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ANIMAL SCIENCE

Endemicity Analysis of the Ichtyofauna of the Rio Doce Basin, Southeastern Brazil

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Abstract: The Rio Doce is a very important freshwater system in Brazil running through the Atlantic Forest, however available information about its biodiversity is scarce. In 2015, the Rio Doce basin was damaged by a burst of Fundão tailing dam in Mariana (Minas Gerais) causing an extraordinary environmental damage, with consequences still incompletely known. In the present paper we analyzed 6042 latitude/longitude records of 208 fish species from the Rio Doce deposited in collections prior to November 2015, in order to identify areas of endemism in the river before the burst. Several areas of endemism were identified along the basin, most of them describing small and novel patterns. Our analyses helped to identify areas of major diversity along the basin as well as information gaps concerning fish sampling. We hope this contribution will help obtaining quantitative measures on the impact caused by the Fundão dam catastrophe on fish biodiversity and will be useful to orient general actions towards the restoration of the basin.

Key words: Fish, conservation, Endemism, biogeography, Atlantic Forest.

INTRODUCTION

The Rio Doce is one of the most important freshwater systems in southeastern Brazil, crossing 888 kms between the states of Minas Gerais and Espírito Santo, flowing into the Atlantic Ocean (PIRH Rio Doce 2010). Despite its great extension, detailed information about its biodiversity is scarce and scattered, mostly unpublished or included in mining and hydroelectric reports. The headwaters of the Rio Doce are located in the Serra do Espinhaço and, along its trajectory, the river crosses high valleys as the Caraça Mountain on Minas Gerais, lowlands and open areas with lake systems as the Parque Estadual do Rio Doce and the Lagoa Juparanã which is the largest coastal lake in Brazil (Sarmento-Soares et al. 2017). The Rio Doce has long been a utilitarian river, characterized by the intense economic exploitation of timber, mining, siderurgy and hydroelectric power, among others. The economic development of the Rio Doce valley is limited to some areas, while other areas are characterized by poverty and social vulnerability. The Iron Quadrangle, an extense area rich in iron located in the headwaters, is central to the economy of the upper valley. According to the Agência Nacional de Mineração (2017) this zone has at least 86 mining dams stocking the rejects generated by the process of iron extraction, while only a few protected areas have place in the area. Currently, around ten hydroelectric dams are in work, some of which involve flooded areas, reaching a million cubic meters of water in the reservoir (CEMIG 2015). Historically, the Rio Doce valley has been inhabited by indigenous people Krenak (also called Aymorés, or ‘Botocudos’) whose populations are currently retracted to a few villages, due to the loss of vegetation and the invasive economic expansion (Dean 1996).

On November 5th 2015 a break up in Fundão tailings dam, controlled by enterprises Samarco (Companhia Vale do Rio Doce and BHP Biliton), located in Mariana (Minas Gerais), released near 60 millions of tons of iron waste into the Rio Doce, later swept along until the Atlantic Ocean (IBAMA 2016, Wanderley et al. 2016). This catastrophe caused an unprecedent social and environmental damage (Zhouri et al. 2016; see Comitiva de Atingidos da Bacia do Rio Doce 2018) very likely extirpating several species from the Rio Doce main channel (Sarmento-Soares & Martins-Pinheiro 2017).

Although many papers studying different aspects of the event and its impact on several taxonomic groups have been published since the accident (Fernandes et al. 2016, Dos Santos & Milanez 2017, da Silva Junior et al. 2018, Miranda & Marques 2016, Loureiro Fernandes et al. 2020, Souza Passos et al. 2020, among many others), much more information is need in order to deeply understand its consequences on biodiversity.

This paper aims to summarize the available information on fish diversity present in museums collections before the Samarco's accident (i.e., before 2015) and to describe patterns of endemism along the basin. Our results describe a general picture of the fish biodiversity and endemism in the "*healthy river*" –i.e., before 2015 - that could be used as baseline to evaluate the changes in the ictiofauna of the basin after the Samarco's catastrophe. The obtained results could be of relevance to diagnose the general situation of the Rio Doce, as well as to orientate restoration actions.

MATERIALS AND METHODS

Study area

The Rio Doce is the fifth largest river system in Brazil. Its headwaters are located between the Serra de Mantiqueira and Serra de Espinhaco (Minas Gerais) and its mouth flows into the Atlantic Ocean in the state of Espírito Santo. The Rio Doce has a trajectory of 888 km and represents the major river basin in the brazilian southeastern region covering an area of 84000 km^2, and it is mostly extended across the Mata Atlantica, an extremely vulnerable biome (Supplementary Material – Figure S1).

In order to compare the areas of endemism here identified, we adopted a classification of the Rio Doce basin in Units of analysis (UA/UAs) based on the main sub-basins and administrative divisions of the territory (ANA 2006, PIRH Rio Doce 2010, Figure S1b): UA Piranga, UA Piracicaba, UA Santo Antônio, UA Suaçuí, UA São José, UA Santa Maria do Rio Doce, UA Guandu, UA Manhuaçu and UA Caratinga. Most of these UAs correspond to entire sub-basins, named as the main tributary (for ex: Piranga, Piracicaba, Santo Antônio, Guandu). Some UAs include the main watershed and additional smaller independent river basins draining to main channel of Rio Doce (Suaçuí, Caratinga, Manhuaçu, Santa Maria do Rio Doce, São José). Each UA differs in extension, geologic/hydrographic traits and degree of protection (see Table I).

Distribution data

We gathered distributional data from 6.042 lots of fish from the Rio Doce from museum collections. The examined material (53.945 specimens) belongs to the following fish collections: Instituto Nacional da Mata Atlântica (former Museu de Biologia Mello Leitão) (MBML-Peixes), Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande

Table I. **Nine Units of analysis (UA) along the Rio Doce (Source: PIRH Rio Doce 2010).**

UA	Surface	Main river sub-basins	Original vegetation	Number of protected areas included
1. Piranga	17565 km²	Piranga, Casca, Matipó	Semi-deciduous forest (Zona da Mata)	1-6
2. Piracicaba	5463 km²	Piracicaba, Doce	Cerrado	6- 7
3. Santo Antônio	10761 km²	Santo Antônio, Preto, Doce	Campo rupestre, Cerrado, Atlantic Forest	8-12
4. Suaçuí	21557 km²	Suaçuí Grande, Suaçuí Pequeno, Corrente Grande	Semi-deciduous forest (Zona da Mata)	12-13
5. Caratinga	6483 km²	Ribeirão do Boi Ribeirão do Bugre	Semi-deciduous forest (Zona da Mata)	-
6. Manhuaçu	9643 km²	Manhuaçu, Barroso, Barrosinho	Semi-deciduous forest (Zona da Mata); Atlantic Forest	14-15
7. Guandu	2468 km²	São Domingos, Guandu	Atlantic Forest	-
8. Santa Maria do Rio Doce	3031 km²	Rio Santa Maria do Rio Doce, Santa Joana	Atlantic Forest	16-18
9. São José	9751 km²	Mutum Preto, Pancas	Atlantic Forest, Muçununga	19-20

