophysionomy: ranging from grassland with small and sparse shrubs, savanna with predominant woody vegetation (trees of approximately 6-7 m height) and discontinuous tree cover, to forest formation with a canopy height of 12–15 m (Coutinho 2006, Sano et al. 2008). This heterogeneity is indicated by a high endemism rate (44%), representing about 12% of all Brazilian species (Klink & Machado 2005). The cerrado *sensu stricto* is considered one of the most common phytophysiognomies, occurring in approximately 70% of all territorial extensions of its domain (Eiten 1972).

From 1990 to 2010, the net loss rate of Cerrado natural vegetation was around 117.870 km^2 (Beuchle et al. 2015). However, more recent data from the Brazilian Annual Land Use and Land Cover Mapping Project (MapBiomas, Collection 5.0; https://mapbiomas.org) showed that the accumulated losses, ranging from 1985 to 2019, is in reality, 408,6 thousand hectare.

It is estimated that the Cerrado ecosystem may disappear by 2050 (Strassburg et al. 2017) if extensive farming expansion, including agriculture and cattle ranching, is not contained and if conservation and preservation programs of biodiversity are not adopted (Strassburg et al. 2017). In addition, a recent study concluded that both the hydrology and ecology of the Cerrado will be strongly affected considering climate change in the near future (Rodrigues et al. 2020).

The state of São Paulo presents the lowest area of Cerrado remnant cover indices, around 13% of the original distribution (Sano et al. 2010). Public and multi-stakeholder conservation programs can change this imminent extinction scenario, cooperating with national and international biodiversity safeguarding goals in the Cerrado (Strassburger et al. 2016, 2017). The first step to improving conservation and restoration actions is conducting plant inventories to assess species diversity and differences in the community composition and structure among areas through time and biogeographic patterns (Lima et al. 2020). After that, acquiring functional traits, for example, running qualitative or quantitative studies of pollination and seed dispersal syndromes associated with vegetation stratification is essential to preserve Cerrado's dynamics (Gottsberger & Silberbauer-Gottsberger 2018) and guide future restoration actions (Buisson et al. 2020).

The remnant Cerrado studied was fragmented nearly 30 years ago. The total density is 15,522 individuals per hectare – with the largest diameter and maximum registered being 34.7 cm and 12 m, respectively. Myrtaceae, Fabaceae and Malpighiaceae as the richest families and *Bauhinia rufa* (Bong.) Steudel, *Xylopia aromatica* (Lam.) Mart., *Miconia rubiginosa* (Bonpl.) A.DC, *Virola sebifera* Aubl. and *Myrcia guianensis* (Aubl.) DC. are the species with highest abundance (Reys et al. 2013). In the cerrado sensu stricto studied, the edge effect and cardinal orientation intensifies the reproductive phenophases and synchronizes individuals of *Xylopia aromatica* and *Myrcia guianensis* (Camargo et al. 2011, Vogado et al. 2016). Also, the edges influence the structure of the liana community, increasing the species richness, abundance, and host occupancy (Melis et al. 2021). Climate seasonality directly affects leaf fall and flush, flowering, fruiting and germinative strategies (Camargo et al. 2013, 2018, Escobar et al. 2018, 2021, Martins et al. 2021), certainly shaping the floristic diversity found in the area. Although several ecological aspects of the cerrado study area have been investigated, an accurate list of the flowering plants, however, has not yet been published.

Here, we presented a checklist of vascular plants, and associated pollination and seed-dispersal systems, based on 12 years of inventory carried in a cerrado sensu stricto remnant on Southeastern Brazil, described as a priority area for survey and conservation in the state of São Paulo by Metzger & Rodrigues (2008). This study aims to provide a starting point to implement public policies to management, land use, conservation, restoration and future ecological studies.

Material and Methods

1. *Study site*

The study area is a remnant of Cerrado located in a private land at Itirapina municipality, state of São Paulo (22°10'31.41" S; 47°52'26.3" W), southeastern Brazil (Figure 1a). The average altitude of the area is 760 meters above sea level. The Cerrado is described as a savanna biome composed by different vegetation physiognomies, including the woody savanna, widespread in the neotropical region (Coutinho 2006). The cerrado *sensu stricto* is a typical dominant woody vegetation of the Cerrado (Coutinho 2006) and at the study area (Reys et al. 2013). The study area is a rectangular fragment that has been anthropized for nearly 30 years, with sides facing the four cardinal points: west: a highway; east: a remnant of Cerrado and a pasture; south and north: sugarcane crops (Figure 1b).

The cerrado vegetation surveyed presents a discontinuous tree cover around 6-7 meters high (emergent trees reaching up to 12 meters) and discontinuous herbaceous layer with grasses and some herbs, bromeliads, and palms (Camargo et al. 2011, Reys et al. 2013) (Figure1c-d). The average canopy openness varies from 24% (edges) to 15% (interior) (Reys et al. 2013). The climate of the cerrado study area is seasonal, with a dry cold season from April to September and a rainy warm season from October to March (Camargo et al. 2018, Escobar et al. 2018). The mean annual temperature is 20 °C, with a maximum of 32 °C (February) and a minimum of 18 °C (July). The mean total annual rainfall is 1524 mm (Camargo et al. 2018, Escobar et al. 2018). Soil is classified as Latosol-Argisol according to the Brazilian Soil Classification System (EMBRAPA 1999, Reys et al. 2013).

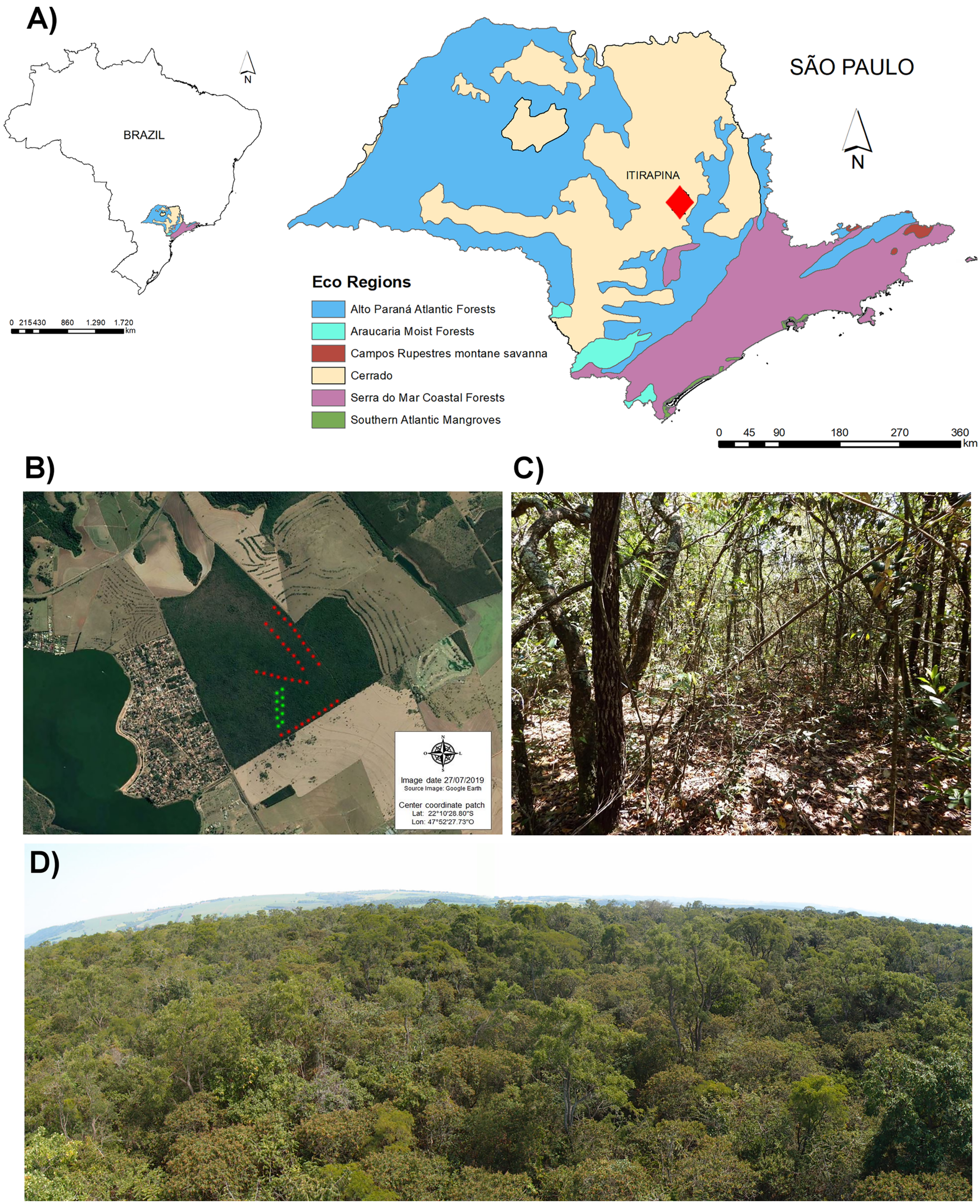

Figure 1. Map and vegetation of the study site. (A) Geographical location of the Itirapina municipality, belonging to the Cerrado Eco Region (Olson et al. 2001), São Paulo State, Southeastern Brazil; (B) Satellite image of the cerrado *sensu stricto* fragment studied with a scheme of the sampled plots (red dots: sample plots according to Reys et al. (2013); green dots: sample plots of this study), Fazenda São José da Conquista; (C) Photograph showing the vegetation interior; (D) Photograph taken from the top of a phenological tower, showing the cerrado sensu stricto fragment from above. Red diamond represents the Itirapina municipality on the São Paulo State map. (Photographs by G. M. Marcusso and B. Alberton, respectively).

2. *Data collection*

First, we established 36 plots of 25 m x 2 m at least 50 meters apart and equally distributed throughout the south and east sides of the fragment: east edge (10 plots), south edge (10 plots), east interior (8 plots), and south interior (8 plots), as described by Reys et al. (2013) (Figure 1b). The plots were arranged in two parallel lines on both sides, with one line on the edge – defined as the area of contact with the matrix – and another line 100 meters from the edge. Within the 36 plots we marked, sampled and identified all trees and scrubs with a diameter ≥ 3 cm at 30 cm from their ground base (Reys et al. 2013). Later, in 2015, we added 10 more plots (2 m x 50 m, 20 m apart) in the same study area, adopting the rapid sampling method (Gentry´s 0.1-ha transects) created and used by Gentry (1982), including all woody individuals with diameter at breast height ≥ 2,5 cm (Figure 1b).

Next, we collected floriferous branches and reproductive structures of tree, shrub, herb, and climber species during unsystematic walks, aiming to fully cover all Cerrado areas through monthly field trips from 2004 to 2018. We identified taxa according to specialized literature and taxonomic experts, compared them with herbarium collections and deposited fertile voucher specimens in the Herbarium Rioclarense (HRCB). We verified the species and family names using Flora do Brasil (2020). The results are presented under APG (2009) and APG IV (2016). Finally, we divided species into four groups according to life form (trees, shrubs, sub-shrub and herbs, and climbers) and dispersal system (Escobar et al. 2018, Van der Pijl 1982). We classified all species as “typical” Cerrado species or belonging to other physiognomies in accordance with Durigan et al. (2004, 2012). We checked the degree of threaten plants in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and in the Official Red List of Endangered Species of the Brazilian Flora (Flora do Brasil 2020) and classified in (EW – Extinct in the Wild, CR – Critically Endangered, EN – Endangered, VU – Vulnerable, NT – Near Threatened, DD – Data Deficient, LC – Least Concern).

We characterized diaspores of the surveyed species and fit them into the dispersion syndromes as self-, wind- and animal-dispersed diaspores according to Escobar et al. (2018, 2021) and Van der Pijl (1982). The inference of pollinators was made based on an extensive bibliographic survey, searching by the pollinators of Cerrado species from our study site performed by Martins (2019) and Martins et al. (2021).

Results

The list of plants included a total of 195 species belonging to 54 families and 131 genera (99 monospecific). We could not identify to the species level nine plant morphotypes. The richest families were Fabaceae (25 species), Asteraceae (16), Myrtaceae (16), Rubiaceae (11), Bignoniaceae and Malpighiaceae (10), Melastomataceae (9), Erythroxylaceae, Sapindaceae and Annonaceae (6), corresponding to 58% of the total surveyed species. Moreover, 27 of the 54 families surveyed had only one species (50%), and 12 families (22%) had only two species (Table 1). Considering only taxa identified to the genus level, there was also a predominance of arboreal and shrub life forms (34%, 64 spp each), followed by lianas (12%, 23 spp), sub-shrub (10%, 20 spp), and herbs (10%, 19 spp) (Figures 2 and 3). Within our Cerrado remnant, there were 27 species considered as Least Concern (LC), one as Near Threatened (NT) – *Bowdichia virgilioides* Kunth -, and 157 as Data Deficient (DD) (Table 1).

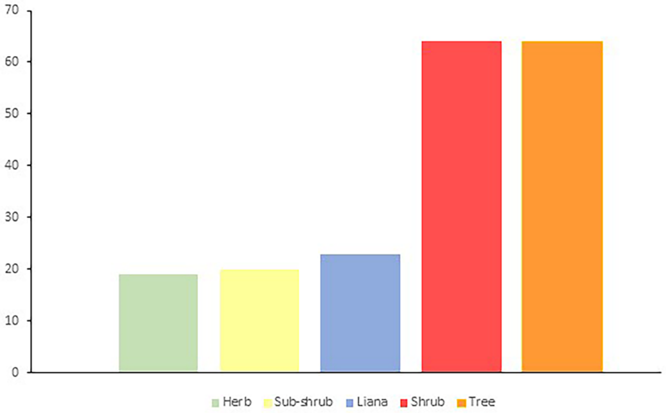

Figure 2. Number of plant species by life form surveyed in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil. Number of species = 195.

Table 1. List of plant species recorded in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil, and their respective voucher number, life form, dispersion syndrome, pollinator system, typical Cerrado species according to Durigan et al. (2004, 2012) and threat status according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and in the Official Red List of Endangered Species of the Brazilian Flora (Flora do Brasil, 2020) and classified in (EW – Extinct in the Wild, CR – Critically Endangered, EN – Endangered, VU – Vulnerable, NT – Near Threatened, DD – Data Deficient, LC – Least Concern and typical cerrado species. Dispersal syndromes: Self= self-dispersed, Wind = wind-dispersed, Animal= Animal-dispersed; Pollination: butt = butterfly; dvi = diverse insects; hum = hummingbird, sph = sphingidae; ND = not determined, Y = yes, N= no. *most frequent species of the southern cerrado based on the woody flora list provided by Bridgewater et al. (2004). ** pollinator based on plant genera.

Family	Species	Voucher HRCB	Life form	Dispersion syndrome	Pollinator system	Typical cerrado species	Threat status
Amaranthaceae	*Froelichia procera* (Seub.) Pedersen	65975	Herb	Self	wind	N	DD
	Gomphrena sp.	66008	Herb	Self	bee	Y	DD
Anacardiaceae	*Anacardium humile* A.St.-Hil.	65976	Tree	Animal	bee	Y	LC
Annonaceae	*Annona coriacea* Mart.*	Reys et al. (2013)	Shrub	Animal	beetle	Y	LC
	Duguetia furfuracea (A.St.-Hil.) Saff.*	65977	Tree	Animal	beetle	Y	DD
	Duguetia lanceolata A.St.-Hil.	65978	Tree	Animal	beetle	N	LC
	Guatteria australis A.St.-Hil.	65979	Tree	Animal	beetle	N	LC
	Xylopia aromatica (Lam.) Mart.*	65980	Tree	Animal	beetle	Y	LC

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Family	Species	Voucher HRCB	Life form	Dispersion syndrome	Pollinator system	Typical cerrado species	Threat status
Apocynaceae	*Aspidosperma tomentosum* Mart. & Zucc.*	Reys et al. (2013)	Tree	Wind	moth	Y	LC
	Ditassa sp.	65981	Liana	Wind	beetle	N	DD
	Mandevilla hirsuta (A. Rich.) K. Schum.	65983	Liana	Wind	moth**	Y	LC
	Oxypetalum appendiculatum Mart.	65982	Liana	Wind	wasp	Y	DD
	Temnadenia violacea (Vell.) Miers	65984	Liana	Wind	hum	Y	LC
Araliaceae	*Didymopanax vinosus* (Cham. & Schltdl.) Marchal	65985	Shrub	Animal	bee	Y	DD
Arecaceae	*Syagrus flexuosa* (Mart.) Becc.*	Reys et al. (2013)	Herb	Animal	bee	Y	DD
Aristolochiaceae	*Aristolochia labiata* Willd.	65987	Liana	Wind	fly	N	LC
Asteraceae	*Acanthospermum* sp.	65988	Herb	Wind	bee**	N	DD
	Asteraceae	66007	Herb	Wind	bee	N	DD
	Baccharis dracunculifolia DC.*	65989	Shrub	Wind	bee	Y	DD
	Bidens gardneri Baker	65990	Herb	Animal	butt	Y	DD
	Calea cuneifolia DC.	65991	Sub-shrub	Wind	bee	Y	DD
Asteraceae	*Chresta sphaerocephala* DC.	65993/74534	Shrub	Wind	bee	Y	LC
	Chromolaena laevigata (Lam.) R. M. King & H. Rob.	65994	Shrub	Wind	butt	N	DD
	Chromolaena maximilianii (Schrad. ex DC.) R.M.King & H.Rob.	65996	Shrub	Wind	bee	N	DD
	Eupatorium sp.	65997	Sub-shrub	Wind	bee	N	DD
	Gochnatia pulchra Cabrera*	66001	Tree	Wind	bee	Y	DD
	Heterocondylus alatus (Vell.) R. M. King & H. Rob.	65998	Shrub	Wind	bee**	N	DD
	Lepidaploa psilostachya (DC.) H. Rob.	66005	Sub-shrub	Wind	bee	N	DD
	Moquiniastrum barrosoae (Cabrera) G. Sancho	65999	Shrub	Wind	butt	N	DD
	Piptocarpha rotundifolia (Less.) Baker*	66002	Tree	Wind	bee	Y	DD
	Vernonanthura ferruginea (Less.) H. Rob.	66003	Shrub	Wind	bee	N	DD
	Vernonia sp.	66006	Sub-shrub	Wind	bee	N	DD
Bignoniaceae	*Adenocalymma axillare* (K. Schum.) L. G. Lohmann	66015	Liana	Wind	bee	Y	DD
	Amphilophium elongatum (Vahl) L. G. Lohmann	66009	Liana	Wind	bee	Y	DD
	Anemopaegma sp.	66010	Liana	Wind	bee	N	DD
	Fridericia florida (DC.) L. G. Lohmann	66018	Liana	Wind	bee	Y	DD
	Fridericia platyphylla (Cham.) L. G. Lohmann	66011	Liana	Wind	bee	Y	DD
	Fridericia samydoides (Cham.) L. G. Lohmann	66013	Liana	Wind	bee	N	DD
	Handroanthus ochraceus (Cham.) Mattos	Reys et al. (2013)	Tree	Wind	bee	Y	DD
	Jacaranda caroba (Vell.) DC.*	66014	Shrub	Wind	bee	Y	DD
	Jacaranda rufa Silva Manso	Reys et al. (2013)	Shrub	Wind	hum	Y	DD
	Pyrostegia venusta (Ker Gawl.) Miers	66017	Liana	Wind	bee	Y	DD
Bixaceae	*Cochlospermum regium* (Mart. ex Schrank) Pilg.	66019	Shrub	Wind	dvi	Y	LC
Burseraceae	*Protium heptaphyllum* (Aubl.) Marchand*	66020	Tree	Animal	dvi	Y	DD
	Protium sp.	66021	Tree	Animal	bee	N	DD
Calophyllaceae	*Kielmeyera grandiflora* (Wawra) Saddi	66023	Tree	Wind	bat	Y	DD
Caryocaraceae	*Caryocar brasiliense* Cambess.*	66024	Tree	Animal	bee	Y	LC
Celastraceae	*Peritassa campestris* (Cambess.) A. C. Sm.	66025	Shrub	Animal	bee	N	DD
	Plenckia populnea Reissek*	Reys et al. (2013)	Tree	Wind	fly	Y	DD
	Tontelea micrantha (Mart. ex Schult.) A. C. Sm.	66027	Shrub	Animal	bee	N	DD
Chrysobalanaceae	*Licania humilis* Cham. & Schltdl.	Reys et al. (2013)	Tree	Animal	bee	Y	DD
Commelinaceae	*Commelina benghalensis* L.	66028	Herb	Self	bee	N	DD
	Commelina diffusa Burm.f.	66029	Herb	Self	bee	N	DD
Connaraceae	*Connarus suberosus* Planch.*	Reys et al. (2013)	Shrub	Animal	bee	Y	DD
	Rourea induta Planch.*	66030	Shrub	Animal	bee	Y	DD
Convolvulaceae	*Ipomoea procurrens* Meisn.	66031	Liana	Self	wind	Y	DD
Cyperaceae	*Cyperus* sp.	66032	Herb	Self	bee	N	DD

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Family	Species	Voucher HRCB	Life form	Dispersion syndrome	Pollinator system	Typical cerrado species	Threat status
Dilleniaceae	*Curatella americana* L.	66033	Shrub	Animal	bee	Y	DD
	Davilla elliptica A.St.-Hil.*	66034	Shrub	Animal	sph	Y	DD
Ebenaceae	*Diospyros lasiocalyx* (Mart.) B.Walln.	66035	Tree	Animal	bee	Y	DD
Erythroxylaceae	*Erythroxylum buxus* Peyr.	Reys et al. (2013)	Shrub	Animal	bee	Y	DD
	Erythroxylum cuneifolium (Mart.) O. E. Schulz*	66037	Shrub	Animal	wasp	Y	DD
	Erythroxylum microphyllum A.St.-Hil.	66036	Shrub	Animal	bee	Y	DD
	Erythroxylum pelleterianum A.St.-Hil.	74536	Shrub	Animal	bee	Y	LC
	Erythroxylum suberosum A.St.-Hil.*	66038	Shrub	Animal	wasp	Y	DD
	Erythroxylum tortuosum Mart.*	66040	Shrub	Animal	wasp	Y	DD
Euphorbiaceae	Euphorbiaceae	66043	ND	ND		N	DD
	Manihot tripartita subsp. *humilis* (Müll.Arg.) D.J.Rogers & Appan	66041	Shrub	Animal	bee**	Y	DD
	Sapium glandulosum (L.) Morong	66042	Shrub	Animal	dvi	N	DD
Fabaceae	*Anadenanthera colubrina* (Vell.) Brenan*	66044	Tree	Wind	bee	Y	DD
	Anadenanthera peregrina var. *falcata* (Benth.) Altschul*	66045	Tree	Wind	bee	Y	DD
	Andira humilis Mart. ex Benth.	66046	Tree	Animal	bee**	Y	DD
	Bauhinia rufa (Bong.) Steud.*	66047	Shrub	Self	bat	Y	DD
	Bowdichia virgilioides Kunth*	74539	Tree	Wind	bee	Y	NT
	Chamaecrista campestris H. S. Irwin & Barneby	66050	Sub-shrub	Self	bee	N	DD
	Chamaecrista desvauxii (Collad.) Killip	66051	Sub-shrub	Self	bee	Y	DD
	Chamaecrista flexuosa (L.) Greene	66053	Sub-shrub	Self	bee	Y	DD
	Copaifera langsdorffii Desf.	74530	Tree	Animal	bee	Y	DD
	Crotalaria martiana Benth.	66055	Shrub	Self	bee**	Y	DD
	Dalbergia miscolobium Benth.*	Reys et al. (2013)	Tree	Wind	bee	Y	DD
	Dimorphandra mollis Benth.*	66056	Tree	Zoochoric	bee	Y	DD
	Fabaceae sp	66070	ND	ND		N	DD
	Leptolobium dasycarpum Vogel	Reys et al. (2013)	Shrub	Wind	bee	Y	DD
	Machaerium acutifolium Vogel*	66057	Tree	Wind	bee	Y	DD
	Machaerium brasiliense Vogel	66058	Tree	Wind	bee	Y	DD
	Mimosa debilis Humb. & Bonpl. ex Willd. var. debilis	66060	Sub-shrub	Self	bee	Y	DD
	Mimosa gracilis var. *capillipes* (Benth.) Barneby	66059	Herb	Self	bee	N	DD
	Plathymenia reticulata Benth.*	66061	Tree	Wind	bee	Y	LC
	Pterodon emarginatus Vogel	Reys et al. (2013)	Tree	Wind	bee	N	DD
	Pterodon pubescens (Benth.) Benth.*	66062	Tree	Wind	bee	Y	DD
	Senna rugosa (G. Don) H. S. Irwin & Barneby*	66063	Sub-shrub	Self	bee	Y	DD
	Stryphnodendron rotundifolium Mart.	66065	Tree	Animal	bee	Y	DD
	Stryphnodendron sp.	66067	Tree	Animal	bee	N	DD
	Stylosanthes acuminata M. B. Ferreira & Sousa Costa	66069	Sub-shrub	Animal	bee	Y	DD
Iridaceae	*Trimezia juncifolia* (Klatt) Benth. & Hook.	66071	Herb	Self	bee	Y	DD
Lacistemataceae	*Lacistema hasslerianum* Chodat	66072	Tree	Animal	wind	Y	DD
Lamiaceae	*Aegiphila verticillata* Vell.	66074	Tree	Animal	bee	Y	DD
	Hyptis campestris Harley & J.F.B. Pastore	74537	Herb	Self	bee	N	DD
Lauraceae	*Ocotea corymbosa* (Meisn.) Mez	66076	Tree	Animal	dvi	Y	DD
Lauraceae	*Ocotea pulchella* (Nees & Mart.) Mez*	66077	Tree	Animal	fly	Y	LC
Loganiaceae	*Strychnos brasiliensis* Mart.	66082	Liana	Animal	moth	Y	DD
	Strychnos pseudoquina A. St.-Hil. *	66083	Shrub	Animal	moth	Y	DD

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Family	Species	Voucher HRCB	Life form	Dispersion syndrome	Pollinator system	Typical cerrado species	Threat status
Malpighiaceae	*Banisteriopsis argyrophylla* (A. Juss.) B. Gates	66084	Sub-shrub	Wind	bee**	N	DD
	Banisteriopsis campestris (A. Juss.) Little	66085	Sub-shrub	Wind	bee	Y	DD
	Banisteriopsis stellaris (Griseb.) B. Gates	66088	Liana	Wind	bee	N	DD
	Byrsonima basiloba A. Juss.*	66090	Shrub	Animal	bee	Y	DD
	Byrsonima coccolobifolia Kunth*	Reys et al. (2013)	Shrub	Animal	bee	Y	LC
	Byrsonima crassifolia (L.) Kunth*	Reys et al. (2013)	Shrub	Animal	bee	N	DD
	Byrsonima intermedia A. Juss.*	66091	Shrub	Animal	bee	Y	DD
	Malpighiaceae	66095	ND	ND		N	DD
	Malpighiaceae	66096	ND	ND		N	DD
	Peixotoa reticulata Griseb.	66094	Liana	Wind	bee	N	DD
Malvaceae	*Eriotheca gracilipes* (K.Schum.) A. Robyns*	66097	Tree	Wind	bee	Y	DD
	Peltaea polymorpha (A.St.-Hil.) Krapov. & Crist¢bal	66098	Shrub	Self	bee	Y	DD
Melastomataceae	*Leandra solenifera* Cogn.	Reys et al. (2013)	Shrub	Animal	bee	N	DD
	Miconia albicans (Sw.) Triana*	66100	Tree	Animal	bee	Y	DD
	Miconia fallax DC.	Reys et al. (2013)	Shrub	Animal	bee	Y	DD
	Miconia ligustroides (DC.) Naudin*	66103	Shrub	Animal	bee	Y	DD
	Miconia paucidens DC.	66104	Shrub	Animal	bee	N	LC
	Miconia pepericarpa DC.	66108	Shrub	Animal	bee	Y	DD
	Miconia rubiginosa (Bonpl.) DC.*	66106	Tree	Animal	bee	Y	DD
	Miconia stenostachya DC.*	66109	Tree	Animal	bee	Y	DD
	Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	66110	Tree	Wind	bee	Y	DD
Moraceae	*Brosimum gaudichaudii* Trécul*	66111	Tree	Animal	wind	Y	DD
	Ficus citrifolia Mill.	Reys et al. (2013)	Tree	Animal	wasp	N	DD
Myristicaceae	*Virola sebifera* Aubl.*	66112	Tree	Animal	bee	Y	DD
Myrtaceae	*Blepharocalyx salicifolius* (Kunth) O.Berg*	Reys et al. (2013)	Shrub	Animal	bee	Y	LC
	Campomanesia pubescens (Mart. ex DC.) O. Berg*	66114	Shrub	Animal	bee	N	LC
	Eugenia bimarginata DC.*	66116	Tree	Animal	bee	Y	DD
	Eugenia punicifolia (Kunth) DC.	Reys et al. (2013)	Shrub	Animal	bee	Y	DD
	Eugenia pyriformis Cambess.	66120	Shrub	Animal	bee	N	DD
	Myrcia bella Cambess.	66121	Shrub	Animal	bee	Y	DD
	Myrcia guianensis (Aubl.) DC.	66122	Tree	Animal	bee	Y	LC
	Myrcia splendens (Sw.) DC.	66123	Tree	Animal	bee	Y	DD
	Myrcia tomentosa (Aubl.) DC.*	Reys et al. (2013)	Tree	Animal	bee**	Y	DD
	Myrcia venulosa DC.	66125	Tree	Animal	bee	N	LC
	Myrtaceae	66128	ND	Animal	ND	N	DD
	Myrtaceae	66129	ND	Animal	ND	N	DD
	Psidium australe Cambess.	66126	Shrub	Animal	bee**	N	DD
	Psidium grandifolium Mart. ex DC.	Reys et al. (2013)	Shrub	Animal	bee**	Y	LC
	Psidium sp.	66127	Shrub	Animal	bee**	N	DD
	Siphoneugena crassifolia (DC.) Proença & Sobral	Reys et al. (2013)	Tree	Animal	bee**	N	DD
Nyctaginaceae	*Guapira noxia* (Netto) Lundell	66130	Tree	Animal	bee**	Y	DD
	Guapira opposita (Vell.) Reitz	74533	Tree	Animal	bee**	Y	DD
Ochnaceae	*Ouratea spectabilis* (Mart.) Engl.*	66131	Tree	Animal	bee**	Y	LC
Orchidaceae	*Galeandra montana* Barb. Rodr.	66132	Herb	Wind	bee**	Y	DD
	Ionopsis utricularioides (Sx.) Lindl.	74535	Herb	Wind	bee**	N	LC
	Pelexia laminata Schltr.	66133	Herb	Wind	bee**	Y	DD

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Family	Species	Voucher HRCB	Life form	Dispersion syndrome	Pollinator system	Typical cerrado species	Threat status
Oxalidaceae	*Oxalis hirsutissima* Mart. & Zucc.	66134	Sub-shrub	Self	bee**	Y	DD
Passifloraceae	*Passiflora foetida* L.	66135	Liana	Animal	bee**	Y	DD
Peraceae	*Pera glabrata* (Schott) Poepp. ex Baill.*	66136	Tree	Animal	bee**	Y	DD
Poaceae	*Ichnanthus inconstans* (Trin. ex Nees) D”ll	66138	Herb	Animal	bee**	N	DD
	Urochloa brizantha (Hochst. ex A. Rich.) R. D. Webster	66137	Herb	Animal	bee**	N	DD
Polygalaceae	*Bredemeyera floribunda* Willd.*	Reys et al. (2013)	Shrub	Animal	bee**	Y	DD
	Securidaca rivinifolia A.St.-Hil. & Moq.	66139	Sub-shrub	Wind	bee**	Y	DD
Primulaceae	*Myrsine guianensis* (Aubl.) Kuntze	66141	Tree	Animal	bee**	Y	DD
	Myrsine umbellata Mart.	Reys et al. (2013)	Tree	Animal	bee**	Y	DD
Proteaceae	*Roupala montana* Aubl.	Reys et al. (2013)	Shrub	Wind	bee**	Y	DD
Rubiaceae	*Amaioua guianensis* Aubl.	66143	Tree	Animal	bee**	N	DD
	Coccocypselum lanceolatum (Ruiz & Pav.) Pers.	66145	Herb	Animal	bee**	Y	DD
	Cordiera sessilis (Vell.) Kuntze	66146	Shrub	Animal	bee**	N	DD
	Declieuxia fruticosa (Willd. Ex Roem. & Schult.) Kuntze	66149	Shrub	Animal	bee**	Y	LC
	Palicourea sp.	66151	Sub-shrub	Animal	bee**	N	DD
	Palicourea r¡gida Kunth	66150	Sub-shrub	Animal	bee**	Y	DD
	Palicourea racemosa (Aubl.) Borhidi	66156	Sub-shrub	Animal	bee**	N	DD
	Psychotria hoffmannseggiana (Willd. ex Schult.) Mll. Arg.	66152	Shrub	Animal	bee**	N	DD
	Psychotria trichophora Mll. Arg.	66157	Shrub	Animal	bee**	N	DD
	Rubiaceae	66160	ND	ND		N	DD
	Tocoyena formosa (Cham. & Schltdl.) K. Schum.*	66158	Shrub	Animal	sph	Y	DD
Salicaceae	*Casearia* Jacq.	66162	Shrub	Animal	fly**	N	DD
	Casearia sylvestris Sw.*	66161	Shrub	Animal	fly	N	DD
Sapindaceae	*Serjania lethalis* A. St.-Hil.	66165	Liana	Wind	bee	N	DD
	Serjania meridionalis Cambess.	66167	Liana	Wind	bee	N	DD
	Serjania regnellii Schltdl.	66168	Liana	Wind	bee**	N	DD
	Serjania sp. 1	66163	Liana	Wind	bee	N	DD
	Serjania sp. 2	66164	Liana	Wind	bee	N	DD
	Talisia angustifolia Radlk.	74532	Shrub	Animal	dvi**	N	LC
Sapotaceae	*Pouteria ramiflora* (Mart.) Radlk.*	66173/74538	Tree	Animal	butt	Y	DD
	Pouteria torta (Mart.) Radlk.*	66177	Tree	Animal	butt	Y	LC
Siparunaceae	*Siparuna guianensis* Aubl.*	66179/74531	Tree	Animal	fly	Y	DD
Smilacaceae	*Smilax brasiliensis* Spreng.	66182	Shrub	Animal	fly	N	DD
Solanaceae	*Cestrum* sp.	66183	Shrub	Animal	sph	N	DD
	Solanum lycocarpum A.St.-Hil.*	66184	Shrub	Animal	bee	Y	DD
	Solanum paniculatum L.	66185	Shrub	Animal	bee	Y	DD
Styracaceae	*Styrax ferrugineus* Nees & Mart.*	66186	Shrub	Animal	bee	Y	DD
Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	66140	Herb	Self	bee	N	DD
Verbenaceae	*Lippia lupulina* Cham.	66188	Sub-shrub	Self	butt	Y	DD
	Lippia origanoides Kunth	66189	Sub-shrub	Self	butt	N	DD
	Lippia sp.	66187	Sub-shrub	Self	butt**	N	DD
Vitaceae	*Cissus erosa* Rich.	66191	Liana	Animal	fly	Y	DD
Vochysiaceae	*Qualea dichotoma* (Mart.) Warm.*	Reys et al. (2013)	Tree	Wind	bee	N	DD
	Qualea grandiflora Mart.*	66192	Tree	Wind	sph	Y	DD
	Qualea multiflora Mart. *	66194	Tree	Wind	bee	Y	DD
	Vochysia cinnamomea Pohl*	66195	Tree	Wind	bee	Y	DD
	Vochysia tucanorum Mart. *	66196	Tree	Wind	bee	Y	DD

