UNIVERSIDADE FEDERAL DE VIÇOSA

OTÁVIO LUIZ FERNANDES

INTEGRATIVE APPROACH OF THE KNOWLEDGE OF EPHEMEROPTERA (INSECTA) FROM BRAZIL: THREATS, CURRENT STATUS AND DATABASE DEVELOPMENT

VIÇOSA – MINAS GERAIS

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

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"Infinitamente belos, insuportavelmente efêmeros.

Assim são os bons momentos da vida."

(Adaptado de Rubem Alves)

RESUMO

FERNANDES, Otávio Luiz, M.Sc., Universidade Federal de Viçosa, março de 2021. **Abordagem integrativa do conhecimento de Ephemeroptera (Insecta) do Brasil: ameaças, estado atual e desenvolvimento de banco de dados.** Orientador: Frederico Falcão Salles.

Um dos principais obstáculos para a conservação da biodiversidade é a falta de conhecimento adequado da taxonomia descritiva e da distribuição dos organismos, o que é especialmente verdadeiro para a ordem de insetos Ephemeroptera no Brasil. Espécies de Ephemeroptera são amplamente endossadas como bioindicadoras da qualidade da água e integridade ecológica, pois são sensíveis aos efeitos das atividades humanas nos ambientes aquáticos. No entanto, quase todo o conhecimento disponível para a ordem no Brasil é derivado de estudos taxonômicos e está fragmentado na literatura especializada. Nesse contexto, este estudo teve três objetivos principais. Primeiramente, integrar e disponibilizar, por meio de um website, conhecimentos de taxonomia básica e distribuição de Ephemeroptera do Brasil. Em segundo, avaliar o status do conhecimento e revelar as implicações de tal conhecimento para a conservação da ordem. E, terceiro, determinar o nível de concordância entre os especialistas sobre as ameaças ambientais que afetam a persistência das populações de Ephemeroptera no Brasil. Em nossos resultados, fornecemos acesso aberto a informações taxonômicas e distributivas básicas de Ephemeroptera por meio de um banco de dados online interativo. Revelamos que os estudos taxonômicos da ordem aumentaram enormemente nas últimas duas décadas, com grande expansão no número de descrições de espécies (166 em 2004 para 414 em 2020) e seus distintos semaforontes. Porém, todos os registros de ocorrência da ordem estão distribuídos em apenas 33% do Brasil, elucidando vieses para determinadas áreas do país. Além disso, a opinião de especialistas mostrou que as populações de Ephemeroptera sofrem com diferentes pesos e conjuntos de ameaças ambientais em cada bioma brasileiro, sendo as relacionadas à agricultura consideradas proeminentes em quase todos os biomas. Concluímos que é necessário direcionar esforços para diminuir os déficits distribucionais e taxonômicos e, direcionar estudos ecológicos sobre as ameaças ambientais em curso no território brasileiro e que afetam os Ephemeroptera.

Palavras-chave: Insetos aquáticos. Banco de dados. Déficits de conhecimento. Viés de amostragem. Ameaças ambientais.

ABSTRACT

FERNANDES, Otávio Luiz, M.Sc., Universidade Federal de Viçosa, March, 2021. **Integrative approach of the knowledge of Ephemeroptera (Insecta) from Brazil: threats, current status and database development.** Advisor: Frederico Falcão Salles.

One of the main obstacles to the conservation of biodiversity is the lack of adequate knowledge of the descriptive taxonomy and the distribution of organisms, which is especially true for the insect order Ephemeroptera from Brazil. Ephemeroptera species are widely endorsed as bioindicators of water quality and ecological integrity, as they are sensitive to the effects of human activities in aquatic environments. However, near all the knowledge available for the order in Brazil is derived from taxonomic studies and is fragmented in the specialized literature. In this context, this study had three main objectives. First, to integrate and make available, through a website, basic taxonomic and distributional knowledge of Ephemeroptera from Brazil. Second, to assess the knowledge status and to reveal the implications of such knowledge to the conservation of the order. And, third, to determine the level of agreement between mayflies' experts about environmental threats that affects the persistence of Ephemeroptera populations in Brazil. In our results, we provide open access to basic taxonomic and distributional information for Ephemeroptera through an interactive online database. We revealed that taxonomic studies of the order increased enormously in the last two decades, with a major expansion in the number of species descriptions (from 166 in 2004 to 414 in 2020) and its distinct semaphoronts. However, all occurrence records of the order are distributed in only 33% of Brazil, elucidating biases towards certain areas of the country. Additionally, experts' opinion showed that mayflies suffer with different weights and sets of environmental threats in each Brazilian biome, with agriculture related ones considered prominent in almost all biomes. We conclude that is needed to direct efforts towards diminishing distributional and taxonomic shortfalls, and to address ecological studies regarding the ongoing environmental threats in the Brazilian territory that affect Ephemeroptera.