do Sul (MCP-Peixes), Museu de Ciências da Pontifícia Universidade Católica de Minas Gerais (MCNIP), Museu de Zoologia da Universidade Estadual de Londrina (MZUEL-Peixes), Museu Nacional/ UFRJ (MNRJ), Museu de Zoologia da Universidade Federal da Bahia (UFBA), Museu de Zoologia da Universidade de São Paulo (MZUSP) and Coleção Zoológica do Norte Capixaba da Universidade Federal do Espírito Santo (CZNC). Collections evaluated through database are: The Academy of Natural Sciences (ANSP-Ichthyology), Departamento de Zoologia e Botânica da Universidade Estadual Paulista, Campus de São José do Rio Preto (DZSJRP-Pisces), Laboratório de Ictiologia de Ribeirão Preto da Universidade de São Paulo (LIRP), Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá (NUP), Universidade Federal do Rio Grande do Sul (UFRGS), Museu de Zoologia da Universidade Estadual de Campinas (ZUEC-PIS) and Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS-PIS). Specimens evaluated in museum collections have their identifications checked with curatorial team through morphological examination and expert consultation when necessary. For taxonomic classification of specimens we followed Fricke et al. (2020). Collection data with missing geographic coordinates was georrefered based on information of localities included in the specimens' labels. Databases are available at Centro de Referência em Informação Ambiental-(CRIA) and collections repositories.

Fish assemblage categories

To build a general picture of the ecological diversity of fish along the basin, we categorize the species in the functional groups proposed by Elliott et al. (2007) for estuarine fish (Marine migrants (MM); Marine stragglers (MS); Estuarine species (ES); Amphidromous (AM); Freshwater species (FW) and Alien freshwater species (AS); see Supplementary Material – Table SI).

Sampling coverage

To illustrate the Wallacean shortfall along the Rio Doce basin, we measured sampling coverage by counting sampling points per 100 km^2 along the whole basin. The sampling coverage of the entire basin is calculated and compared to the sampling coverage of each Unit of Analysis (UA). If the coverage value for an UA is between +or-25% of the value calculated for the basin, the UA sampling level is considered within the mean: if above, it would be considered as high sampling level and if below, a low sampling level.

Analysis of Endemism

For the identification of AEs we use the software NDM-VNDM version 2.5 (Goloboff 2005) that implements the optimization criteria proposed by Szumik et al. (2002) and Szumik & Goloboff (2004). This algorithm evaluates the degree of congruence of the distributional range of species to a predefined area (an array of cells) by calculating an Index of Endemicity of Species (IEs). The values of IEs vary from 0 to 1; being 1 for a species homogeneously distributed in the evaluated area and absent outside it ("perfect fit"). During the calculation of IEs both observed (i.e: empirical information) and potential records were considered. Potential records of species were assigned by hand using the fill options and the presence of a species was assumed in those cells where the taxon could potentially occurr according to the ecological knowdlege and our previous observations of the species in the territory. By default, observed occurrences have higher value than potential occurrences in the analysis, having a major impact on the IEs.

The Endemicity Index of an Area (IEA) is equal to the sum of IEs of their endemic species; then, a certain group of cells will be better supported as an AE as more endemic species includes and as higher their EIs index are (Szumik & Goloboff 2004). The Individual Areas of endemism (IA/IAs) obtained were summarized in Consensus Areas (CAs) in order to facilitate the analyses of results. Similarly, to consensus trees in phylogeny, CAs summarizes the common information contained in IAs helping to visualize general patterns in a synthetic way. Here we applied the flexible consensus rule described in Aagesen et al. (2013), including in each CA those IAs that present at least 40% of endemic species in common.

Two strategies of analyses were followed: (A) analyzing the complete list of species (complete dataset, including 208 species), and (B) analyzing freshwater species only (reduced dataset including 123 species, near the 59% of total species). These differential strategies were defined in order to test whether there are AEs characterized exclusively by freshwater species that could remain hidden when analyzing the whole dataset and - if that was the case- to check if these AEs differ from those defined by a mix of species with different ecological requirements. The search parameters were kept constant in all the performed analysis (Minimum number of endemic species per area: 2; Minimum IEs: 0.5; Minimum IEA: 1; Minimum percentage of unique species per area: % 30; Loose Consensus with 40% of minimum species similitude), while the species filling was manually assigned and modified according to the grid size used.

Grid sizes

We analyzed the data under a wide range of latitude/longitude grid sizes, in order to observe possible variations in the patterns of endemism occurring at different geographic scales (0.07° x0.07°, 0.10°x0.10°, 0.15°x0.15°, 0.20°x0.20°, 0.30°x0.30°, 0.40°x0.40° and 0.50°x0.50°). The results discussed here, are mostly those obtained from 0.15°, 0.30°and 0.50° that happened to resume the variation found under the complete spectrum of grid sized tested. Complete results are included in the Supplementary Material – Results S1-S7 and presence/absence matrices are available upon request.

RESULTS

Taxonomic and functional diversity along the Rio Doce

A total of 208 species of fish included in 54 families and 20 orders were recorded for the Rio Doce basin (Table SI). The species composition in terms of abundance per family indicates a clear predominance of the families Loricariidae and Characidae. These families concentrate the 26% of the species present in the basin, while Sciaenidae represents 5% of the total of species (11 species), Cichlidae and Trichomycteridae with 4% of the total (9 species each). Each of the remaining 49 families represents less than the 3% of the species richness of the basin (Figure S2).

Regarding the functional diversity of fish, following the classification of Elliot et al. (2007), the Rio Doce basin presents 20 species marine stragglers (MS); 37 marine migrants (MM); 12 estuarine species (ES) and one Amphidromous species (AM). Additionally, 121 species are freshwater (FW) and 17 species are considered as alien freshwater species (AS), all of them of commercial or aquarium value. (See details in Table SI).

Sampling coverage

The UA Piranga (3.03/0.81), Suaçuí (1.93/0.45), Caratinga (0.70/0.19) and Manhuaçu (2.29/0.52) present a low sampling value when compared with the main values for the Rio Doce basin (6.44/1.15), while the UA Piracicaba (8.94/2.48), Santo Antônio (9.39/1.80), Guandu (10.49/1.78), Santa Maria do Rio Doce (34.04/3.53) and São José (15.98/2.09) presented a high value of sampling relative to the main values for the basin (Table II).

Table II. Sampling level calculated for each Unit of analysis (UA) at Rio Doce, considering the amount of lots in fish collections and the sampling localities per area. Calculated sampling index considered as high; middle or low, in order to illustrate the Wallacean shortfall along Rio Doce basin. High (above 1.43); middle (between 1.3 – 0.8) or low (below 0.8).