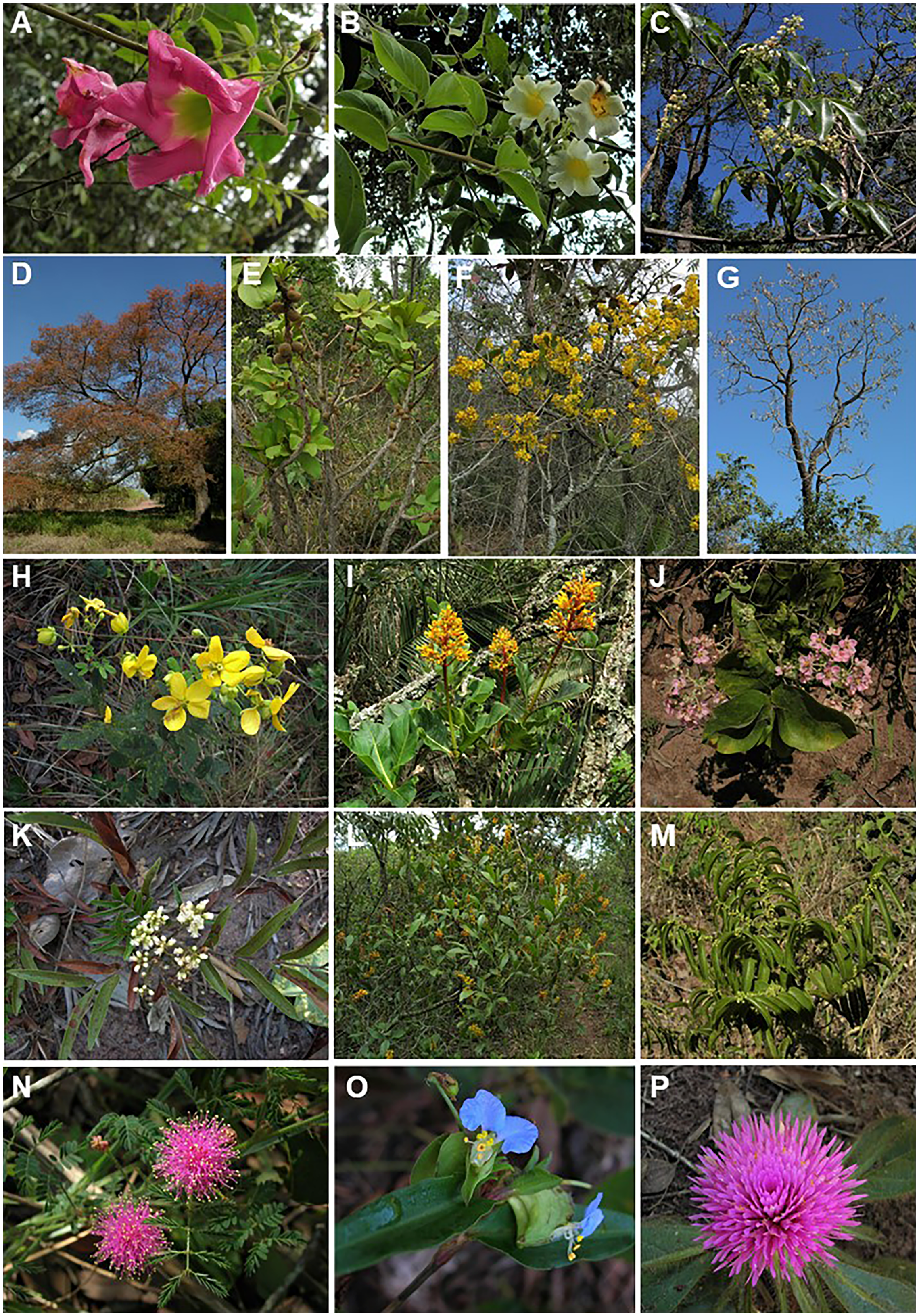

Figure 3. Diversity of plant species and life-forms in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil. Liana: (A) *Temnadenia violacea* (Vell.) Miers - hummingbird pollination, (B) *Amphilophium elongatum* (Vahl) L.G.Lohmann - bee pollination and (C) *Serjania lethalis* A.St.-Hil. - bee pollination; Trees: (D) *Copaifera langsdorffii* Desf. - bee pollination, (E) *Pouteria torta* (Mart.) Radlk. - diverse insects' pollination, (F) *Ouratea spectabilis* (Mart.) Engl. - bee pollination and (G) *Anadenanthera peregrina* (L.) Speg.- bee pollination; Sub-shrub: (H) *Senna rugosa* (G.Don) H.S.Irwin & Barneby - bee pollination, (I) *Palicourea rigida* Kunth - hummingbird pollination and (J) *Banisteriopsis campestris* (A. Juss.) Little - bee pollination; Shrub: (K) *Talisia angustifolia* Radlk. - diverse insects' pollination, (L) *Byrsonima intermedia* A. Juss. - bee pollination and (M) *Casearia sylvestris* Sw. - fly pollination; Herb: (N) *Mimosa gracilis* Benth. - bee pollination., (O) *Commelina erecta* L. - bee pollination and (P) *Gomphrena macrocephala* A.St.-Hil. – bee pollination. (Photos MGG Camargo).

Besides that, the typical Cerrado species according to Durigan et al. (2004, 2012) found in our study area are 62.7% of the species (123) surveyed at the remnant.

Bee pollination were the dominant system, corresponding to 133 plant species (68.2%), followed by diverse insects (6.2%, 12 spp), butterflies (3.1%, 6 spp), flies (3.6%, 7 spp), beetles (3.1%, 6 spp), moths (3.6%, 7 spp), wind (1.5%, 3 spp), wasps (2.6%, 5 spp), hummingbirds (1.5%, 3 spp), sphingid moths (2.1%, 4 spp) and bats (1%, 2 spp) (Figures 4 and 5).

As for the dispersal systems, the animal-dispersed diaspores predominated (55.4%, 108 spp), followed by wind (33.3%, 65 spp) and self-dispersion (11.2%, 22 spp). Animal seed dispersal was predominant in trees and shrubs (Figure 6) and presented birds as the main dispersion agents (LPC Morellato and collab. Unpublished information).

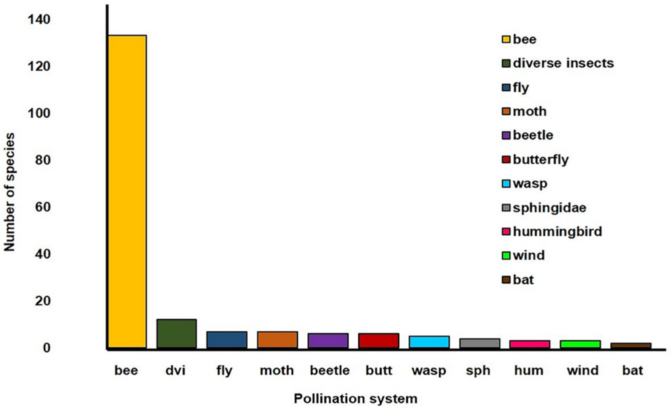

Figure 4. Number of species by pollination system recorded in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil. Pollination systems: bee, diverse insects (dvi), fly, moth, beetle, butterfly, wasp, sphingidae (sph), hummingbird (hum), wind and bat

Figure 5. Examples of species by seed dispersal syndromes collected in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil. Wind-dispersed: (A) *Piptocarpha rotundifolia* (Less.) Baker, (B) *Banisteriopsis stellaris* (Griseb.) B. Gates, (C) *Eriotheca gracilipes* (K.Schum.) A. Robyns; Self-dispersed: (D) *Bauhinia rufa* (Bong.) Steud., (E) *Anadenanthera peregrina* var. *falcata* (Benth.) Altschul - with unripe fruit and a detail of the ripe fruits, (F) *Lippia origanoides* Kunth – with unripe fruit and a detail of the ripe fruits; Animal-dispersed: (G) *Myrcia guianensis* (Aubl.) DC., (H) *Miconia rubiginosa* (Bonpl.) DC., (I) *Erythroxylum pelleterianum* A.St.-Hil. (Photos MGG de Camargo).

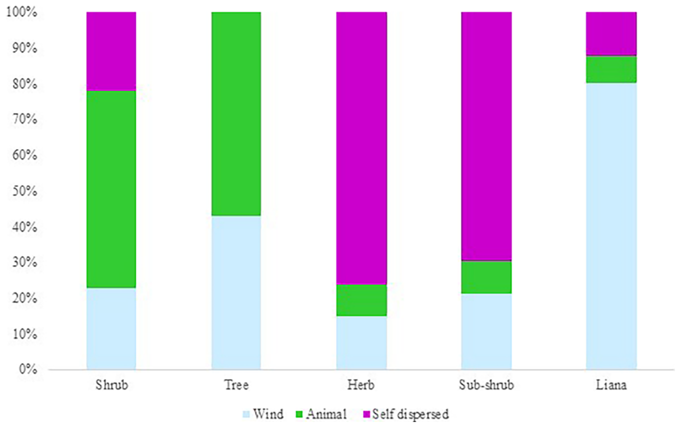

Figure 6. Number of plant species by seed dispersal syndromes according to the life forms collected in the cerrado *sensu stricto*, Itirapina, São Paulo State, Southeastern Brazil

Discussion

We found a species richness (195) similar to other studied remnants of cerrado *sensu stricto* in São Paulo state, which used comparable sampling effort and inclusion criteria. For instance, 254 species were listed in Assis, São Paulo, by Durigan et al. (1999), 141 in Santa Rita do Passa Quatro by Waiser & Godoy (2001)*,* and 177 species in Botucatu by Ishara et al. (2008). However, the taxonomy used in those papers did not follow the APG III classification (2009), limiting a comprehensive floristic comparison. The proportion of species distribution by family found in our cerrado checklist was very similar to the pattern found in previous studies carried out in Brazilian Cerrado areas (Ratter et al. 2003, Gottsberger & Silberbauer-Gottsberger 2006, Mantovani & Martins 1993, Felfili et al. 2002, Batalha & Martins 2001, Weiser & Godoy 2001, Durigan et al. 2001, Fidelis & Godoy 2003, Ishara et al. 2008, Carvalho et al. 2010, Reys et al. 2013), with Fabaceae, Malpighiaceae and Rubiaceae always among the most representative families. Compared to Reys et al. (2013) survey, the present species list has increased to 40% the number of species, indicating the need for long-term, extensive surveys.

We surveyed 59 out of the 100 most frequent woody species listed to the Cerrado in Southern floristic province in Brazil (include São Paulo, Paraná and Minas Gerais states) based on Bridgewater et al. (2004). Among the species described on the Bridgewater et al. (2004) list as the most common species in number of individuals we found in our site: *Qualea grandiflora* Mart., *Byrsonima coccolobifolia* Kunth, *Piptocarpha rotundifolia* (Less.) Baker, *Erythroxylum suberosum* A.St.-Hil., *Caryocar brasiliense* Cambess., *Xylopia aromatica* (Lam.) Mart., *Byrsonima intermedia* A. Juss., *Casearia sylvestris* Sw., *Annona coriacea* Mart., *Ocotea pulchella* (Nees & Mart.) Mez and *Qualea multiflora* Mart. It is important to highlight that most of these plants have been studied due to therapeutic properties as a result of their chemical composition. For example, *Qualea grandiflora* presents bioactivity against *Plasmodium falciparum* (Cordeiro et al. 2017). Flavonoids isolated from *Casearia sylvestris* and *Byrsonima coccolobifolia* have been described as possible leishmanicidal (Antinarelli et al. 2015, Souza et al. 2014). Antioxidant, anxiolytic, antiulcer, insecticide, and antiparasitic properties of *Annona coriacea* – a species which presents a diversity of secondary metabolites may be promising for pharmacological use (Rocha et al. 2020) – have begun to be studied. *Erythroxylum suberosum* has been reported to have antifungal and antibacterial activities (Violante et al. 2012), as well. These results reinforce the assumption that the high biodiversity of the Cerrado found even in small fragments like our study site, can be a source of new compounds with possible applications in therapeutic resources and further solidify the argument that the studied area must be preserved.

The only species belonging to a Near Threatened (NT) class found in our study site was the *Bowdichia virgilioides* Kunth (Table 1) a species distributed across the Amazon Rainforest, Caatinga, Central Brazilian Savanna (Cerrado), Atlantic Rainforest and Pantanal domains (Flora do Brasil 2020). Circumstances such as deforestation and over-extraction (due to the use in construction and furniture), —associated with biological characteristics such as low density and dormancy of its seeds — contributes to its endangered status (Rosa-Magri & Meneghi 2014). Extinction of plant species leads to a loss of many ecological functions, community stability, and resilience, aside from secondary extinctions as a function of loss of key interactions (Rossati et al. 2015).

Our study highlights the elevated number of plant species surveyed classified as Data Deficient (DD) (Table 1), showing the relatively low amount of available data about this domain species and the necessity of many complementary studies. However, some studies have shown that the data-deficient species described in many inventories are of extreme conservation concern, usually including species with a great risk for extinction (Bland et al. 2015), or naturally rare (Corlett 2016, Roberts et al. 2016). In addition, the Cerrado of South America has the highest number of rare species showing the urgent need to include them in conservation planning (Maciel & Martins 2021). Due to the great heterogeneity within the Cerrado domain and the alarming rate of destruction in recent years (MapBiomas, Collection 5.0), it is imperative that we conduct additional studies to provide more floristic and functional data for the remaining remnants. A more comprehensive floristic survey will improve the knowledge, and fill gaps in biodiversity data (Roberts et al. 2016) on this domain, allowing us to propose better management strategies and contribute to improve models of restoration for the Cerrado (Pelizzaro et al. 2017, Buisson et al. 2017, 2018). The proportions of plants in each life form category were consistent with most of the previous studies that describe cerrado *sensu stricto* as a vegetation dominated by trees and shrubs (50% of wood cover) (Coutinho 2006, Silva et al. 2015). However, in an inventory carried out in Pratania, SP, Carvalho et al. (2010) listed 37.5% shrubs, followed by herbs (27.5%), trees (23%), and lianas (12%). These conflicting results are possibly due to the level of preservation of the studied area and frequency of fires (Durigan et al. 2007).

A recent inventory has mapped only 1% of remnant areas of Cerrado vegetation protected for the São Paulo State (Instituto Florestal 2020). This is alarming data, highlighting the need for more protective measures of conservation from what has left of this important vegetation domain. In terms of importance, even for a small patch of vegetation, our study has reported the occurrence of several endemic cerrado species, as: *Caryocar brasiliense* Cambess., *Anacardium humile* A.St.-Hil., *Aspidosperma tomentosum* Mart. & Zucc., *Licania humilis* Cham. & Schltdl., *Erythroxylum tortuosum* Mart., *Dalbergia miscolobium* Benth., *Dimorphandra mollis* Benth., *Ouratea spectabilis* (Mart.) Engl. e *Stryphnodendron rotundifolium* Mart. Plant species of a wider distribution, such as: *Duguetia lanceolata* A.St.-Hil., *Guatteria australis* A.St.-Hil., *Peritassa campestris* (Cambess.) A. C. Sm., *Sapium glandulosum* (L.) Morong, *Pterodon emarginatus*

Vogel e *Campomanesia pubescens* (Mart. ex DC.) O. Berg., found in this study area, can also be observed in other vegetation physiognomies (e.g.: semideciduous forest and Rain Forest) and vegetation domains (e.g.: Atlantic rainforest and Pantanal) (Flora do Brasil 2020), since our cerrado site belongs to the ecotone region of Cerrado and Atlantic Rainforest.

Our cerrado *sensu stricto* species were pollinated mainly by bees, with more than a half of species presenting bee-pollinated flowers (Figure 4), as expected for Cerrado and other tropical vegetation systems (Gottsberger & Silberbauer-Gottsberger 2006, Monteiro et al. 2021, Genini et al. 2021) and stressing the relevance of preserving Cerrado remnants for this key ecosystem service. For the same community, Martins et al. (2021) found that plant species with different flower colors presented distinct flowering peaks over the year but maintained color diversity over time. For example, while white flowers peaked in the transition between dry and wet season, matching with the community flowering peak, yellow flowers were distributed all year long, being an important resource during the dry season, when a reduced number of species is flowering (Martins et al. 2021). The observed flowering pattern provide functional diversity over time, contributing to the presence of different groups of pollinators such as bees, small insects, flies and hummingbirds, and nocturnal pollinators such as Sphingidae moths and bats (Gottsberger & Silberbauer-Gottsberger 2006, Amorim et al. 2009, Martins et al. 2021).

The observed predominance of seed dispersal by animals is expected for woody-dominated Cerrado phytophysiognomies (Weiser and Godoy 2001, Gottsberger & Silberbauer-Gottsberger 2006, Gottsberger & Silberbauer-Gottsberger 2018) and previously indicated for our study area (Camargo et al. 2013, Escobar et al. 2018). Wind-dispersed species are the second most important seed dispersal system, followed by self- dispersed seeds. The fruiting pattern of our community is seasonal according to the dispersal system (Camargo et al. 2013, Escobar et al. 2018). Animal-dispersed fruits are produced all over the year, but mainly during the wet season (Camargo et al. 2013, Escobar et al. 2018). Some animal-dispersed species such as *Miconia rubiginosa* (Bonpl.) DC., *Pouteria torta* (Mart.) Radlk., *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. and *Xylopia aromatica* (Lam.) Mart. produce fruits even in the dry season and are important to guarantee resources for the frugivores in the area (Escobar et al. 2018). Fruiting peaks of self- and wind-dispersed species are observed at the dry season, when the wind dispersal is more efficient (Camargo et al. 2013, Escobar et al. 2018). The proportion of seed dispersal function groups and fruiting time has been recently related to germination strategies (Escobar et al. 2018, 2021), a matter of key relevance for restoration and that needs further investigation for Cerrado species.

The cerrado remnant studied can be considered a conservation priority due to the high diversity of data-deficient species and the importance for ecosystem services. Our checklist pointed out a high diversity of data-deficient species, a category considered equivalent to threatened by extinction by some authors (Bland et al. 2015, Corlett 2016, Roberts et al. 2016). Consequently, our study area may represent a conservation priority based on the degree of knowledge gap and extinction risk of some species, associated with high diversity and potential ecosystem services provided. Our survey also indicated that even a small remnant can congregate a richest collection of species, plant functional types and life forms representatives of the Cerrado and concentrate an enormous value for biodiversity conservation, ecosystem services and restoration, holding several culturally important species (Pellizaro et al. 2017, Lemes et al. 2020).

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Author Contributions

RB, MTGG, MGGC and LPCM designed the study; all authors collected data in the field and helped in data compilation. MTGG curated the data, LPCM resource acquisition and management, RB and MTGG wrote the manuscript. All authors read the manuscript and contributed with suggestions.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Article

Antennal sensilla in *Cyclocephala literata* Burmeister, 1847 (Coleoptera: Scarabaeidae: Dynastinae)

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Abstract: Adults of the beetle *Cyclocephala literata* Burmeister, 1847 are important pollinators to some Magnoliaceae. Is known that insects could find host plants by detecting volatiles through antennal sensilla. *Cyclocephala* has its three distal antennomeres lamellate, and the surface of each lamella has sensilla trichodea, chaetica, placodea, coeloconica, basiconica and ampullacea. Three kinds of sensilla placodea were found (type I, II and III), and two kinds of sensilla coeloconica were observed (type I and II). Females have on average 10,776 sensilla, of which 10,214 are sensilla placodea, 536 are sensilla coeloconica, and 26 are sensilla basiconica. Males have on average 10,386 sensilla, of which 9,873 are sensilla placodea, 464 are sensilla coeloconica, and 49 are sensilla basiconica. Males and females have similar quantities of sensilla, and sensilla placodea are predominant. The differences observed in the number of sensilla of males and females were found in other beetles and were attributed to the detection of cospecific sexual pheromones by one of the sexes, or to the detection of plant volatiles. The antennal sensilla of *C. literata* is described and quantified in present study, and some perspectives about the differences kind of chemical communication, pollination, and antennae dimorphism is discussed.

Keywords: *chemical receptors; Cyclocephalini; masked chafer; morphology; ultrastructure.*

Sensilas antenais em *Cyclocephala literata* Burmeister, 1847 (Coleoptera: Scarabaeidae: Dynastinae)

Resumo: Adultos do besouro *Cyclocephala literata* Burmeister, 1847 desempenham importantes funções de polinização em plantas da família Magnoliaceae. É conhecido que muitos insetos encontram plantas hospedeiras pela detecção de voláteis pelas sensilas antenais. *Cyclocephala* possui os três antenômeros distais lamelados e na superfície de cada lamela possuem sensila trichodea, caética, placódea, coelocônica, basicônica e ampulacea. Foram encontrados três tipos de sensilas placódeas (tipo I, II e III), e dois tipos de sensilas coelocônicas (tipo I e II). Fêmeas apresentam em média 10.776 sensilas, das quais 10.214 são sensilas placódeas, 536 são sensilas coelocônicas e 26 são sensilas basicônicas. Os machos apresentaram em média 10.386 sensilas, das quais 9.873 são sensilas placódeas, 464 são sensilas coelocônicas e 49 são sensilas basicônicas. Machos e fêmeas apresentam quantidades semelhantes de sensilas e as sensilas placódeas são predominantes. As diferenças encontradas nas quantidades de sensilas em machos e fêmeas também foram encontradas em outros besouros e foram atribuídas a detecção de feromônios coespecíficos por um dos sexos, ou para detecção de voláteis de plantas. A sensila antenal de *C. literata* é descrita e quantificada no presente estudo, e algumas perspectivas sobre as diferenças entre os tipos de comunicação química, polinização e dimorfismo antenal é discutido.

Palavras-chave: *receptores químicos; Cyclocephalini; escaravelho; morfologia; ultraestrutura.*

Introduction

Scarab beetles (Coleoptera: Scarabaeidae) are a diverse group with a wide range of adult feeding habits, including phytophagous species that consume leaves, flowers, and fruits of diverse plant species; some species cause damage to cultivated plants (Solís 2004, Maia & Schlindwein 2006, Shaughney & Ratcliffe 2015, Rodrigues et al. 2016, Ferreira et al. 2018). Moreover, larvae of some phytophagous scarabs feed on roots and also may cause economic damage to crops (Santos & Ávila 2009, Cherman et al. 2011, Coutinho et al. 2011). The antenna of adult scarab beetles has several minute sensorial structures that detect plant volatiles, gases, and pheromones (Kim & Leal 2000, Larsson et al. 2001). These sensilla are associated with beetle orientation, resource location, aggregation behavior, and mating (Schneider 1964, Leal & Mochizuki 1993, Larsson et al. 2001, Romero-López 2016).

The phytophagous scarab beetles are also known as Pleurosticti (basal spiracles in the connective membrane, partly in the urosternites, the last pair not being covered by the elytra, according to Lima (1953)), a group that includes speciose scarabaeoid subfamilies, such as Cetoniinae, Dynastinae, Melolonthinae, Rutelinae, and other smaller taxa (Morón 2004). Pleurosticti is recovered as monophyletic in cladistic analyses (Browne & Scholtz 1998, Hunt et al. 2007, Ahrens & Vogler 2008, 2011, Bocak et al. 2014, Cherman & Morón 2014, Mckenna et. al. 2015, Šípek et al. 2016).

Within this large group, some studies found evidence of the volatiles detection by the antennal sensilla, helping the insect to find potential mating partners, host plant, or other specimens to form an aggregation (Kim & Leal 2000, Larsson et al. 2001, Rodrigues et al. 2014). Regarding of phytophagous scarab beetles, it is seemed that both sexual pheromone and plant volatiles are used to guide beetles to sites (the host plant) were mating and feeding occurrences (Gottsberger 1989, Maia et al. 2013, Moore & Jameson 2013), such as in some Rutelinae (e.g., Hansson et al. 1999, to *Phyllopertha diversa* Waterhouse, 1875; Larsson et al. 2001, to *Anomala cuprea* (Hope, 1839)), and in Melolonthinae (e.g., Sun et al. 2014, to *Holotrichia oblita* (Faldermann, 1835); Ruther et al. 2000, to *Melolontha hippocastani* Fabricius, 1801).

The importance of plant volatiles to scarab beetles may elucidate why the host plant flowering and beetle swarming are concomitant processes (e.g., Rodrigues et al. 2014, to the ruteline *Anomala testaceipennis* Blanchard, 1856; Rodrigues et al. 2017, to the melolonthine *Liogenys bidenticeps* Moser, 1919). Sexual pheromones and plant volatiles are also used by Dynastinae beetles, and plant volatiles are especially important to pollinators as some species of the tribe Cyclocephalini (see Schiestl & Dötterl 2012, Vuts et al. 2014).

The genus *Cyclocephala* Dejean, 1821 (Dynastinae: Cyclocephalini) is a group of New World phytophagous scarabs, of which 123 species are known to occur in Brazil (Grossi & Vaz-de-Mello 2019). Adults sometimes swarm on plant and consume leaves and flower parts (Oliveira & Ávila 2011, Moore & Jameson 2013, Dias & Rodrigues 2018), and they use the plant as a mating site (Gottsberger 1989, Munin et al. 2008, Maia et al. 2013, Costa et al. 2017). Within the genus, the antennal sensilla is known to and undetermined species (Bohacz et al. 2020) and to *Cyclocephala putrida* Burmeister, 1847 (Saldanha et al. 2020).

Within the genus, *Cyclocephala literata* Burmeister, 1847 was registered to São Paulo, Rio de Janeiro, and Santa Catarina States, in the Atlantic Forest and Cerrado (Brazilian Savanna), mainly in gallery forests (Endrödi 1985; Gottsberger 1986). This specie is the only known pollinator of *Magnolia ovata* (A.St.-Hil.) Spreng. and an occasional flower visitor to *Annona crassiflora* Mart. (Magnoliaceae; Gottsberger 1986, Gottsberger et al. 2012). Gibbs et al. (1977) provisionally identified the pollinator of *M. ovata* as the cyclocephaline beetle *Augoderia nitida* Burmeister, 1847 or *Cyclocephala* aff. *emarginata* Endrödi, 1966 but Seymour et al. (2010) named the species as *C. literata* based in personal communication with two dynastine specialists. *Magnolia ovata* (“baguaçu”) is an important plant to Brazilian forest ecology specially in gallery forests (Cazetta et al. 2002), a medicinal plant (Stefanello et al. 2005, Kassuya et al. 2009), used in several human activities (to building constructions, craftsmanship, and other usages) and is an endangered species (Carvalho 2003).

Gottsberger et al. (2012) described the attraction of *C. literata* to volatiles produced by *M. ovata*. To Seymour et al. (2010) the floral thermogenesis could increase the volatilization of plant odorants and the heat itself is an attractant (energy reward). Regarding the above-mentioned biological importance of the *Cyclocephala literata*, the present work aims to describe the antennal sensilla of the beetle.

Material and Methods

This study was conducted at the Universidade Estadual de Mato Grosso do Sul (UEMS), Campus of Cassilândia, MS, Brazil. Adults of *C. literata* were obtained by using two methods: 1) collecting and rearing larvae and 2) capturing adults attracted to a light trap model “Luiz de Queiroz” (Silveira Neto & Silveira 1969). Larvae were collected from decomposing organic matter in the soil from January to December 2018. A total of 161 larvae was collected and reared in laboratory. Each larva was kept separately in a plastic container (500 mL) filled with the original substrate (2/3 of the container volume). A total of 35 adults were obtained, 18 males and 17 females. Furthermore, 40 adults (15 males and 25 females) were collected using a light trap in a pasture area dominated by *Urochloa decumbens* (Stapf) Webster (Poaceae).

Males and females were sexed by the dimorphism of the protarsi (Figure 1): males have tarsomere V enlarged and claws strongly curded regarding females. The specimens were preserved in 70% ethanol and are deposited in the UEMS entomological collection, campus Cassilândia. Adult reared voucher specimens were sent to Prof. Dr. Paschoal Coelho Grossi (Universidade Federal Rural do Pernambuco, Recife, Brazil) for species identification. The antennae of 10 males and 10 females were detached from the head and prepared according to the procedures described by Tanaka et al. (2006). Each sample (lamella) was successively dehydrated in 80% ethanol and 90% ethanol for 15 minutes each and 100% ethanol for 20 minutes. The samples were dried by CO_2 critical point drying with a Leica® CPD300 dryer before imaging was taken with a Zeiss® EVO LS15 scanning electron microscope (SEM) at the Departamento de Física e Química da Universidade Estadual Paulista, campus Ilha Solteira, state of São Paulo, Brazil. Were taken images of the external surface of lamellae when it is closed and also the inner surface contact between lamellae. Images were obtained with magnifications of 100, 20 and 10 µm. Sensilla terminology follows Keil (1999). The images obtained in SEM were subjected to image enhancement filters available in the software Image-Pro Plus 6.0. The sensillae were quantified in the images obtained by SEM.

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Figure 1. *Cyclocephala literata* Burmeister, 1847. A) male B) female.

Results

The outer surface of the lamellae of *C. literata* have several sensilla chaetica and sensilla trichodea (Figure 2A, C, D, E and F, Figure 3A, C and E). Both sensilla are hair-like, but sensilla chaetica are short and grouped on inner surface of proximal lamella and sensilla trichodea are long. The inner surface of proximal and distal lamella, and both sides of medial lamella have sensilla placodea, sensilla ampullacea (= pores) (Figure 3F), sensilla basiconica, sensilla coeloconica, and some fovea that enclose sensilla placodea I and sensilla basiconica (Figure 2−3).

Three types of sensilla placodea are identified (Figure 3B and F). Type I (Meinecke 1975: G2; Bohacz et al. 2020: placodea F) is a plate surrounded by a furrow, the surface is irregularly reticulate, and mean diameter is 7.67 µm (5.51−9.28 µm). Type II (Meinecke 1975: G1; Bohacz et al. 2020: placodea A) is similar to type I but has a smooth surface and mean diameter of 12.34 µm (5.71−17.85 µm). Type III (Meinecke 1975: J4; Bohacz et al. 2020: placodea A) is almost smooth, the peripheral furrow (or ditch) is absent, and mean diameter is 11.51 µm (6.89−15.17 µm).

The sensilla coeloconica are divided into type I (with pointed apex; Meinecke 1975: L1) and II (with blunt apex; Meinecke 1975: L2). Both sensilla are sparsely distributed (Figure 3F). Sensilla basiconica (Meinecke 1975: L4) resemble minute setae enclosed in a depressed pit, with the apex projected outside the pit. They are sparse (Figure 3F), mainly grouped in foveae on the medial lamella, and present on distal margins of lamellae (Figure 3B and D). The sensilla basiconica present in foveae are bigger than those placed in surface. The sensilla ampullacea are noted as pores and are sparsely distributed on lamellae surface.

The inner surface of proximal and distal lamella and both surfaces of medial lamella have two well defined area (Figure 2B), the posterior area mainly formed by sensilla placodea type III, and the anterior area with heterogeneously distributed sensilla basiconica, sensilla coeloconica (type I and II), and sensilla placodea (type I and II).

Females have on average 10,776 sensilla on all lamellae, of which 10,214 (94.8%) are sensilla placodea, 536 (5.0%) are sensilla coeloconica, and 26 (0.2%) are sensilla basiconica (Table 1). Males have on average 10,386 sensilla in lamellae, of which 9,873 (95.0%) are sensilla placodea, 464 (4.5%) are sensilla coeloconica, and 49 (0.2%) are sensilla basiconica (Table 1). There is a slightly difference on the number of sensilla between sexes, but the dimorphism is inconspicuous.

Discussion

Sensilla trichodea are found on the outer side of the proximal and distal lamellae and on the edges of the medial lamella, whereas sensilla chaetica are mainly distributed in a brush-like structure in outer (proximal) side of proximal lamella. A similar distribution of these sensilla is found in *Cyclocephala putrida* Burmeister, 1847 (Saldanha et al. 2020). An undetermined species of *Cyclocephala*

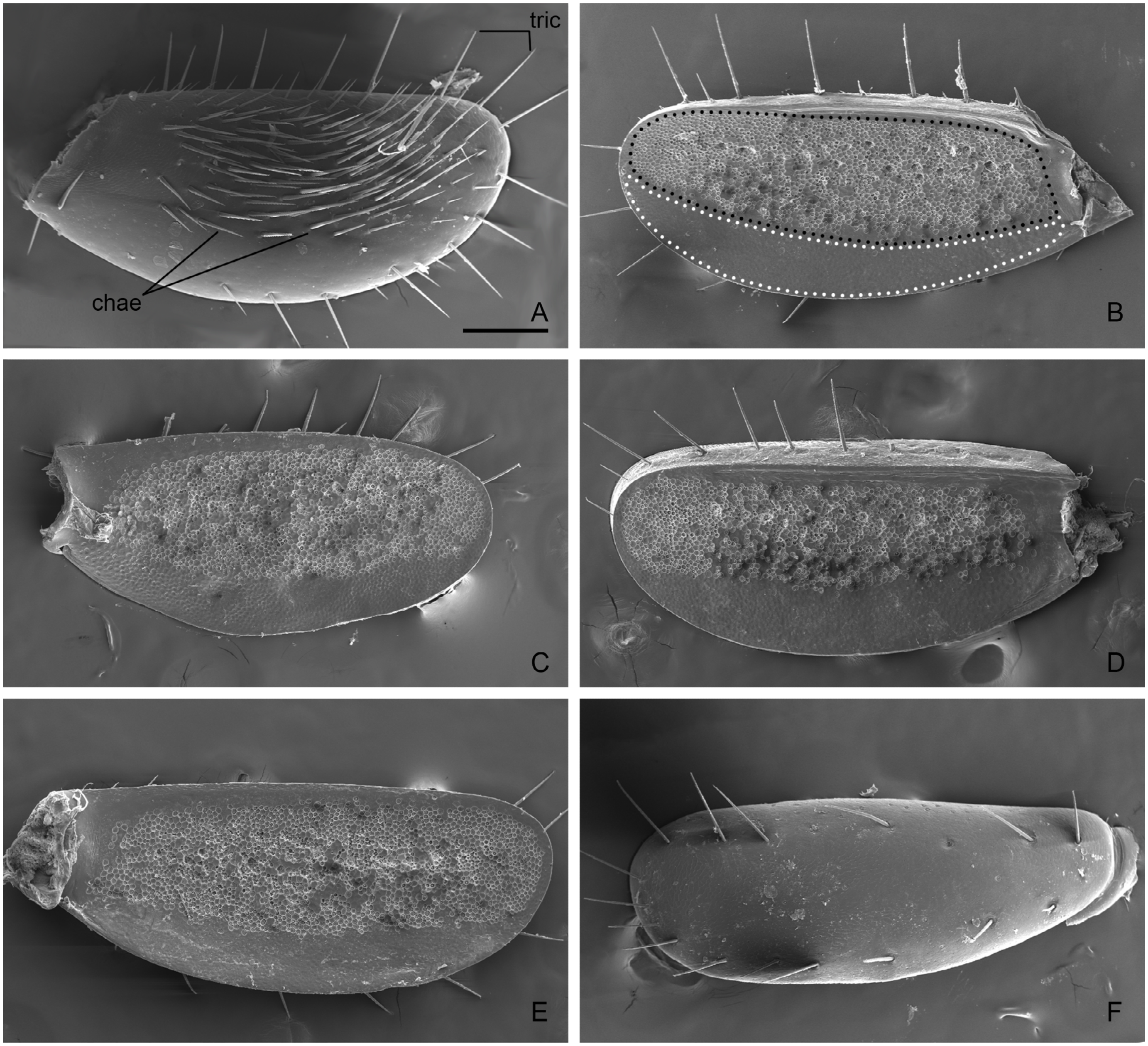

Figure 2. *Cyclocephala literata* Burmeister, 1847; antennal lamella of female. A-B) proximal lamella (outer and inner side, respectively) C-D) medial lamella (inner and outer side, respectively). E-F) proximal lamella (inner and outer side, respectively). Anterior area (black dotted line) with sensilla placodea type I and II, sensilla coeloconica type I and II, and sensilla basiconica. Posterior area (white dotted line) with type I homogeneously distributed. Tric = sensilla trichodea; Chae = sensilla chaetica. Scale = 200 µm.

studied by Bohacz et al. (2020) was described with a "brush-like sensilla trichodea". Hair-like sensilla grouped in a brush-like structure (or "field of setae") occur in Dynastinae (Bohacz et al. 2020) and at least in some Cetoniinae (Bohacz et al. (2020) to *Valgus hemipterus* (Linnaeus, 1758); Costa et al. (2021) to two species of *Hoplopyga*). Otherwise, in other phytophagous scarab as *Anomala inconstans* Burmeister, 1844 (Rutelinae) the proximal lamella have not a brush-like structure and sensilla chaetica are sparce on the outer side of proximal lamella (Rodrigues et al. 2019). Hair-like sensilla were identified as mechanoreceptors (Romero-López et al. 2004, 2010, Mutis et al. 2014), but sensilla trichodea were also identified as gustative sensilla (contact chemoreceptor; Keil 1999). The function of the brush-like structure is still unknown.