Keywords: Aquatic insects. Database. Knowledge shortfalls. Sampling bias. Environmental threats.

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SUMMARY

1. General Introduction

1.1. Biodiversity

First coined by Walter G. Rosen, the term *biodiversity* was idealized in 1985 as a contraction for *biological diversity*, while planning a national forum on that subject (Sarkar, 2002). Since then, both terms have been synonymized and used interchangeably (Harper & Hawksworth, 1994). “The” biodiversity concept is a very broad one that encompasses a great variety of phenomena (see e.g. CBD — United Nations, 1992, Maclaurin & Sterelny, 2008; Magurran, 2013; Mayer, 2006). The International Union for Conservation of Nature (IUCN) managed a consistent definition that includes different interpretations of traditional types of biological variety: “biodiversity is the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems” (CBD, 1992). The concept of biodiversity and the consensus among scientists and activists about the urgency of preventing biodiversity from continuing to be destroyed by the excesses of the human species led to a shift in the way we address the issue of nature conservation (Franco, 2013).

Without the products and services provided by the natural environment, human beings would not be able to survive and prosper (Rolston, 1985). It provides a wide range of materials derived from plants and animals useful for agriculture, fisheries, forestry, building industries and tourism (Rolston, 1988). Verma (2016) states a social value for biodiversity in which customs, religion and psycho-spiritual aspects of people are expressed. Many animals like cow, bull, peacock, owl, snake etc. also have a significant place in our psycho-spiritual arena and thus hold special social importance. In addition, in a non-instrumental approach, biodiversity is valuable because it supports the life of other living things such as animals and plants, and simply because it exists, regardless of its usefulness for human beings (Gaia & Jones, 2017; Justus et al., 2009; Naess, 1973).

Despite the infinity of relationships between human well-being and the maintenance of biodiversity, human activities have impacted nature in such a way that a movement arouse in the beginning of the century to name a new geological era, ‘the

Anthropocene' (McGill et al., 2015). The term is an important consequence of the recognition of increasing intensity of the degree to which humans are driving patterns in global biogeochemistry and biodiversity (Gibbard & Walker, 2014). The present trend in biodiversity loss threatens our opportunities for biotechnology, wood, medicines, energy, water supply, climate quality, with interference in essential ecological functions, all representing important values for human society (Alho, 2008). As a result, the urgency of conserving biodiversity spans multiple sectors, from governments and academia to environmental and development non-government organizations (NGOs), to businesses and community groups (Seddon et al., 2016).

1.2. Ephemeroptera Hyatt & Arms, 1890

Mayflies (order Ephemeroptera) date from Carboniferous and Permian times and represent the oldest order of the existing winged insects. They are unique among pterygote insects in having two winged adult stages, the subimago and imago (Brittain & Sartori, 2009). Globally, this order contains 442 genera and fewer than 3700 species, according to Jacobus, Macadam & Sartori (2019). In Brazil, 10 families have been recorded up to now, containing 82 genera and approximately 400 valid species (Salles & Boldrini, 2020). Mayflies are found in almost all types of freshwater habitats throughout the world and display an amphibiotic life cycle, that is, the larval stage is aquatic and the alate stage is terrestrial (Salles et al., 2004).

The nymphal stage of mayflies is the dominant life history stage, and is always aquatic. The nymphs undergo a series of moults as they grow, the precise number being variable within a species, depending on external factors, such as temperature, food availability and current velocity (Brittain & Sartori, 2003). Typically, nymphs have up to seven pairs of abdominal gills, usually three caudal filaments and mouthparts generally adapted for collector/gatherer or deposit feeding. A few species are predaceous; some are scrapers, and other filter feeders (Barber-James et al., 2008).

Historically, mayflies are considered unskilled dispersers (Brittain, 1982). There are two main modes of movement: as nymphs, individuals are dispersed in a passive way by downstream movements (i.e., drift) and, as winged stages, by air (actively or not) (Lancaster & Briers, 2008). Their drift shows a strong diel periodicity, with a peak during the hours of darkness. Factors that have been shown to influence mayfly drift include changes in current velocity and discharge, increased sediment loading, temperature changes, oxygen conditions, density, food availability, and predators (Brittain & Sartori, 2009). Because most species live only a few hours as winged adults and with the sole purpose of mating, active flight dispersal is likely to occur in shorter distances than in drift movements (Monaghan et al., 2005). Some other feasible mechanisms such as wind-blown dispersal of females, eggs attached to the feet or bodies of birds, and wind-blown eggs are believed to occur. Mayflies have been trapped as high as 2000 feet above the ground, but they are readily killed by desiccation. However, dying females usually extrude their eggs and possibly wind-blown dead females can be propagules (Edmunds, 1972).