Units of analysis (UA)	Lots	Localities	Area (km²)	Lots/ 100 km²	Localities/100 km²	Sampling level
Piranga	532	143	17 565	3.03	0.81	low
Piracicaba	509	141	5 696	8.94	2.48	high
Santo Antônio	1 011	194	10 761	9.39	1.80	high
Suaçuí	415	98	21 557	1.93	0.45	low
Caratinga	44	12	6 248	0.70	0.19	low
Manhuaçu	221	50	9 643	2.29	0.52	low
Guandu	259	44	2 468	10.49	1.78	high
Santa Maria do Rio Doce	1032	107	3 032	34.04	3.53	high
São José	1.558	204	9 751	15.98	2.09	high
Total values	5 581	993	86 722	6.44	1.15	middle

Areas of Endemism

The presented results are focused in the analyses performed under grid sizes of 0.15°x 0.15°, 0.30°x0.30° and 0.50°x0.50°; however, some interesting or unique CAs found under other scales are also included (complete results are included in Results S1-S7). Each CA is followed by a subscript indicating the corresponding grid size of analysis. The letters "*all*" or "*fw*" indicate whether the results were obtained from the complete or reduced dataset (freshwater species exclusively), respectively.

Areas of Endemism and Units of analysis

The analyses conducted resulted in the identification of several AEs along the Rio Doce basin, some of which are congruent with different UAs, while others do not relate with these, representing original patterns (Table SII). A CA ($CA1_{0.50ºfw}$), recovers the whole basin as an AE (Figure S1c) defined by 21 wide distribution species detailed in Table SII.

Most Rio Doce species are regionally endemic, with records in neighbor river basins. Several CAs found were located at the UAs Piranga, Santo Antônio, Santa Maria do Doce and São José, and most CAs identified by the complete dataset were also recovered when analyzing the reduced dataset (freshwater species). The UA Piranga was evaluated together with UA Piracicaba, as they present faunal sobreposition. The same situation was observed in UA Santa Maria do Doce and UA Guandu.

UA Piranga and Piracicaba

The UA Piranga and the UA Piracicaba were not recovered as complete AEs in our analysis, but small AEs were identified inside each UA when using grids of 0.5º. Two main AEs were identified in the UA Piranga, located in the Southeastern and Northwestern portions of the sub-basin (Figure 1a). The Southeastern pattern was recognized only when analyzing the reduced dataset ($CA0_{0.50ºfw}$) and it is defined by *Characidium alipioi* and *Pareiorhaphis nasuta*. The Northwestern pattern was identified for the complete and reduced datasets ($CA4_{0.50ºall}$ and $CA9_{0.50ºfw}$), although both defined only for the freshwater species *Hemigrammus marginatus*, *Pareioraphis proskynita* and *Parotocinclus robustus*. These Northwestern and Southeastern patterns are adjacent but geographically separated by the Rio Piranga (geologically related to the uplifts of Serra da Mantiqueira), each one associated to the smaller watercourses running in opposite sides of the river (Figure S3). Another AE, related to the Piracicaba's headwaters at uplifts of Serra do Espinhaço, defined by *Hyphessobrycon santae* and *Pareiorhaphis proskynita* were identified for the complete and reduced datasets ($CA9_{0.50ºall}$ and $CA11_{0.50ºfw}$). Details about the endemic species and their EIs are included in Table SII.

UA Santo Antônio

Two patterns congruent with the UA Santo Antonio were recognized in the analyses of 0.20º and 0.40º (Figure 1b). The CAs identified by using the complete and reduced datasets are identical in terms of species composition and spatial extension. $CA6_{0.20ºall}$ and $CA4_{0.20ºfw}$ are extended along the headwaters of Rio Santo Antonio at Serra do Espinhaço), characterized by the presence of *Hypomasticus thayeri* and *Pareiorhaphis vetula*. $CA1_{0.40ºall}$ and $CA3_{0.40ºfw}$ are also defined by these species plus *Henochilus wheatlandii*.

UA Manhuaçu

$CA6_{0.30ºall}$ and $CA7_{0.30ºfw}$ are congruent with the UA Manhuacu (Figure 2a). These AEs are identical and defined by the presence of *Characidium vidali* and *Pareiorhaphis sp. aff. P. garbei*. These species inhabit the Caparaó Mountains in the Rio

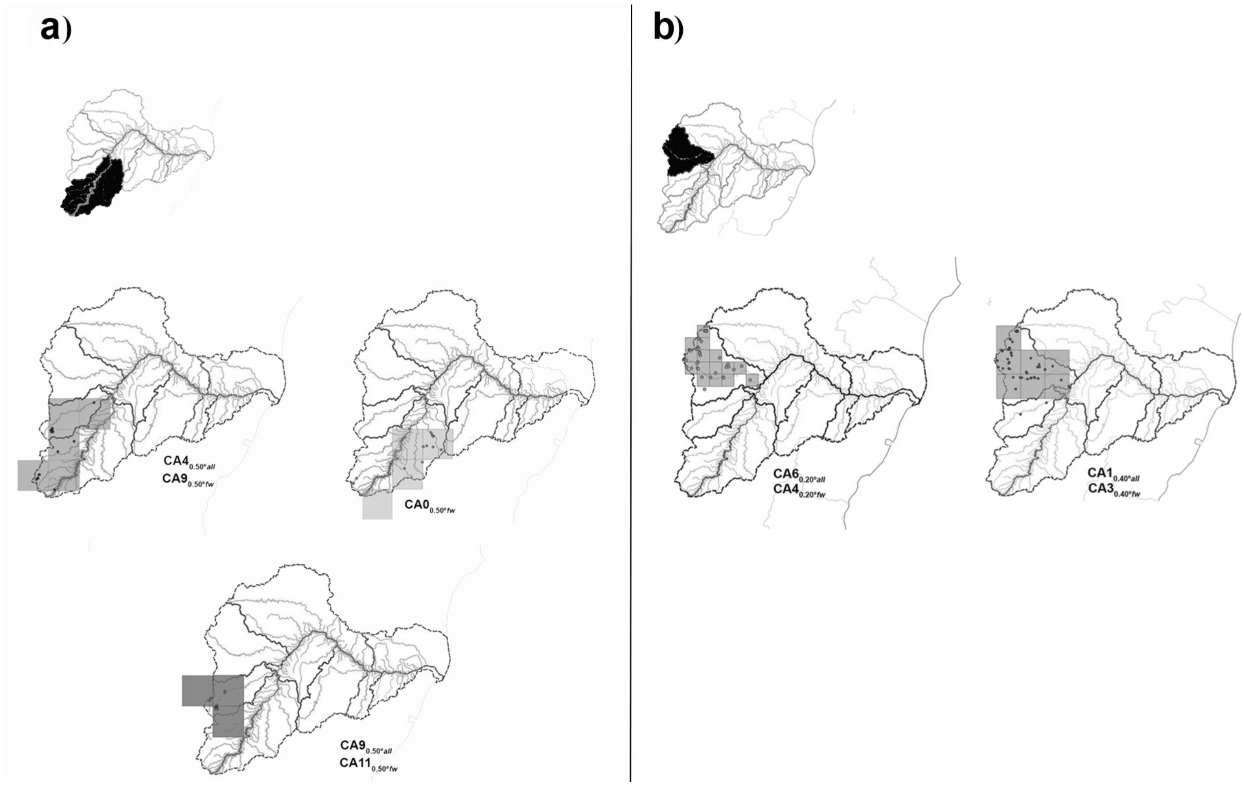

Figure 1. a) Consensus areas related to the Units of analysis Piranga resulting from the 0.50º latitude-longitude grid based analyses for the reduced (*fw*) and complete datasets (*all*): Note that both CAs are contiguous but not continuous, and are both separated by the main channel of the Rio Piranga; b) Consensus areas related to the UA Santo Antônio resulting from the 0.20º and 0.40º latitude-longitude grid based analyses for the reduced (*fw*) and complete datasets (*all*).