Two types of sensilla coeloconica occur on inner side of antennal club of *C. literata*, and are sparsely distributed over inner side of lamellae (5.0% in females and 4.5% in males). The sparce sensilla coeloconica are common to phytophagous scarab, but they are absent in the lamellae of some melolonthine as Ablaberini, Heteronicini, Liparetrini, Maechidiini, Phyllotocidiini, Sericini, and Sericoidini (Bohacz et al. 2020). Interestingly, these tribes are sometimes recovered as sisters groups to all other phytophagous scarabs (Ahrens & Vogler 2011). This fact must by checked in more species but it

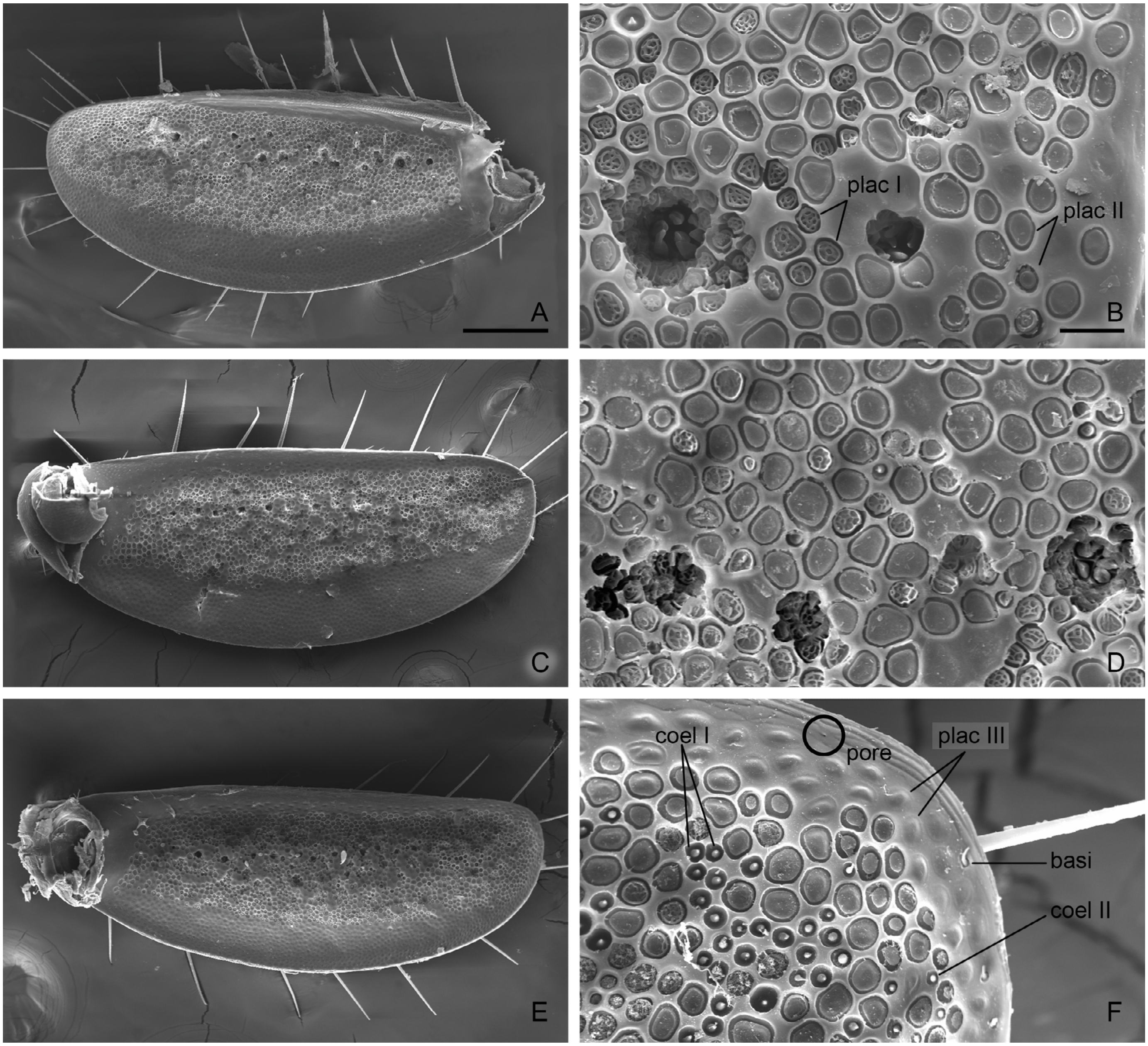

Figure 3. *Cyclocephala literata* Burmeister, 1847; antennal lamella of male. A-B) proximal lamella (inner side and detail of area with foveae, respectively). C-D) medial lamella (inner side and detail of area with foveae, respectively). E-F) distal lamella (inner side and detail of distal area, respectively). Basi = sensilla basiconica, Coel I = sensilla coeloconica type I, Coel II = sensilla coeloconica type II, Plac I = sensilla placodea type I, Plac II = sensilla placodea type II, Plac III = sensilla placodea type III; Pore = sensilla ampullacea. Scale of A, C, D = 200 µm; scale of B, D, E = 20 µm.

could be an important step to the evolution of phytophagous scarabs. To a species of Sericini (*Maladera orientalis* Motschulsky, 1857), the sensilla coeloconica are present in pedicel but not in lamellae, and its was conjectured the as hygroreceptors and thermoreceptor by Shao et al. (2019).

Sensilla basiconica are scares, present in inner surface of club, and are predominant in some fovea (see below). Romero-López et al. (2004) suggests that the sensilla basiconica (named as coeloconica) was related with plant volatiles detection. The sensilla basiconica is found in all scarab beetles (Scarabaeoidea) but the foveae in compassing sensilla basiconica is found in Dynastinae, Rutelinae and some Melolonthinae (Bohacz et al. 2020). Both sensilla coeloconica and sensilla basiconica have similar distribution in *C. literata* and *C. putrida* (Saldanha et al. 2020).

Sensilla ampullacea were associated with detection com CO_2 in some insects (Keil 1999). The attraction of beetles to CO_2 released by *M. ovata* is not known yet, but *C. literata* was certainly attracted to *M. ovata* volatiles and probably is also attracted to the heat produced at night by flowers (Gottsberger et al. 2012).

Sensilla placodea are the main sensilla of lamellae and represents about 95% of all sensilla in males and females of *C. literata*. The dominance of sensilla placodea is usual to scarab beetles (Meinecke

Table 1. Mean number of three sensilla types on the antennal lamellae of adult Cyclocephala literata.

Sensillum	proximal lamella		medial lamella		distal lamella	
	outer	inner	outer	inner	outer	inner
			Female (*n* = 10)			
Placodea	0	2,475 ± 19.40	2,461 ± 20.68	2,478 ± 25.23	0	2,800 ± 23.73
Coeloconica	0	147 ± 2.10	108 ± 1.78	114 ± 4.21	0	167 ± 4.16
Basiconica	0	3 ± 0.21	6 ± 0.5	8 ± 0.59	0	9 ± 0.47
Total	0	2,625 ± 20.69	2,575 ± 20.27	2,600 ± 27.6	0	2,976 ± 23.12
			Male (*n* =10)			
Placodea	0	2,714 ± 30.84	2,436 ± 22.85	2,512 ± 23.15	0	2,211 ± 24.16
Coeloconica	0	91 ± 1.67	92 ± 2.91	81 ± 2.41	0	200 ± 3.71
Basiconica	0	10 ± 0.57	0	0	0	39 ± 1.47
Total	0	2,815 ± 29.93	2,528 ± 22.99	2,593 ± 24.07	0	2,450 ± 25.84

1975, Romero-López et al. 2004, 2010, Tanaka et al. 2006, Mutis et al. 2014, Martínez-Bonilla et al. 2015, Rodrigues et al. 2019, Bohacz et al. 2020) and the sensilla is related to pheromone detection in *Popillia japonica* Newman, 1841 (Kim & Leal 2000) or related both with sexual attractants (peripherical ones) and plant volatiles detection (central ones) in *Anomala cuprea* (Hope, 1839) (Larsson et al. 2001). *C. literata* do not have an evident sexual dimorphism in antennae, and the amount of sensilla placodea is similar between sexes. It could be evidence that the beetle uses plant volatiles (and possibly heat and CO_2 as discussed in above sentence) as the main attractant, and sexual pheromones could have a minor hole or even by entirely absent. *C. literata* is a pollinator as above mentioned and does not have the sexual dimorphism regarding the amount and pattern of lamellar sensilla. Otherwise, *C. putrida* have an evident antennae dimorphism, females have more sensilla than males, and the beetle is not seemed as a pollinator (Saldanha et al. 2020).

Kim & Leal (2000) suggested that the sexual dimorphism in antennae is related to the detection of sexual attractant. Hallett et al. (1995) and Renou et al. (1998) show that a dynastine beetle (*Oryctes rhinoceros* L., 1758) does not have antennal dimorphism, and uses aggregative semiochemicals, not pheromones to find potential partners.

It is not known how many variables are involved on the attraction of beetle pollinator to flower chamber of host plants, but it is accepted that the flower attractants (odorants, heat, gases) play the main role in the beetle aggregation, and the conspecific sexual pheromones are partially or entirely suppressed, at least to cyclocephaline pollinators (Beach 1982, Pellmyr & Thien 1986, Dieringer et al. 1999, Gibernau et al. 1999). Otherwise, not pollinators cyclocephaline, that usually matting on the ground, grass leaves or shrubs, used sexual pheromones as attractant (Haynes & Potter 1995).

The relationship between dimorphism, pollination, and the kind of chemical communication in *Cyclocephala* must be checked and the rules of sexual pheromones or aggregative odorants must be clarified in the genus.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Article

Fish assemblage patterns in a subtropical estuary in southern Brazil

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Abstract: In this study, the relationship between fish assemblage structure and environmental factors was analyzed in a bay in southern Brazil. Fish were collected every two months between February and December 2002 at six sampling sites using bottom trawl nets. Abiotic data (salinity, temperature, rainfall, and depth) and biotic data (number of individuals, biomass, and total length of individuals from each species) were obtained. In total, 56 fish species representing 27 families were collected. Assemblage structure varied with seasonality, as was evidenced by the variation in temperature and rainfall in each season. Catches showed a high abundance of demersal fishes, particularly *Genidens genidens, Eucinostomus gula,* and *E. argenteus.*

Keywords: *Coastal area; spatio-temporal variation; fish fauna; southwest Atlantic.*

Padrões da assembleia de peixes em uma baía subtropical do sul do Brasil

Resumo: Neste estudo, a relação entre a estrutura da assembleia de peixes e fatores ambientais foi analisada em uma baía no sul do Brasil. Os peixes foram coletados a cada dois meses entre fevereiro e dezembro de 2002 em seis locais de amostragem usando redes de arrasto de fundo. Dados abióticos (salinidade, temperatura, precipitação e profundidade) e dados bióticos (número de indivíduos, biomassa e comprimento total de indivíduos de cada espécie) foram obtidos. No total, 56 espécies de peixes representando 27 famílias foram coletadas. A estrutura da assembleia variou com a sazonalidade, conforme evidenciado pela variação da temperatura e precipitação em cada estação. As capturas mostraram grande abundância de peixes demersais, principalmente *Genidens genidens, Eucinostomus gula e E. argenteus.*

Palavras-chave: *Área costeira; variação espaço-temporal; ictiofauna; Atlântico Sudoeste.*

Introduction

Bays, estuaries, and lagoons are coastal transition environments between fresh and saltwater (Mclusky & Elliott 2004, Basset et al. 2013). These environments, in tropical and subtropical coastal areas, provide a variety of ecosystem services that have strong implications for their conservation and management, including the provision of fishing resources, protection of the coast, areas of tourism, and rich biodiversity (Lotze et al. 2006, Sheaves et al. 2014).

In coastal environments, abiotic and biotic conditions are constantly changing, with rapid variations in salinity, temperature, oxygen, and turbidity (Elliott & Hemingway 2002). In addition to these physical and chemical factors, the reproductive biology of species, recruitment and/or migration patterns, and biological interactions, such as predation and competition, can also influence the spatial and temporal distribution of fish fauna (Mclusky & Elliott 2004, Whitfield & Elliott 2011, Potter et al. 2015).

Although they are unstable environments, coastal environments, especially estuaries, are among the most productive natural habitats, as the accumulation of sediments from the sea and adjacent rivers forms a rich source of food that supports a large number of animals (Mclusky & Elliott 2004). Knowledge of biological patterns is essential for understanding the coastal system as a whole (Barletta et al. 2010). Fish are indicators of environmental status, and it is essential to understand the dynamics and distribution of fish assemblages to formulate strategies for managing the effects of human activities on coastal environments (Whitfield & Elliott 2002, Mérigot et al. 2017).

Thus, several studies have investigated the patterns of spatial and temporal variation in fish assemblages and their relationship with habitats and physical conditions in these environments (Azevedo et al. 2007, Favero et al. 2019, Cattani et al. 2020). Most fishes are not adapted to spend their entire life cycle in estuarine environments. These environments are usually inhabited by seasonal members or by species that use this habitat strictly as a migration route between feeding and spawning areas. This results in a fish fauna assemblage consisting mainly of species that occur on the adjacent continental shelf (Blaber et al. 1995).

In this context, the aim of this study was to quantify the spatiotemporal distribution of estuarine fish and their key abiotic associations in a subtropical bight in southern Brazil. This may improve our understanding the ecosystem functioning, which is an important consideration for adopting conservation and preservation measures.

Material and Methods

1. Data collection

Fish were collected every two months between February and December 2002 from six sampling sites. The samplings were carried out in the Saco dos Limões cove, state of Santa Catarina, Brazil (Figure 1). The Saco dos Limões cove is located on the inner side of Santa Catarina Island, on the east of the South Bay. The cove is shallow, with depths less than 1 meter in its southern portion, and a little deeper in the northern portion. Moving away from the cove towards the center of the bay, to the west, there is a slope with a depth of more than 3 meters. To the north, in the region of the strait between the North and South bays, the depth is greater than 10 meters. Has a sandy-muddy bottom with large amount of biodetritic material, with a predominance of the fine sediments fraction in the innermost region of the cove, while the sandy fraction is found in the nearby shallows to the Rio Tavares Mangrove (Schettini et al. 2002, Souza-Conceição & Schwingel 2011). Sites 1, 2, and 6 were furthest from the coast, with deeper water and greater marine influence than the remaining three sites, at which water was shallower, under less marine influence, and the input of continental waters was greater (Figure 1). At each sampling site, one simultaneous double trawling lasting 10 min was carried out at a speed of 2 knots, using two identical bottom trawl nets with 4.5, 7.5, and 9 m footrope, a mesh size of 14 mm in the top and bottom panels, and a mesh size of 12 mm at the cod-end. Before each trawl, depth data were collected using an echo sounder and bottom water temperature and salinity data were collected using a Horiba U-10 multi-parameter water quality meter. Rainfall data were provided by the AGRI/CIRAM meteorological station in Florianópolis (27°34′41.89″ S and 48°30′32.79″ W). The caught specimens were identified based on taxonomic keys (Figueiredo & Menezes 1978, 1980, Fischer 1978, Menezes & Figueiredo 1980, 1985, Marceniuk 2005). Taxonomic classification and nomenclature of fish species were confirmed by comparison with information by Eschmeyer (2020).

2. Data analysis

Multivariate permutational analysis of variance (PERMANOVA) was used to assess temporal and spatial differences in fish abundance (Anderson et al. 2008). In case of rejection of the null hypothesis in PERMANOVA, the factors with significant differences ($p < 0.05$) were subjected to pairwise PERMANOVA, and were visualized through the canonical analysis of principal coordinates (CAP) using Spearman's correlation at 0.5 (Anderson et al. 2008).

PERMANOVA was also used to test temporal and spatial differences in environmental variables, while distance-based linear models (DistLM), using the Akaike selection criterion (AIC), assessed the influence of environmental variables on fish data variability. For graphic visualization of the influence of predictor variables on the spatial

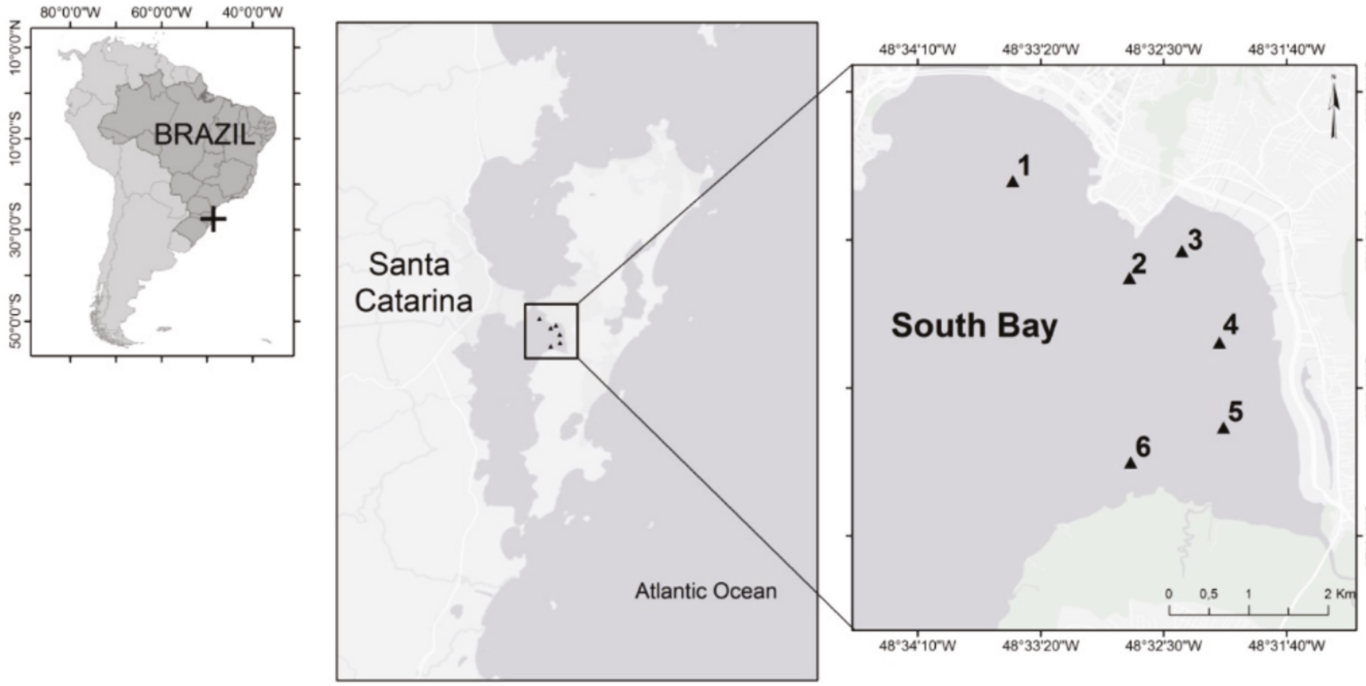

Figure 1. Map of Santa Catarina Island, with details of the collection sites.

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grouping of the samples, distance-based redundancy analysis (dbRDA) was applied (Anderson et al. 2008).

To identify differences in the taxonomic structure (genuine diversity) of fishes among the seasons, the average taxonomic distinctness (Delta+ or AvTD) and variation in taxonomic distinctness (Lambda+ or VarTD) indices were calculated based on a matrix of species, gender, family, class, and order as taxonomic hierarchies. Biplots and funnel charts were used to assess whether the index values (Delta+ and Lambda+) of the seasons were within the expected ranges of variation (Clarke & Warwick 1994). Taxonomic differences between the seasons were tested using a one-way PERMANOVA in which the dependent variables were the species richness and the values of AvTD and VarTD, and the fixed factor was season (Anderson et al. 2008).

Results

1. *Environmental variables*

There were no significant differences in salinity among the seasons and sampling sites (Figure 2a). Mean temperature differed significantly among seasons (Pseudo-F = 12.672; $p = 0.0006$). Pairwise comparisons revealed differences between summer and fall (t = 2.849; $p = 0.0254$), fall and winter (t = 3.4821; $p = 0.0122$), fall and spring (t = 3.7009; $p = 0.0035$), and winter and spring (t = 4.8468; $p = 0.0035$). Mean temperatures were the highest in spring (mean ± standard deviation; 25.7 ± 2.14 °C), followed by summer (24.7 ± 1.6 °C), fall (22.1 ± 1.45 °C), and winter (18.92 ± 0.49 °C) (Figure 2b).

Depth differed significantly among sampling sites (Pseudo-F = 46.67; $p = 0.0001$) and seasons (Pseudo-F = 7.7778; $p = 0.0038$). Pairwise comparisons revealed significant differences in depth between sites 1 and 2, 1 and 3, 1 and 4, 1 and 5, 2 and 6, 3 and 6, 4 and 6, and 5 and 6 (Table 1). Depth also differed significantly between fall and winter, and between winter and spring (Table 1). The highest mean depth values were detected in fall at site 1 (6 m), in spring at sites 1 and 6 (5.5 ± 0.7 m), and in summer at sites 1 and 6 (5 m). The lowest mean depth values (2 m) were observed in winter at sites 4 and 5, fall at site 4, spring at sites 3 and 4, and in summer at sites 3, 4, and 5 (Figure 2c).

Significant differences in rainfall were detected among seasons based on values extrapolated and applied to all sampling sites (Pseudo-F = 7.5865; $p = 0.001$). Pairwise comparison indicated that only winter differed from other seasons (Table 1). The highest mean rainfall was observed in summer (232 mm), followed by spring (185.05 ± 29.51 mm), fall (127.25 ± 85.98 mm), and winter (113.2 mm) (Figure 2d).

2. *Fish assemblage*

A total of 11,327 specimens were collected, distributed across 27 families and 56 species (Table 2). The families represented by the highest richness of species in our study were Sciaenidae (11), Carangidae (7), Gerreidae and Tetraodontidae (4 each), Paralichthyidae and Epinephelidae (3 each), and Serranidae, Ariidae and Mugilidae (2 each) (Table 2). All other families were represented by only one species. The families with the highest catch numbers (five families totaling 85.04%) were Gerreidae (39.75%), Ariidae (32.87%), Paralichthyidae (7.29%), Carangidae (5.13%), and Tetraodontidae (3.87%). The families

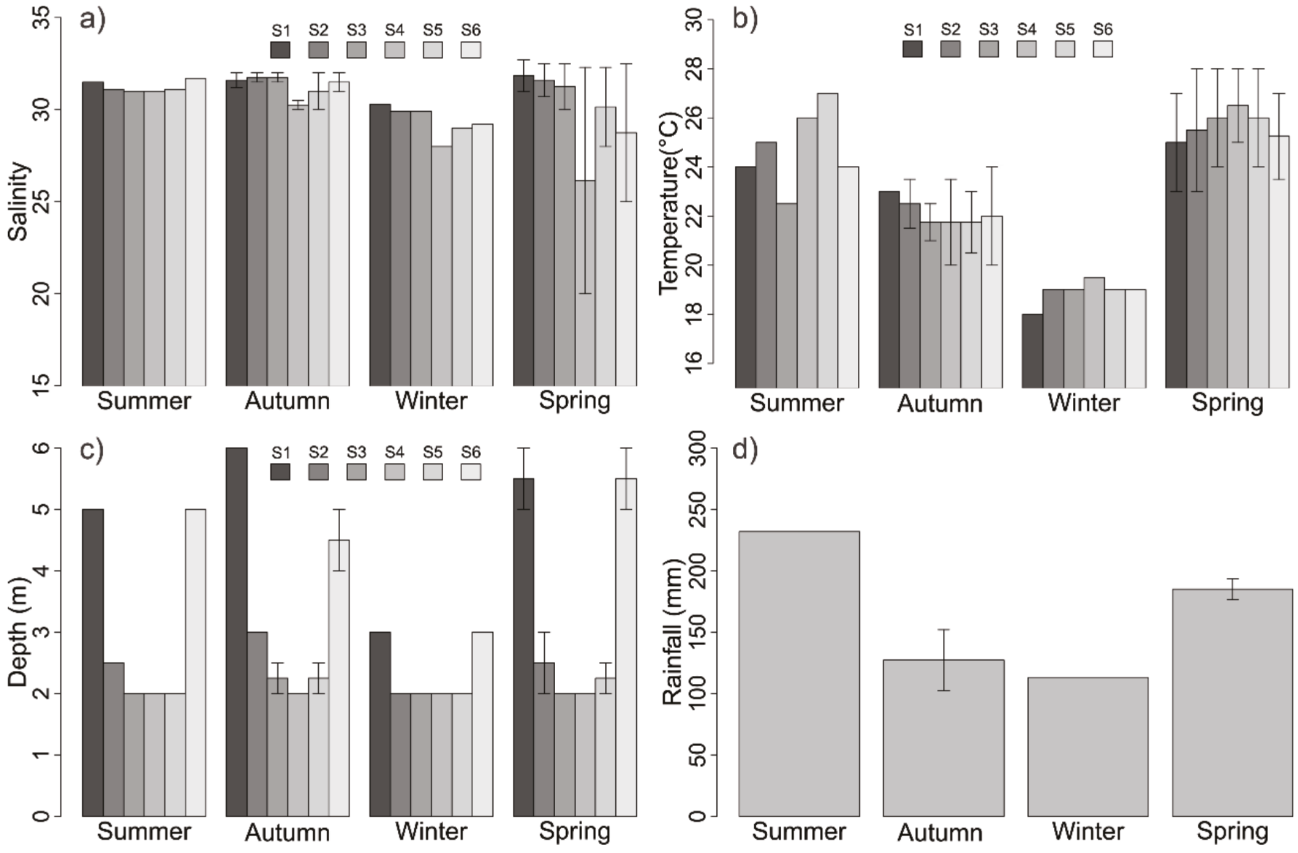

Figure 2. Average values (standard error) in the salinity (a), temperature (b), depth (c) and rainfall (d) bars, comparing the seasons of the year at the six sample sites.

Table 1. PERMANOVA pairwise based on the Euclidean distance from the depth (normalized) between the sites and the seasons, with the t-values (Student's t test) and the permutation *p*-value [p (perm)]. In bold, variables with significant *p*-value.

Groups	T	*p*(perm)
1, 2	**7.7567**	**0.0008**
1, 3	**11.619**	**0.0004**
1, 4	**13.279**	**0.0004**
1, 5	**10.371**	**0.0009**
1, 6	1	0.3576
2, 3	1.8074	0.1434
2, 4	2.3094	0.0844
2, 5	1.4142	0.2254
2, 6	**5.3333**	**0.0072**
3, 4	0.57735	0.6288
3, 5	0.33333	0.7615
3, 6	**7.5056**	**0.0025**
4, 5	0.8165	0.4541
4, 6	**8.165**	**0.0017**
5, 6	**6.9378**	**0.0028**
Summer, Autumn	1.4142	0.2339
Summer, Winter	**3.74821**	**0.0096**
Summer, Spring	0.80064	0.4519
Autumn, Winter	**5.6569**	**0.002**
Autumn, Spring	0.22942	0.8438
Winter, Spring	**3.6829**	**0.0083**

with the heaviest catch weights (five families, 84.39%) were Gerreidae (30.11%), Ariidae (28.22%), Tetraodontidae (10.55%), Sparidae (6.54%), Sciaenidae (5.05%), and Paralichthyidae (4.92%).

The most common species in this study were *Genidens genidens* (29.30%), *Eucinostomus gula* (15.50%), *E. argenteus* (15.03%), *Diapterus rhombeus* (8.88%), *Citharichthys spilopterus* (6.34%), *Chloroscombrus chrysurus* (4.10%), and *Genidens barbus* (3.58%). Together these species represented 82.73% of the individuals captured. Only one individual each was captured from the species *Elops saurus*, *Lutjanus synagris*, *Paralichthys orbignyanus*, *Scorpaena plumieri*, *Stellifer brasiliensis*, *S. rastrifer*, and *Trachinotus carolinus* (Table 2).

The total catch weight was 260,822.7 g (Table 2). The catch weights for *G. genidens* (26.33%) was the highest, followed by *E. gula* (11.99%), *D. rhombeus* (9.92%), *Sphoeroides testudineus* (9.02%), *E. argenteus* (7.64%) and *Archosargus rhomboidalis* (6.54%). Together these represented 71.44% of the total catch weight.

Thirty species occurred in all seasons and 12 species occurred in only one season. The greatest richness was observed in fall and spring (45 species each), followed by summer (38 species), and winter (31 species) (Table 2). Twenty-five species occurred at six sites, and 14 species occurred at only one site. The highest number of species occurred at site 4 (42 species), followed by sites 6 (39), 5 (37), 3 (36), 2 (35), and 1 (31) (Table 2).

Forty-two species were found during both day and night trawls. Eight species were found only during night trawls and six species were found only during day trawls (Table 2). Species richness was greater in the nighttime (50 species) than in the daytime (48 species). Additionally, greater abundance occurred at night than at day; 7,256 fishes (64.06% of the total catch) and 4,071 fishes (35.94%) were captured in the nighttime and daytime, respectively. Twenty-six of the species occurring in both periods were more abundant at night, while 13 were more abundant during the day, and three were equally abundant in both periods (Table 2).

Mean abundance differed significantly among the seasons, periods, and sites. PERMANOVA detected significant differences ($p<0.05$) for the three factors (Table 3). However, pairwise comparisons (PERMANOVA pairwise test), revealed that the differences were not significant between summer and winter, and fall and winter. Mean abundance also did not differ significantly between sites 1 and 2, 1 and 3, and 2 and 3 (Table 4).

Mean abundance was the highest in fall at site 4 (311.75 ± 97.83), followed by winter at site 4 (280 ± 251.73), fall at sites 5 (259.5 ± 258.59) and 6 (258.75 ± 97.8), spring at sites 5 (239.75 ± 64.86) and 4 (200.75 ± 94), winter at site 6 (194 ± 59.4), and spring at site 6 (188.5 ± 98.89). Mean abundance was the lowest in summer at site 1 (33), followed by winter at sites 1 (48.5 ± 54.45) and 2 (50.5 ± 2.12), spring at site 1 (52.33 ± 19.65), summer at sites 2 (56 ± 4.24) and 3 (74.5 ± 4.95), fall at site 1 (82.5 ± 37.22), and spring at site 3 (93.33 ± 19.65) (Figure 3a). The highest number of fish was captured at night in fall (259.92 ± 155.03) and winter (219 ± 148.97), and the lowest during the day in winter (66 ± 53.21) and summer (70.17 ± 42.49) (Figure 3b).

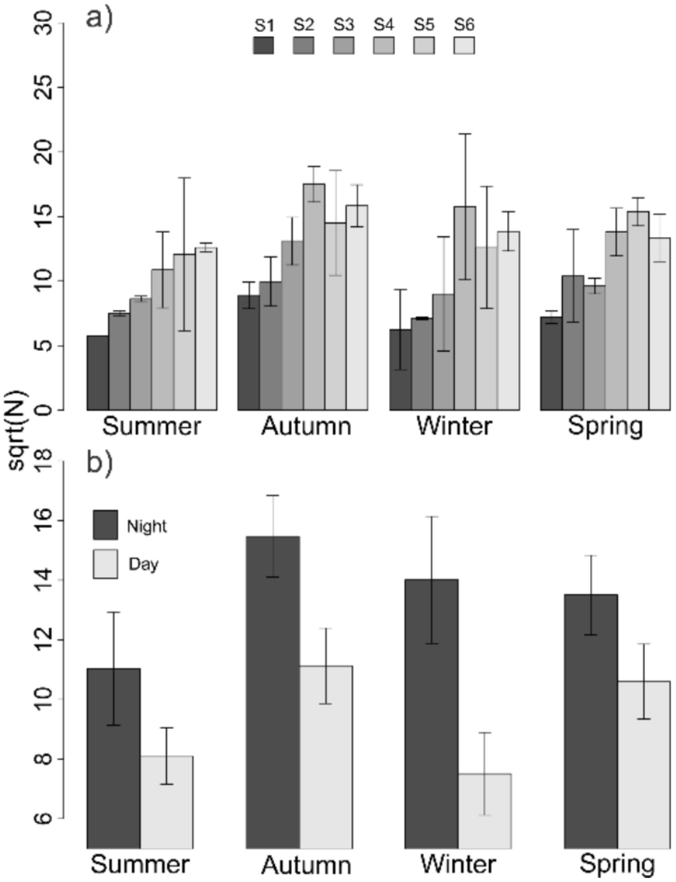

Figure 3. Mean values (standard error in the bars) of the square root of the abundance of fish caught in the seasons at sites 1, 2, 3, 4, 5 and 6 (a) and between day and night (b).

Table 2. List of species, number of individuals (n), weight (W), average, minimum and maximum of the total length (TL), season (S = spring, Su = summer, A = autumn, and W = winter), sites and period (D = day, N = night) of the fish caught (* species present in only one site). The fish classification follows Van der Laan et al. (2020).