This weak dispersal power of mayflies also results in a high percentage of endemism. Many species colonizing warm running waters in the Neotropics are found nowhere else (Salles et al., 2020a,b; Ulmer, 1920). The isolation of this continent for millions of years is one of the most important reasons why 2/3 of the Neotropical genera and more than 90% of the species are endemic from this realm (Gattolliat & Nieto, 2009). Vicariance, radiation, and dispersal events must be taken into account to explain present faunal composition and distribution of mayflies throughout all realms (Barber-James et al., 2008).

In combination with the dominant aquatic nymphal stage, high levels of diversity and endemicity, mayflies are considered a keystone group and their presence is believed to be an important environmental indicator of oligotrophic to mesotrophic (i.e. low to moderately productive) conditions in running waters (Bauernfeind & Moog, 2000). They are widely endorsed as bioindicators of water quality and ecological integrity (Barbour et al., 1999; Menetrey et al., 2008) due to a high sensitivity to oxygen depletion, acidification, and various contaminants including metals, ammonia and other chemicals (Beketov, 2004; Hubbard & Peters, 1978). Subsequently, many of the biological water quality assessment methods for streams include Ephemeroptera, as for example the EPT index (Hamid & Rawi, 2017; Kietzka et al., 2019), Biological Monitoring Working Party (BMWP), Average Score per Taxon (ASPT), Trent Biotic Index (TBI) (Woodiwiss, 1964), and Family Biotic Index (FBI) (Hilsenhoff, 1988). In addition to Ephemeroptera, most indices are known to use other two orders of aquatic invertebrates: Plecoptera, and Trichoptera. All three orders and their families, as a rule, have the highest scores of sensitivity and, hence, have a pronounced effect on the absolute value of the indices (Semenchenko & Moroz, 2005).

Mayflies constitute a major part of the macroinvertebrate biomass and production in freshwater habitats. Several species, including a range of other invertebrates (especially species of Arachnida and Odonata), birds, lizards and other reptiles, amphibians, bats and other mammals—feed upon mayflies (Baxter et al., 2005; Domínguez, 2001; Macadam & Stockan, 2015). Arguably, the most important of these predator-prey relationships is the contribution of mayflies to the diet of fish. While some fish species casually or incidentally feed on mayflies, other fish species rely almost exclusively on them (Macadam & Stockan, 2015). In being an integral part of the diet of fish and other aquatic animals, mayflies serve as a link in the flow of energy between primary producers and secondary consumers (Jacobus et al., 2019). Mayflies also play an important role in the cycling and transfer of nutrients and carbon between aquatic and terrestrial habitats. Winged mayflies emerge from the water and enter the terrestrial realm, where they may be consumed by many riparian species such as birds, bats, spiders, lizards, and other invertebrates (see Baxter et al., 2005). Partly, this typifies their role in transferring matter from aquatic to terrestrial systems. In this role, however, it is important to consider that they also serve as a “biotransporter” of potentially problematic substances, such as waterborne contaminants, to terrestrial ecosystems (Walters et al., 2008).

Despite their relevance, research on Ephemeroptera is still represented by four main biodiversity shortfalls: Wallacean, that is, lack of data on the distribution of species (Lomolino, 2004); Linnaean, due to the lack of taxonomic information (Brown & Lomolino, 1998); Darwinian, regarding the lack of phylogenetic data on species relationships (Diniz-Filho et al., 2013); and the Haeckelian shortfall, referring to the lack of knowledge about the distinct semaphoronts of the individuals that represent the species (Faria et al., 2020). The majority of published studies in recent years have focused on reducing these deficiencies: Linnean (Nascimento et al., 2017; Gonçalves et al., 2017; Molineri & Salles, 2017; Salles et al., 2015); Wallacean (Boldrini et al., 2012; Raimundi, 2020; Salles et al., 2010); Darwinian (Cruz et al., 2018; Molineri et al., 2015; Nieto et al., 2020; Salles et al., 2016); and Hackealian (Boldrini et al., 2013; Cruz et al., 2014; Salles et al., 2016a, b). However, much of this knowledge is fragmented or dispersed in the specialized literature, which hampers the access to all the knowledge available for the order.

Given the importance of biodiversity and the relevance of mayflies as an important component of freshwater ecosystems, the aims of this work are i) to integrate, ii) to assess the knowledge status of the order Ephemeroptera in Brazil, iii) to reveal the implications of such knowledge to the conservation of the order, and, iv) to determine the level of agreement between mayflies' experts about environmental threats that affects the persistence of Ephemeroptera populations in Brazil. To do so, this dissertation is divided into four chapters: Chapter one is a dataset of Ephemeroptera from Brazil, including taxonomic and distributional information of the order. Chapter two concerns with the assessment of the knowledge presented in the previous chapter and its implications to the conservation of the species. As a complement to discuss some conservation aspects of the order, chapter three brings information regarding environmental threats that are detrimental to the persistence of the species of mayflies occurring in Brazil.