José Pedro and on headwaters of Rio Manhuaçu, living in stream environments with moderate to rapid current and sandy or rocky bottom. These species also occur at the lower stretch of Rio Paraíba do Sul, and the records at Rio Doce are assumed as its northern limits of distribution.

UA Santa Maria do Rio Doce/Guandu

The UA Santa Maria is recognized as an AE under the smaller grid size (0.15º), for the complete and reduced datasets (CA4$_{0.15\text{º}all}$; CA2$_{0.15\text{º}fw}$, Figure 2b-i), defined by *Astyanax* sp. aff. *A. microschemos* and *Phalloceros elachistos* as endemic species. These are freshwater riverine fish inhabiting both versants of Santa Teresa Mountains: the Rio Santa Maria in the Rio Doce valley and the shorter river basins draining to the sea, as Rio Reis Magos and Rio Piraque Açu. When using grids of 0.30° two wider AEs are identified that represent the UA Santa María and the UA Guandu together: CA2$_{0.30\text{º}fw}$, characterized by *Astyanax* sp. aff. *A. microschemos, Characidium timbuiense, Microglanis parahybae, Neoplecostomus espiritosantensis, Phalloceros elachistos*, and CA1$_{0.30\text{º}all}$ defined by the same species plus *Pterygoplichthys etentaculatus* and *Pygocentrus piraya*, both exotic. When enlarging the grid size to 0.50° three AEs, spatially related but not perfectly congruent to the UAs Santa Maria Doce and Guandu are identified (see Figure 2b-ii). The CA10$_{0.50\text{º}all}$ presents a high congruence with the UA Santa Maria and it is defined by the same species identified as endemic for this UA in smaller grid sizes (*Microglanis parahybae* and *Phalloceros*

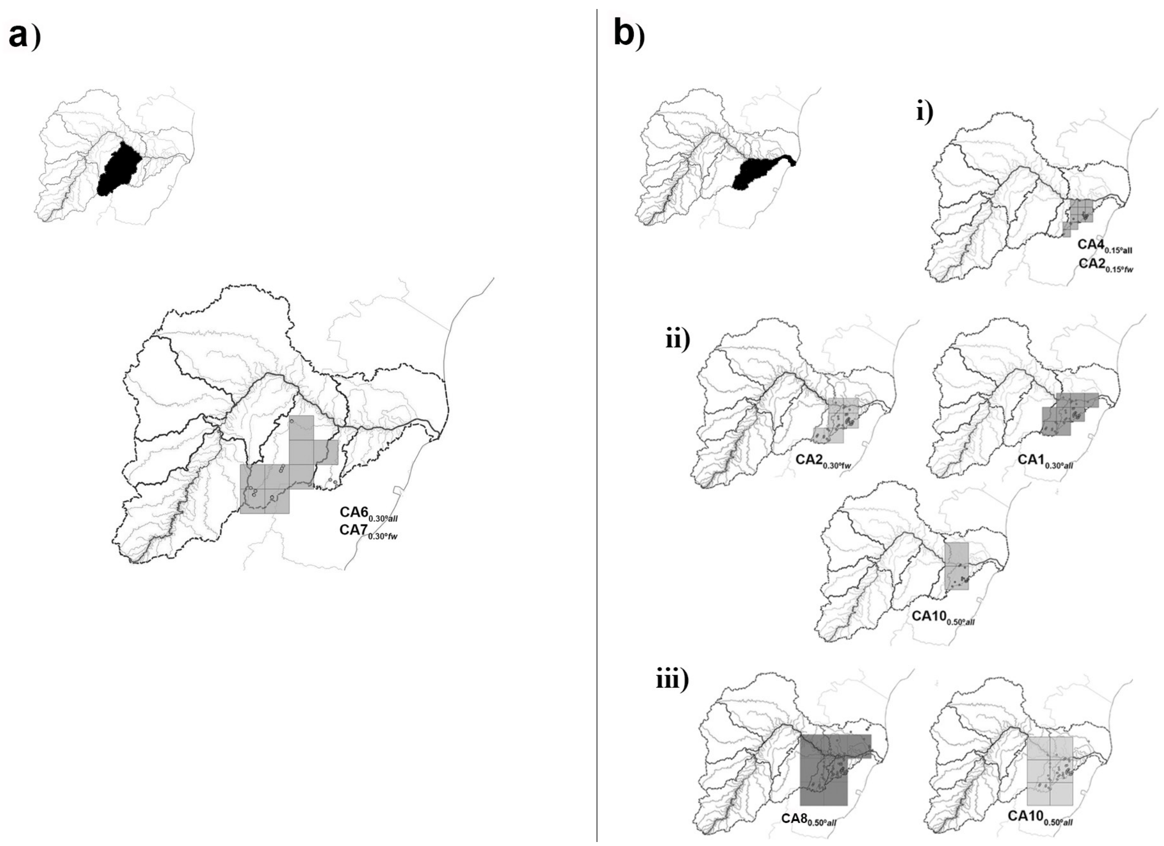

Figure 2. a) **Consensus areas related to the UA Manhuaçu resulting from the 0.30º latitude-longitude grid based analysis for the reduced (*fw*) and complete datasets (*all*); b) Consensus areas related to the UA Santa Maria of Rio Doce resulting from the 0.15° (i), 0.30º (ii) and 0.50º (iii) latitude-longitude grid based analyses, for the reduced (*fw*) and complete datasets (*all*).**

elachistos). CA8$_{0.50º all}$ is wider and includes the same set of endemic species, plus *Characidium timbuiense*, *Neoplecostomus espiritosantensis*, *Parotocinclus maculicauda* and *Serrasalmus brandtii* and CA10$_{0.50º fw}$ is characterized by a broader set of endemic species, including also *Awaous tajasica*, *Corydoras nattereri* and *Pimelodella aff. harttii* (Figure 2b-iii).