Family/Specie	n	W(g)	Average TL (mm)	Mín-Máx TL (mm)	Season	Sites	Period
ELOPIDAE							
*Elops saurus**	1	6.38	100.00	100-100	W	5	D
OPHICHTHIDAE							
Ophichthus gomesii	6	766.46	494.33	390-610	S, Su	2, 4, 5	D, N
ARIIDAE							
Genidens barbus	405	4950.27	110.02	42-213	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Genidens genidens	3318	68665.10	123.17	47-125	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
SYNODONTIDAE							
Synodus foetens	114	5234.00	197.13	217-469	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
BATRACHOIDIDAE							
*Porichthys porosissimus**	2	6.57	70.50	60-81	S, A	6	N
POMATOMIDAE							
Pomatomus saltatrix	22	841.25	151.05	105-253	S, Su, A	1, 2, 3, 4, 5, 6	D, N
TRICHIURIDAE							
Trichiurus lepturus	8	266.62	369.50	65-538	S, Su, A	4, 6	D, N
GOBIIDAE							
Gobionellus oceanicus	19	516.16	179.39	132-247	S, Su, A, W	1, 3, 4, 5	D, N
CENTROPOMIDAE							
*Centropomus parallelus**	6	864.02	226.67	68-346	S, Su, A, W	6	D, N
SPHYRAENIDAE							
Sphyraena guachancho	5	41.09	115.80	95-145	A	3, 4	D, N
PARALICHTHYIDAE							
Citharichthys spilopterus	718	11812.06	94.87	11-385	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Etropus crossotus	107	726.44	85.29	41-142	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
*Paralichthys orbignyanus**	1	299.34	310.00	310-310	S	2	D
ACHIRIDAE							
Catathyridium garmani	25	328.80	80.92	35-121	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
CYNOGLOSSIDAE							
Symphurus tessellatus	84	1713.09	139.53	90-261	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
CARANGIDAE							
Caranx latus	3	56.83	102.33	91-125	W	5, 6	N
Chloroscombrus chrysurus	464	2314.65	69.84	34-171	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Oligoplites saliens	7	154.96	143.29	107-182	S, Su, A, W	5	D, N
Oligoplites saurus	22	226.35	109.86	40-161	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Selene setapinnis	51	941.70	108.53	56-162	S, Su, A, W	1, 2, 3, 4, 6	D, N
Selene vomer	33	1033.79	111.58	37-218	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
*Trachinotus carolinus**	1	209.21	248.00	248-248	Su	4	N
MUGILIDAE							

Family/Specie	**n**	**W(g)**	**Average TL (mm)**	**Mín-Máx TL (mm)**	**Season**	**Sites**	**Period**
Mugil curema	42	4404.37	213.67	156-334	S, Su, A, W	1, 2, 3, 4, 5	D, N
Mugil platanus	16	4009.85	295.63	238-379	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
LUTJANIDAE							
*Lutjanus synagris**	1	402.23	315.00	315-315	S	4	D
GERREIDAE							
Diapterus rhombeus	1006	25870.13	99.73	35-709	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Eucinostomus argenteus	1703	19932.59	83.84	34-251	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Eucinostomus gula	1756	31289.34	97.08	10-203	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Eucinostomus melanopterus	37	1431.18	144.57	102-207	Su, A, W	1, 2, 3, 4, 5, 6	D, N
HAEMULIDAE							
Orthopristis ruber	40	1683.13	122.55	55-227	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
SPARIDAE							
Archosargus rhomboidalis	179	17045.82	167.12	84-279	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
SCIAENIDAE							
*Bairdiella ronchus**	4	313.07	180.00	150-223	S, Su	5	N
Ctenosciaena gracilicirrhus	14	492.84	122.71	56-225	Su, A	1, 3, 6	D, N
Cynoscion leiarchus	36	1287.32	133.36	43-225	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Cynoscion microlepidotus	2	11.08	91.00	91-91	W	1, 3	N
Isopisthus parvipinnis	41	324.10	80.71	40-204	A, W	2, 3, 4, 6	D, N
Menticirrhus americanus	3	603.13	261.33	251-278	Su, A	2, 4	D, N
*Menticirrhus littoralis**	2	1275.00	369.50	334-405	A	3	N
Micropogonias furnieri	245	8815.89	133.41	25-262	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
*Stellifer brasiliensis**	1	7.87	88.00	88-88	S	6	D
*Stellifer rastrifer**	1	8.26	99.00	99-99	S	6	D
Stellifer sp.	2	35.26	94.00	94-94	S	6	D
SERRANIDAE							
Diplectrum radiale	64	2291.10	131.39	63-199	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Rypticus randalli	4	211.77	153.00	119-183	S, A	2, 4	D, N
EPINEPHELIDAE							
Mycteroperca acutirostris	13	904.93	159.54	57-270	S, Su, A	2, 3, 4, 5, 6	D, N
*Mycteroperca bonaci**	2	805.61	309.00	271-347	S, A	4	D, N
Mycteroperca microlepis	5	713.40	209.20	134-261	S, Su, A	4, 5	N
SCORPAENIDAE							
*Scorpaena plumieri**	1	22.43	98.00	98-98	A	6	N
TRIGLIDAE							
Prionotus punctatus	156	3780.50	115.69	33-302	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
EPHIPPIDAE							
Chaetodipterus faber	81	3065.24	96.09	23-135	S, Su, A, W	1, 2, 3, 4, 5	D, N
TETRAODONTIDAE							

Family/Specie	n	W(g)	Average TL (mm)	Mín-Máx TL (mm)	Season	Sites	Period
Lagocephalus laevigatus	55	1991.12	102.73	52-273	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
Sphoeroides greeleyi	71	734.17	72.17	33-120	S, Su, A	1, 2, 3, 4, 5, 6	D, N
Sphoeroides spengleri	36	1271.93	97.44	30-223	S, Su, A	2, 3, 4, 5, 6	D, N
Sphoeroides testudineus	277	23517.30	136.41	46-274	S, Su, A, W	1, 2, 3, 4, 5, 6	D, N
MONACANTHIDAE							
Stephanolepis hispida	9	295.65	105.56	50-192	S, Su, A, W	4, 6	D, N

Table 3. PERMANOVA based on the Bray-Curtis similarity of abundance (transformed by the square root) comparing the collection points, seasons and periods (day and night). d.f = degrees of freedom; MS = sum of the mean squares; *p* (perm) = permutation p-value.

Variation source	d.f	MS	Pseudo-F	*p*(perm)
Site	5	4359.2	3.9294	0.0001
Season	3	4695.6	4.2326	0.0001
Period	1	5506.8	4.9638	0.0002
SitexSeason	15	1282.0	1.1556	0.1746
SitexPeriod	5	1280.9	1.1546	0.2688
SeasonxPeriod	3	1478.6	1.3328	0.1593
SitexSeasonxPeriod	15	792.8	0.7146	0.9689
Residue	22	1109.4		

High abundances of *M. furnieri*, *C. spilopterus*, and *G. genidens* at site 6 and *E. argenteus*, *D. rhombeus*, and *E. gula* at site 5 (Figure 4) were responsible for the spatial clusters observed in CAP. High abundances of *S. greeleyi* in the spring samples, *S. foetens*, *D. radiale,* and *E. crossotus* in the fall samples, and *C. chrysurus* in the winter samples were responsible for the seasonal clusters observed in CAP (Figure 5).

In the linear model developed by DistLM, the predictor variables that were most important were temperature (AIC = 269.91) and rainfall (AIC = 270.87). Salinity and depth did not significantly explain the variation in fish community composition (Table 5). dbRDA showed the greatest association between rainfall and summer and fall samples with axis 1, and temperature and spring samples with axis 2 (Figure 6).

PERMANOVA detected significant differences in Delta+ (average taxonomic distinctness) associated with species richness, but not in Lambda+ (variation in taxonomic distinctness) (Table 6). Pairwise PERMANOVA revealed significant differences between the spring and summer and fall and summer samples. However, despite the difference in the number of species (Figures 7a and 7b), the values of Delta+ and Lambda+ for all four seasons were very similar. The average taxonomic distinctness was greater than the simulated average for all four seasons, while the variation in distinctness was below average (Figures 7a and 7b). The biplot graph of both indices revealed a greater differentiation in Lambda+ values, with very close values of Delta+ (Figure 7c). The value of Lambda+ for the spring was especially high, and varied among samples.

Table 4. PERMANOVA pairwise based on the Bray-Curtis similarity of abundance (transformed by the square root) comparing the sites, with the t-values (Student's t test) and the permutation *p*-value [*p* (perm)]. In bold, variables with significant *p*-value.

Grups	t	*p*(perm)
1, 2	1.2362	0.1886
1, 3	1.3100	0.1808
1, 4	**2.1656**	**0.0052**
1, 5	**2.6586**	**0.0015**
1, 6	**2.8788**	**0.0015**
2, 3	1.1583	0.2681
2, 4	**1.8302**	**0.0137**
2, 5	**2.0974**	0.0056
2, 6	**2.0460**	**0.0064**
3, 4	**1.6457**	**0.0370**
3, 5	**2.4455**	**0.0036**
3, 6	**2.0042**	**0.0066**
4, 5	**1.5482**	**0.0482**
4, 6	**1.7946**	**0.0122**
5, 6	**2.5396**	**0.0019**

Discussion

Significant differences in salinity were detected between both the seasons and the sampling sites; this is expected for an exposed area under constant influence of the continental shelf water (Veado & Resgalla 2005, Nakayama et al. 2020). Temperature also differed significantly between the seasons. However, distLM detected a significant p-value only for rainfall and temperature, such that summer and fall samples were positively associated with rainfall and spring samples were positively associated with temperature.

Based on the results of the analysis of environmental variables, our results indicate that both temperature and rainfall are important drivers of variability in fish fauna. Although salinity does not have statistical significance in explaining the variability of fish in the present study, it is an important determinant of fish assemblage structure in marine and estuarine environments (Barletta et al. 2005; 2008, Bot et al. 2018). The importance of rainfall detected by the analyses directly reflects salinity patterns. In environments with fluctuations in salinity such as coastal

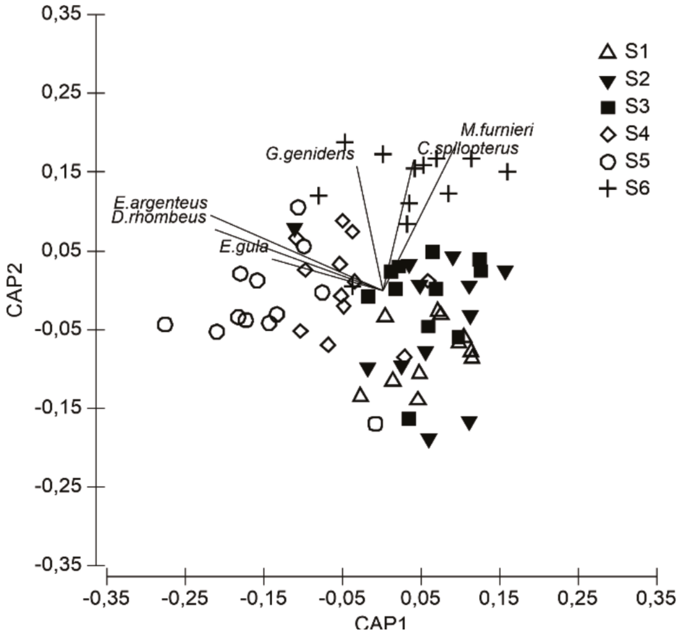

Figure 4. Result of the canonical analysis of main coordinates (CAP), with the species that contributed to the differences between the sites (1 to 6). Species vectors elaborated based on Spearman's correlation with index above 0.5 (p> 0.5). The canonical correlation of the two axes obtained by the analysis was δ1 = 0.7986 and δ =20.7452.

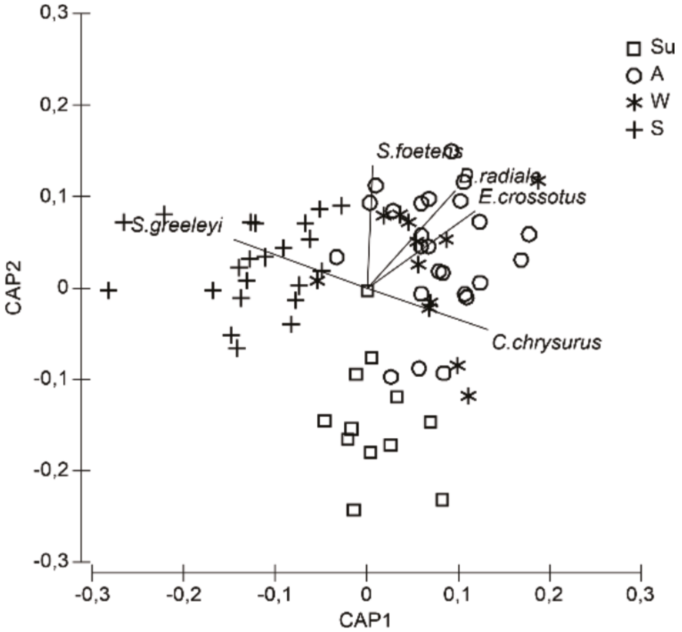

Figure 5. Result of the canonical analysis of main coordinates (CAP), with the species that contributed to the differences between summer (Su), autumn (A), winter (W) and spring (S). Species vectors elaborated based on the Spearman correlation with an index of 0.5 (p> 0.5). The canonical correlation of the two axes obtained by the analysis was δ1 = 0.8506 and δ2 = 0.743

and estuarine environments, fish migrate to areas that do not have high variation in salinity during times of high rainfall, which results in an influx of freshwater to the sea. For example, along the east-west axis of the Paranaguá Estuarine Complex, fish assemblages migrated to the median areas of the estuary (where salinity varies relatively little) during rainy seasons (Barletta et al. 2008).

In a previous study of demersal fish fauna in a region close to the area of this study (Cattani et al. 2016b), and in studies at lower latitudes, such as in Paraná (25°S) (Schwarz Jr. et al. 2006, Barletta et al. 2008, Possato et al. 2017) and Ubatuba, São Paulo (23°S) (Rossi-Wongtschowski & Paes 1993), a high number of species of *Sciaenidae* were observed. This predominance is common in Brazil (Reis-Filho et al. 2010, Vilar et al. 2011) and in estuaries worldwide and is due to the transition between marine/euryhaline environments throughout the evolutionary history of the family. This suggests that fishes in this adapt easily to changes in salinity, which facilitates their stay in estuarine regions (Lo et al. 2015).

Table 5. Result of the DistLM analysis with permutation p-value and the proportion of explanation of the variables for the selected model. In bold, variables that had a significant p-value.

Variable	P (Perm)	Proportion
Rainfall	**0.0002**	**9.3519E-02**
Temperature	**0.0018**	**7.7931E-02**
Salinity	0.4187	2.8904E-02
Depth	0.1508	4.0931E-02

The dominance of a few demersal fish species in the fish assemblages was observed in this study. Gerreidae and Ariidae were of the greatest abundance in this area. The high abundance of Ariidae in estuarine environments demonstrates the high adaptive capacity of these fish, which allows them to survive in these environments in different ontogenetic phases, despite variation in e.g. salinity, temperature, turbidity, and dissolved oxygen (Azevedo et al. 2007, Barletta et al. 2008, Cattani et al. 2016a, Possato et al. 2017). Gerreidae species are not typically more abundant than are Ariidae and Sciaenidae in estuaries (Queiroz et al. 2007, Barletta et al. 2005, Pinheiro et al. 2008).

Three species in the genus *Eucinostomus* (*E. argenteus, E. gula,* and *E. melanopterus*) were found in greater abundance in Guaratuba Bay during the period of low rainfall (May October), when salinity was nearly 35, and in lesser abundance during rainy periods, when salinity

Table 6. Result of PERMANOVA of richness, average taxonomic distinction (AvTD) and variation of taxonomic distinction (VarTD), considering the season. df = degrees of freedom; MS = sum of the mean squares; *p* (perm) = permutation *p*-value.

Variation source		df	MS	Pseudo-F	*p*(perm)
Richness	Season	3	122.6	2.4947	0.0634
	Residue	66	49.1460		
AvTD	Season	3	0.0429	3.6281	0.0175
	Residue	66	0.0118		
VarTD	Season	3	2.2997	0.9697	0.4140
	Residue	66	2.3714		

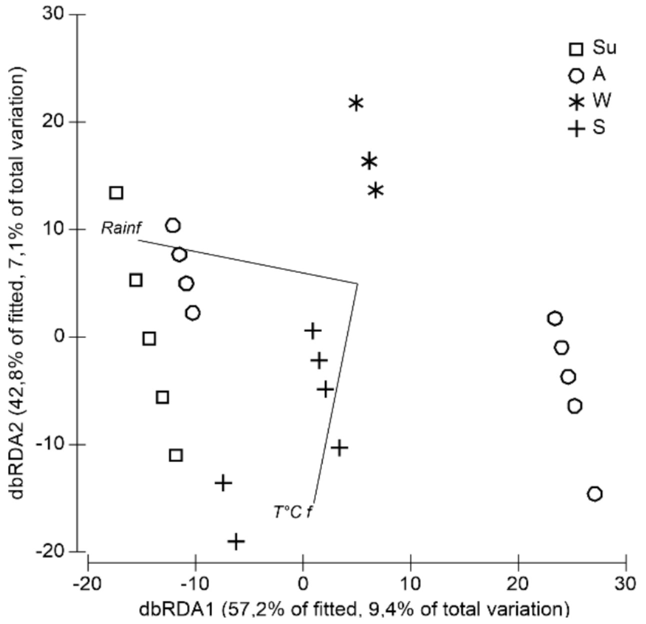

Figure 6. Result of the redundancy analysis based on the linear model (dbRDA), with the predictor variables that were most important for the linear model. Su = summer, A = autumn, W = winter, S = spring.

was nearly 5 (Chaves & Otto 1998). The high occurrence of Gerreidae in this study may have been associated with the generally high salinity values in that region; salinity values were almost always above 30, particularly in the summer and fall.

The present study indicated that catch is higher during the night. However, for shallow areas, such as beaches and tidal creeks, fish abundance seems to be greater during the day (Oliveira-Neto et al. 2010, Ignácio & Spach 2009, Ribeiro et al. 2014). The displacement of demersal species to shallower areas can interfere with abundance patterns between periods (Oliveira-Neto et al. 2010).

Although there are behavioral differences between species during the day and the night, demersal assemblages are well-adapted to low visibility conditions, with light being a secondary factor for structuring assemblages, particularly during the post-larval stages (Oliveira-Neto et al. 2010). However, in Sepetiba Bay, there were no major differences in assemblage structure between day and night (Pessanha & Araujo 2003). Possibly, for demersal fishes, differences in abundance between periods are more linked to the probability of catch, which is greater at night because it is more difficult for fish to see the net (Johnson et al. 2008). This would justify the greater abundance at night observed in the present study.

We also observed seasonal variation in fish fauna in this study. In particular, we did not observe seasonality in the taxonomic structure of the community, rather, seasonality was due mainly to different occurrence patterns for some species. The average taxonomic distinctness and variation in taxonomic distinctness indicate that taxonomic complexity did not differ among seasons.

However, the main regulatory mechanism for fish assemblages in this area is not clear. Despite seasonal differences directly reflecting the physical and chemical parameters of the water column, which in turn influence the distribution and occurrence patterns of demersal assemblages (Whitfield et al. 2012, Possato et al. 2017), the environmental gradients in the present study were not well demarcated.

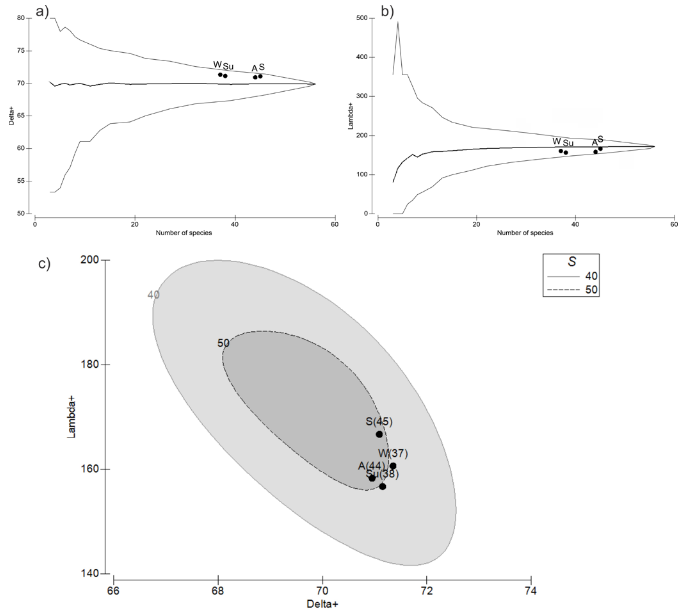

Figure 7. Average taxonomic distinction (AvTD - Delta +) (a) and variation of the taxonomic distinction (VarTD - Lambda +) (b) calculated for the Saco dos Limões by season (S = spring, Su = summer, A = autumn, and W= winter). For both indexes, the expected average is represented by the central dotted line and the limit of the 95% confidence interval by the solid line of the surroundings, in the form of a funnel. Biplot graph of Lambda + and Delta + (c). The ellipse represents the value of the 95% confidence interval of probability of finding 40 and 50 species respectively.

It is possible that the processes of reproduction, spawning, and recruitment have a strong influence on assemblage structure because of the large abundance of small individuals belonging to a small number of species.

Considering the size (e.g. total lenth) at first maturity of the three most abundant species, 155 mm to *G. genidens* (Mishima & Tanji 2018), 120 mm to *E. argenteus* (Corrêa & Vianna 2016), and 110 mm to *E. gula* (Froese & Pauly 2021), which together account for 60% of the total abundance, its suggests that there is a predominance of young individuals in our study (see Table 2). The abundance of juveniles of these species highlights the ecosystem function of the coastal environment as a growth zone for juvenile fish (Elliott et al. 2007), due to the high biological productivity generated by the inflow of the Tavares River (Souza-Conceição & Schwingel 2011).

The essential role in the nursery function, particularly for marine fishes (Strydom et al. 2003), could be associated with the availability of food and refuge from predators (Elliot & Hemingway 2002). The importance of this study area to juvenile fishes may also indicate that juveniles are valuable for assessing ecological conditions in transitional waters.

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André Pereira Cattani: Substantial contribution to the idea and design of the study, and contribution to the analysis and interpretation of data, the writing of the paper, and critical review (adding intellectual content).

Yuri Gerke: Contribution to the analysis and interpretation of data and the writing of the paper.

Helen Audrey Pichler: Contribution to the analysis and interpretation of data and the writing of the paper.

Johnatas Adelir-Alves: Contribution to the analysis and interpretation of data, the writing and preparation of the paper.

Henry Louis Spach: Contribution to the analysis and interpretation of data, the writing of the paper, and critical review (adding intellectual content).

Paulo Ricardo Schwingel: Substantial contribution to the idea and design of the work, and contribution to data collection, the analysis and interpretation of data, and the writing of the paper.

Conflicts of Interest

The authors declare no conflict of interest related to the publication of this manuscript.

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Thematic Review

Building knowledge to save species: 20 years of ichthyological studies in the Tocantins-Araguaia River basin

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Abstract: The Tocantins-Araguaia River basin is the largest basin located entirely in the Brazilian territory. The high degree of endemism of its ichthyofauna has been revealed in several studies, with the upper Tocantins River having the largest absolute number of endemic taxa within the Amazon basin. Here we provide an accurate review of the ichthyofauna of the Tocantins-Araguaia River basin, based on collections made between 2000-2020, including an extensive list of valid fish species occurring in the basin and a discussion of the major threats to its ichthyofauna. Ichthyofauna diversity was further refined based on web searches. Protected Areas and hydropower plants were mapped using shape files or coordinates from the responsible government agencies. 751 species of fishes are currently known from the Tocantins-Araguaia River basin. A considerable increase in fish diversity knowledge occurred in the last 20 years, in parallel with significant anthropic alterations in the basin and its surroundings. Dams constructed along the basin are ranked as the major threats to ichthyofauna. Although the drainage-basin holds several conservation units and indigenous lands, they have not been sufficient to guarantee the preservation of fish species. Our compilation emphasizes that the upper Tocantins River must be considered as a priority area to preserve fish species. Some mitigation actions that may achieve satisfactory results concerning ichthyofauna conservation are proposed.
Keywords: *Amazon basin; Cerrado; Conservation; Diversity; Ichthyofauna.*

Construindo conhecimento para salvar espécies: 20 anos de estudos ictiológicos na bacia do rio Tocantins-Araguaia

Resumo: A bacia do rio Tocantins-Araguaia é a maior bacia localizada completamente no território brasileiro. O elevado nível de endemismo de sua ictiofauna foi atestado em vários estudos, com o alto rio Tocantins possuindo o maior número absoluto de táxons endêmicos da bacia Amazônica. Aqui, fornecemos uma acurada revisão sobre o conhecimento da ictiofauna da bacia do rio Tocantins-Araguaia entre 2000-2020; uma extensa lista das espécies válidas de peixes ocorrentes na bacia, assim como uma discussão sobre as maiores ameaças para sua ictiofauna. Dados sobre a diversidade da ictiofauna foram refinados por meio de buscas na internet. O mapeamento das Áreas Protegidas e das hidrelétricas foi realizado utilizando os arquivos de área ou coordenadas fornecidas pelas agências governamentais responsáveis. 751 espécies de peixes são atualmente conhecidas para a bacia do rio Tocantins-Araguaia. Um aumento considerável no conhecimento sobre a diversidade de peixes ocorreu nos últimos 20 anos em paralelo com significativas alterações antrópicas na bacia e seu entorno. As represas ao longo da bacia são consideradas a maior ameaça à ictiofauna. Embora a região hidrográfica abrigue diversas unidades de conservação e terras indígenas, estas não têm sido suficientes para garantir a preservação das espécies de peixes. Nossa compilação enfatiza que o alto rio Tocantins precisa ser considerado como área prioritária para a conservação das espécies de peixes. Algumas ações mitigatórias, que podem atingir resultados satisfatórios em relação à conservação da ictiofauna, são também propostas.
Palavras-chave: *Bacia Amazônica; Cerrado; Conservação; Diversidade; Ictiofauna.*

Introduction

The Neotropical fish fauna is extremely rich, harboring 20 to 25% of world freshwater fish diversity. More than 6,000 known species and an expected 2000–3000 species left to be described in the Neotropics (Reis et al. 2016; Malabarba & Malabarba 2020). Most of this diversity is in the Amazon River basin, home to the richest freshwater ichthyofauna on Earth, with more than 2,700 known species (Dagosta & de Pinna 2019). The Tocantins-Araguaia River basin is the largest Brazilian exclusive basin (MMA 2006; ANA 2020). Two thirds of its waters drain the Cerrado domain (IBGE 2014) to discharge directly to the Atlantic Ocean, in the Amazon domain. The composition of the ichthyofauna of the Tocantins-Araguaia River basin is traditionally considered closely related to the Amazon basin, especially in its lower course (Goulding et al. 2003). Recently, most of the composition of the Tocantins-Araguaia fish fauna was proposed as more closely related to Amazon-draining Brazilian Shield rivers, forming a biogeographical region together with the Xingu, Tapajós, and some shield tributaries of the rio Madeira (Lima & Ribeiro 2011; Dagosta et al. 2020). According to these authors, the upper Tocantins and the upper Araguaia represent two smaller bioregions of the Amazonian fish fauna. Although with fish richness knowledge far from complete and sharing a number of species with other Amazonian rivers, the high degree of endemism of the Tocantins-Araguaia River basin is corroborated in several studies (*e.g.* Santos et al. 2004; Hubert and Renno 2006; Lucinda et al. 2007; Abell et al. 2008; Bertaco & Carvalho 2010; Carvalho et al. 2010; Bertaco et al. 2011; Hales and Petry 2013; Dagosta and de Pinna 2017, 2019). In fact, the upper rio Tocantins holds the largest absolute number of endemic taxa within the Amazon basin (Dagosta & de Pinna 2017, 2019) and the Araguaia River, the major fluvial artery of central Brazil and the Amazon-Cerrado ecotone, is home to more fish species than any other basin in the Cerrado (Latrubesse et al. 2019).

The Cerrado consists of tropical savannah mainly along the northern slop of the Brazilian Shield. It is well documented that the Cerrado is among the most threatened domains in the American continent (*e.g.* Silva & Bates 2002; Strassburg et al. 2017; Latrubesse et al. 2019; Colli et al. 2020). Concerningly, the rich and endemic ichthyofauna from the Tocantins-Araguaia River basin is also under severe threats from anthropic action, which has increased in the last two decades with the construction of several dams, expansion of agriculture and mining, introduction of exotic species, and waterway projects (*e.g.* Claro-Garcia & Shibatta 2013; Lees et al. 2016; Lima et al. 2016; Akama 2017; Pelicice et al. 2014; Pelicice et al. 2017; Latrubesse et al. 2019; Dagosta et al. 2020; Pereira et al. 2020; Perônico et al. 2020; Azevedo-Santos et al. 2021; Pelicice et al. 2021). Along with the huge hydropower plants advanced, the area drained by the Tocantins-Araguaia River basin is inserted on the newest Brazilian agricultural frontier, the MATOPIBA region. This new frontier was created from the Republic Presidency decree (nº. 8,447/2015), which provides for the Brazilian agricultural development plan in regions of the States of Maranhão, Tocantins, Piauí, and Bahia; whose intensive occupation for agricultural production began in the 1980s and has been increasing (Araújo et al. 2013; Barros & Stege 2019). Several reports have indicated major changes to fish species composition as a result of dams, an increase in species considered at risk, and a reduction of commercial species as a result of restriction and even loss of migratory species (*e.g.* Santos et al. 2004; Lucinda et al. 2007; Mérona et al. 2010; Bartolette et al. 2017; ICMBio 2018; Perônico et al. 2020). On the other hand, the scientific knowledge of the ichthyofauna from the Tocantins-Araguaia River basin and the consequences of the aforementioned environmental alterations are far from satisfactory (*e.g.* Hunke et al. 2014; Akama 2017; Pereira et al. 2020), although an increase of the number of publications investigating fish species under the influence of dams is notable. Pereira et al. (2020) highlighted gaps of scientific research on the matter, particularly involving the potential cumulative impacts of dams on phylogenetic diversity, and they pointed to the need of studies focusing on these areas. According to Agostinho et al. (2009), the growth in the number of hydroelectric dams was faster than that of scientific knowledge about ecological aspects of the Tocantins-Araguaia River basin, including its fish fauna. Therefore, the disparity between the rate of scientific knowledge of diverse aspects of the ichthyofauna and the increasing threats in this basin needs to be evaluated. A review of the knowledge of the ichthyofauna of the Tocantins-Araguaia River basin in the last 20 years is provided in the present study. We aim to evaluate the growing knowledge of the ichthyofauna in parallel with the increase in anthropic alterations in the basin and its surroundings. Additionally, a list of valid fish species occurring in the basin is also provided and the major threats to its ichthyofauna is discussed.

Materials and Methods

1. *Study area*

As the name states, the Tocantins-Araguaia River basin is composed mainly of the Tocantins and Araguaia rivers. The hydrographic region is the largest basin located entirely in the Brazilian territory, comprising 918,273 km^2 (about 11% of the Brazilian territory), encompassing the States of Goiás, GO (26.8%), Tocantins, TO (34.2%), Pará, PA (20.8%), Maranhão (3.8%), Mato Grosso, MT (14.3%), and the Federal District, DF (0.1%). Most of the basin is located in the Midwest region, where its headwaters are formed. Downstream from the confluence of the Tocantins and Araguaia rivers, the basin enters the North region until its mouth (MMA 2006; ANA 2020).

The Tocantins River is formed by the das Almas and Maranhão rivers, constituting one of the main rivers in the Cerrado of Central Brazil. From its headwaters, in the Goiás Plateau, about 1,000 m of altitude, to its mouth in the Atlantic Ocean, this river runs about 2,400 km. Major right margin tributaries are the Bagagem, Tocantinzinho, Paranã, dos Sonos, Manoel Alves, and Farinha rivers; major left margin tributaries are the Santa Teresa, Araguaia and Itacaiúnas rivers (MMA 2006; ANA 2020). Traditionally, the Tocantins River is divided in three stretches: the upper Tocantins, which extends from its headwaters to the Lajeado rapids, 1,060 km and an elevation change of 925 m (about 0.87 m/km); the median Tocantins, between Lajeado rapids and São João do Araguaia waterfalls, 980 km and an elevation change of 149 m (about 0.15 m/km); and the lower Tocantins, that runs from São João do Araguaia until its mouth, 360 km and an elevation change of 26 m (about 0.07m/km) (Paiva 1982; Agostinho et al. 2009; ANA 2020). However, although the aforementioned traditional division of the Tocantins River is widely used, here we follow what was proposed by Dagosta & de Pinna (2019), which divided the entire system in three stretches: lower Tocantins (downstream of Imperatriz, State of Maranhão, MA, and Itaguatins, State of Tocantins, TO), upper Tocantins (upstream Imperatriz and Itaguatins), and Araguaia (include upper, median, and lower stretches).

The upper course environment is frequently composed of rapid waters and waterfalls, while the lower course, especially downstream of Tucuruí, has a low gradient, which allow the formation of large backwaters and flood plains (Paiva 1982; Agostinho et al. 2009).

The Araguaia River, the principal tributary of the left margin of the Tocantins River, is 2,600 km long and originates in the Brazilian central Plateau. The biggest fluvial island in the world is located in the Araguaia River system, the Ilha do Bananal (350 km long by 80 km wide). The Araguaia River runs parallel to the Tocantins River, until they meet at the city of Marabá, Patá State (Goulding et al. 2003; ANA 2020).

The Tocantins River originally had many rapids and waterfalls environments, because of that it has been a target of the hydroelectric sector and several projects of this type have been implemented in the last decades. On the other hand, the geography of the Araguaia River basin, without major differences in altitudes or accidents, has been preferred for waterways and agriculture.

2. *Ichthyofauna diversity*

The list of fishes occurring in the Tocantins-Araguaia River basin includes species described after the last update provided by Dagosta and de Pinna (2019) and others that were missing in their study, with updates on synonyms and species occurrence. New records were based on the ichthyological collection of Universidade Federal do Tocantins and literature sources (*e.g.* Bichuette & Trajano, 2003; Miranda & Mazzoni, 2003; Benedito-Cecílio et al. 2004; Agostinho et al. 2009; Lima & Caires 2011; Lucinda et al. 2007; Soares et al. 2009; Silva et al. 2019; and taxonomic descriptions). Authorships of the species analyzed are available in the supplementary material (see Supplementary file 1).

Since *Apareiodon machrisi*, *Archolaemus blax*, *Astyanax goyacensis*, *Leporinus bimaculatus*, and *Stictorhinus potamius* were recorded in basins other than Tocantins-Araguaia by Dagosta & de Pinna (2019) they were not considered herein as endemic to the Tocantins-Araguaia River basin. In the list, the subfamily category was applied only for Characidae and Loricariidae, which are the most species-rich families. Classification follows Nelson et al. (2016) for orders and the Eschmeyer's Catalog of Fishes for families (Fricke et al. 2020). Families and subfamilies were organized in alphabetical order. Endangered species were screened from the most recent list of the Threatened Brazilian Fauna (Fishes) (ICMBio 2018). For the distribution map of these species, we included type localities based on the original descriptions plus additional distribution data provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). These data were obtained during the assessment process of Brazilian fauna using the Red List method led by the ICMBio between 2009 and 2014 (ICMBio 2018).