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2. Chapter One – EphemBrazil: a dynamic and online database of Ephemeroptera from Brazil

2.1. INTRODUCTION

Cayuela et al. (2012) indicated that one of the main obstacles to the conservation of biodiversity is the lack of adequate knowledge of the descriptive taxonomy and the distribution of organisms (the so called Linnean and Wallacean shortfalls respectively (see Hortal et al., 2015); which is especially true for Ephemeroptera in Brazil. There is, for example, no catalogue of Brazilian fauna or South American (only lists of species) and the most important literature regarding the taxonomy of Ephemeroptera in the region, the book 'Ephemeroptera of South America', had its first and only edition published in 2006 (Domínguez, 2006). Since then, the number of species from this area increased from 400 to near 700, mostly due to the advances regarding the group's taxonomy, both in Brazil and in the Neotropical Region (Domínguez & Dos Santos, 2014). As a consequence of the growing number of studies regarding the order, the knowledge generated, specially distributional records, is dispersed in specialized literature. A situation that for non-specialists, such as ecologists, hampers any attempt to promote studies and conservation actions.

Additionally, for some of the previously described species of Ephemeroptera, we only know a few locations where they are distributed, if not a single locality (Campos et al., 2019; Cruz et al., 2014; Cruz & De-Souza, 2014; De-Souza et al., 2011; Mayo, 1972). This can be related to endemism, of course, but most of the time is related to the lack of knowledge in certain areas - some areas are simply not sampled; some have been sampled but the identification of their taxa is above the species level; while some have been sampled, the taxa have been properly identified, but the list is restricted to a museum or a collection. The compilation of good distributional data is the first step in any systematic conservation planning exercise (Margules & Pressey, 2000). Without reasonable information on where the species live, it is impossible to know which species are in danger and where to concentrate efforts to preserve them (Cardoso et al., 2011). A complete repertoire of information about the extent of geographical distribution, area of occupation, and about fragmentation of habitats, is crucial for the process of assessing the risk of extinction of a species and which category of endangerment it is in (Bland et al., 2017).

Given the ideas presented above, the main objectives of this chapter are: to assemble a dataset of taxonomic and distributional information of the species of Ephemeroptera from Brazil; to provide, based on an interactive and easily updated website, open access to this dataset; and finally, to provide dynamic distributional maps for species, genera and families of Brazilian mayflies. This database will facilitate the reunion and access to distributional and basic taxonomic information for the order.

2.2. MATERIAL AND METHODS

2.2.1. Taxonomic data

In order to build an updated list of species of mayflies from Brazil, we accessed current species lists available in the Taxonomic Catalog of the Brazilian Fauna (CTFB) (http://fauna.jbrj.gov.br/fauna/listaBrasil/ConsultaPublicaUC/ConsultaPublicaUC.do), the List of South American Ephemeroptera Species (Domínguez et al., 2018; http://ibn-conicet.gob.ar/), and Ephemeroptera of South America (Salles et al., 2020; http://ephemeroptera.com.br/). Based on these data we assembled a preliminary list of species recorded from Brazil. During the assessment of extinction risk of Ephemeroptera from Brazil in November, 2019 promoted by ICMBio, several experts on mayfly taxonomy reviewed the validity of each species in the preliminary list and, in a consensus, invalidated some species or records due to taxonomic issues. Additionally, some other species listed under the CTFB have been invalidated in taxonomic reviews, either due to synonymization of names or designation as *nomina dubia.* A table of invalid species or records is presented with the justification for the invalidation of each species, together with the reference for such. The names of the mayfly experts that participated on this discussion are also included. Species considered invalid by researchers will have as reference “researchers’ consensus”. Finally, a list of valid species was built by assessing all species described or recorded from Brazil until November 2020 and excluding invalidated ones.

To quantitatively access the proportions of descriptions of each life stage of Ephemeroptera (egg, nymph, adult male and adult female), we compiled all stages described for each species using the website Ephemeroptera of the World (www.insecta.bio.spbu.ru/z/Eph-spp/Contents.htm), and scientific papers published until November 2020, by searching on Google Scholar for each species name.

2.2.2. Distributional data

A digital database called Biodiversity Conservation Status Assessment System (SALVE), developed by ICMBio to organize and facilitate information compilation for extinction risk assessments was initially used to integrate all distributional information of Brazilian mayflies. Part of the compilation was conducted by the team of the Regional Museum of Entomology at Federal University of Viçosa, Brazil. The bibliographic research on Ephemeroptera was made by accessing all scientific publications between 1843 (year of the first publication mentioning a species of mayfly from Brazil) and November 2020. The following websites were consulted in order to access these papers: Ephemeroptera Galactica (http://www.ephemeroptera-galactica.com/), Google Scholar (https://scholar.google.com.br/), and Zootaxa (www.zootaxa.com.br). In addition, the coordinates presented by (Molineri et al., 2019, 2020) were very useful in building the distributional database. In case of absence of coordinates in papers, a search using the description of collection sites was made to find the most accurate coordinates on Google Maps (https://www.google.com.br/maps).