UA São José

Several AEs identified across the different scales are located in different zones of the UA São José (Figure 3). When using grids of 0.50º two AEs were identified, with the reduced and complete datasets, covering almost the complete UA São José: CA6$_{0.50º fw}$, defined by *Aspidoras virgulatus; Australoheros capixaba; Awaous tajasica; Corydoras nattereri; Microglanis pataxo; Otothyris travassosi; Metynnis lippincottianus; Synbranchus sp. n.* sensu Roberts as endemic species, and CA7$_{0.50º all}$ characterized by the same species (with exception of *Metynnis lippincottianus*) plus *Centropomus parallelus; Eleotris pisonis; Genidens genidens; Microphis lineatus; Mugil curema; Potamarius grandoculis; Trinectes paulistanus*. A diversity of AEs were found when using smaller grid sizes: CA0$_{0.15º all}$, CA3$_{0.15º all}$ and CA3$_{0.30º all}$ are related to the coast, and CA2$_{0.30º all}$, CA3$_{0.30º fw}$ and CA6$_{0.50º all}$, also associated to the coast but extended to the inlands. When using grids of 0.15º, some AEs were identified in the central-north part of the

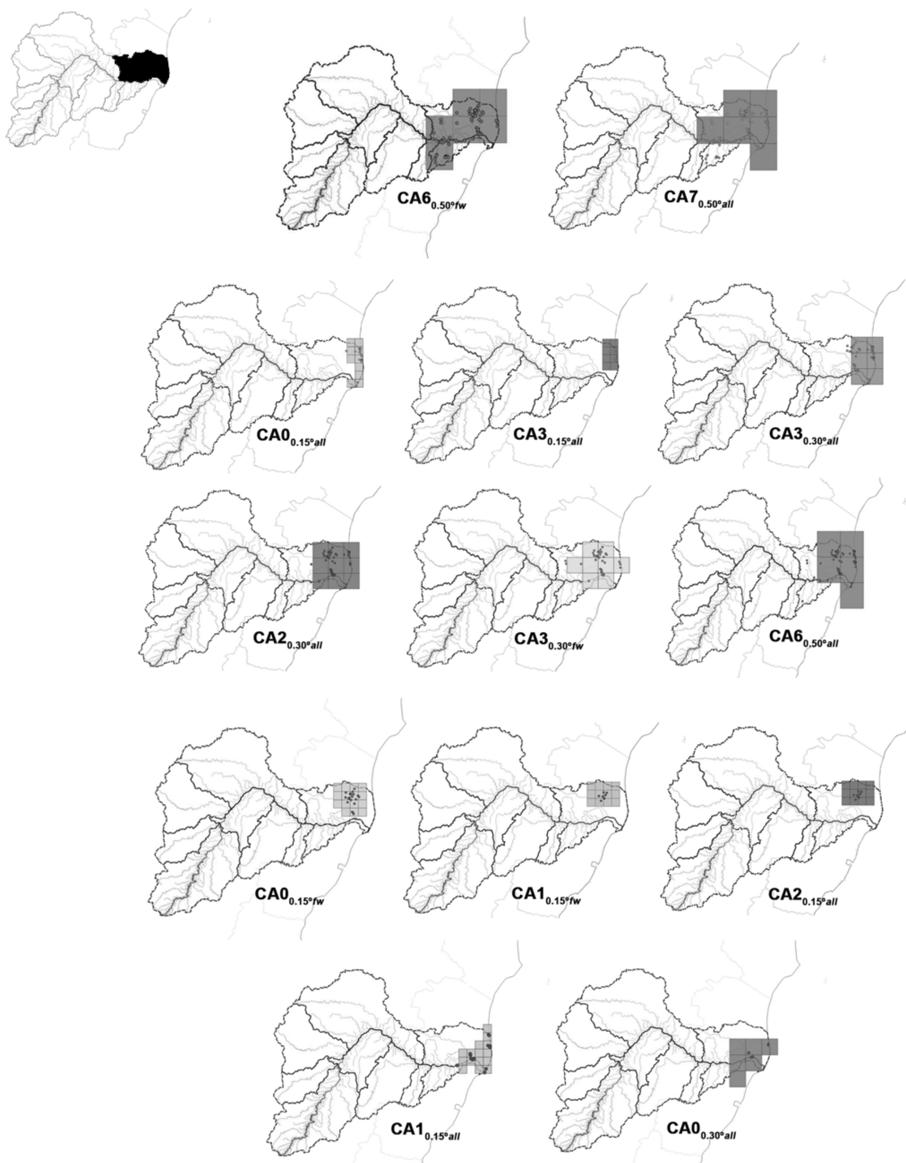

Figure 3. Consensus areas related to the UA São José, resulting from the 0.15º, 0.30º and 0.50º latitude-longitude grid based analyses, for the reduced (*fw*) and complete datasets (*all*).

UA (CA0$_{0.15º fw,}$ CA1$_{0.15º fw}$ and CA2$_{0.15º all}$). These AEs are characterized by *Aspidoras virgulatus, Brycon ferox* and *Otothyris travassosi*. CA1$_{0.15º all}$ and CA0$_{0.30º all}$ follow the coastline and are extended to the south, occupying the southeastern portion of the UA São José. These CAs share two endemic species in common: *Centropomus parallelus* and *Trinectes paulistanus*. Table SII includes a detailed taxonomic list of the species at Rio Doce.

No AE found in our analyses were congruent -neither complete or partially- or related to the UAs Suaçuí nor Caratinga (see Discussion for further details).

Areas of Endemism not related to UAs

Some AEs identified in our analyses do not show relation with any of the UAs proposed for the Rio Doce basin. We named these patterns according to their geographic location and following is presented a brief description.

Headwaters region

The analyses of the complete and reduced dataset for grids of 0.30º and 0.50º allowed the recognition of an AE related to the headwaters of the Rio Doce (Figure 4). This AE is represented by CA7$_{0.30º all}$, CA6$_{0.30º fw}$, CA3$_{0.50º all}$ and CA4$_{0.50º fw}$, and it is defined by *Characidium* sp. n. "alvorada" a potentially new species under study (S. Santos, pers comm.; Santos 2017) and *Parotocinclus robustus*. On the other hand, CA4$_{030º fw}$, characterized by *Henochilus wheatlandii* and *Phalloceros uai*, constitutes a disjuct AE with a portion ocurring on the headwater zone and other portion located northern and associated to the Rio São Francisco on western versant of Serra do Espinhaço mountains (*P. robustus*, *P. uai*).

Western patterns

A set of AEs were found located on the Western side of the Rio Doce basin (Figure 5). An AE, represented by CA5$_{0.50º fw}$, CA9$_{0.30º fw}$ and CA5$_{0.30º all}$ and defined by the endemic species *Astyanax* sp. aff. *A. scabripinnis*, *Geophagus* sp. n. sensu Mattos and *Trichomycterus brasiliensis*. Another AE located in the western central zone was found, represented by CA0$_{0.50º all}$; CA1$_{0.50º all}$;