3. *Data survey*

To gather information about the ichthyofauna from the Tocantins-Araguaia River basin over the last 20 years, a search of the literature published between January 2000 and December 2020 was conducted on the following research platforms: Google Scholar, Scientific Electronic Library Online (SciELO), and Web of Science (Thomson Reuters). In addition, Eschmeyer's Catalog of Fishes (Fricke et al. 2020) was consulted to confirm the validity of the species. Searches for taxonomic studies including new taxa and/or taxonomic review articles were conducted with the words: "new species AND [order] AND Tocantins AND/OR Araguaia''; "Taxonomic review AND [order]"; "Taxonomic revision AND [order]", in which all orders listed for the basin were included: Myliobatiformes, Anguiliformes, Osteoglossiformes, Clupeiformes, Characiformes, Gymnotiformes, Siluriformes, Batrachoidiformes, Gobiiformes, Cichliformes, Beloniformes, Cyprinodontiformes, Synbranchiformes, Pleuronectiformes, Acanthuriformes, and Tetraodontiformes. For each article were recorded: number of species described, author(s), year, type-locality, coordinates of the holotype, stretch of occurrence (upper Tocantins, lower Tocantins, and/or Araguaia), species with occurrence in other basins, and journal where it was published. Literature searches for phylogenetic studies were conducted using the following word combinations: "Phylogeny OR Systematic Phylogeny OR Systematic OR Phylogenomic AND [order]". In [order], were included all orders listed above. Only articles that analyzed specimens or samples of tissue of vouchers from the Tocantins-Araguaia River basin were recorded. Search for ecological studies were conducted using the following words combination: "Araguaia-Tocantins River Basin Fish Ecology"; "Tocantins Fish Ecology"; "Araguaia Fish Ecology"; "Fish communities Tocantins"; "Fish communities Araguaia"; "Fish assemblages Tocantins Basin"; "Fish assemblages Araguaia Basin"; "Ichthyofauna Inventory Tocantins"; "Ichthyofauna Inventory"; "Feeding habits Fish Tocantins"; "Feeding Habits fish Araguaia"; "Trophic Guilds Fish Tocantins"; "Trophic Guilds Fish Araguaia"; "Reproductive habits fish Tocantins"; and "Reproductive habits Araguaia". The literature search included both articles and books/or book chapters.

4. *Hydroelectric plants*

Information on hydroelectric plants in operation through December 2020 was taken from databases of the Agência Nacional de Energia Elétrica (ANEEL 2020). Dams were categorized as follows: Hydroelectric Plant Station (UHE), with energy production capacity between 5.000 and 50.000 KW greater than 30 MW and requires granting authorization or concession large reservoirs; Small Hydroelectric Central (PCH), with production capacity between 5.000 and 30.000 MKW, and reservoirs of up to 13 km^2; and Hydraulic Power Plant (CGH), with generation capacity of up to 5.000 MKW, with or without dams but without a reservoir (ABRAPCH 2020; ANEEL 2020). The map was created based on these categories and localities (coordinates) supplied by ANEEL's data records (ANEEL 2020).

5. *Mapping of Protected Areas (conservation units, CUs and indigenous lands, ILs)*

All conservation units (CUs) in which total or partial area is included in the Tocantins-Araguaia hydrographic region were plotted (regardless of category). Thus, CUs from both Cerrado and Amazon domains were included. Area shape files (.kml) for CUs and ILs were obtained from the ICMBio website (https://www.icmbio.gov.br/portal/unidadesdeconservacao/biomas-brasileiros). Some State CUs that were missing in the ICMBio shape files, were also included. For CUs without shape area files, a single point was plotted at the city/area of occurrence.

6. *Institutional abbreviations*

Auburn University (AU), Museu Paraense Emílio Goeldi (MPEG), Museu de Zoologia da Universidade de São Paulo (MZUSP), Universidade Estadual de Londrina (UEL), and Universidade Federal do Pará (UFPA).

Results

1. *Ichthyofauna diversity and conservation*

The ichthyofauna of the Tocantins-Araguaia River basin is composed of 751 species, in 314 genera, 51 families, and 16 orders (Supplementary file 1). The most species-rich orders are Characiformes (303 species, 40.3% of the ichthyofauna), Siluriformes (249 species, 33.2%), and Cichliformes (58 species, 7.7%) (Figure 1a). The most representative families are Characidae (138 species, 18.4%), Loricariidae (86 species, 11.5%), and Cichlidae (58 species, 7.7%), respectively (Figure 1b).

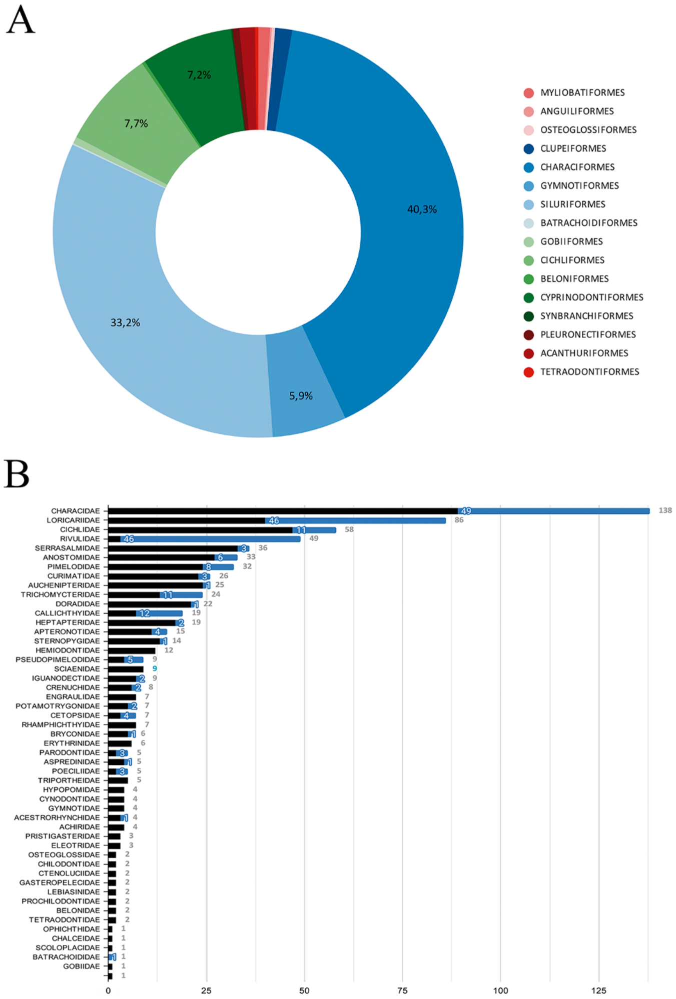

Figure 1. Freshwater fish species recorded per **A.** order in the Tocantins-Araguaia River basin, and **B.** family, where total number of species in gray, non-endemic species in black, endemic species in blue.

There are 229 fish species endemic to the Tocantins-Araguaia River basin from 26 families, corresponding to 30.5% of the total number of fish species from the basin (see examples of endemic species in Figure 2). Characidae is the family with the highest absolute number of endemic species (49 of 138 species, 35.5%), followed by Rivulidae (46 of 49, 93.9%) and Loricariidae, with (46 of 86, 53.5%). It is important to highlight the case of *Hypsolebias brunoi* (Costa), with type locality said to be at "Brazil: Estado de Goiás: temporary pool near the city of Vila Boa, ribeirão Canabrava floodplains, upper rio Urucuia drainage, rio São Francisco basin (15°0'0.4"S, 47°04'3.3"W; 449 m above sea level)" (see Costa 2003, pg.55). However, coordinate data indicate that it occurs in the upper portions of the Tocantins River basin. If this is correct, its type locality is at Canabrava River at the Tocantins-Araguaia River basin and, as far as we know, the species is only known from the type material. Considering each river stretch, the largest absolute number of endemic species is that of the upper Tocantins, which harbors 91 endemic species, followed by the Araguaia River and lower Tocantins stretches, with 69 and 21 endemic species exclusive to each, respectively.

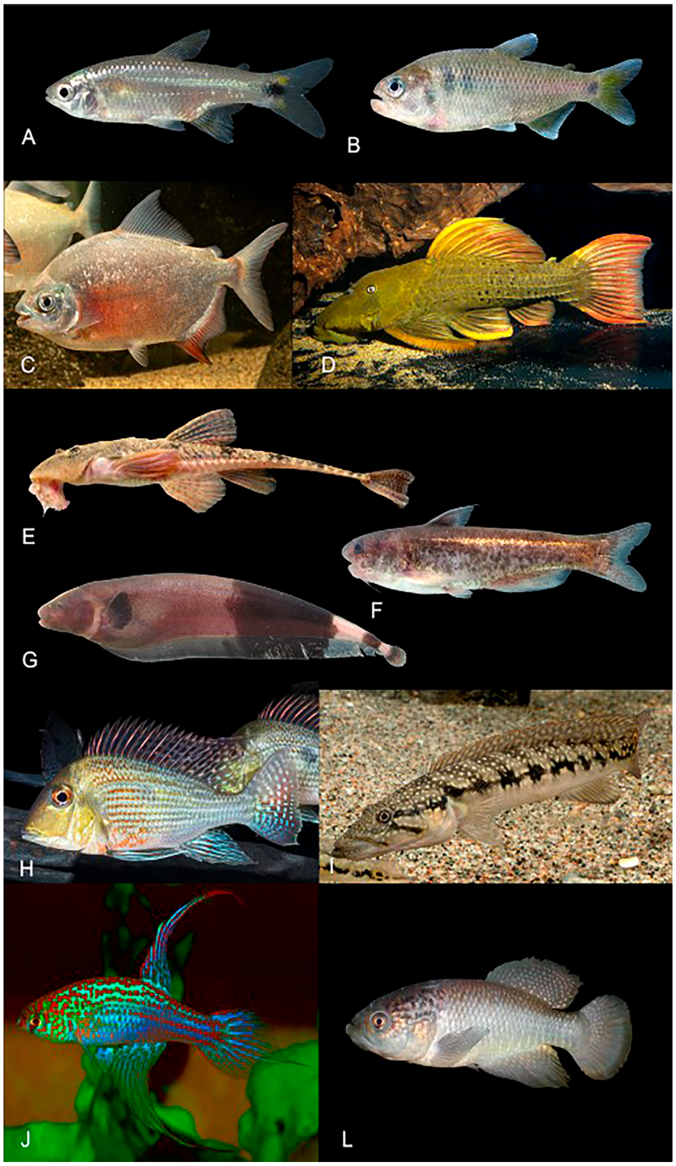

Figure 2. Some species endemic to the Tocantins-Araguaia River basin. **A.** *Ctenocheirodon pristis* MZUSP 113680, upper Tocantins River at Monte Alegre De Goiás, **B.** *Moenkhausia dasalmas* MZUSP 113910, upper Tocantins River at Alto Paraíso de Goiás, **C.** *Mylesinus paucisquamatus*, aquarium specimen not preserved, **D.** *Pseudacanthicus pitanga*, aquarium specimen not preserved, **E.** *Rineloricaria osvaldoi* MZUSP 114137, upper Tocantins River at Arraias, **F.** *Cetopsis arcana* MZUSP (uncatalogued), **G.** *Apteronotus camposdapazi*, MZUSP 114134, upper Tocantins River at Arraias, **H.** *Geophagus sveni*, aquarium specimen not preserved, **I.** *Crenicichla jegui*, aquarium specimen not preserved, **J.** *Maratecoara lacortei*, aquarium specimen not preserved, **L.** *Cynolebias griesei*, aquarium specimen not preserved. Photos by José Birindelli (A, B, E, F, G), Oliver Lucanus (C, D, H, I), and André Carletto (J, L).

Regarding endangered fish fauna, 51 threatened species occur in the Tocantins-Araguaia River basin according to the Brazilian Red List (ICMBio 2018). Of them, 47 (92.1%) are endemic to the basin. A list of threatened species from the Tocantins-Araguaia River basin, categorized according to the International Union for Conservation of Nature (IUCN) methodology, with distribution data, type-locality, and main threats is provided in Table 1. Regarding the IUCN threat categories, 22 species (43.1%) are considered vulnerable (VU); 22 (39.2%) endangered (EN), and nine (17.6%) critically endangered (CR). The majority of the threatened species belong to the orders Cyprinodontiformes (22, 43.1%) and Siluriformes (19, 37.3%). Regarding families, most threatened species belong to Rivulidae (22, 43.1%), Loricariidae (seven, 13.7%), Trichomycteridae (five, 9.8%), and Pimelodidae (four, 7.8%). Other families are represented by less than three species (6%). Threatened fish species in the basin are mostly from the upper Tocantins River (27, 52.9%).

Several threatened species belong to genera represented by only one species in the basin, such as: *Aguarunichthys* Stewart, *Cynolebias* Steindachner, *Mylesinus* Valenciennes, *Potamobatrachus* Collette, *Rhynchodoras* Klausewitz & Rössel, *Roestes* Günther, *Sartor* Myers & Carvalho, *Scobinancistrus* Isbrücker & Nijssen, *Simpsonichthys* Carvalho, and *Teleocichla* Kullander. In the case of *Potamobatrachus*, *P. trispinosus* Collette is the unique representative of the order Batrachoidiformes in the basin. Genera with few representatives in the basin include *Baryancistrus* Rapp Py-Daniel and *Lamontichthys* Miranda Ribeiro (two species each, both threatened); *Pimelodella* Eigenmann & Eigenmann and *Trigonectes* Myers (two species each, one threatened); *Maratecoara* Costa (three species, two threatened). Thus, 42 of 51 (82.3%) threatened species have restricted genera diversity, most of them occurring in the upper Tocantins River stretch.

Table 1. List of threatened fish species from the Tocantins-Araguaia River basin with IUCN category, distribution along the basin, type-locality, and main threats to each one. Species with (*) are additionally found outside the basin: *Brycon nattereri* (Paraná River), *Hyphessobrycon coelestinus* (upper São Francisco River), *Rhynchodoras xingui* (Xingu River), and *Scobinancistrus pariolispos* (Xingu and Tapajós rivers). Sources of information include ICMBio (2018), original descriptions, and Catalog of Fishes/CAS (Fricke et al., 2020).

Threatened species	IUCN Category	Distribution	Type-locality	Main threats
CHARACIFORMES				
Anostomidae				
Sartor tucuruiense Santos & Jégu, 1987	EN	Lower	Tucuruí, PA	UHEs Tucuruí and Lajeado
Bryconidae				
Brycon gouldingi Lima, 2004	EN	Upper/Lower/Araguaia	Parauapebas, Serra dos Carajás, PA	Successive dams along the species distribution, sport and commercial fishing
Brycon nattereri Günther, 1864 (*)	VU	Upper	Oriçanga, SP	Dams and deforestation of ciliary forests
Characidae				
Hyphessobrycon coelestinus Myers, 1929 (*)	EN	Upper	Lagoa Bonita,São Bartholomeu, GO	Urban expansion
Serrasalmidae				
Mylesinus paucisquamatus Jégu & Santos, 1988	EN	Upper/Lower	Jatobal, PA	Successive dams along the species distribution, mining
Cynodontidae				
Roestes itupiranga Menezes & Lucena, 1998	VU	Lower	Itupiranga, Lago Grande, PA	UHEs Tucuruí and Marabá
SILURIFORMES				
Doradidae				
Rhynchodoras xingui Klausewitz & Rössel, 1961 (*)	EN	Upper Tocantins/Araguaia	Upstream of Xingu River	Successive dams along the species distribution
Heptapteridae				
Pimelodella spelaea Trajano, Reis & Bichuette, 2004	EN	Upper	São Bernardo Cave, São Domingos, GO	Non-organized tourism in the cave area, trampling and silting the river
Loricariidae				
Ancistrus cryptophthalmus Reis, 1987	EN	Upper	Passa Três cave, São Domingos, GO	Non-organized tourism in the cave area, trampling and silting the river
Ancistrus minutus Fisch-Muller, Mazzoni & Weber, 2001	EN	Upper	Córrego Batéias, Minaçu, GO	UHE serra da Mesa, urban and agriculture expansion, mining
Baryancistrus longipinnis (Kindle, 1895)	CR	Upper/Lower/Araguaia	Tocantins River	Successive dams along the species distribution
Baryancistrus niveatus (Castelnau, 1855)	CR	Upper/Lower/Araguaia	Araguaia River, GO	Successive dams along the species distribution
Lamontichthys avacanoeiro Paixão & Toledo-Piza, 2009	EN	Upper	Serra da Mesa, GO	Successive dams along the species distribution
Lamontichthys parakana Paixão & Toledo-Piza, 2009	CR	Lower	Tucuruí, PA	UHE Tucuruí
Scobinancistrus pariolispos Isbrücker & Nijssen, 1989 (*)	VU	Lower Tocantins/Araguaia	Jatobal, PA	Successive dams along the species distribution

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Threatened species	IUCN Category	Distribution	Type-locality	Main threats
Pimelodidae				
Aguarunichthys tocantinsensis Zuanon, Rapp Py-Daniel & Jégu, 1993	EN	Upper/Lower/Araguaia	Rapids above Marabá, PA	Successive dams along the species distribution
Pimelodus halisodous Ribeiro, Lucena & Lucinda, 2008	VU	Upper	Paranã River, Fazenda Traçadal, Paranã, TO	Successive dams along the species distribution
Pimelodus joannis Ribeiro, Lucena & Lucinda, 2008	VU	Upper	Ipueiras, TO	Successive dams along the species distribution
Pimelodus sterwartii Ribeiro, Lucena & Lucinda, 2008	VU	Upper	Paranã River, Fazenda Traçadal, Paranã, TO	Successive dams along the species distribution
Pseudopimelodidae				
Microglanis robustus Ruiz & Shibatta, 2010	CR	Lower	Jatobal, Tucurí, PA	UHEs Tucuruí
Trichomycteridae				
Ituglanis bambui Bichuette & Trajano, 2004	CR	Upper	Angélica Cave, Parque Estadual da Terra Ronca, São Domingos, GO	Non-organized tourism in the cave area, trampling and silting the river
Ituglanis epikarsticus Bichuette & Trajano, 2004	VU	Upper	São Mateus Cave, Parque Estadual da Terra Ronca, São Domingos, GO	Non-organized tourism in the cave area, trampling and silting the river
Ituglanis mambai Bichuette & Trajano, 2008	EN	Upper	Lapa do Sumidouro Cave, Posse, GO	Non-organized tourism in the cave area, trampling and silting the river
Ituglanis passensis Fernandez & Bichuette, 2002	VU	Upper	Passa Três cave, São Domingos, GO	Non-organized tourism in the cave area, trampling and silting the river
Ituglanis ramiroi Bichuette & Trajano, 2004	VU	Upper	São Bernardo Cave, Parque Estadual da Terra Ronca, GO	Non-organized tourism in the cave area, trampling and silting the river
BATRACHOIDIFORMES				
Batrachoididae				
Potamobatrachus trispinosus Collette, 1995	EN	Lower Tocantins/Araguaia	Jatobal, PA	UHE Tucuruí
CICHLIFORMES				
Cichlidae				
Crenicichla cyclostoma Ploeg, 1986	CR	Lower	Tucuruí, PA	UHE Tucuruí, UHE Santa Isabel (preview)
Crenicichla jegui Ploeg, 1986	EN	Lower	Itupiranga, PA	UHE Tucuruí, UHE Santa Isabel (preview), possible illegal ornamental exportation
Teleocichla cinderella Kullander, 1988	EN	Lower Tocantins/Araguaia	Tucuruí, PA	UHE Tucuruí, UHEs Santa Isabel and Marabá (preview)

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Threatened species	IUCN Category	Distribution	Type-locality	Main threats
CYPRINODONTIFORMES				
Rivulidae				
Cynolebias griseus Costa, Lacerda & Brasil, 1990	CR	Upper	Nova Roma, GO	Urban expansion
Hypsolebias flammeus (Costa, 1989)	EN	Upper	Arraias, TO	UHE Paranã
Hypsolebias marginatus (Costa & Brasil, 1996)	CR	Upper	Barro Alto, GO	Pastures and/or agriculture expansion
Hypsolebias multiradiatus (Costa & Brasil, 1994)	CR	Upper	Brejinho de Nazaré, TO	UHE Lajeado
Hypsolebias notatus (Costa, Lacerda & Brasil, 1990)	EN	Upper	Alvorada do Norte, GO	Urban expansion
Hypsolebias tocantinensis Nielsen, Cruz & Baptista, 2012	EN	Upper	Lajeado River, Campestre do Maranhão, MA	Pastures and/or agriculture expansion
Maratecoara formosa Costa & Brasil, 1995	VU	Upper	Brejinho de Nazaré, TO	UHE Lajeado, Pastures and/or agriculture expansion
Maratecoara splendida Costa, 2007	VU	Upper	Canabrava River, between Alvorada and Peixe, TO	Pastures and/or agriculture expansion
Melanorivulus crixas Costa, 2007	VU	Araguaia	Crixás Mirim River, Nova Crixás, GO	Pastures and/or agriculture expansion
Melanorivulus karaja (Costa, 2007)	VU	Araguaia	Tributary to rio Dueré River, Formosa River drainage, TO	Pastures and/or agriculture expansion
Melanorivulus kayapo (Costa, 2006)	VU	Araguaia	Upper Caiapó River, Jataí GO	Pastures and/or agriculture expansion
Melanorivulus kunzei Costa, 2012	VU	Araguaia	Upper Caiapó River, Jataí, GO	Pastures and/or agriculture expansion
Melanorivulus litteratus (Costa, 2005)	VU	Araguaia	Ribeirão do Sapo, Araguaia River, MT	Pastures and/or agriculture expansion
Melanorivulus pindorama Costa, 2012	VU	Upper	Small tributary to Gameleira River, Sono River, TO	Pastures and/or agriculture expansion
Melanorivulus planaltinus (Costa & Brasil, 2008)	VU	Upper	Coca River floodplains, Planaltina de Goiás, GO	Pastures and/or agriculture expansion
Melanorivulus rubromarginatus (Costa, 2007)	VU	Araguaia	Stream tributary to Espingarda River, Peixe River drainage, GO	Pastures and/or agriculture expansion
Melanorivulus salmonicaudus (Costa, 2007)	VU	Araguaia	Crixás River Mirim, Nova Crixás, GO	Pastures and/or agriculture expansion
Melanorivulus ubirajarai Costa, 2012	VU	Araguaia	Tributary of Babilônia River, Mineiros, GO	Pastures and/or agriculture expansion
Plesiolebias canabravensis Costa & Nielsen, 2007	VU	Upper	Canabrava River floodplains, TO	Pastures and/or agriculture expansion
Plesiolebias xavantei (Costa, Lacerda & Tanizaki, 1988)	EN	Upper	Tocantins River, Porto Nacional, TO	UHE Lajeado, UHE Ipueiras (preview), Pastures and/or agriculture expansion
Simpsonichthys cholopteryx Costa, Moreira & Lima, 2003	EN	Araguaia	Ribeirão do Sapo, MT	Pastures and/or agriculture expansion
Trigonectes strigabundus Myers, 1925	EN	Upper	Porto Nacional, TO	UHE Lajeado, extraction of clay and gravel, urban expansion

2. *Knowledge on ichthyofauna*

Studies on the ichthyofauna from the Tocantins-Araguaia River basin over the last 20 years were published in a total of 278 articles and five books, including species descriptions and taxonomic reviews (123 articles), phylogenetic studies (60 articles), species inventory (14 articles and five books), and ecological studies (53 articles). Between January 2000 and December 2020, 185 new species of fishes were described based on material from the Tocantins-Araguaia River basin. Of them, 167 (90.3%) are currently considered endemic to the system. Recently described species are from seven orders, 25 families, and 78 genera. The most representative orders were Siluriformes (73 species, 39.5%), Characiformes (67, 36.2%), and Cyprinodontiformes (31, 16.8%); other orders were less representative (less than 5%) (Figure 3a). Most representative families were Characidae (50 species, 27%), Loricariidae (35, 18.9%), and Rivulidae (29, 15.7%); while other families were less representative (less than 5%) (Figure 3b). In addition to the 185 new species described from the system, 29 species originally described from other basins had their distributions later expanded to the Tocantins-Araguaia River basin.

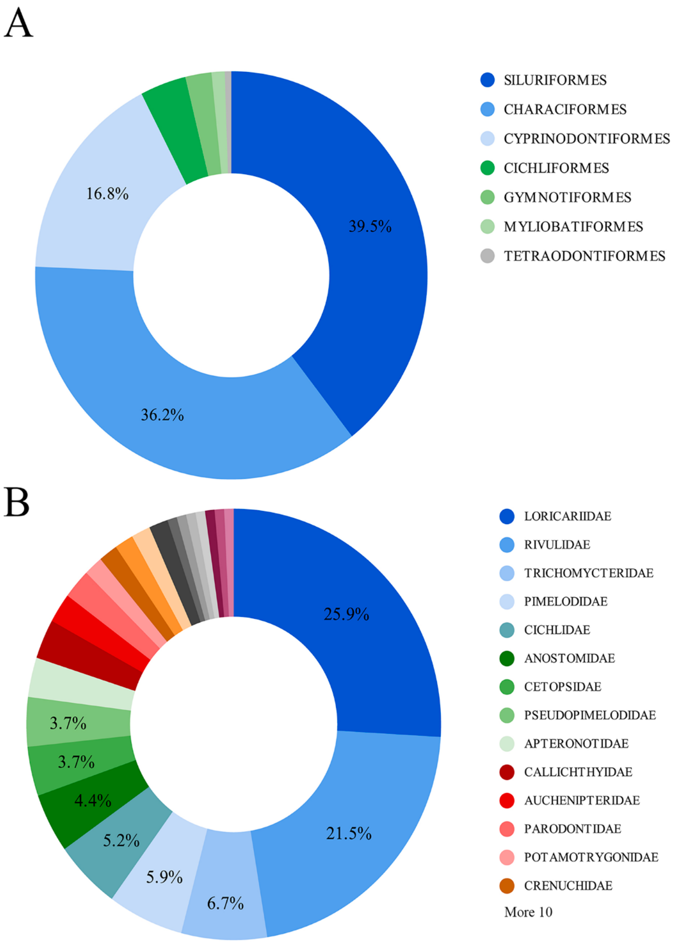

Figure 3. Described species in the last 20 years from the Tocantins-Araguaia River basin per **A.** order, and **B.** family.

The upper Tocantins River is the stretch with the highest number of species described in the last 20 years (Figure 4a). Of the 185 species described, 91 (49.2%) were described based on material from that stretch, corresponding to 24.7% of the total species richness of that basin. That preponderance is followed in descending order by the Araguaia River stretch (42 species, 22.7%), the lower Tocantins River stretch (17, 9.2%), the upper Tocantins plus Araguaia River stretches (15, 8.1%), the upper plus lower Tocantins River stretches (10, 5.4%), the whole system (nine, 4.9%), and the lower Tocantins plus Araguaia stretches (one, 0.5%). Since 2001, at least five species were described for the whole system per year, except in 2019 (four species). Higher numbers of species descriptions were observed in 2005, 2007, and 2008 with 14 species described per year, followed by 2003 and 2010 with 13 species described each year, and 2016 with 12 species described (Figure 4b). Species descriptions were published in a total of 22 journals, concentrated mainly in four: Neotropical Ichthyology (49 articles with new species, 26.5%), Zootaxa (40, 21.6%), Ichthyological Exploration of Freshwaters (37, 20%), and Ichthyology & Herpetology (formerly Copeia) (14, 7.6%). Other journals are less representative, with less than eight articles with species description (less than 4%).

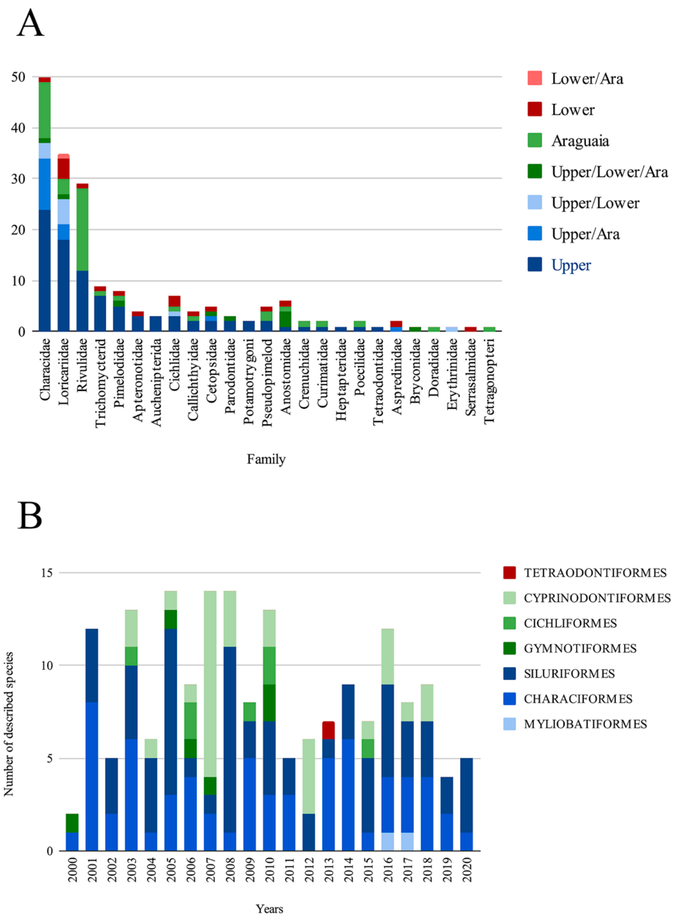

Figure 4. Described species in the last 20 years at Tocantins-Araguaia River basin per **A.** family and stretches, and **B.** order and year.

Ecological studies on the fishes from the Tocantins-Araguaia River basin published in the last 20 years included the following subjects: general ecology and community studies (19 articles, 35.8%), dam effects (14, 26.4%), feeding habitats and/or reproductive biology (10, 19.9%), fisheries (five, 9.4%), and others (five, 9.4%). Most ecological studies were conducted in the upper Tocantins River stretch (37, 69.8%), followed by lower Tocantins (nine, 17%), and Araguaia stretches (seven, 13.2%).

3. *Major threats to the ichthyofauna*

Considering all main threats to the endangered species of fishes from the Tocantins-Araguaia River basin, the major threat is the hydroelectric plants. Twenty-six of 51 (50.9%) of endangered species are directly affected by dam construction (Figure 5a). The second major threat is habitat loss by expansion of pastures and/or agriculture activities (affecting 15 species, 29.4%), followed by unorganized tourism activities (seven, 13.7%), which primarily affect cave species, and by urban expansion (three, 5.9%). Mining activities and sport and commercial fishing were also listed as threats for endangered fish species from this basin. Concerning mining activities, most of them are concentrated in the Carajás region (Silva et al. 2014), which could directly impact the Itacaiunas River basin. Another important factor is that the basin has always been considered for the use of its main watercourses with axis for waterway transport (PNE 2030).

There are 73 hydroelectric plants in operation along the Tocantins-Araguaia River basin, of which, eight are UHEs, 29 are PCHs, and 36 are CGHs, distributed in five Brazilian states (Goiás, Tocantins, Mato Grosso, Maranhão, and Pará). Most hydroelectric plants (43) are located in the upper Tocantins River stretch (seven UHEs, 20 PCHs, and 16 CGHs), 29 are located in the Araguaia River stretch (nine PCHs and 20 CGHs), and only one in the lower Tocantins River stretch (UHE Tucuruí) (Figure 5b, Supplementary file 2).

The majority (60%) of hydroelectric power generation projects in the Tocantins-Araguaia River basin started to operate in the last 20 years. The UHE Tucuruí (lower stretch, Pará State), the largest enterprise in the system, is the first significant dam in operation since 1984. The UHE São Domingos (upper stretch, Goiás State) started operations in 1991 and, in 1998, UHE Serra da Mesa (upper stretch, Goiás State), the second largest of the basin, started operations. Since then, five others UHEs are now in operation: Lajeado (upper stretch, Tocantins State) since 2001, Cana Brava (upper stretch, Goiás State) since 2002; Peixe Angical (upper stretch, Tocantins State) since 2006, São Salvador (upper stretch, Tocantins State) since 2009; and Estreito (limit of lower and upper stretch, Maranhão State) since 2011. Further five dams are under construction or will be built in the short term in the Tocantins-Araguaia River basin: four PCHs in the upper Tocantins and one CGH in the Araguaia stretch. In addition to those already in operation or under construction, there are 51 new hydroelectric projects being studied for possible implementation in the Tocantins-Araguaia River basin, being 42 PCHs (21 in Tocantins River and 21 in Araguaia River) and nine UHEs (seven in the Tocantins and two in the Araguaia).

4. *Protected areas (CUs and ILs)*

According to our survey, there are at least 41 CUs in the Tocantins-Araguaia hydrographic region (Figure 5a, Supplementary file 3). Among them, 13 are of integral protection, *i.e.* units conserved free from human interference, where only the indirect use of their natural attributes are allowed (see SNUC - Brazilian law 9,985/2000). Most of the CUs (28) are located along the Tocantins River and six along the Araguaia River.

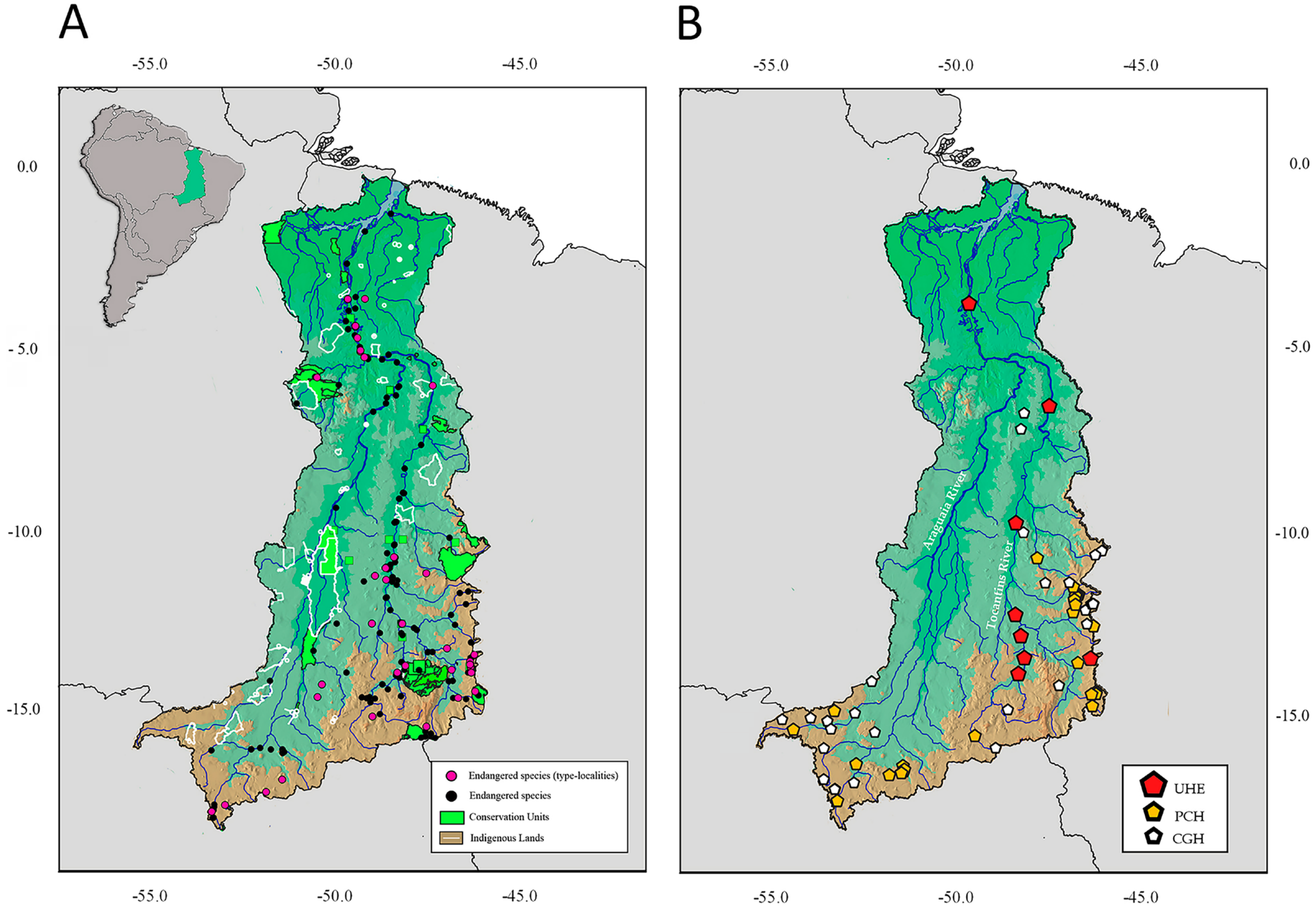

Figure 5. Distribution maps of **A.** endangered species*, Conservation Units, and Indigenous Lands, and **B.** hydroelectric plants already installed at Tocantins-Araguaia River Basin. (*) One point could represent more than one locality.