As standardized information, year and author of description and coordinates of occurrence were tabulated for each species. A resume table was automatically generated by SALVE containing all geographic coordinates for each species containing respectively country, state and municipality addressed by each coordinate. The system also automatically searched for the occurrence in protected areas. Additionally, we used software QGIS 3.10 to cross all coordinates with a shape file of Brazilian biomes to find out to which biome each coordinate addresses.

The final dataset is compiled in an online spreadsheet composed of two sheets to be used in the online database: a) distributional data, which includes taxonomic information of valid species (order, family, genera, scientific name, author and year), coordinates of occurrence (latitude, longitude, datum), and the information derived from each coordinate (federal and non-federal protected area, private protected area, country, state, municipality, title of scientific paper containing the coordinate, author and year of record). And b) dataset of general information, including taxonomic information (family, genera, scientific name, and stages described), endemicity, and IUCN extinction risk status (ICMBio, 2018). The database will be constantly updated as new information is available - especially the IUCN status for all the species, as the results of the last assessment have not been published yet - new distributional records, new species, and new information on life stages.

2.2.3. Database website

A website (html) interface to consult the database constructed as a Google sheet™, was developed by a collaborator using a range of R packages such as gsheet, tidyverse, DT, flexdashboard and distill (R Core team, 2021).

2.3. RESULTS

The updated list of Ephemeroptera from Brazil, until November 2020, contains 10 families, 82 genera and 414 species. We accounted 19 invalid species, five by researchers' consensus and fourteen based on scientific studies published until that date **(Table 2.1)**. Three different justifications were found to invalidate the species record: misidentification when recording a species from the country; synonymization (Syn.) of names designated for the same organism; and designation as *Nomem dubium*, due to the impossibility of identifying these species (usually because of inadequate description, poor condition of type specimens, and even loss of type material).

At total, our dataset contains 3472 coordinates of occurrence records with georeferences of 410 species of Ephemeroptera from Brazil; all of them are available at the database 'EphemBrazil' (https://ephembrazil.netlify.app/). *Caenis uruzu* Molineri & Malzacher, *Asthenopodes picteti* Hubbard, *Leptohyphes mollipes* Needham & Murphy, and *Tricorythodes cristatus* Allen, have no occurrence coordinates in our records, as the articles of descriptions present inconsistencies in their distribution and there are no additional records of the species in scientific publications.

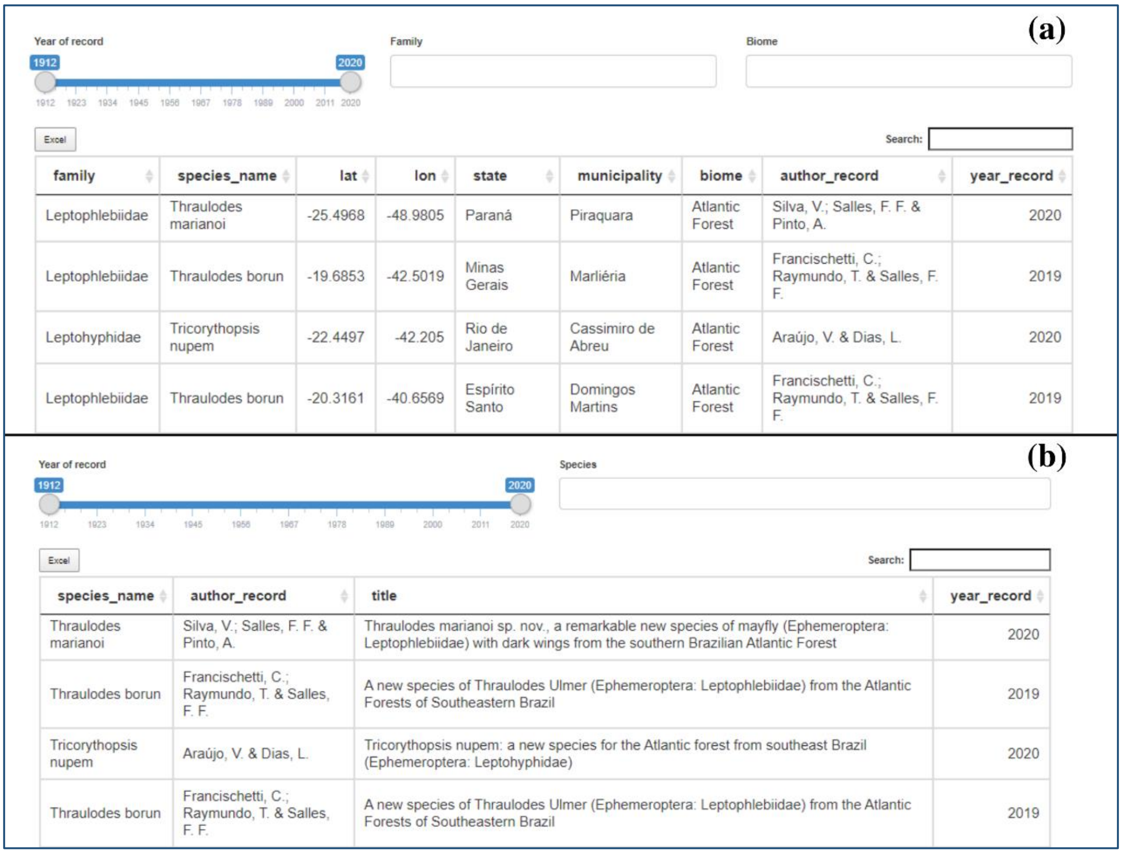
Data on EphemBrasil is available in two forms. First, raw data is presented in grids divided in two tabs: Distributional data and Bibliographic data **(Figure 2.1)**. In both tabs, filters can be applied to search for specific information, such as species, genus, family, biome, and/or by restricting the year of the record. Also, a search box is available to filter data using specific words within each grid, and both grids can be downloaded after filtering or as a whole. Additionally, all records of the database can be viewed in an online spreadsheet (https://docs.google.com/spreadsheets/d/1_FKOEwXyNBgc8MnzMVoQvFsI1Llae_VGNjgj5K0CaH8/edit). Secondly, the data can be accessed using the interactive map view **(Figure 2.2)**, in which all distributional records are represented with colored dots according to the families. This interactive map view can also be filtered as explained above for the grids **(Figure 2.3)**. Each bullet point, when clicked, opens a balloon with details of the species record **(Figure 2.4)**. In addition, three different layers can be applied in map view: default, aerial or terrain, with close up view as near as 20 meters in default and 100 meters in aerial and terrain. Moreover, we present a tab of summary graphs of all records in the database by family, biome, Brazilian state, year and, latitude and longitude **(Figure 2.5)**.