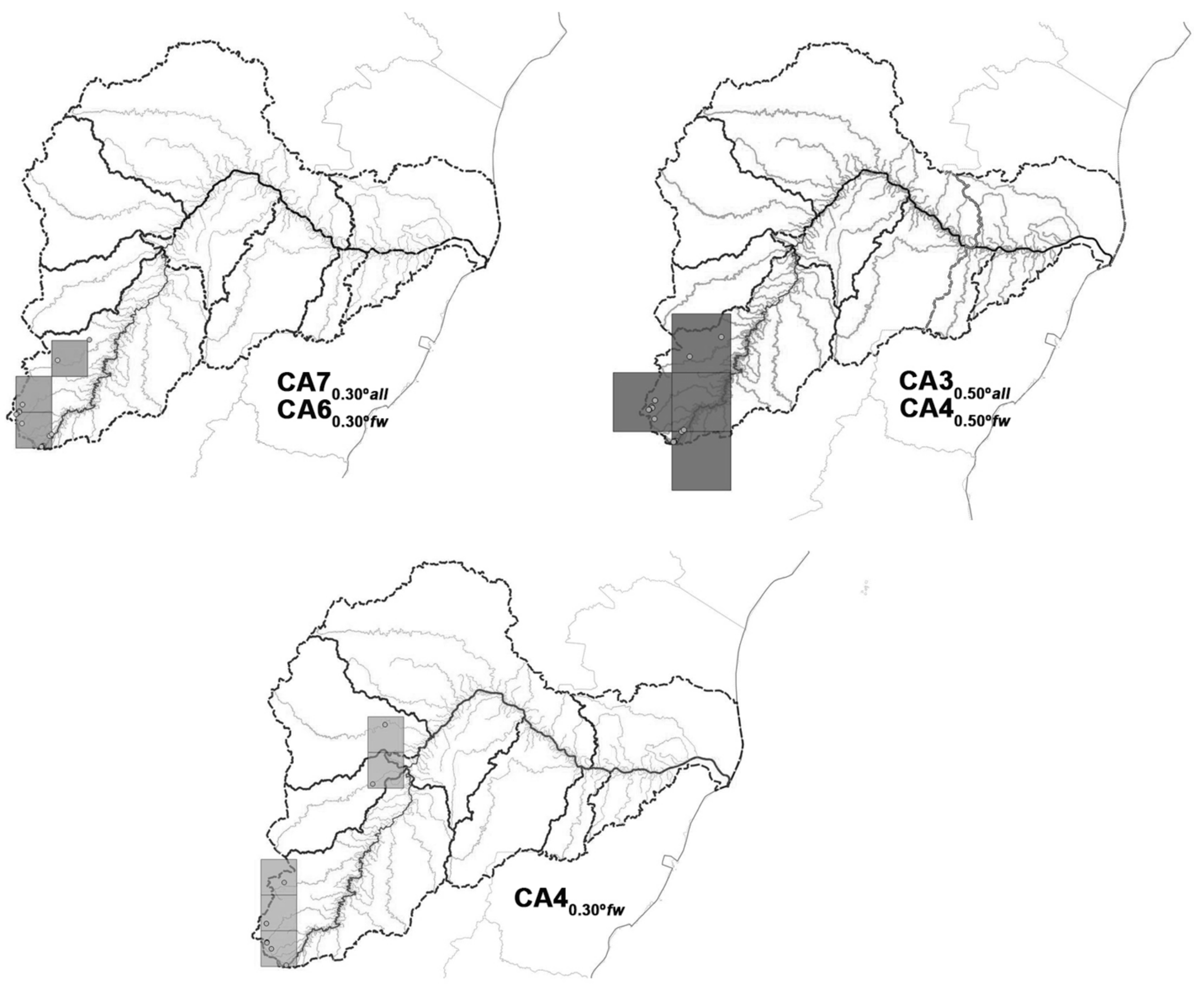

Figure 4. Headwaters pattern, resulting from the 0.30° and 0.50º latitude-longitude grid based analyses for the reduced (*fw*) and complete datasets (*all*).

CA2$_{0.50ºfw}$; CA3$_{0.50ºfw}$; CA2$_{0.50ºall}$; CA8$_{0.30ºall;}$ CA8$_{0.30ºfw}$; CA0$_{0.30ºfw}$, CA4$_{0.30ºall}$, and CA1$_{0.30ºfw}$. Several species characterize these CAs, among others *Astyanax* sp. aff. *A. scabripinnis*; *Brycon opalinus*; *Characidium* sp. n. "alvorada"; *Corydoras* sp.n. sensu Tencatt; *Deuterodon pedri*; *Harttia* sp.n. sensu Vieira; *Hasemania nana*; *Henochilus wheatlandii*; *Hoplias intermedius*; *Hypomasticus thayeri*; *Oligosarcus solitarius*; *Pareiorhaphis vetula*; *Phalloceros uai*; *Trichomycterus* sp. aff. *T. trefauti*; *Trichomycterus astromyterus*. The full list of endemic species is detailed in Table SII.

West-East Disjunction

A disjunct AE was identified when analyzing the reduced dataset (CA5$_{0.30ºfw}$ and CA7$_{0.50ºfw}$). This AE presents a West and East portion, and it is defined by *Astyanax* sp. aff. *A. lacustris (B), Deuterodon intermedius, Characidium cricarense, Gymnotus capitimaculatus, Hypostomus scabriceps, Phalloceros ocellatus, Trichomycterus pradensis* in CA5$_{0.30ºfw}$ while an extended list of endemic species defines CA7$_{0.50ºfw}$ (see details in Table SII and Figure S4a). Several species distributed in the eastern portion of UA São José and UA Santa Maria do Rio Doce, are distinctive from the upstream ones (at UA Suaçuí and UA Santo Antônio). This marked west-east disjunction is evidenced by separate species of the same