Concerning CUs along the Tocantins River stretch (28), half of them (11) are distributed among areas near the Federal District area (PARNA de Brasília, APA Planalto Central), areas further north in Goiás State (PARNA Chapada dos Veadeiros; and RPPNs Soluar, Serra do Tombador I and II) and the remaining four in the east corner of the basin (FLONA Mata Grande, PES Terra Ronca, RESEX Recanto das Araras de Terra Ronca, and APA Nascentes do rio Vermelho). To the north, along the Tocantins River course, there are sequential but disconnected CUs, most of them created as compensation to the UHEs implemented in the region (APAs Lago de São Salvador, Lago de Peixe Angical, Lago de Palmas and Serra do Lajeado). To the east of Palmas city, there are other important CUs such as PES do Jalapão, ESEC Serra Geral do Tocantins, and PARNA Nascentes do rio Parnaíba. Information about CUs are summarized in Supplementary file 3. Furthermore, there are at least 36 Indigenous Lands along the Tocantins-Araguaia River basin, 26 of them along the Araguaia River and 13 along the Tocantins River, in which six are in the upper and seven in the lower stretches of this river.

Discussion

1. *Building knowledge on fish diversity*

The actual ichthyofaunal composition of the Tocantins-Araguaia River basin, as well as the whole Amazon basin, is a result of historical geomorphological processes and landscape changes (Dagosta & de Pinna 2019) that, together with ecological factors and evolutionary processes, molded the great fish diversity we find nowadays. Dagosta & de Pinna (2019) conducted an extensive study of distribution and biogeographical patterns of Amazon fishes and recorded 705 species for the entire Tocantins-Araguaia River basin. Our results show an absolute number of 751 species, which corresponds to 27.6% of the species richness of the whole Amazon basin (2,716 species according to Dagosta & de Pinna 2019) and 23.8% of the Brazilian freshwater fish species (3,148 according to ICMBio 2018). Interestingly, exotic and/or invasive species were not recorded in the literature as a substantial problem in the Tocantins-Araguaia River basin. For instance, *Oreochromis niloticus* (Linnaeus) has been detected in the basin, but it has not since been recaptured and the species is not established, probably as a consequence of the high biotic resistance provided by the elevated diversity of the system (Agostinho et al. 2017).

The fishes from the Tocantins-Araguaia River basin are more diverse than those from entire geographic regions such as Oceania, Central America, and Europe (see Dagosta & de Pinna 2019). Furthermore, our data show that 22% of the entire ichthyofauna of this basin is endemic, corroborating its high degree of endemism repeatedly mentioned in the literature (*e.g.* Santos et al. 2004; Hubert & Renno 2006; Lucinda et al. 2007; Abell et al. 2008; Bertaco & Carvalho 2010; Carvalho et al. 2010; Bertaco et al. 2011; Hales & Petry 2013; Dagosta & de Pinna 2017, 2019).

The outstanding number of species records in the Tocantins-Araguaia River basin is continuously increasing due to new species descriptions. According to our results, 185 new species were described from the basin in the last 20 years, and another 20 probably new species are in the process of description by ourselves and other Brazilian taxonomists (*e.g.* Guilherme Dutra, Naércio Menezes, 2020, pers. comm.). Such an increase of species description in the last 20 years could be explained by multiple factors. One of them is the increase of ichthyofaunal inventories, which are required as part of the documentation necessary for the installation of hydroelectric plants and other enterprises.

In the Tocantins-Araguaia system, 60% (five of eight) of the UHEs were installed in that period, and all of them in the upper stretch of the basin (ANNEL 2020). Among the 185 described species in the last 20 years, 43 (23.2%) descriptions were based on material (considering only holotype and/or paratypes) from inventory or monitoring associated to UHEs installed in the Tocantins River basin, since there is no UHE in the Araguaia River basin. Considering only the upper Tocantins stretch, where most impoundments were installed, this number increased considerably to 37 (40.6%) descriptions out of 91 species described for this stretch.

Another determining factor is certainly the existence of a higher number of taxonomists examining fishes from the basin. Until 2015, there was high incentive from the Brazilian government for research in taxonomy, such as the Support Program for Research Projects for Capacity and Training of Human Resources in Biological Taxonomy (PROTAX) and the Biodiversity Research Program (PPBio), both supported by the Ministério da Ciência, Tecnologia e Inovações (MCTI). In addition, in that period, there were many public tenders as a response to the demand created by the Support Program for Federal University Restructuring and Expansion Plans (REUNI), which ensured that many taxonomists settled in the northern university centers in the country (MEC 2020).

Although these incentives have been essential to the increase in taxonomists and teaching/research institutions in the country, the current situation of Brazilian science is worrying. The drastic reduction of the research budget proposed by the Brazilian government in 2019 caused significant financial cuts in the MCTI, which directly affected support for Brazilian research (see Escobar, 2019; Santos & Carbayo, 2021). According to recent data from the Instituto de Pesquisa Econômica Aplicada (IPEA), investments in science and technology have been systematically reduced in the last few years (De Negri & Koeller 2019; Santos & Carbayo, 2021) and the forecast for next years is for more budget cuts (Sociedade Brasileira para o Progresso da Ciência; portal.sbpcnet.org.br, consulted in Dec 2020 and Jan 2021). Environmental policies are also catastrophic, the Ministério do Meio Ambiente (MMA) has been operating on an extremely reduced budget. In addition, there are several procedures that impact the local environment directly, such as end of land demarcations and permission to mine in Indigenous Lands; flexible environmental licensing; dismantling of environmental defense agencies; changes in the Forest Code and increased allowance for the use of hazardous pesticides. Unfortunately, this scenario represents a setback for the growth of Brazilian science, which could have negative consequences for the formation and settlement of taxonomists in general (see Santos & Carbayo, 2021) including ichthyologists.

2. *Major environmental impacts affecting ichthyofauna*

Endangered species of the Tocantins-Araguaia River basin are directly affected by two major threats: dam constructions and habitat loss by expansion of pastures and/or agriculture activities (Table1).

2.1. Dams

Many developing countries, such as Brazil, have adopted economic developmental policies based on major infrastructure works (Latrubesse et al. 2017; Winemiller et al. 2016). The Brazilian option for hydroelectric energy can be explained by the great hydroelectric potential still available in the nation (Moretto et al. 2012; Serra & Oliveira 2020). Currently, there has been a great expansion of hydroelectric projects in the midwest and northern regions by virtue of the large volume of water discharge of the drainage channels, because rivers in these regions maintain minimum flows for the supply reservoirs throughout the year (Barletta et al. 2010; Moretto et al. 2012; Fearnside 2015; Serra & Oliveira 2020). According to the Brazilian National Energy Plan (PNE 2030), the total energetic potential of the Tocantins-Araguaia River basin is 11,297 MW, however just about 8% of this potential could be utilized without environmental restrictions. The Brazilian Ten-Year Energy Expansion Plan (PDE 2017-2021) included UHE Marabá (PA, made operational this year) and other hydroelectric plants that are approved or in viability studies, such as UHEs Ipueiras (TO), Serra Quebrada (TO/MA), and Tupirantins (TO). In the PDE 2021-2030, the scenario is even more drastic with five hydroelectric plants potentially being built in the tributaries: UHE Buriti Queimado (Almas River, GO), UHEs Maranhão and Porteiras (Maranhão River, GO), UHEs Mirador (Tocantizinho River, GO), and Paranã (Paranã River, TO).

The increased demand for electricity and the option to produce it using water sources has led to the exploration not only of large water bodies, but also to a recent increase in exploring new possibilities, such as small rivers and tributaries (Barletta et al. 2010; Frederico et al. 2021). Although the damming of rivers is one of the main human activities that cause the reduction of fish diversity, there are several gaps in the knowledge on the biological impact of small dams when compared to large ones (Pereira et al. 2020).

In addition to the large dams on the Tocantins River, the Tocantins-Araguaia River basin has many smaller dams installed on its upper stretch (Figure 5b), and several others are under study to be installed in the basin (ANEEL 2020). These small dams are often located in streams which generally harbor restricted-range species of fishes, and which may be important bioindicators of anthropogenic changes in environments. When are installed in sequence, these smaller dams may create a cascade effect, which increases negative environmental impacts in the structure and function of fish communities (Alexandre & Almeida 2010; Pereira et al. 2020; Teresa & Casatti 2017).

Regardless of size and complexity, the presence of a barrier is often associated with changes in the physical structure of rivers, mainly causing the homogenization of several micro-habitat characteristics such as current speed, depth, and substrate among other changes (Alexandre & Almeida 2010). Consequently, according to the authors, any change in habitat stability can alter the life cycle of fish species and the local structuring of their assemblages. Lees et al. (2016) carried out a survey of studies with generalized impacts for several aquatic and terrestrial taxa across the Amazon lowlands, such as the habitat loss and degradation, regional climate changes stimulated by deforestation and accentuated by increased methane output. Specifically, regarding fish assemblages, drastic alterations are notorious, because the environmental changes occur right after the reservoir filling phase, with a reduction time of water renewal and the consequent transformation of a lotic environment to a lentic ecosystem (Agostinho et al. 2007).

Migratory fish perform seasonal migrations to spawn, which requires free stretches of rivers. The reproductive success of these species is related to access to free-flowing spawning areas upstream (in the main channel or tributaries) and nursery areas (downstream floodplain) (Agostinho et al. 2008). The construction of physical barriers blocks the fish movement, preventing the dispersion, isolating populations and breaking the sequence of displacements and stimuli necessary for reproduction for migratory species (Barthem et al. 1991; Agostinho et al.2005; Barletta et al. 2010; Pelicice et al. 2014). Successive dams along the river are even worse, interrupting the migration routes for migratory fish species, which are unable to complete their life cycle, which leads to large decrease in their populations or even local extinctions (Ribeiro et al. 1995; Lees et al. 2016).

Particularly within the upper Tocantins River stretch, Perônico et al. (2020) demonstrate that fish diversity patterns changed significantly after construction of the Peixe Angical dam, with several shifts in the taxonomic assemblages in the first five years after the impoundment. These authors reported a significant change in composition and abundance of the fish fauna, for example, a total of 27 species of migratory fishes were recorded in the stretch. However, 23 of these species were recorded before the river regulation and only 12 after seven years of the impoundment. Abundance of migratory fish also declined consistently (87%) and several species that were abundant, before the impoundment, had their abundance decline by 90% or even completely disappeared (*e.g. Argonectes robertsi*, *Hemisorubim platyrhynchus*, *Myleus setiger*, *Myleus torquatus*, *Pimelodus blochii*, *Pinirampus pirinampu*, *Prochilodus nigricans*, *Oxydoras niger*, and *Rhaphiodon vulpinus*).

For the lower Tocantins River stretch, Santos et al. (2004) reported a decline in the population of 22 commercial species of fishes after the Tucuruí dam construction. Later, Mérona et al. (2010) concluded that the Tucuruí Lake formation resulted in major changes in the composition of fishes in the extension of the river studied, and also demonstrated that the disruption of migratory routes is one of the main factors that negatively affected the fish community. The presence of the dam accentuated the isolation of the river downstream, preventing displacement upstream of migratory species for reproduction and limiting the recolonization of the lower portions of the river by juveniles dispersing from the upstream area.

Fishways were employed as a main solution in several impoundments to mitigate impacts over the migration and dispersion dynamics, especially for long distance migratory species. In the Tocantins River, fish ladders were installed in Lajeado and Peixe Angical dams (Pompeu et al. 2012). However, studies have demonstrated that these ladders are not effective and fail to support the downstream and upstream passage for both migratory and non-migratory fishes (*e.g.* Agostinho et al. 2007, 2012; Pelicice & Agostinho 2012). In fact, there is no scientific evidence that any fish passage existing plays an efficient role in fish and fisheries conservation (Pompeu et al. 2012; Pelicice et al. 2014).

2.2. Agribusiness

Undoubtedly, agribusiness plays a huge importance to the Brazilian economy and it has been a great challenge to reconcile environmental conservation with economic development. The Cerrado domain is the largest and richest Neotropical savanna, considered a hotspot conservation area (Myers et al. 2000). Despite accounting for 30% of Brazilian biodiversity, only a small portion of the Cerrado is protected (Françoso et al. 2015).

The expansion of commodity monocultures and pastures is ranked as the major cause of Cerrado deforestation and land degradation, while hydroelectric plants and urban expansion constitute secondary issues (Faleiro et al. 2013).

The Tocantins-Araguaia River basin holds about 90% of its area in this domain, with the remaining 10% composed of the ecotone area between the Cerrado and Amazon domains. In the year 2000, there were 52,259,267.15 ha (56.9%) of natural forest (including forest and savanna formations) in the Tocantins hydrographic region, in 2019, this area was reduced to 43,479,134.27 ha (47.3%). On the other hand, the agricultural area grew from 29,323,418.73 ha (31.9%), in 2000, to 38,473,772.61 ha (41.9%), in 2019 (Souza et al. 2020).

In the heart of Cerrado, at the MATOPIBA region, soybean agriculture expanded 253% from 2001 to 2014 (Carneiro-Filho & Costa 2016) and is expected to expand by 318% by 2050 compared to 2015 (Soterrone et al. 2019). In 2018, most of the deforestation of the Cerrado occured in the Tocantins, a state that holds 34.2% of the Tocantins-Araguaia River basin, with 153,320 ha, 23% of total forest loss. A total of 947,287 ha of natural Cerrado formations were converted to soy cultivation and livestock pastures between 2008 and 2017, with soy and beef being the main commodities that lead to deforestation in Tocantins State in the period (Drost et al. 2019).

Deforestation reflects directly in hydrology through the soil erosion and silting of the rivers and loss of natural riparian forests related to surface temperature increase. Other concerns about land transformation for agricultural practices is the change of pH, density and availability of P and K in the soil (Hunke et al. 2014; Latrubesse et al. 2019). Hunke et al. (2014) reviewed field studies in Cerrado areas, between 1977 and 2012, and found that most soil and water parameters were affected by land use changes caused by crops, specifically parameters related to soil hydraulic properties, such as pH and soil phosphorus content, as well as nutrient (mainly nitrogen) and pesticide contamination in surface waters. Such changes in water parameters directly affects fish fauna (Dala-Corte et al. 2016; Teresa & Casatti, 2017). Furthermore, the removal of riparian vegetation affects species adapted to shaded streams that feed mainly allochthonous items provided by the forest (Menezes et al. 2007; Teresa et al. 2015).

3. *Threatened species*

The Tocantins-Araguaia River basin is home to a high number of threatened fish species. According to the most recent list of the Brazilian endangered fishes, 311 continental fish species are considered threatened (ICMBio 2018); 51 of them (16.4%) occur in the Tocantins-Araguaia River basin, with 47 endemic to the system and 27 restricted exclusively to the upper Tocantins stretch (Figure 6, Table 1). In addition, it is important to highlight that most of the threatened species (42; 82.3%) have restricted generus-level diversity in the basin, mostly also from the upper Tocantins River stretch. Therefore, it is important to reinforce the area with priority of conservation actions, targeted to ichthyofauna, to avoid loss of phylogenetic and taxonomic diversity.

As mentioned above, dam construction is the main threat for the ichthyofauna. It is responsible for the decrease of populations of most threatened characiforms, siluriforms, and all threatened cichliforms. Rapids and bedrock background environment are crucial for these taxa, such as the anostomid *Sartor tucuruiensis*, the siluriforms *Aguarunichthys tocantinensis* (rare species), *Baryancistrus longipinnis*, *B. niveatus*, *Lamontichthys avacanoeiro*, *L. parakana*, and *Microglanis robustus*; and the cichlids *Crenicichla cyclostoma*, *C. jegui*, and *Teleocichla cinderella*.

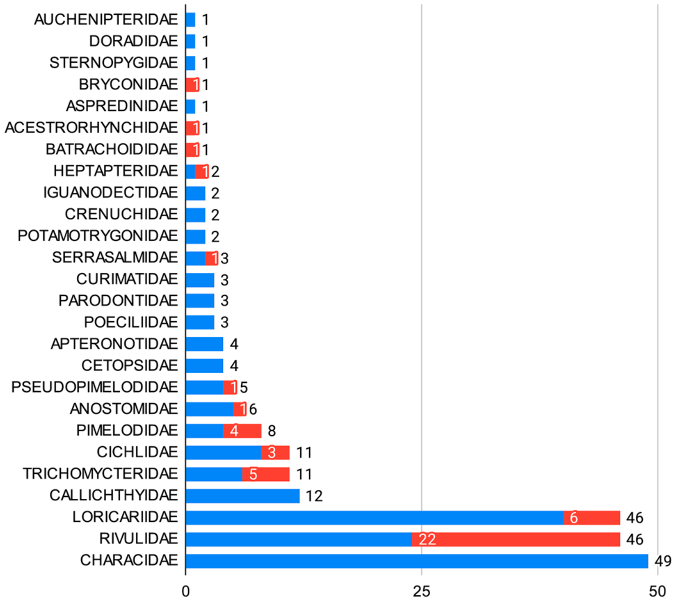

Figure 6. Total of species endemic to the Tocantins-Araguaia River basin per family. Number of threatened species are shown in red.

All these species were reported from strong rapids. In the case of *M. robustus*, despite the species description being relatively recent, only the type specimens collected before the construction of the UHE Tucuruí reservoir are known (Ruiz & Shibatta 2010). However, no sample effort has been made since then to evaluate occurrence, abundance, and conservation status of the species (ICMBio 2018). Similarly, *L. parakana* was described in 2009 based solely on three specimens (Paixão & Toledo-Piza 2009) collected in 1984, when the Tucuruí reservoir was filled. Since then, the species was exclusively manually collected by diving under about 18 m deep in 2019, in a field expedition to Pedral do Lourenço (Marabá, PA). Other threatened species (Figure 7) such as *Baryancistrus longipinnis*, *Crenicichla jegui*, *Potamobatrachus trispinosus*, *Sartor tucuriensis* and *Teleocichla cinderella*, were also collected in the same event. (Alberto Akama, 2020, pers. comm.). The Pedral do Lourenço seems to be the main bedrock refuge to these species in the lower Tocantins and is in danger of being channelized to make the river navigable during the dry season.

In addition, migratory species were also affected by dam construction in the basin of interest, resulting in large decreases in their populations. The three threatened species of *Pimelodus* (*P. halisodous*, *P. joannis*, and *P. sterwartii*) are short to medium distance migratory species (Tiago Costa e Silva, 2020, pers. comm.). Today, they are uncommon and not abundant, with restricted occurrence to the upper Tocantins River (Ribeiro et al. 2008; ICMBio 2018), a stretch directly affected by the UHEs Peixe Angical, São Salvador, and Lajeado dams. Species of the order Characiformes are also impacted by the disruptive routes, such as *Brycon* spp. and *Mylesinus paucisquamatus*. The latter is considered rare, with just 81 specimens reported along 200 km in the upper Tocantins stretch over a decade of monitoring between 1998 and 2009 (Victorino Júnior et al. 2016).

The agribusiness plus urban expansion are responsible for the population decrease of most rivulids occurring in the Tocantins-Araguaia River basin and the characid *Hyphessobrycon coelestinus* (Table 1). Rivulidae includes 22 (43%) of the threatened species in the basin.

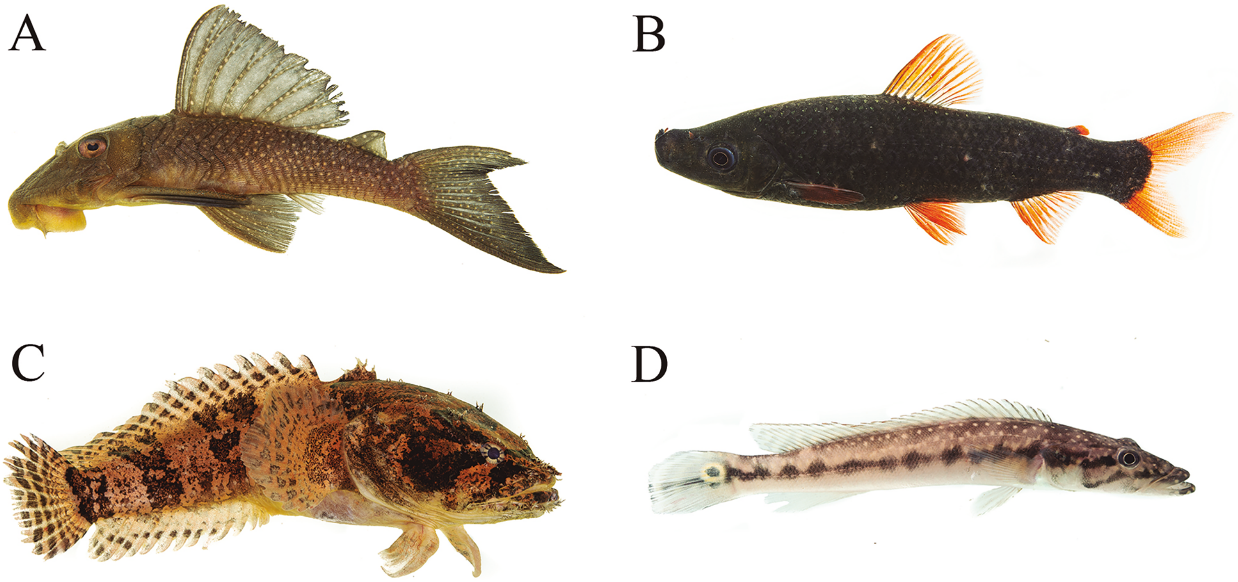

Figure 7. Some threatened species from Pedral do Lourenço, lower Tocantins River. **A.** *Baryancistrus longipinnis*, **B.** *Sartor tucuruiensis*, **C.** *Potamobatrachus trispinosus* and **D.** *Crenicichla jegui.* All images by Leandro Sousa.

Rivulids are highly adapted to seasonal ponds and floodplains that completely dry out during the dry season, with an annual life-history strategy with eggs resistant to drought and embryonic development with up to three diapauses, phases where development and growth is reversibly suspended (Loureiro et al. 2018). This means that rivulids may complete their life cycle within a single, seasonal pond, that depends on the vegetation to be formed in the rainy season. Deforestation for pasture, agriculture, urbanization, and dam construction has caused habitat loss and threatened 44.8% of all rivulid species of the Tocantins-Araguaia basin. In fact, the high endemism of the group along with strong human impacts impelled Rivulidae to be the most endangered fishes not only in the system, but in Brazil (Rosa & Lima 2008; Volcan et al. 2011; ICMBio 2018). Thus, conservation actions to protect annual fishes should be directed not only to their restricted range area, but also to create large protecting areas that encompass river corridors and associated wetlands, to enable connectivity and dispersal populations (Volcan et al. 2011).

Remaining threatened species are cave inhabitants impacted by unorganized tourism in these areas. All of them are from the karst area of Mambaí and São Domingos region (Goiás State), that is formed by subterranean streams of the Vermelho River, tributary of Paranã River (upper Tocantins River). This region hosts seven threatened siluriforms species: *Ancistrus cryptophthalmus* (Loricariidae), *Ituglanis bambui*, *I. epikarsticus*, *I. mambai*, *I. passensis*, and *I. ramiroi* (Trichomycteridae), and *Pimelodella spelaea* (Heptapteridae). Concern about conservation policies in the region were expressed by Bichuette & Trajano (2008) and Rizzato & Bichuette (2014), specifically for *Ituglanis boticario*, which occurs in the same region of *I. mambai*, but at a smaller population density. The species is not considered threatened, possibly because the most recent list (ICMBio 2018) was made after the species description.

According to Trajano (2000), subterranean ecosystems have special issues for conservation due to their fragility and distinctive features, such as the high degree of endemism associated with morphological, ecological, and behavioral differences among stygobiotic Such fragility is an outcome of the low biological diversity of these ecosystems, which generally rely on nutrients from the surface and the susceptibility to climatic fluctuations because these animals have evolved in a relatively stable environment. Thus, in karst areas other threats, besides deforestation, have to be considered, such as limestone quarrying, pollution from mining, pesticides and even domestic sewage, as well as human visitation due to topoclimatic changes by hot light sources and the opening of artificial passages during cave exploration and management, favoring the introduction of alien epigean organisms and organic matter, soil compaction of sediment banks and direct disturbance and trampling of cavernicole organisms (Trajano 2000).

4. *Protect Areas at the Tocantins-Araguaia River basin*

Conservation units play an essential role in the efforts to reduce degradation of natural environments and maintaining biodiversity (ICMBio 2020; Oliveira et al. 2017). Historically, conservation efforts and policies are concentrated in land habitats and terrestrial vertebrates. However, freshwater ecosystems are even more sensitive and are among the most threatened environments in the world (Azevedo-Santos et al. 2019; Abell & Harrison 2020; Tagliacollo et al. 2021). Leal et al. (2020) demonstrated that terrestrial target conservation strategies provided limited advantages for freshwater species. When freshwater species are prioritized, more terrestrial species benefit than in the reverse. This suggests that a terrestrial-freshwater conservation approach provides maximum achievable benefits rather than targeting only one domain.

As defined by the National Nature Conservation Units System (SNUC, Brazilian law 9,985/2000), Biodiversity or Ecological Corridors are instruments of management and planning in order to secure the integrity of the ecological processes in the CUs areas of connection, allowing the free gene flow and dispersal between these protected natural areas (ICMBio 2020). Although the idea of Ecological Corridors has been considered by the MMA since 2000, projects such as the Ecological Corridor of the Jalapão area (which include PES do Jalapão, ESEC da Serra Geral do Tocantins, and PARNA Nascentes do rio Parnaíba) and the Araguaia Corridor of Biodiversity, have not been fully implemented (Latrubesse et al. 2019; ICMBio 2020).

Along the Araguaia River course, five CUs (APA Meandros do rio Araguaia, PARNA Araguaia, PES do Cantão, and RPPNs Canguçu and Bico do Javaés) could be included in the project of the Araguaia Corridor. This project aims to interlink CUs with Indigenous lands (at least 11), that will guarantee more protection of the Ilha do Bananal, the Araguaia floodplain, and the wetland of the rio das Mortes. Meanwhile, the Ecological Corridor of the Jalapão Region aims to connect National and States CUs located in three important headwaters drainages: Tocantins-Araguaia, Parnaíba, and São Francisco River basins (ICMBio 2020). The importance of these projects is mainly to reinforce the conservation of these regions' ecosystems and strengthen CUs integration. It is important to highlight that the Araguaia River holds 26 of the 36 Indigenous Lands of the basin, most of them near Ilha do Bananal. It means that these lands are probably more efficient to protect the ichthyofauna than the CUs along the basin because this stretch holds 17.6% of the endangered species.

The Tocantins-Araguaia River basin is known for its high endemic ichthyofauna (*e.g*. Lima & Caires 2011; Dagosta & de Pinna 2019). Our results demonstrate that most endangered species are endemic to the upper Tocantins River stretch (27 species, 52.9%). Although most CUs (25) are also located in the same stretch, most threatened species occur outside these areas (Figure 5a). Previous studies have demonstrated that threatened or almost threatened species are most commonly found outside formally protected areas (Azevedo-Santos et al. 2019; Tagliacollo et al. 2021). Thus, either these CUs are not properly inventoried, or they are not efficient in ensuring the preservation of threatened species. In fact, Oliveira et al. (2017) have demonstrated that only 1% of total Protected Areas (including CUs and ILs) in Brazil are well sampled, with 50% of them not sampled at all. Furthermore, the largest absolute number of endemic species is from the upper Tocantins stretch, (91 out of 229 species, 39.7%). Therefore, it is crucial that ichthyofaunal surveys are carried out within the CUs in the upper Tocantins River stretch to make sure that threatened species and endemic species are being protected.

For example, the Mambaí karsts area is inserted from the limit of the PES Terra Ronca (São Domingos, GO) up to the APA das Nascentes do rio Vermelho (Mambaí, GO), where most threatened cavefish species occur. Although the region includes these two CUs, it has not been effective to protect the subterranean systems and the epigean areas nearby (Rizatto & Bichuette 2014), since unorganized tourism still represents a substantial risk to cavefishes.

Likewise, the presence of CUs along the basin seems to not be very effective in protecting migratory Siluriformes (*e.g. Pimelodus* spp.), Characiformes (*e.g. Brycon gouldingi*, *Roestes itupiranga*), and other species that depends on free river stretches, as cichlids (*e.g.*, *Crenicichla* spp., *Teleocichla cinderela*), loricariids (*e.g. Ancistrus minutus, Lamonthichthys* spp., *Scobinancistrus pariolispos*), and pseudopimelodids (*e.g. Microglanis robustus*) because the already installed UHEs constitute a major risk to these species (see more on the UHEs discussion section). Conversely, for annual rivulid species, expansion of CUs, as well as new projects to connect CUs in Ecological Corridors, specially between free stretches of the Paranã and Tocantins rivers, will contribute to the preservation of the floodplains and, thus, ensure that these species can complete their life cycles.

Conclusions

A considerable increase in fish diversity knowledge in the Tocantins-Araguaia River basin occurred in the last 20 years, in parallel with major changes in the basin and its surroundings in the same period. More than a half (54.9%) of the threatened species were described between 2001 and 2012. Meanwhile, considerable urban and agricultural expansion, along with several hydroelectric plants were installed in the basin during this period. The process to get permission to construct dams that includes samplings and some ichthyofaunal reports has contributed, in some way, in generating knowledge on the fish fauna, but unfortunately the environmental alterations due to those enterprises are far more disastrous to the fish community. In view of the anthropic environmental alterations already present in the Tocantins-Araguaia River basin discussed herein, together with the available knowledge on the high endemicity and highly threatened nature of the fish fauna, some mitigation actions should be implemented to avoid an irreversible loss of species. Actions should include: compliance to the environment legislation (*e.g.* Forest Code); compliance to the Indigenous Lands demarcations; expanding soy moratorium for the Cerrado; full implantation of planned Ecological Corridors, with a terrestrial-freshwater conservation approach (see Leal et al. 2020); establishment of new Ecological Corridors in the upper Tocantins-Araguaia basin; preservation of free stretches of rivers for migratory fish routes, especially in the upper Tocantins River; raising awareness of freshwater fish diversity and ecological services (provided by these ecosystems) to the population in general; and increase in investment in science, particularly on biodiversity studies. In addition, actions directed to aquatic biodiversity conservation that were proposed by WWF-Brasil (2016) in the Tapajós River basin, could be implemented in the Tocantins-Araguaia River basin. Among these actions is the Systematic Conservation Planning (PSC) approach, which is based on an information and analysis system that identifies priority areas for conservation and indicates free river stretches that are crucial to maintain the natural flow regimes. Within this context, the Brazilian government should proceed with an integrated strategic plan that defines scenarios and indicators on the conservation state of large rivers and their main tributaries, as well as define a set of rivers to be preserved before the accumulation of countless hydroelectric plants generates disastrous and irreversible impacts.

Supplementary Material

The following online material is available for this article:

Supplementary file 1 - List of 751 freshwater fish species from the Tocantins-Araguaia River basin and their occurrence. X: occurrence according to a taxonomic publication or to direct exam of specimens in collections by Dagosta & de Pinna (2019) or by us, S: secondary literature source (*e.g.* inventories), ?: doubtful occurrence, R: species restrict/endemic to the Tocantins-Araguaia river basin, E. Endangered species.

Supplementary file 2 - List of the enterprises in operation at the Tocantins-Araguaia River basin. All data collected from ABRAPCH (2020) and ANEEL (2020). Brazilian States: GO = Goiás, MA = Maranhão, MT = Mato Grosso, PA = Pará, and TO = Tocantins.

Supplementary file 3 - List of the conservation units at the Tocantins-Araguaia River basin. All data collected from ICMBio and ISA (https://uc.socioambiental.org/pt-br). CUs of integral protection include: National Parks (PARNA), State Parks (PES) Biological Reserves (REBIO), Ecological Estation (ESEC) and Natural Monument (MN). CUs of sustainable use include: Protect Environment Areas (APA), Extractivist Reserves (RESEX), National Forest (FLONA) and Natural Heritage Private Reserves (RPPN). Brazilian States: BA = Bahia, GO = Goiás, MA = Maranhão, MT = Mato Grosso, PA = Pará, PI = Piauí, TO = Tocantins, and Federal district, DF = Distrito Federal, ha = hectare.

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Priscila Camelier: Contribution to the concept and design of the study, data collection, data analysis and interpretation, the manuscript preparation and to critical revision, adding intelectual content.

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Ilana Fichberg: Contribution to the concept and design of the study, data collection, and to critical revision, adding intelectual content.

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Conflicts of Interest

The authors declared no conflict of interest.

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Relative condition factor and predictive model for the presence of the invasive snail *Achatina* (*Lissachatina*) *fulica* in Sergipe, Northeast Brazil

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Abstract: *Achatina fulica* is among the world's 100 most impactive invasive species, and is now found in almost all Brazilian states, including Sergipe. This exotic snail is known to have negative impacts, not only on the environment, due primarily to the rapid growth of its populations, but also on public health, given that it is an intermediate host of nematodes that cause zoonotic diseases. However, relatively little is known of the development of this snail, including its relative condition factor. We investigated the occurrence of *A. fulica* in 24 municipalities distributed in the eight subregions of the state of Sergipe in the dry and rainy season. Furthermore, we present here a predictive model for the occurrence of *A. fulica* based on the variation in climate and soil chemistry. This snail was more frequent on soils with a pH of 6.5–7.5. A negative correlation was found between the growth of *A. fulica* and the soil pH, then, the more acidic the soil, the more allometric the growth of *A. fulica*. The relative condition factor indicated differences in the development pattern of *A. fulica* among the eight subregions. The influence of rain in increasing the frequency of *A. fulica* showed a significant correlation. As well, higher temperatures influenced the resting behavior of *A. fulica*. The mathematical model used to identify the potential presence of *A. fulica* presented a high degree of agreement. This is the first ecological study of *A. fulica* to verify the association between the body mass-length relationship and the relative condition factor, and the results indicate that the development of this exotic land snail in Sergipe is influenced by climatic factors and principally, the soil pH. The predictive mathematical model provides valuable insights into the biotic and abiotic factors associated with the presence of *A. fulica*, and the influence of climatic variables and the chemical parameters of the soil on the occurrence of this species. These findings provide important guidelines for the development of measures for the control of *A. fulica* populations, which will contribute to both public and environment health.