Table 2.1. - Invalid species of Ephemeroptera recorded from Brazil

Species	**Justificative**	**Reference**
Baetodes proiectus Mayo 1973	Misidentification	Researchers' consensus
Callibaetis fluminensis Cruz, Salles & Hamada, 2009	*Syn.*	(Cruz et al., 2017)
Callibaetis gregarious Navás, 1930	*Nomen dubium*	(Cruz et al., 2017)
Cloeodes barituensis Nieto & Richard, 2008	Misidentification	(Kluge, 2017)
Cloeodes redactus Waltz & McCafferty, 1987	Misidentification	(Kluge, 2017)
Moribaetis comes Navás, 1912	*Nomen dubium*	(Cruz & Salles, 2014)
Brasilocaenis intermedia Malzacher, 1986	*Nomen dubium*	(Lima et al., 2019)
Campylocia dochmia Berner & Thew 1961	*Syn.*	(Gonçalves et al., 2017)
Euthyplocia hecuba Hagen, 1861	Misidentification	Researchers' consensus
Leptohyphes petersi Allen, 1967	Misidentification	(Molineri, 2020)
Farrodes maculatus Needham & Murphy, 1924	Misidentification	Researchers' consensus
Lisetta ernsti Thomas, Dominique & Orth, 2005	Misidentification	Researchers' consensus
Thraulodes limbatus Návas, 1936	*Nomen dubium*	Researchers' consensus
Campsurus brasiliensis Traver, 1944	*Syn.*	(Molineri & Emmerich, 2010)
Campsururs duplicatus Spieth, 1943	*Syn.*	(Molineri & Salles, 2017)
Campsurus longicauda Navás, 1931	*Nomen dubium*	(Molineri et al., 2015)
Campsurus quadridentatus Eaton, 1871	*Nomen dubium*	(Molineri et al., 2015)
Campsurus striatus Needham & Murphy, 1924	*Nomen dubium*	(Molineri et al., 2015)
Campsurus zikani Navás, 1934	*Nomen dubium*	(Molineri et al., 2015)

Researchers responsible for species validity review: Fabiana Criste Massariol, Frederico Falcão Salles, Inês Corrêa Gonçalves, Lucas Ramos Costa Lima, Luiza Hoehne Mattos de Oliveira, Márcia Regina de Souza, Paulo Vilela Cruz, Rafael Boldrini, Rodolfo Mariano Lopes da Silva and Yulie Shimano Feitoza.