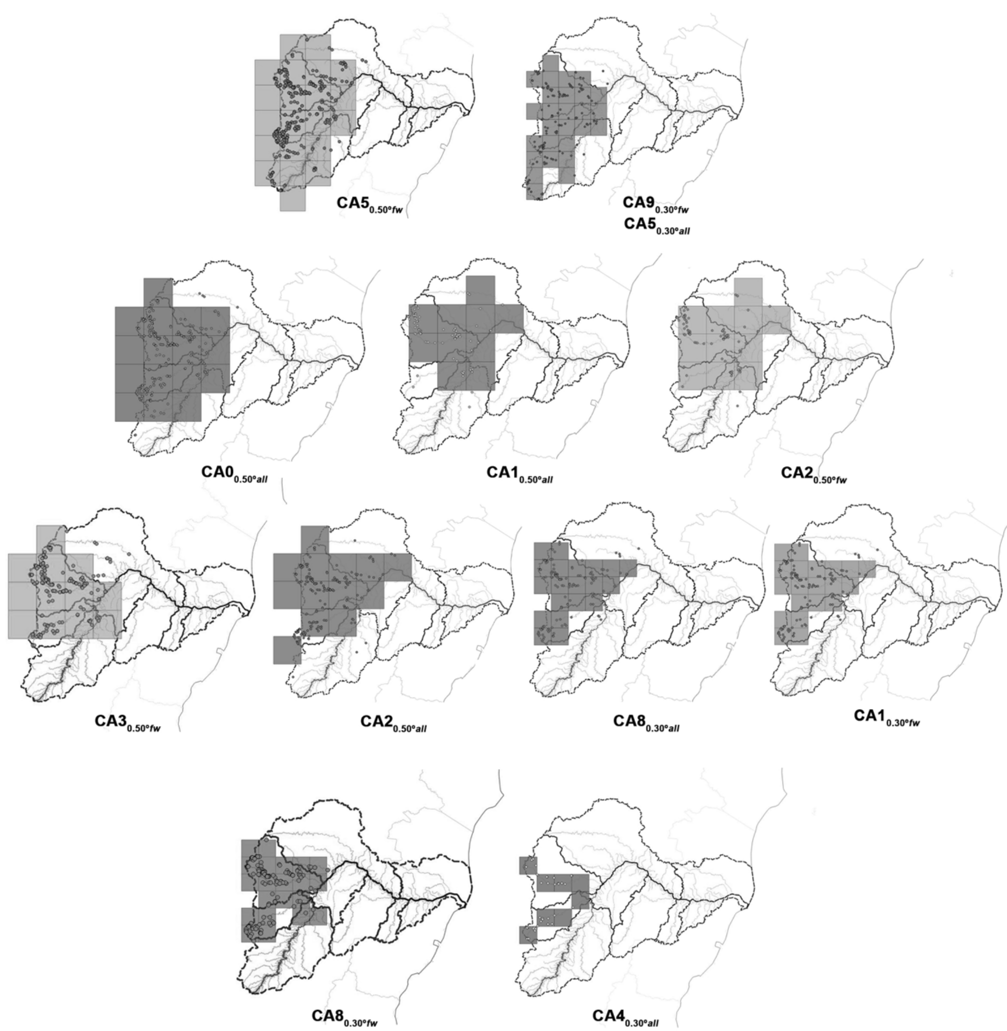

Figure 5. Western endemism pattern, resulting from the 0.30º and 0.50° latitude-longitude grid based analyses, for the reduced (*fw*) and complete datasets (*all*).

genus in the west and east areas. The western portion of the Rio Doce, at UA Suaçuí and UA Santo Antônio, is characterized by *Astyanax* sp. aff. *A. scabripinnis*; *Brycon opalinus*; *Characidium* sp. "alvorada" and *Phalloceros uai,* lineages observed in rivers at upper Rio Doce; while the eastern portion is defined as an area of endemism by *Astyanax sp. aff. A. microschemos*; *Brycon ferox*; *Characidium cricarense*; and *Phalloceros* elachistos at UA Santa Maria do Rio Doce and/or São José, lineages observed in rivers along lower portions of Rio Doce at Espírito Santo. Most of western and eastern portion lineages are allopatric, with no overlap in their distribution, indicating local endemism. The species widely distributed are mostly migratory, and these fishes probably illustrate a common history between Rio Doce and neighbor basin as Paraíba do Sul and coastal Espírito Santo as Itapemirim and Itabapoana.

Southern Pattern

Two CAs were found in the Southeastern part of the basin ($CA5_{0.50º\mathit{all}}$ and $CA8_{0.50º\mathit{fw}}$), characterized by *Characidium alipioi, Characidium vidali, Neoplecostomus microps* and *Pareiorhaphis sp. aff. P. garbei* (Figure S4b).

Grid sizes and Areas of Endemism

The total number of CAs increased when increasing the grid size used during the analyses. The maximum number of CAs was found when using cells of 0.40º and decreased again when enlarging grid size to 0.5º. This trend was observed for both, the complete and reduced datasets, and the number of IAs obtained for the complete and reduced datasets under different grid showed little variation (see Figure S5).

DISCUSSION

Sampling coverage

There is a large discontinuity between distinct sections of the basin, illustrating the lack of a specific planning regarding obtention of ichthyological data. In practice, sampling is determined by specific interests in certain geographical areas or related to area accessibility, proximity of research centers, etc. Additionally, enterprises in need of environmental evaluations also contribute to sampling in certain areas.

Areas of Endemism

Our data allowed the identification of several AEs characterized by particular sets of species along the Rio Doce basin, revealing fish as good indicators of freshwater endemism. Some of the recognized AEs are congruent with the UAs proposed by PIRH Rio Doce (2010) and several others do not show relation to these units and represent novel biotic patterns for the basin; also some UAs are totally unrelated to the AEs here identified. Although units defined on geo-political bases can be useful for administrative purposes, our results alert about the risks of using them as guide for conservation. Since these units are implicitly considered homegeneus in terms of its biotic and environmental conditions, it could be assumed that preserving a fraction of it would guarantee the preservation of its biodiversity; however our results show that smaller local patterns -defined by distinctive species composition- ocurr inside these units. A good example of this is the UA São José that allocates several AEs identified at different scales. The numerous endemisms found in the UA São José are probably related to the heterogeneity of habitats of the area - including a huge lacustrine system (Sarmento-Soares et al. 2017) -, and the large preserved lowlands that host diverse small sized fishes associated to riparian vegetation, as well as the coastal tablelands inhabited by endemic species as *Microglanis pataxo*, *Otothyris travassosi*, *Australoheros capixaba*, *Synbranchus sp. n.* sensu Roberts. Our results call to examine the use of UAs as tools for conservation, since it may lead to a simplification of the biodiversity present in the basin, and to support the use of natural patterns as units in conservation planning (see Crisci et al. 2003).

The UA São José is of maximum importance regarding freshwater fish diversity, hosting endangered species as *Acentronichthys leptos*, *Ituglanis cahyensis*, *Mimagoniates sylvicola*, *Rachoviscus graciliceps*. It is noticeable that none of these species contributed to define endemisms in this analysis, possibly due to their scarce representation in the data set. The lacustrine fish *Australoheros capixaba* and *Brycon ferox*, as well as fishes inhabiting clean water estuaries as *Dormitator maculatus*, and the diadromous *Awaous tajacica* a marine species specially adapted to freshwaters, are indicative endemisms at São José.

Species distributions and ecology

Rheophilic species at Rio Doce inhabit rapids and waterfalls and have a strong biogeographical signal, reinforcing the identification of the main Rio Doce as a unique pattern (CA1 Rio Doce basin - Figure S1). Some rheophilic specialists are migratory, circulating along large river channels within the Rio Doce, and many species as the anostomids *Hypomasticus mormyrops*, *Leporinus copelandii*, *Megaleporinus conirostris* are migratory fish found in lotic environments, presenting behavioral adaptations to occupy fast flowing river stretches (Vieira & Birindelli 2008). Additionally, the loricariids *Hypostomus affinis*, *Loricariichthys castaneus* inhabit moderate to rapid water and remain swimming in rapids sheltered from direct current.

On the other hand, small-sized freshwater fish as Characidae, Callichthyidae, Heptapteridae, Gymnotidae, are mostly sedentary and have life strategies and ecological attributes associated to very particular habitats. These species are generally dependent on of riparian vegetation for their food, shelter, and breeding sites. Although not presenting high biomasses, these small-sized species represent most of the fish biodiversity at the neotropics (Castro & Polaz 2020). The thirteen CAs found at UA São José (Figure 3) are mostly supported by the presence of small fishes living in association to riparian environments as *Aspidoras virgulatus*, *Corydoras nattereri*, *Microglanis pataxo*, *Otothyris travassosi*.

Marine influenced species inhabit the Rio Doce at UA São José and are indicative of endemisms when analyzing the complete dataset (Figure 3). These marine and estuarine species move along the Rio Doce lower valley and some are of great economic importance (*Mugil curema*, *Centropomus parallelus*). A large amount of marine fishes penetrate the Rio Doce circulating in mangrove areas and/or lacustrine system, in search for food, shelter or even reproduction and nursery, and are indicators of endemism as *Achirus lineatus*, *Anchovia clupeoides*, *Atherinella brasiliensis*, *Caranx latus*, *Chloroscombrus chrysurus*, *Diapterus rhombeus*, *Eleotris pisonis*, *Eucinostomus melanopterus*, *Eugerres brasilianus, Gobionellus oceanicus, Microphis lineatus, Polydactylus virginicus, Sphoeroides greeleyi, Sphoeroides testudineus, Trinectes paulistanus*. The mugilid *Mugil curema*, the centropomid *Centropomus parallelus* are found far from the Rio Doce mouth and are indicative of CA at UA São José.