Keywords: *Achatina fulica*; Invasive exotic snail; Relative Condition Factor; Environmental factors.

Fator de condição relativo e modelo preditivo para a presença do caracol invasor *Achatina* (*Lissachatina*) *fulica* em Sergipe, Nordeste do Brasil

Resumo: *Achatina fulica* está entre as 100 das piores espécies invasoras em todo o mundo, e no Brasil está presente em quase todos os estados, incluindo Sergipe. Este caracol exótico é conhecido por ter impactos negativos, não só no meio ambiente, devido principalmente ao rápido crescimento de suas populações, mas também na saúde pública, uma vez que é um hospedeiro intermediário de nematodeos causadores de doenças zoonóticas. No entanto, pouco se sabe a respeito do desenvolvimento dessa espécie, incluindo o fator de condição relativo. Investigamos a ocorrência de *A. fulica* em 24 municípios distribuídos nos oito territórios do estado de Sergipe no período seco e chuvoso. Além disso, apresentamos aqui um modelo preditivo para a ocorrência de *A. fulica* baseado na variação do clima e da química do solo. Esse caracol é mais frequente no solo com pH de 6,5 à 7,5, sendo esse padrão ideal para o crescimento isométrico. O crescimento alométrico de *A. fulica* apresentou correlação negativa com o pH do solo, quanto mais ácido for o solo, maior será o crescimento de *A. fulica*. O fator de condição de *A. fulica*, apresentou diferença no desenvolvimento nos oito Territórios. A influência da chuva na frequência de *A. fulica* apresentou correlação significativa. Além disso, temperaturas mais elevadas influenciaram no comportamento de repouso de *A. fulica*. O modelo matemático para identificar a possível presença de *A. fulica* apresentou uma concordância forte.

Este é o primeiro estudo ecológico de *A. fulica* a verificar a associação entre a relação massa-comprimento e o fator de condição relativo, sendo possível evidenciar que essa espécie exótica em Sergipe sofre alterações no desenvolvimento, por fatores climáticos e principalmente pelo pH do solo. O modelo matemático preditivo fornece informações valiosas sobre os fatores bióticos e abióticos associados à presença de *A. fulica* e a influência de variáveis climáticas e dos parâmetros químicos do solo na ocorrência desta espécie. Esses achados fornecem importantes diretrizes para o desenvolvimento de medidas de controle de populações de *A. fulica*, que poderão contribuir para a saúde pública e ambiental.

Palavras-chave: *Achatina fulica; Caracol exótico invasor; Fator de condição Relativo; Fatores ambientais.*

Introduction

The Giant African land snail *Achatina* (*Lissachatina*) *fulica* Bowdich, 1822 is native to East Africa, although human interference, combined with the efficient dispersal capacity of the species, has led to its distribution throughout much of the tropical and subtropical regions of the world, including Africa, the Americas, eastern and southern Asia, and Oceania (Thiengo et al. 2007, Silva & Omena 2014). It is considered an invasive species, which is generally found in dense populations and compete for food and space with native snail species (Raut & Barker 2002). This snail also acts as an intermediate host of parasitic nematodes that represent a threat to public health and veterinary medicine. Examples are the nematodes *Angiostrongylus cantonensis* (Chen 1935), an etiological agent of eosinophilic meningitis (EM) in humans (Zanol et al. 2010), and *Aelurostrongylus abstrusus* (Railliet 1898), which causes pneumonia in both domestic and wild felines (Thiengo et al. 2008).

In recent years, several studies have investigated the dispersal, invasion, distribution, and abundance of *A. fulica* in different countries around the world (e.g., Tomiyama 1992, 1993, 1994, Cowie 1998, Zanol et al. 2010, Fontanilla et al. 2014, Sarman et al. 2015, Gbadeyan et al. 2020, Oliveira et al. 2020; Silva et al. 2020). Large-scale eradication programs have been established in many regions, which include the manual collection and destruction of the snails and their eggs (Smith et al. 2013) and the use of traps to capture *A. fulica* (Roda et al. 2018). However, data on the influence of climatic variables and soil chemistry on the development and behavior are still scarce, and little is known of the relative condition factor (KR) of this species (Bolger 1989). The relative condition factor expresses the relationship between body mass and length, which provides important insights into the behavioral features of a species and the influence of biotic and abiotic factors in its development (Le Cren 1951). Albuquerque et al. (2009) concluded that the understanding of the factors that influence the body length and mass of this mollusk, and its condition factor, would provide a valuable tool for the management and control of *A. fulica* populations. The relationship between the body mass and length of a species provides valuable insights into the influence of environmental conditions on the organism and its development stages (Ghisi et al. 2012). The KR parameter also indicates the wellbeing of the individual in its environment and provides the potential for systematic comparison between two or more populations occurring under different conditions (Araujo et al. 2011).

According to Fischer & Colley (2005), abiotic factors, such as the chemical composition of the soil, may also influence the development of *A. fulica* and the establishment of its populations. Raut & Barker (2002) found that this species can exploit different types of soil for the extraction of nutrients, and as a refuge, with the type of soil having both quantitative and qualitative effects on the growth rate of this snail, its size shell, mass and coloration. *Achatina fulica* uses the soil for behaviors such as resting, burrowing and estivation (Fischer 2009), which should be considered for the development of measures for the control and eradication of the species (Roda et al. 2018). Climatic variables should also be considered, given that the frequencies of the different types of its defensive behavior are related directly to relative humidity and rainfall (Miranda et al. 2015, Pilate et al. 2017, Silva et al. 2020).

In 2015, the State Committee for the Control of the African Snail reported the presence of *A. fulica* in 19 municipalities in Sergipe/Brazil (IBAMA 2021). However, few data are available on its development and behavior in northeastern Brazil, much of which is relatively arid, with a long dry season and high temperatures. Given this, understanding the effects of biotic and abiotic factors on the development of *A. fulica* will be essential for the development of predictive models, which can be applied to the evaluation of potential dispersal patterns and demographic parameters (Johnson & Omland 2004). Fischer et al. (2010) consider the ability of *A. fulica* to adapt to different types of habitats to be a major concern and emphasize the need to understand its ecological characteristics in order to develop the most effective strategies for the control of this invasive species. The research into the behavior of this snail will also be important for the development of more effective control strategies, as well as providing parameters for the evaluation of the risks posed by this invasive species for the native land snails of a given area (Pilate et al. 2017).

The present study investigates of the occurrence of *A. fulica* in subregions of the Brazilian state of Sergipe. We try to answer the following questions (i) does it occur in all eight subregions of Sergipe? (ii) is the body mass-length relationship of *A. fulica*, including the relative condition factor, correlated with climatic variables and soil chemistry? (iii) does the development of *A. fulica* vary between the rainy and dry seasons? and (iv) can climatic variables or soil chemistry be used to compile a predictive model of the occurrence of *A. fulica* in Sergipe?

Methods

1. *Study area*

Sergipe is located in northeastern Brazil, and covers an area of 21,925.42 km², with an estimated population of 2,298,696 inhabitants (94.36 individuals/km²) in 2019 (IBGE 2020). The study area comprised 24 municipalities distributed in the eight subregions of the Brazilian state of Sergipe – Greater Aracaju, East Sergipe, the Lower São Francisco River, South Sergipe, the Eastern Plateau, Western Plateau, Central Highlands, and South Central Sergipe (IBGE 2011; see Fig. 1, Appendix S1). The climate in the state of Sergipe is divided into three distinct regions according to temperature and rainfall. Humid tropical region, with high temperature and high humidity along the coast (East); Sub-humid tropical region or drier intermediate region (Agreste), and inland semi-arid region (Semi-arid). The humid tropical region is characterized by high precipitation (average of 1,355 mm/year) and high relative humidity (annual average of 80%). In the Agreste, rainfall is slightly below that observed in the Tropical Humid region, with values around 1,000 mm/year, with a similar monthly distribution. The semiarid region in the interior of the state of Sergipe is considerably drier than the coastal region. The average rainfall is less than 700 mm/year, with values below 30 mm in the summer months (SEMARH, 2010). The 100-year means up to 2012 (Santos & Souza 2018) indicate that the months from April through August are the rainiest of the year in this region, although March and September are also considered to be part of the rainy season, albeit with slightly lower mean precipitation. The dry season proper extends between October and February, although over the past 12 years, the mean precipitation recorded in Aracaju in February was 59±41mm, while that in September was 63±29 mm (INIMET 2021).

2. *Sampling*

The *A. fulica* specimens were collected in February (the end of dry season) and September (end of the rainy season), in 2019 and 2020. The sampling points (plots) were established based on the records of the occurrence of *A. fulica* in Sergipe presented by the state's municipal authorities in the 2015 meeting of the State Committee for the Control of the African Snail during which, 19 municipalities reported the infestation of the urban zone by *A. fulica* (IBAMA 2021). The specimens were collected using the plot method, adapted from Pereira et al. (2015). A 20 m x 10 m plot was established in each of the 24 study municipalities. The plots were demarcated with wooden stakes, which were driven into each corner, with all the *A. fulica* individuals found within the perimeter during a 10-minute search being collected by the researcher.

3. *Environnemental analyses*

During each survey, the characteristics of the environment were noted on a field chart, including data on the weather (sunny, cloudy, rainy), the presence of household garbage., rubble, domestic animals or sewage, the characteristics of vegetation, the upkeep of the area, the characteristics of the soil (humid or dry), the behavior of *A. fulica* (active, resting), and the presence of *A. fulica* eggs (Appendix S2). The meteorological data, that is, the mean monthly temperature, relative humidity, and precipitation were obtained from the automatic and traditional meteorological stations maintained by the Brazilian National Meteorological Institute (INMET) in the different subregions of the state of Sergipe (INMET 2021). The meteorological data presented here refer to the following months: February and September of 2019 and 2020, when the snails were collected in Sergipe.

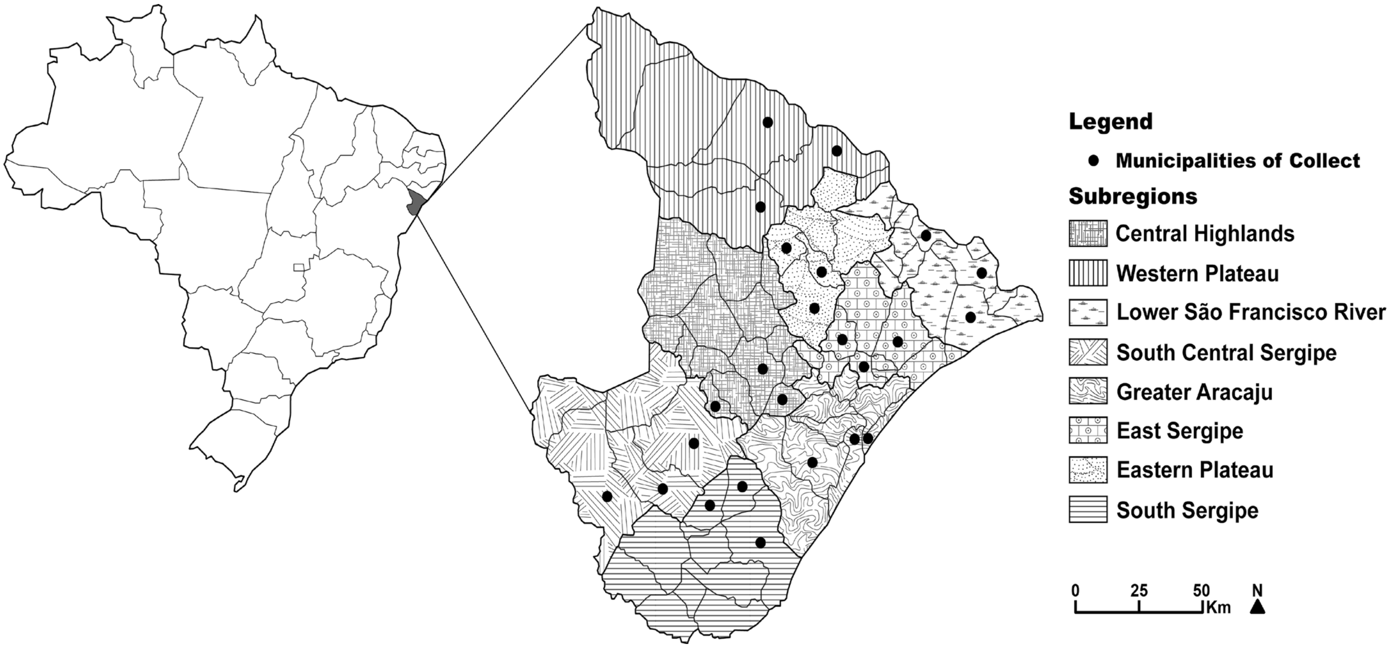

Figure 1. Map of the political divisions of the Brazilian state of Sergipe, showing the municipalities in which the *Achatina fulica* populations were surveyed in 2019 and 2020. The black dots represent the municipalities surveyed in the present study.

Soil samples were collected from each of the 24 plots after the collection of the *A. fulica* specimens. The samples were obtained at a depth of up to 5 cm using a stainless-steel spatula at the four corners of the plot, and from one "variable" point within the plot, to provide a total sample of 500 g. The variable point was selected based on the presence of the *A. fulica* specimens observed within the plot, either buried or in the aggregation phase, when these snails gather together at a single point (Fischer et al. 2012; Almeida et al. 2016). When no *A. fulica* were observed in the plot, the fifth sample was obtained from the center of the plot (Appendix S3). Once collected, the samples were mixed, homogenized and stored in 500-ml pots. The soil samples were analyzed using the method described by Camargo et al. (2009), which consists of the measurement of the pH in water, Calcium (Ca) and organic matter (OM).

4. *Biometry, allometric growth (b), and the relative condition factor*

Achatina fulica specimens were taken to the laboratory, where the total length (Lt) of the shell of each individual was measured using a digital Vernier calliper (0.01 mm precision) and the total mass (Wt) was determined using a digital balance (0.0001 g precision). These data were used to calculate the mean morphometric parameters of the specimens, and to determine the body mass-length relationship and the relative condition factor. The body mass-length relationship is based on the equation $Wt = {}_{a.}\, Lt^{b,}$ where Wt = total mass, Lt = total length, and *a* and *b* are the growth parameters (Le Cren 1951, Bolger & Connoly 1989). The value of the allometric coefficient (*b*) was used to determine whether growth was isometric ($b = 3$), that is, with a symmetrical relationship between the variation in mass and length, positively allometric ($b > 3$), when mass increases more quickly than length, or negatively allometric ($b < 3$), when length increases more quickly than mass (Araujo et al. 2011). The relative condition factor (KR) was then calculated by the equation $KR = M.obs/M.exp$, where *M.obs* = the observed body mass (the weight of the specimen), and *M.exp* = the expected mass, as determined by the slope of the body mass-length relationship. When KR =1, body mass is considered ideal, when KR >1, the individual is above its expected mass, and when KR <1, it is below its expected mass (Le Cren 1951). The *A. fulica* size (shell length) classes were adapted from Fischer & Colley (2005) and Almeida (2013): infants (< 1.00 cm), juveniles (1.01– 4.00 cm), young adults (4.01–7.00 cm) and adults (> 7.00 cm).

5. *Data analysis*

The variation in the relative condition factor (KR) of the *A. fulica* specimens among the eight subregions of the state of Sergipe was analysed using Pearson's nonparametric Chi-square. For the analysis of the numerical variables, the normality of the data was evaluated using the Lilliefors test, associated with the Kolmogorov-Smirnov test, to classify the distribution of each variable as either parametric (homogeneous distribution) or nonparametric (heterogeneous distribution), assigned to four conditions: 1) all the numerical variables that did not group with any independent variable (the condition factor [KR], variation in condition factor, the allometric growth of *A. fulica*, relative humidity and the variation in humidity, the temperature and the variation in temperature, rainfall and the variation in rainfall, the soil pH, calcium, and organic matter); for the conditions (2,3,4) numerical variables were treated as dependent and categorical variables as independent; 2) the association between rainfall and the presence of *A. fulica*, 3) the association between the soil pH and the presence of *A. fulica*), and 4) The relative condition factor, which was analysed per subregion. In the case of condition 1, as all the variables were classified as parametric, the analyses were based on Pearson's parametric correlation coefficient (*r*). The variables in conditions 2 and 3 were classified as nonparametric, so in this case, the analyses were based on the nonparametric Mann-Whitney *U* test. Condition 4 was evaluated using the Kruskal-Wallis nonparametric analysis of variance.

As a significant correlation was observed (condition 1), the soil pH was the variable used to describe the linear equation, based on a simple linear regression, which evaluated the degree of influence and the linear relationship between the soil pH and the allometric growth of the species. A binary logistic regression (Mendes & Veja 2011) was then used to obtain a predictive model of *A. fulica* based on the environmental factors (temperature, relative humidity, rainfall, and soil pH). These variables have been chosen because they showed an influence or correlation value with the categorical variable presence of the species, through principal component analysis (PCA). From then on, the waste disposal model was included in the model, as well as the outlier values, thus avoiding compromising the explanatory power of the model. The result of the equation will steam from the replacement of the letters by their respective values, the result of the equation being 0 and close to 0 corresponding to the absence of *A. fulica*, while values close to 01 or 01 refer to the presence of this snail (being always considered the result module). Regarding the categorical variable (period) it considers the value of 01 for dry and 02 for rainy. The Kappa index was then applied to the results of this equation to compare the model (binary logistic regression) with the empirical results survey.

Results

1. *Characteristics and distribution of Achatina fulica in Sergipe*

The occurrence of *A. fulica* was confirmed in 18 of these plots, from which 735 snail specimens were collected. The *A. fulica* population was dominated by juvenile (n = 423; 57.5%) and young adult snails (n = 282; 38.3%) in all the study periods (Fig. 2). The majority (n = 649; 88.2%) of the snails were at rest, while 67 (9.3%) were estivating and 19 (2.5%) were active.

During the dry season of 2019, 124 specimens were collected from nine plots (Table 1). These specimens had a mean length of 5.36±1.11 cm (range: 2.8–9.0 cm). In the rainy season of 2019, 258 specimens were collected from 18 study plots. The mean length of the specimens collected during this period was 3.44±1.41 cm (range: 1.5–7.7 cm). A total of 90 specimens were collected during the dry season of 2020, from 12 plots. These specimens had a mean length of 4.02±1.17 cm (range: 0.9–6.7 cm). In the rainy season of 2020, 263 specimens were collected from 17 plots. These specimens had a mean length of 3.07±1.46 cm (range: 0.6–8.4 cm).

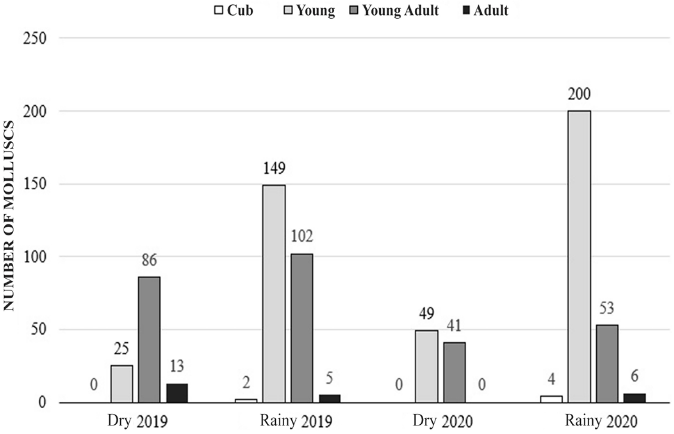

Figure 2. Distribution of the size classes of the *Achatina fulica* specimens collected in the dry and rainy seasons in 26 municipalities of the Brazilian state of Sergipe, in 2019 and 2020.

2. *Relationship between frequency of Achatina fulica and climatic characteristics*

During the dry season of 2019 (Fig. 3, 4), the largest numbers of *A. fulica* specimens were collected in the East Sergipe (n = 48 snails, 38.7% of the total number of specimens collected in the period) and Greater Aracaju subregions (n = 37, 29.8%). In the rainy season of this year, the largest samples were collected in the South Central (n = 73, 28.2%), East Sergipe (n = 58, 22.4%) and South Sergipe subregions (n = 45, 17.4%). In both seasons, there was a significant correlation (p = 0.01) between rainfall and the number of *A. fulica* specimens collected. In the dry season of 2020 (Fig. 3, 4), *A. fulica* was most frequent in the plots in the Greater Aracaju (n = 28, 31.1%), South Central (n = 21, 23.3%) and South Sergipe subregions (n = 20, 22.2%). In the rainy season of this year, *A. fulica* was most frequent in the South Central (n = 72, 27.3%) and South Sergipe subregions (n = 70, 26.6%).

Table 1. Variation in the body mass (g) and total length (cm) of the *Achatina fulica* specimens collected in the dry (February) and rainy (September) seasons of 2019 and 2020, in the eight subregions of the Brazilian state of Sergipe.

Season	Number of *A. fulica* specimens	Body length (cm)				Body mass (g)			
		Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Dry 2019	124	2.8	9.0	5.36	±1.11	2.5	68.3	15.87	±9.85
Rainy 2019	258	1.5	7.7	3.44	±1.41	0.5	54.4	6.86	±8.97
Dry 2020	90	0.9	6.7	4.02	±1.17	0.01	39.5	12.52	±9.11
Rainy 2020	263	0.6	8.4	3.07	±1.46	0.3	86.1	9.47	±13.33

Min = Minimum, Max = Maximum; SD = Standard Deviation.

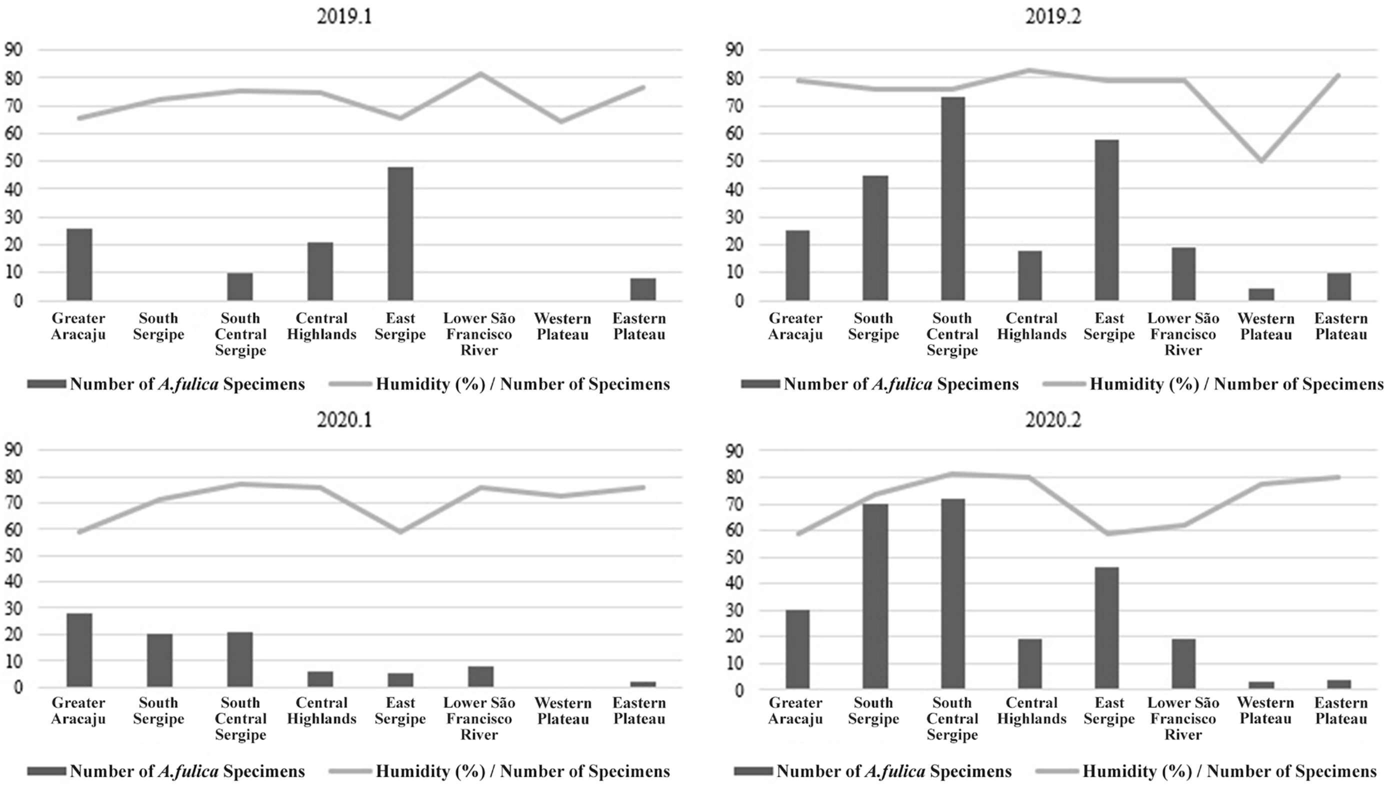

Figure 3. Number of *Achatina fulica* specimens and the relative humidity (%) recorded in the different subregions of the Brazilian state of Sergipe in the dry and rainy seasons of 2019 and 2020.

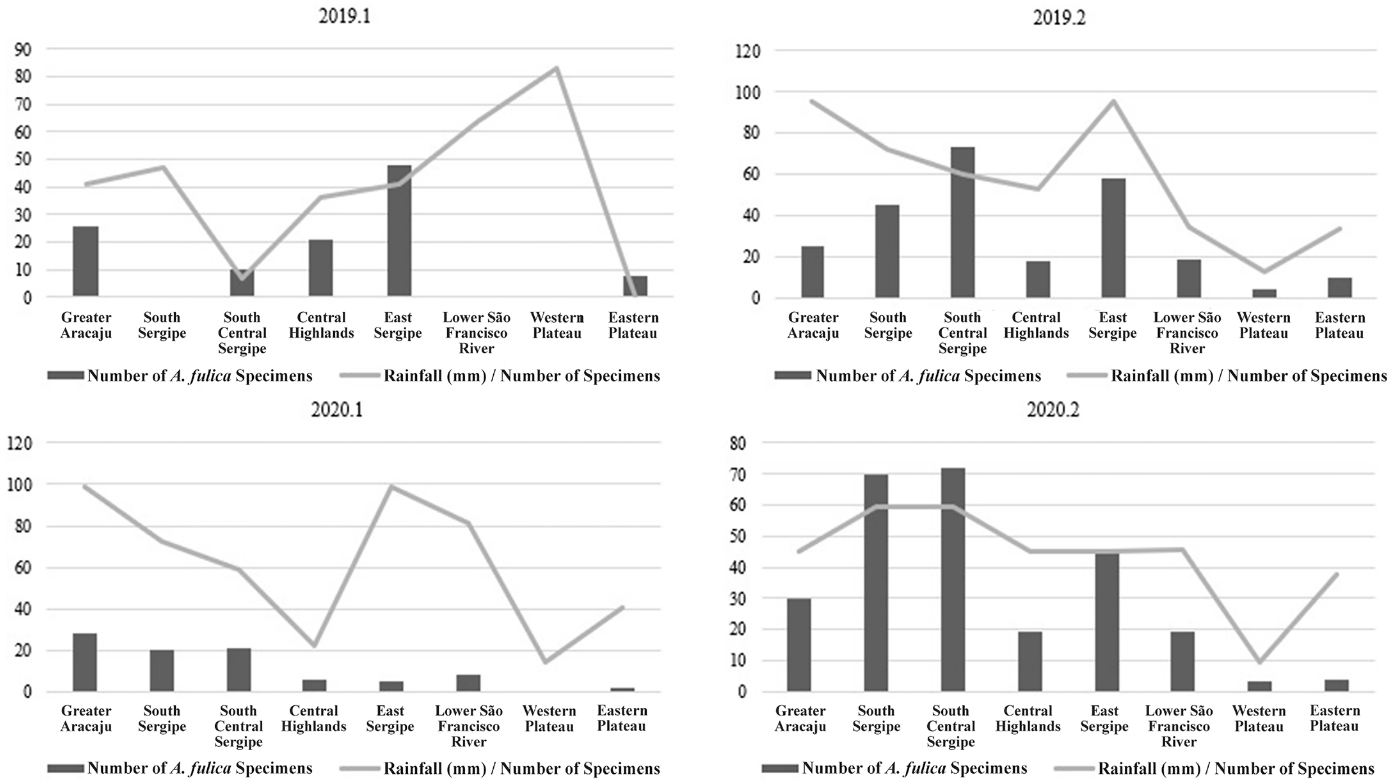

Figure 4. Number of *Achatina fulica* specimens collected (columns) and the rainfall (gray lines, in mm) recorded in the different subregions of the Brazilian state of Sergipe in the dry (2019.1 and 2020.1) and rainy (2019.2 and 2020.2) of the present study period.

During the dry season of 2019, relative humidity was highest in the Lower São Francisco subregion (81.6%), and lowest (64.0%) on the Western Plateau (Fig. 3). In the rainy season of this year, the highest relative humidity (82.7%) was recorded in the Central Highlands and the lowest (50.0%), once again, on the Western Plateau. In the dry season of 2020, the highest relative humidity (78.8%) was recorded in the Lower São Francisco subregion, while the lowest value (59.2%) was recorded in Greater Aracaju. During the rainy season, relative humidity peaked in South Central Sergipe (81.2%) and the Central Highlands (80.3%), and was lowest in Greater Aracaju (58.7%).

During the present study, a mean precipitation of 34.8±29 mm was recorded in the dry season month of February 2019 and 55.5±29 mm in September, whereas in 2020, the mean for February was 58.6±31 mm, and that for September was 43.3±15 mm. It is important to note, however, that the rainfall observed during the dry season occurred in isolated downpours, with an irregular distribution in February, in particular in 2020. In 2019, the different subregions, the highest monthly rainfall (83.2 mm) was recorded on the Western Plateau subregion, whereas in the rainy season, rainfall was highest (95.6 mm) in Greater Aracaju (Fig. 4). In 2020, the highest values in the dry season, which reached 98.8 mm, were recorded in Greater Aracaju and East Sergipe, whereas in the rainy season, the highest value (59.4 mm) was recorded in South Sergipe, with a similar level being registered in the South Central subregion.

In the dry season of 2019, all the 24 sample plots surveyed in the eight subregions of Sergipe had dry soil and the conditions were sunny, whereas in the rainy season, 13 of the plots had humid soil, and 18 were surveyed on cloudy days. In the dry season of 2020, by contrast, only nine of the plots had dry soil, and 15 were surveyed on cloudy days, while in the rainy season, 21 of the plots had dry soil, and all 24 were surveyed on sunny days. The plots with humid soil in the rainy season of 2019 were all located in the Greater Aracaju, South Sergipe, Central South Sergipe, East Sergipe and the Lower São Francisco subregions, which are all located within the humid coastal zone of the state. In 2020, plots with humid soil were all located in these same subregions, whereas in the rainy season, humid soil was observed only in the plots in Greater Aracaju, South Sergipe and East Sergipe.

The mean temperature recorded in Sergipe in the dry season (February) was 28.2±0.7°C, which is similar to the mean for the rainy season (September) of this year, that is, 26.0±1.3°C. In 2020, the mean temperature of the dry season was 27.9±0.7°C, while it was 24.4±2.7°C in the rainy season. A significant correlation ($p = 0.03$) was found between the resting behavior of *A. fulica* and the ambient temperature, that is, the higher the temperature, the more frequent resting behavior is in *A. fulica*.

3. *Characteristics of the soil in the different subregions of Sergipe*

The most acidic soil in the dry season of 2019 was recorded in the plot in the municipality of Japaratuba (pH = 5.73), which is located in the East Sergipe subregion (Table 2). The most alkaline soil was recorded in Nossa Senhora da Glória (pH = 8.93), on the Western Plateau. In the rainy season of this year, the most acidic soil (pH = 4.6) was recorded in the plot in Itabaiana, in the Central Highlands, while the most alkaline was Porto da Folha (pH = 8.11) on the Western Plateau.

The plots were more neutral in the dry season of 2020, with a mean pH of 7.0±0.4 (range: 6.1–7.8) being recorded in the 24 plots. In the rainy season of this year, the most acidic soil (pH = 4.95) was collected from the plot in Nossa Senhora das Dores (Eastern Plateau), while the most alkaline (pH = 8.14) was in Propriá, in the Lower São Francisco subregion.

The Calcium concentrations of the soil also varied considerably among plots (Table 2). In the dry season of 2019, the lowest value (0.92 $\text{cmol}_c/\text{dm}^3$) was recorded in Cumbe, on the Eastern Plateau, while the highest concentrations were observed in Porta da Folha, on the Western Plateau (12.3 $\text{cmol}_c/\text{dm}^3$) and Barra dos Coqueiros, in the Greater Aracaju subregion (11.4 $\text{cmol}_c/\text{dm}^3$). In the rainy season of this year, the highest Ca concentration (11.3 $\text{cmol}_c/\text{dm}^3$) was also recorded in Porto da Folha. Even higher concentrations were recorded in 2020, reaching 17.1 $\text{cmol}_c/\text{dm}^3$ in Barra dos Coqueiros in the dry season, and 21.2 $\text{cmol}_c/\text{dm}^3$ in this same plot in the rainy season.

The quantity of organic matter also varied considerably among plots. In the dry season of 2019, the lowest concentration (OM = 4.8 g/dm^3) was recorded in Nossa Senhora das Dores (Eastern Plateau), while the highest value (54.7 g/dm^3) was observed in Riachão dos Dantas (South Central Sergipe).

Table 2. Variation among seasons in the characteristics of the soil (pH in water, calcium [$\text{cmol}_c/\text{dm}^3$] and organic matter, g/dm^3) recorded during the survey of *Achatina fulica* in the eight subregions of the Brazilian state of Sergipe, in 2019 and 2020.