Figure 2.1. - General view of (a) Distributional data grid and (b) Bibliographic data grid


(a)

Year of record 1912 2020

Family

Biome

Excel

Search:

family	species_name	lat	lon	state	municipality	biome	author_record	year_record
Leptophlebiidae	Thraulodes marianoi	-25.4968	-48.9805	Paraná	Piraquara	Atlantic Forest	Silva, V.; Salles, F. F. & Pinto, A.	2020
Leptophlebiidae	Thraulodes borun	-19.6853	-42.5019	Minas Gerais	Marliéria	Atlantic Forest	Francischetti, C.; Raymundo, T. & Salles, F. F.	2019
Leptohyphidae	Tricorythopsis nupem	-22.4497	-42.205	Rio de Janeiro	Cassimiro de Abreu	Atlantic Forest	Araújo, V. & Dias, L.	2020
Leptophlebiidae	Thraulodes borun	-20.3161	-40.6569	Espírito Santo	Domingos Martins	Atlantic Forest	Francischetti, C.; Raymundo, T. & Salles, F. F.	2019

(b)

Year of record 1912 2020

Species

Excel

Search:

species_name	author_record	title	year_record
Thraulodes marianoi	Silva, V.; Salles, F. F. & Pinto, A.	Thraulodes marianoi sp. nov., a remarkable new species of mayfly (Ephemeroptera: Leptophlebiidae) with dark wings from the southern Brazilian Atlantic Forest	2020
Thraulodes borun	Francischetti, C.; Raymundo, T. & Salles, F. F.	A new species of Thraulodes Ulmer (Ephemeroptera: Leptophlebiidae) from the Atlantic Forests of Southeastern Brazil	2019
Tricorythopsis nupem	Araújo, V. & Dias, L.	Tricorythopsis nupem: a new species for the Atlantic forest from southeast Brazil (Ephemeroptera: Leptohyphidae)	2020
Thraulodes borun	Francischetti, C.; Raymundo, T. & Salles, F. F.	A new species of Thraulodes Ulmer (Ephemeroptera: Leptophlebiidae) from the Atlantic Forests of Southeastern Brazil	2019

Figure 2.2. - General view of interactive map view tab showing filters on the top and family subtitles in the left corner. Note that no filter is applied and all records are shown.

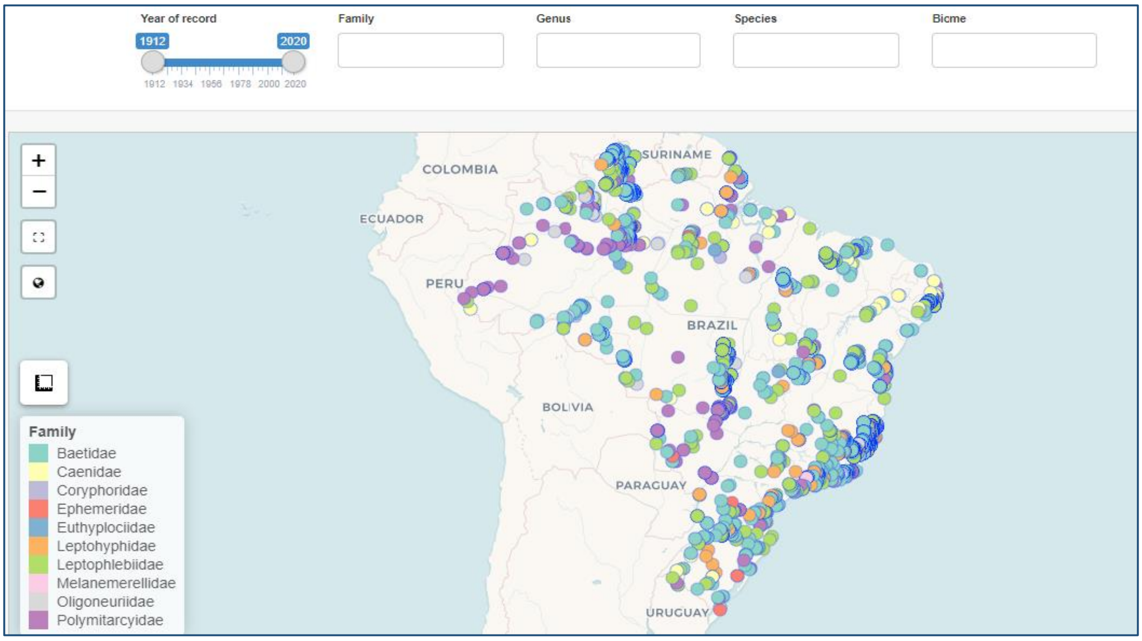


Figure 2.3. - Interactive map view of distributional records in Brazil with different filters applied: (a) family Baetidae; (b) genus *Camelobaetidius* Demoulin; (c) species *Camelobaetidius billi* Thomas & Dominique; (d) *Camelobaetidius billi* Thomas & Dominique records in Atlantic Forest biome.