Endemism Analyses and parameters variation

In concordance with the observed in studies for terrestrial organisms (Aagesen et al. 2009, Casagranda et al. 2009, Salinas et al. 2019, Cabral et al. 2020), variations in the grid size of the analyses, produce noticeable effects on the identification of AEs. While the use of small cells facilitates the identification of small-local patterns (for ex: $CA4_{0.15º\mathit{all}}$; $CA2_{0.15º\mathit{fw}}$; UA Santa Maria do Rio Doce), the use big cells leds to the recognition of patterns of wider extension (see for ex $CA1_{0.50ºfw;}$ whole Rio Doce Basin). The variety of patterns found under different scales –hidden in unique scale analyses– allows to describe different layers of endemism, from local to regional. The diverse AEs found in our analyses reinforce the importance of using multiple grid sizes in order to obtain a comprehensive picture of the endemisms of a region. The origin of AEs identified at different scales could be related to events occurred at different periods of times, representing unique pieces in the historical puzzle of a region.

The AEs found with the reduced dataset were similar in number and composition to the recognized with the complete dataset and some patters were only recognized by analyzng reduced dataset. This result suggests that exclusive freshwater fish present strong biogeographic signal, adding crucial information to the description of historical patterns in freshwater systems. Since these species present a stronger association to riverine environment than species with a broader ecological niche, it is possible that geological and ecological events affecting the basin in past times would have great impact on their distributions, shaping the patterns of sympatry currently observed.

Biogeography of aquatic systems

Biogeographic knowledge is fundamental for biodiversity preservation and several analytical challenges need be overcome to reach a better description of the biogeographic patterns in aquatic systems. Most analytical methods in biogeography have been developed using terrestrial organisms as models, unconsidering the singularities of aquatic biota. Aquatic systems differ from terrestrial in geomorphological aspects (as landscape dynamics and origin) and also, the distribution of aquatic species present singularities given by strong fisiological constraints related to aquatic life, as well as for the geometry of the areas they inhabit (for example, the lineal or dentritic shape of rivers). To consider these particularities during empirical works would be desirable, however, no analytic tools taking these points into account are available to the moment.

Most biogeographic methods use grid systems for the rasterization of data, necessary to compute calculations. This conversion generates different degrees of inaccuracies in the representation of data and the boundaries of the resultant patterns. The cell size choice is a central parameter in biogeographic analyses. In the case of fresh water organisms, an inappropriated cell size election could imply to assume the presence of a species outside the watercourse, or the merging of fauna belonging to different tributary rivers in the same cell, generating major problems in the representation of real distributions.

Our results show that under appropriate analytical parameters NDM is effective for identifying areas of endemism in a freshwater system; however having analytical tools specifically designed for the study of freshwater systems would facilitate a deep exploration of different aspects of its biogeography and provide important data for conservation.

CONCLUSIONS

Areas of endemism are defined by unique combinations of species representing key sites for conservation, and its identification is fundamental step in the understanding of the evolutionary history of taxa (Carvalho 2011, Swenson et al. 2012, Lima et al. 2020). These patterns are not necessarily related to richness hotspots (see for ex: Williams et al. 2000, Hughes et al. 2002) and because of that, including endemism in prioritizarion of areas for conservation has become indispensable to preserve biodiversity singularities (Whittaker et al. 2005).

In this paper we describe by the first time patterns of endemism for the Rio Doce, on the base of fish fauna. We hope our results bring a baseline to future investigations explorating the mechanisms involved in their origin, contributing to a deeper understanding of the evolutionary history of taxa.

Besides the intrinsic value of the presented study, the analyses here performed required the organization and curation of disperse distribution data of the Rio Doce fish, resulting in a distributional dataset that facilitated the observation of general distribution of species, ecological patterns, and information gaps along the basin. Although in recent years several species were described for the Rio Doce fish (i.e., Malanski et al. 2019, Reis et al. 2019) its diversity is still in need of investigation. The poor taxonomic knowledge on the Rio Doce species is reflected in the important number of collection specimens without name and represent an important obstacle for prioritizing freshwater habitats for conservation (Abell et al. 2008, Darwall et al. 2011, Frederico et al. 2018, Hrbek et al. 2018). The missing information also led to the exclusion of many taxa from IUCN evaluations or to being listed as data deficient when evaluated (Collen et al. 2014). In this paper we found 16 unnamed species as endemic to the river, and provisionally named them as: *Pareiorhaphis* sp. (endemic at UA Manhuaçu); *Astyanax* sp. aff. *A. microschemos* plus *Pimelodella sp. aff. P. harttii* (both endemic at UA Santa Maria/ Guandu); *Synbranchus* sp. n. sensu Roberts (endemic at UA São José); *Characidium* sp. n. "alvorada" (endemic at headwaters); *Astyanax* sp. aff. *A. scabripinnis*; *Geophagus* sp. n sensu Mattos; *Harttia* sp.n. sensu Vieira; *Corydoras* sp. n. sensu Tencatt; *Trichomycterus* sp. aff. *T. trefauti plus Rineloricaria* sp. aff. *R. steindachneri* (endemic at western pattern) and also *Pareiorhaphis sp. aff. P. garbei* (southern pattern). The species *Astyanax* sp. aff. *A. lacustris* is recognized as endemic at Rio Doce basin, known to occur in different sections of the river basin. All these species need further taxonomic studies in order to assert its identity.

Our dataset showed sampling gaps in the UAs Suaçuí, Caratinga (both with no CA found in present analysis) and Manhuaçu. This indicates that, along with taxonomic studies, a strong collecting effort is needed in order to improve the knowdlege on the Rio Doce fish fauna (Bini et al. 2006).

The results here described could be used as baseline for comparison to the current distributions of fish in the Rio Doce, in order to obtain a measure of the changes produced by the burst. After 7 years of the Rio Doce mining tragedy, we hope the results and data here presented will help to evaluate the current situation of fish species in the Rio Doce system and to develop a pathway towards the recovery of its diversity. We believe that collective restoration actions – involving local people, scientists and goverment agencies- may be an effective strategy to recover the serious damage caused by this environmental tragedy.

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SUPPLEMENTARY MATERIAL

Figures S1-S5
Results S1-S7
Tables SI, SII

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MDC, LMSS and RFMP designed the general idea of this paper. LMSS identified the species; MDC designed and performed endemism analyses, selected and organized results and generated illustrations. MDC presented preliminary results at the XIII REUNIÓN ARGENTINA DE CLADÍSTICA Y BIOGEOGRAFÍA and defined the general organization of the manuscript. MDC, LMSS and RFMP conducted interpretation of results. All authors contributed substantially to the writing and editing of the manuscript.