Subregion/Municipality	pH	Calcium	Organic Matter	pH	Calcium	Organic Matter	pH	Calcium	Organic Matter	pH	Calcium	Organic Matter
	Season											
	Dry 2019			Rainy 2019			Dry 2020			Rainy 2020		
Greater Aracaju												
Aracaju	7,98	4,69	17,1	7,59	6,26	18,8	7,3	7,62	25,5	7,79	4,86	9,52
Barra dos Coqueiros	5,59	3,13	17,5	7,71	5,72	28,7	6,17	17,1	7,88	6,3	21,2	11,9
São Cristóvão	6,93	11,4	29,4	7,62	6,26	16,8	7,6	5,22	15,2	7,1	6,82	25,4
South Sergipano												
Estância	7,89	3,72	11	6,7	7,19	34,1	6,7	4,23	12,7	7,37	6,87	17,6
Boquim	7,69	6,07	22,2	7,38	5,06	19,1	7,16	7,02	26,5	7,81	6,87	17,6
Salgado	7,22	6,69	26,8	7,88	4,5	12,9	6,76	2,42	11	6,46	3,11	13,3
South Central												
Lagarto	6,83	3,86	16,3	6,7	7,19	34,1	6,12	2	5,26	7,58	7,05	14,9
Tobias Barreto	8,11	10,1	31,8	7,88	4,5	12,9	7,48	6,95	7,23	7,22	6,29	11,2
Riachão dos Dantas	7,93	9,85	54,7	7,38	5,06	19,1	7,39	10,2	22,1	7,93	6,31	11,17
Central Higlands												
Itabaiana	8,17	7,32	12,9	4,65	1,34	9,21	6,76	1,53	10,5	7,75	7,06	16,3
Areia Branca	7,7	7,31	20,1	7,31	5,2	15,3	7,22	7,79	21,1	7,59	9,4	19,6
São Domingos	8,89	3,56	5,79	7,78	4,37	14,5	6,83	5,11	13,9	7,54	5,71	14,6
East												
Rosário do Catete	8,71	2,45	5,49	6,86	7,58	26,1	7,25	5,02	16,2	6,59	3,64	15,8
Siriri	7,69	8,39	28,5	7,07	6,1	21,1	6,96	6,7	28,8	7,91	5,73	9,1
Japaratuba	5,73	3,54	12,6	6,63	4,82	24,4	7,17	5,1	15,4	7,73	5,57	15,4
Easten Plateau												
N. S. das Dores	6,54	2,23	4,85	8,09	4,47	8,09	7,44	3,20	7,44	4,95	1,37	5,89
Cumbe	8,88	0,92	5,57	7,71	5,72	11,2	-	-	-	6,55	2,17	7,86
Feira Nova	7,38	6,92	7,77	7,71	5,72	11,2	6,16	5,00	17,6	7,56	8,23	18,8
Western Plateau												
N.S. da Glória	8,93	3,8	7,89	8,05	5,49	13,4	7,63	11,8	17,8	8,08	8,28	12,6
Porto da Folha	6,8	12,3	9,71	7,79	11,3	16,1	7,07	10,0	8,96	8,11	8,18	6,65
Gararu	7,88	9,87	30,7	7,75	6,89	30,1	7,82	7,05	11,6	7,83	6,24	20,8
Lower São Francisco												
Neópolis	7,88	8,84	23,2	7,83	6,43	28,1	7,29	5,20	13,7	7,61	7,72	13,7
Propriá	8,16	8,58	21,7	8,19	5,08	15,5	7,81	12,2	11,1	8,14	4,66	8,36
Pacatuba	7,74	3,06	6,88	6,78	4,83	17,4	6,24	4,32	10,7	7,33	2,96	6,79

-No data collected.

In the rainy season of this year, the highest values (up to 34.1 g/dm^3) were recorded in Estância (South Sergipe) and Lagarto (South Central Sergipe). In the dry season of 2020, the highest value was recorded in Siriri, East Sergipe (28.8 g/dm^3), while in the rainy season, the highest value (25.4 g/dm^3) was observed in Barra dos Coqueiros (Greater Aracaju).

4. *Allometric growth (b) of Achatina fulica by season*

The body mass-total length relationship of the *A. fulica* specimens did not vary significantly ($p > 0.05$) between 2019 and 2020. However, higher *b* values were recorded in both dry seasons. Negative allometric growth ($b = 2.78$) was recorded in the dry season of 2019, while in the same season of 2020, growth was isometric ($b = 3.04$), based on the equation $Wt = 0.1463\ Lt^{3.04}$ representing, theoretically, the most adequate growth pattern, in which the shell grows in direct proportion to the body mass of the individual (Fig. 5, 6; Appendix S4).

5. *Allometric growth of Achatina fulica in relation to the chemical conditions of the soil*

In 2019, a negative correlation was found between the growth of *A. fulica* and the soil pH ($r = -0.4388$, $p < 0.05$), with allometric growth being greater with decreasing pH. This allowed us to use the soil pH to develop a predictive linear model for the identification of allometric growth in *A. fulica* ($R^2 = 0.156$, $p < 0.05$). In Sergipe, then, the more acidic the soil, the more allometric the growth of *A. fulica* ($b > 3$). However, *A. fulica* was more common, in general, on soils with a pH of 6.5–7.5, which is the ideal condition for isometric growth ($b = 3$). As the soil pH increases, the value of *b* decreases ($b < 3$), inverting the pattern observed where soils are more acidic, i.e., pH < 7 (Appendix S5). The frequency of *A. fulica* decreased significantly ($p < 0.05$) on increasingly alkaline (pH > 7) soils (Fig. 7). In 2020, however, no significant correlation ($p > 0.05$) was found between growth patterns and soil pH.

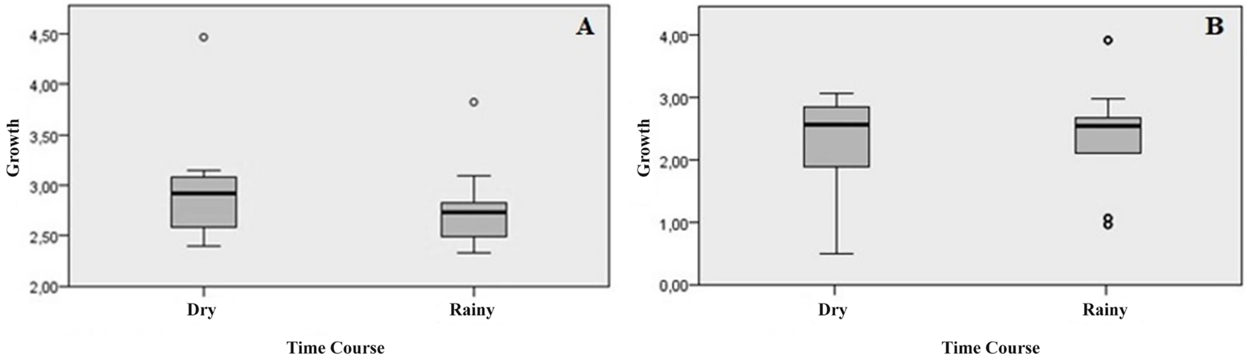

Figure 5. Allometric growth of *Achatina fulica* in the dry and rainy seasons of 2019(A) and 2020(B) in the eight subregions of the Brazilian state of Sergipe. * Kruskall-Wallis.

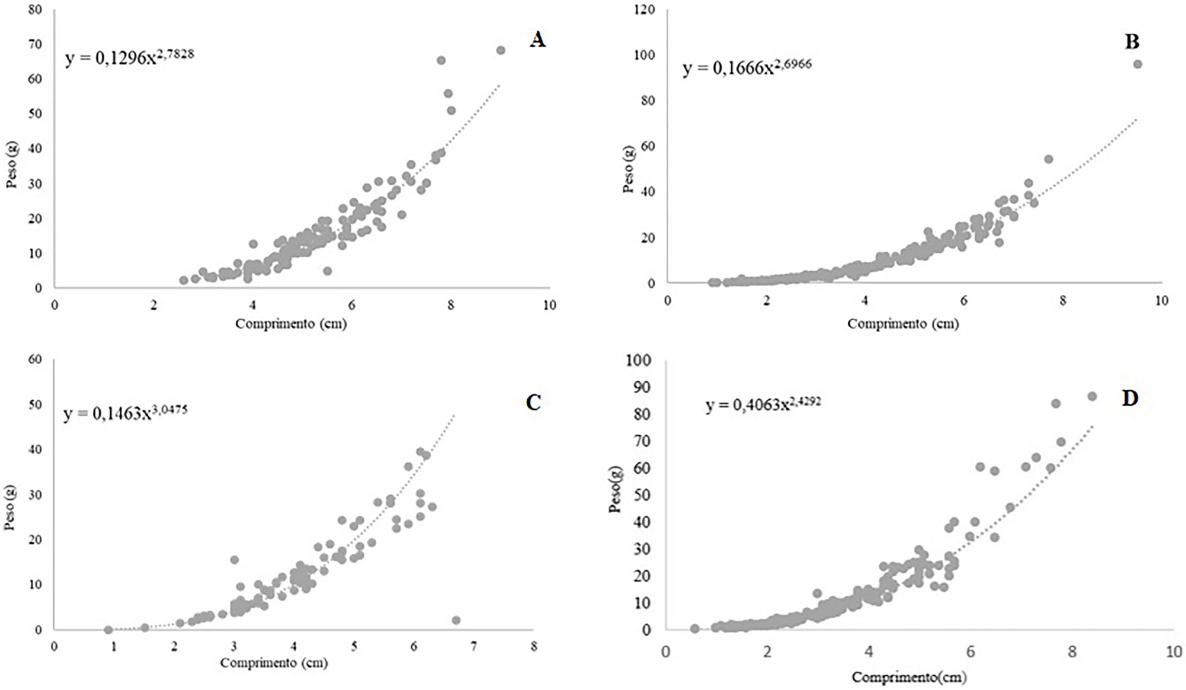

Figure 6. Seasonal variation in the allometric growth of *Achatina fulica* in the eight subregions of the Brazilian state of Sergipe, in 2019 and 2020. (A) 2019 dry period with negative allometric growth of *b*= 2.78. (B) 2019 rainy season with negative allometric growth of *b*= 2.69. (C) 2020 dry period with isometric allometric growth of *b*= 3.04. (D) Rainy season 2020 with negative allometric growth *b*=2.42.

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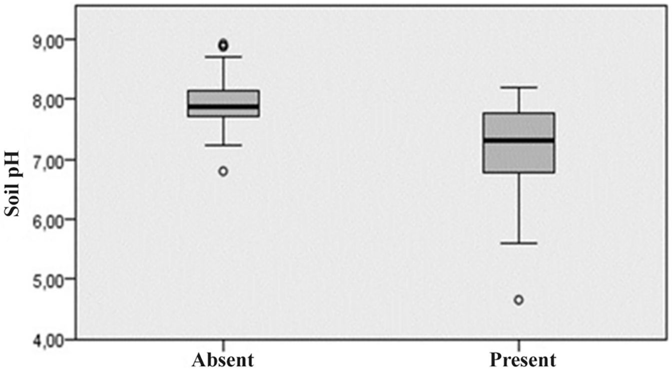

Figure 7. Presence of *Achatina fulica* in relation to the soil pH in the eight subregions of the Brazilian state of Sergipe, 2019.

6. *Relative condition factor of Achatina fulica in the different subregions of Sergipe*

In 2019, the relative condition factor varied significantly ($p = 0.02$) among the eight subregions of Sergipe. This parameter represents the theoretically ideal condition of an individual when its value is 1. In 2019 (Appendix S6), the highest proportion of *A. fulica* specimens with less than ideal body mass (KR <1) was recorded in the Greater Aracaju subregion (n = 29 snails, 41.4% of the total), while the ideal factor (KR = 1) was recorded most on the Western Plateau (n = 3, 75%) and specimens were mostly above the expected body mass (KR >1) in South Sergipe (n = 19, 48.7%).

Significant variation ($p = 0.01$) in the KR of *A. fulica* among subregions was also recorded in 2020 (Fig. 8). The largest proportion of individuals with lower than expected body mass (KR < 1) was recorded in the East Sergipe subregion (n = 41, 80.0%) and on the Western Plateau (n = 4, 57.1%). The highest proportion of snails with ideal body mass (KR = 1) was collected in the Lower São Francisco subregion (n = 7, 25.9%), followed by South Central Sergipe (n = 24, 25.8%). The largest proportion of snails above the expected mass (KR > 1) was recorded in South Sergipe (n = 39, 43.3%), followed by South Central Sergipe (n = 30, 32.3%).

Individual extremes in the relative condition factor were observed in different municipalities in the two seasons of 2019 (Table 3). In the dry season of 2019, the extreme values were recorded in the municipality of Japaratuba, in East Sergipe, where the lowest (KR = 0.34) and highest (KR = 1.99) values were recorded in the same plot. The latter value represents a body mass almost double that expected theoretically. In the rainy season of 2019, the lowest KR value (0.49) was recorded in the plot in Estância, in South Central Sergipe, that is, a body mass less than half that expected, while the highest value in this period was recorded in the same subregion, in Lagarto (KR = 3.16).

In the dry season of 2020, the lowest (KR = 0.17) and highest (KR = 2.36) values were both recorded in the same plot, in São Cristóvão, in the Greater Aracaju subregion. In the rainy season, the lowest KR value was recorded in Boquim (KR = 0.50), in South Sergipe, and the highest, in Riachão dos Dantas (KR = 1.94) in South Central Sergipe.

7. *Seasonal variation in the relative condition factor*

The relative condition factor of *A. fulica* in Sergipe did not vary significantly ($p > 0.05$) between the dry and rainy seasons in 2019. In 2020, by contrast, the condition factor was significantly higher ($p = 0.02$) in the dry season. In the rainy season of 2020, 223 snails were collected, of which, 139 (52.9%) had a body mass lower than expected (KR < 1). In the dry season, 90 *A. fulica* specimens were collected, of which, 20 (22.2%) had an ideal body mass (KR = 1) and 37 (41.1%) were above the expected mass (KR > 1). In both seasons, the larger the number of *A. fulica* specimens collected, the larger the KR values ($p = 0.01$), and in general, the higher the relative condition factor, the larger the proportion of snails observed at rest ($p = 0.001$).

8. *Relative condition factor of Achatina fulica, climatic variables and soil conditions*

The analysis of the relationship between the growth parameters and environmental variables (Table 4) indicated that soil pH was the only factor to have a significant influence on the growth of *A. fulica* ($p < 0.05$), and only in 2019. None of the other variables, including the soil pH in 2020, had any significant effect ($p > 0.05$) on the *A. fulica* growth parameters.

9. *Binary logistic regression associating climatic variables and soil chemistry*

The mathematical model created by the addition of variables such as "season (dry ou rainy), humidity, rainfall and soil pH" presented a significant relationship ($R^2 = 0.858$, $p < 0.001$) with the occurrence of *A. fulica* in the municipalities surveyed in the present study. The equation produced for the prediction of the occurrence of *A. fulica* in a given municipality is shown in Fig. 9. The reliability of this model was confirmed by the Kappa index ($k = 0.849$; $p < 0.001$), and the model provided a correct result for 94.1% of the sample tested (Appendix S7).

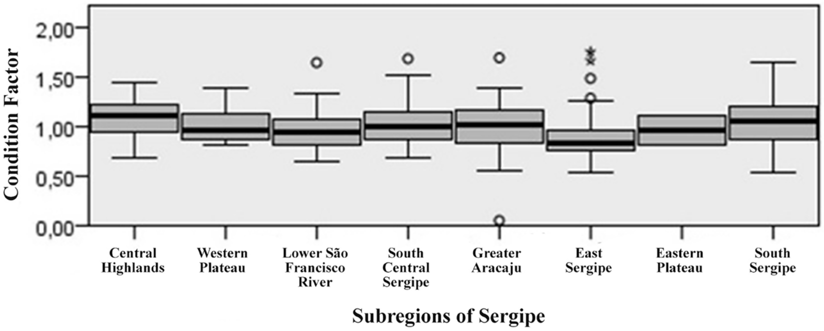

Figure 8. Relative condition factor of the *Achatina fulica* specimens collected in the eight subregions of the Brazilian state of Sergipe in 2020. * p = 0.001(Kruskal-Wallis).

Table 3. Variation in the relative condition factor (KR) of the *Achatina fulica* specimens collected in the different municipalities of the Brazilian state of Sergipe in 2019 and 2020.

Territories/municipalities	Min	Max	Mean±sd	Min	Max	Mean±sd	Min	Max	Mean±sd	Min	Max	Mean±sd
	2019.1			2019.2			2020.1			2020.2		
Greater Aracaju												
Aracaju	0,78	1,18	1,00±0,13	0,84	1,10	1,00±0,09	0,82	1,14	1,00±0,11	0,80	1,33	1,01±0,14
São Cristóvão	0,75	1,47	1,01±0,17	0,75	1,21	1,00±0,13	0,17	2,36	1,12±0,47	0,81	1,33	1,01±0,17
Barra dos Coqueiros	0,94	1,08	1,00±0,05	0,81	1,25	1,01±0,16	1,39†	1,39†	1,39†	0,66	1,32	1,01±0,16
South Sergipe												
Estância	-	-	-	0,49	1,24	1,01±0,13	0,81	1,51	1,00±0,15	0,52	1,77	0,99±0,27
Boquim	-	-	-	0,96	1,03	1,00±0,02	0,83	1,12	0,97±0,20	0,50	1,66	0,97±0,39
Salgado	-	-	-	1,13†	1,13†	1,13†	-	-	-	1,11†	1,11†	1,11†
South Central												
Lagarto	0,71	1,36	1,01±0,19	0,63	3,16	1,02±0,33	0,82	1,17	1,00±0,11	0,73	1,26	1,01±0,12
Tobias Barreto	-	-	-	-	-	-	-	-	-	-	-	-
Riachão dos Dantas	-	-	-	0,91	1,18	1,00±0,08	0,88†	0,88†	0,88†	0,75	1,94	1,02±0,22
Central Higlands												
Itabaiana	0,94†	0,94†	0,94†	0,75	1,20	1,00±0,14	-	-	-	0,85	1,16	1,01±0,12
Areia Branca	0,83	1,13	1,00±0,06	0,92	1,11	1,00±0,60	0,80	1,16	1,01±0,15	0,88	1,21	1,00±0,09
São Domingos	-	-	-	-	-	-	-	-	-	-	-	-
East												
Rosário do Catete	-	-	-	0,84	1,23	1,00±0,09	1,00	1,00	0,99±0,00	0,74	1,67	1,03±0,27
Siriri	0,47	1,64	1,07±0,40	0,91	1,07	1,00±0,69	-	-	-	0,70	1,44	1,03±0,26
Japaratuba	0,34	1,99	1,02±0,24	0,50	1,47	0,95±0,16	0,97	1,04	1,00±0,33	0,71	1,21	1,01±0,17
Easten Plateau												
N. S. das Dores	0,80	1,38	1,01±0,19	0,89	1,17	1,00±0,80	0,82	1,17	1,40±0,11	0,90	1,16	1,00±0,11
Cumbe	-	-	-	-	-	-	-	-	-	-	-	-
Feira Nova	-	-	-	-	-	-	-	-	-	-	-	-
Western Plateau												
N.S. da Glória	-	-	-	0,95	1,05	1,00±0,04	-	-	-	1,00	1,00	1,00±0,00
Porto da Folha	-	-	-	-	-	-	-	-	-	-	-	-
Gararu	-	-	-	-	-	-	-	-	-	-	-	-
Lower São Francisco												
Neópolis	-	-	-	0,84	1,12	1,00±0,10	-	-	-	0,81	1,65	1,02±0,23
Propriá	-	-	-	0,83	1,12	1,00±0,13	0,81	1,27	1,00±0,11	0,85	1,14	1,01±0,13
Pacatuba	-	-	-	0,90	1,10	1,00±0,08	-	-	-	-	-	-

† Only one *A. fulica* specimen collected in this season. – No *A. fulica* specimens collected in the plot in this season. Min = Minimum, Max = Maximum, SD = Standard Deviation.

Table 4. Correlations between environmental factors (soil and climatic parameters) and the growth and relative condition factor (KR) of the *Achatina fulica* specimens collected in the present study in the Brazilian state of Sergipe, in 2019 and 2020.

CORRELATION/YEAR		Variable								
		pH	Calcium	Organic matter	Humidity (%)	Humidity (variation)	Temperature ºC	Temperature (variation)	Rainfall (mm)	Rainfall (variation)
Growth (b) 2019	r	-0.438	-0.252	-0.186	0.065	-0.203	0.207	0.222	-0.272	-0.280
	p	*0.029	0.224	0.348	0.759	0.330	0.320	0.287	0.188	0.174
Condition factor (KR) 2019	r	0.072	0.209	0.224	-0.082	0.164	0.237	0.210	-0.109	-0.146
	p	0.733	0.316	0.283	0.695	0.433	0.197	0.314	0.603	0.486
Variation in the KR 2019	r	-0.322	0.104	0.265	0.077	1.105	0.230	0.104	-0.049	-0.053
	p	0.117	0.622	0.201	0.716	0.618	0.270	0.622	0.817	0.802
Growth (b) 2020	r	-0.040	0.104	0.072	-0.040	-0.043	0.055	-0.192	0.132	0.209
	p	0.764	0.430	0.586	0.775	0.759	0.690	0.391	0.350	0.134
Condition factor (KR) 2020	r	-0.017	0.061	0.158	-0.186	-0.193	-0.064	-0.167	0.086	0.003
	p	0.906	0.666	0.263	0.210	0.194	0.662	0.257	0.570	0.984
Variation in the KR 2020	r	0.105	0.020	0.013	0.037	0.062	-0.121	-0.119	0.006	-0.121
	p	0.429	0.080	0.925	0.789	0.659	0.381	0.391	0.969	0.387

r = Pearson's correlation coefficient. * significant correlation ($p < 0.05$).

$$Presence\ of"Achatinafulica = \frac{e^{(-117.502+[30.548]*a+[0.300]*b+[67.965]*c+[8.307]*d}}{1+e^{(-117.502+[30.548]*a+[0.300]*b+[67.965]*c+[8.307]*d}}$$

Figure 9. Equation of the mathematical model developed to predict the occurrence of *Achatina fulica* in the municipalities of different subregions of the Brazilian state of Sergipe, based on the climatic variables and soil pH recorded in 2019 and 2020. Where *a* = the season, *b* = relative humidity, *c* = rainfall, *d* = soil pH, and *e* = Euller's number.

Discussion

Of the 19 municipalities notified in Sergipe by the State Committee for the Control of African Snails, seven of these are not part of the studied sample (Arauá, Capela, Maruim, Santa Luzia do Itanhi, Umbaúba, Muribeca and Itaporanga d'Ajuda) (IBAMA, 2021). The 18 sites recorded here brings the total number of municipalities in Sergipe known to be infested with *A. fulica* to 25 (see also Carvalho et al. 2012, Ramos-de-Souza et al. 2018, Silva et al. 2020).

In the dry season, *A. fulica* was recorded in nine plots in 2019 and in 12 plots in 2020. These plots were located primarily in the humid coastal and central highland climatic zones (Aragão et al. 2013), which is probably the principal factor determining the presence of the snails in the plots in the dry season.

The vast majority of the *A. fulica* specimens collected during the present study were either juveniles (n = 423, 57.5%) or young adults (n = 282, 38.3%). The gregarious type of behavior pattern was the most commonly observed, and resting (n = 649, 88.2%) was likely a response to the climatic conditions that predominate during the dry season. Fischer et al. (2012) studied the gregarious behavior of *A. fulica* in the field and the laboratory, and found that this behavior is more frequent in urban areas, and more frequent in the infants and juveniles during the dry season. Cook (2001) concluded that one of the principal advantages of aggregation in these snails is the creation of a humid microclimate, which minimizes dehydration by restricting the surface area of the body exposed to evaporation. On Christmas Island, in the eastern Indian Ocean, O'Loughlin & Green (2017) observed that 96.9% of the *A. fulica* specimens were resting during the day, whereas at night, 51.6% were active and on the soil. As the surveys in the present study were conducted during the morning, the timing of the specimen collection may have determined the large number of resting *A. fulica* specimens found in the plots.

The significant association found between the number of *A. fulica* and rainfall would likely account for the larger numbers of snails collected during the rainy seasons. In the metropolitan region of Aracaju, in Sergipe, Silva et al. (2020) found that rainfall had a positive influence on the frequency of *A. fulica*, a pattern also observed in Nigeria by Onyshi et al. (2018), who observed that the conditions of high humidity and rainfall were associated with an increase in the population of *A. fulica*. Despite this, the largest *A. fulica* specimens were collected during the dry season in the present study, reaching a mean of length of 5.36±1.11 cm and mass of 15.87±9.85 g. This may be accounted for by the fact that, even at high temperatures and under low rainfall, *A. fulica*, in particular the larger individuals, use specific behavioral strategies to cope with the relative humidity (Pilate et al. 2017).

The plots surveyed in the present study all presented similar general characteristics, however, that is, vacant lots containing domestic refuse and rubble. The *A. fulica* specimens were encountered under dense tufts of herbaceous vegetation, dead leaves, in piles of litter, and near walls. Silva et al. (2020) found that *A. fulica* was five times more likely to be present in areas with domestic refuse than clean sites. It is important to note, in this case, that three of the study plots, in Aracaju, Barra dos Coqueiros and Nossa Senhora das Dores, did not contain litter at any time during the study period, but in this case, the *A. fulica* specimens were found in the most humid parts of the plot, including tufts of herbaceous vegetation, ornamental plants, tree trunks, shaded locations near walls, and under bricks. Like other land snails, *A. fulica* prefers humid environments and seeks them out actively.

In terms of body size, Civeyrel & Simberloff (1996) identified three phases in the establishment of *A. fulica* in new areas: (i) an initial, exponential phase, characterized by the presence of large, robust individuals, (ii) a second, establishment phase of variable duration, during which the population expands, and (iii) a declining phase, dominated by small individuals with fragile shells. In Sergipe, the survey data indicate that the *A. fulica* populations are currently in the second phase, which is dominated by the presence of juveniles and young adults. This was the scenario observed in the sample plots, and a similar situation has been observed in other urban and peri-urban areas in Sergipe, given the accentuated dispersal capacity of this snail.

The growth data indicate that the most favorable conditions for the growth of *A. fulica* in Sergipe were found in the dry season, in both 2019 (b = 2.78) and in 2020 (b = 3.04), when growth was isometric. This may be accounted for by the relatively high calcium concentrations and the large amounts of organic matter found in the plots in the dry seasons of both years. The soil pH in the rainy season of both 2019 and 2020 was also more acidic, which was associated with negative allometric growth. The negative trends in this growth parameter may be related to a lack of food, predation pressure or even the parasitic infections known to affect *A. fulica* (Almeida 2014). The pH is an indicator of the general chemical conditions of the soil (Silva et al. 2005), with more acidic conditions reflecting a lack of bases, including calcium. The physical and biological properties outlined above may also have had a direct effect on the body length, mass, shape, and shell color of the *A. fulica* specimens (Fischer et al. 2010).

Achatina fulica is tolerant of a wide range of abiotic factors, and is thus able to occupy a diversity of natural habitats and anthropogenic environments (Fisher & Colley 2005). In the present study, a negative correlation ($r = -0.4388$, $p < 0.05$) was found between the soil pH and the allometric growth of *A. fulica*, which supported the inclusion of the soil pH in the predictive linear model of the allometric growth of *A. fulica*. The more acidic the soil, the greater the allometric growth of *A. fulica in* Sergipe ($b > 3$).

This snail is most frequent on soils with a pH of 6.5–7.5, which is the ideal range for isometric growth (*b* = 3). There was also a significant decline ($p < 0.05$) in the number of snails on more alkaline soils.

Raut & Barker (2002) found that the infant and juvenile *A. fulica* specimens tend to have more contact with the soil. In the present study, we can conclude that these individuals may have been affected more negatively by extremes (acidic or alkaline) of soil pH, given that they were usually encountered half-buried in the soil. In the municipality of Valença, in the Brazilian state of Rio of Janeiro, Durço et al. (2013) found a significant negative correlation between the age of the *A. fulica* specimens, and the time spent buried in the soil. Costa (2010) observed a similar pattern, with most of the juvenile snails being buried, while the young adults were observed at rest on the ground and in the bushes. This is consistent with the observations of Almeida et al. (2016), who found that only the infants and juveniles were completely buried in the soil. Fisher (2009) recorded the same scenario in the laboratory, under experimental conditions.

In Sergipe, we recorded the greatest frequency (n = 282, 38.3%) of young adult snails (4.01–7.0 cm) within the amplest range of soil pH (4.5–8.2). This is probably why growth was more allometric in more acidic soils, given that the young adults and adults (> 7.0 cm) are able to rest on other types of substrates. This behavior is probably a strategy that enables the snails to avoid adverse soil conditions, proliferate and develop optimally. In the Brazilian state of Paraná, Fischer & Colley (2005) found that *A. fulica* specimens of different sizes (shell length) rested in different sites, with the larger snails tending to be more frequent on plants and other organic substrates. Pilate et al. (2017) concluded that *A. fulica* adopts specific behavioral strategies to guarantee its survival, such as the retraction of the soft cephalopodal mass and the avoidance of unfavorable sites.

These snails may also present exploratory behavior to identify resources and other features of their environment, given that they have well-developed chemoreceptors, with neurosensory cells on the surface of the body related to environmental perception, feeding, reproductive communication and aggregation (Chase & Tolloczko 1985). In Japan, Tomiyama (1992) investigated the homing behavior of *A. fulica*, and found that the young adults disperse constantly, and over much larger distances than the other individuals, while the adults change resting sites only rarely. Tomiyama & Nakane (1993) found that the young adults produce only sperm, while the mature adults are capable of producing both spermatozoa and ova. This may determine the more ample ranging of the young adults, which disperse in search of reproductive partners, while the adults do not need to disperse to copulate, and may thus remain at rest for much longer periods.

A significant correlation was found between resting behavior and the relative condition factor of *A. fulica*, that is, the higher the relative condition factor, the more these snails remain at rest. Significant differences ($p < 0.05$) were also found in the condition factor of *A. fulica* among the eight subregions of Sergipe. The body mass-length relationship of a species provides important insights into the influence of environmental conditions on the developmental stages of the organism, through estimates of the relative condition factor (Araújo et al. 2011).

Theoretically, the relative condition factor (KR) represents the development as ideal when equal to 1, or otherwise, when the values of KR deviate significantly from 1 (Albuquerque et al. 2009). In 2019, for example, the *A. fulica* specimens presented reduced body mass (KR <1) in the Greater Aracaju subregion, but increased mass (KR >1) in South Sergipe. The scenario observed in Greater Aracaju may have been related to the fact that 21 of the specimens were in a condition of estivation, which may reflect the lack of food in the plot, resulting from the prolonged drought and high temperatures. Estivation is considered to be the most critical phase of the development of *A. fulica* (Raut & Barker 2002), which estivates during the driest parts of the year, when it may secrete an epiphragm, a calcified structure that seals off the shell opening to prevent dehydration (Almeida et al. 2016). During the dry season, then, the number of active snails in an area may decline significantly, while the snails become more active when conditions become more humid (Fischer 2009; Durço et al. 2013). In South Sergipe, the condition factor of *A. fulica* was higher than expected (KR >1), which may be related to the large quantities of refuse and decomposing organic matter in the plots in the municipalities of Estância and Boquim. The soil parameters in Estância were especially favorable during this dry season, with the second highest concentration of organic matter recorded in 2019 (34.1 g/dm^3), as well as a pH of 7.9 and of 7.9 cmol$_c$/dm^3 calcium, which may have contributed to the wellbeing and development of the snail during this period.

In 2020, snails with ideal body mass (KR = 1), were collected in the plots of the Lower São Francisco subregion, while *A. fulica* specimens above the ideal mass (KR >1) were collected in South and South Central Sergipe, with large numbers of snails in this condition. These findings may reflect the conditions in these areas, in particular the Lower São Francisco subregion, where the mean monthly temperature, relative humidity and rainfall were all relatively high during the period when the *A. fulica* specimens were collected. Similarly, the South Central and South Sergipe subregions had high temperatures (28–30°C) and relative humidity (77–81%), as well as frequent precipitation. In India, Sarma et al. (2015) modelled the niche of *A. fulica* and concluded that areas with a hot climate and frequent rainfall have a larger risk of invasion by this species. While this snail loses water through its tegument, it is also able to rehydrate itself through the contact between the tegument and the immediate environment (Cook 2001). Further studies of the development of *A. fulica* and its relationship with climatic and environmental variables will be essential for a better understanding of the factors that influence the development and wellbeing of *A. fulica* (Ghisi et al. 2012).

Predictive environmental models can be used to evaluate how a species will move in both time and space (Albuquerque et al. 2009), and the present study proposes a predictive model for the occurrence of *A. fulica*. The use of predictive environmental models can provide important, systematic insights into the factors that determine observed patterns of dispersal (Johnson & Omland 2004). In the present study, the binary logistic regression returned a significant correlation ($R^2 = 0.858$, $p < 0.01$), representing 85% of our sample from the Brazilian state of Sergipe.

This approach provides an important tool for the projection of future scenarios on the potential occurrence of *A. fulica* in relation to climatic variables and soil pH. Predictive models of this type can be applied to other areas, in particular in Brazil, where *A. fulica* is established over a wide area, and in other tropical and subtropical countries. The predictive model developed in the present study had a 94.1% hit rate for the samples collected in the eight subregions of Sergipe, with a high degree of reliability (Kappa index = 0.849).

Conclusions

This is the first ecological study of *A. fulica* to analyze systematically the body mass-length relationship and relative condition factor in the context of climatic variables and the chemical parameters of the soil, to determine the development pattern and welfare of this snail in the Brazilian state of Sergipe. The analyses also permitted the development of a mathematical model that can be used to determine the potential the presence or absence of *A. fulica* from other areas.

Populations of *A. fulica* were identified in 18 of the 24 municipalities surveyed in the eight subregions of the Brazilian state of Sergipe. These populations were dominated by juveniles and young adults. The patterns observed in the body mass-length relationship and relative condition factor in the different study populations indicate that the development of *A. fulica* is positive in the dry season. The data also indicate that the soil pH may have had the greatest negative effect on the infant and juvenile *A. fulica*, given that these specimens were typically covered in soil when collected. As they are less prone to seek refuge in the soil, the young adult and adult snails may have interacted much less with the substrate, as they were typically found resting on other types of surface.

It is important to note that the Kappa index showed that the variables tested by the predictive model were very reliable (k = 0.849), with a hit rate of 94.1%. The present study also elaborated a predictive mathematical model that should provide a useful analytical tool for the evaluation of other environmental scenarios, based on the biotic and abiotic factors associated with the Giant African land snail, climatic variables and the physicochemical parameters of the soil. These findings should provide fundamental guidelines for the development and improvement of measures for the control of *A. fulica* populations, contributing to improve both public health and environment health.

Supplementary Material

The following online material is available for this article:

Appendix S1 - Location of the 24 fixed plots established for the collection of *A. fulica* specimens in the eight subregions of the Brazilian state of Sergipe, in 2019 and 2020.

Appendix S2 - The field chart used in the present study and the characteristics of the study plots surveyed in the Brazilian state of Sergipe in 2019 and 2020.

Appendix S3 - Layout of the points for the collection of soil samples in the 20 m x 10 m plots surveyed for the presence of *A. fulica* populations in the Brazilian state of Sergipe.

Appendix S4 - Equations and the *a* and *b* parameters of the body mass-length relationships of the *A. fulica* specimens collected in the dry and rainy seasons in the eight subregions of the Brazilian state of Sergipe.

Appendix S5 - Allometric growth of *Achatina fulica* compared with the pH soil recorded in the eight subregions of the Brazilian state of Sergipe, 2019.

Appendix S6 - Relative condition factor of the *A. fulica* specimens collected in the eight subregions of the Brazilian state of Sergipe in 2019 and 2020.

Appendix S7 - Evaluation of the reliability of the mathematical model in comparison with the results of the surveys of the *Achatina fulica* populations conducted in the eight subregions of the Brazilian state of Sergipe in 2019 and 2020.

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Author Contributions

Guilherme Mota da Silva: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Silvana Carvalho Thiengo: Substantial contribution in the concept and design of the study; Contribution to critical revision, adding intelectual content.

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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