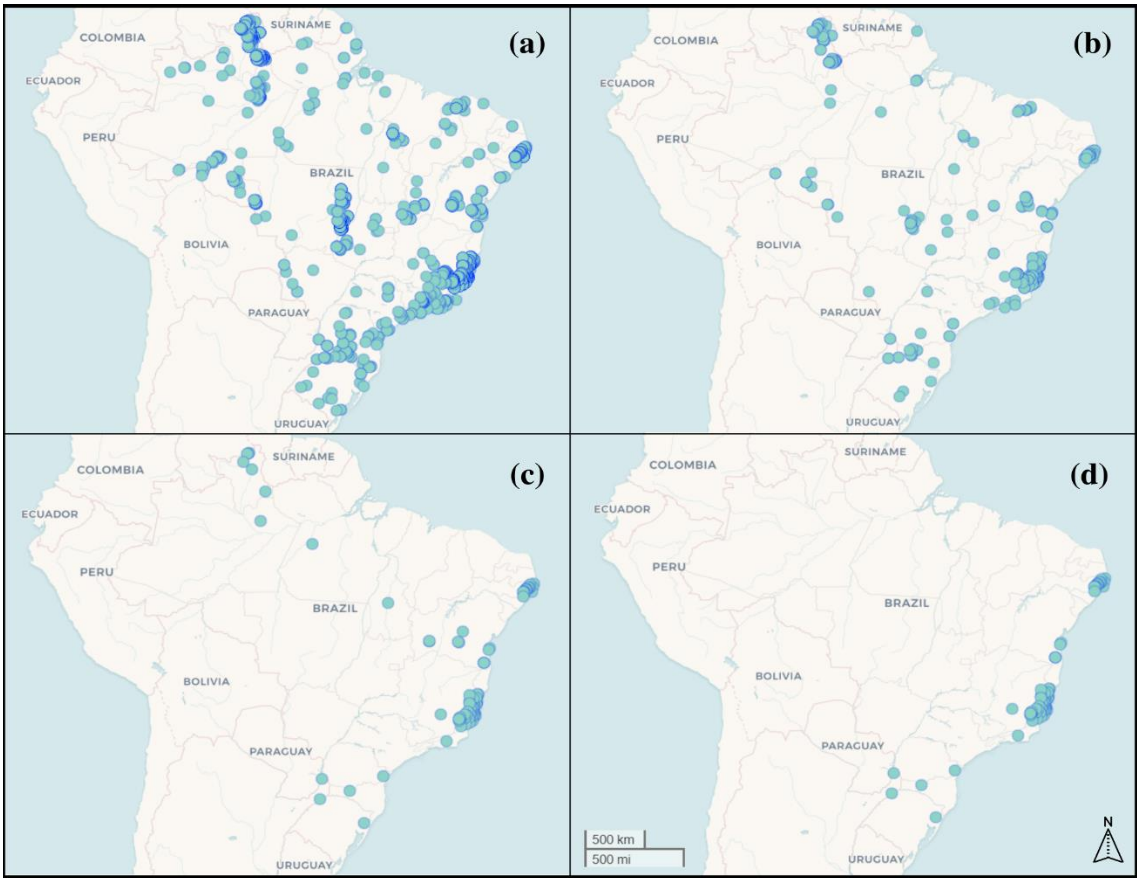


Figure 2.4. - Detailed view of a single record of *Camelobaetidius billi* Thomas & Dominique.

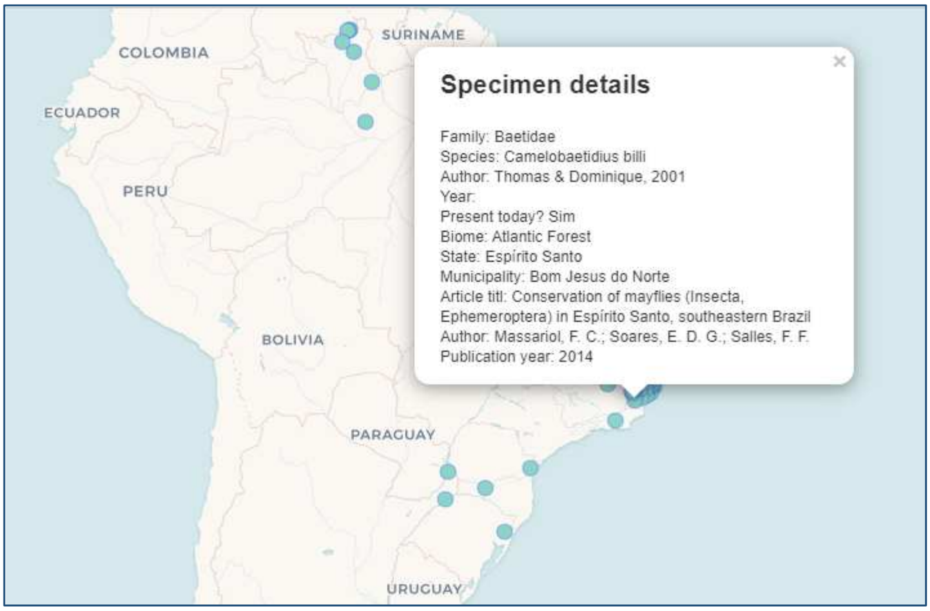
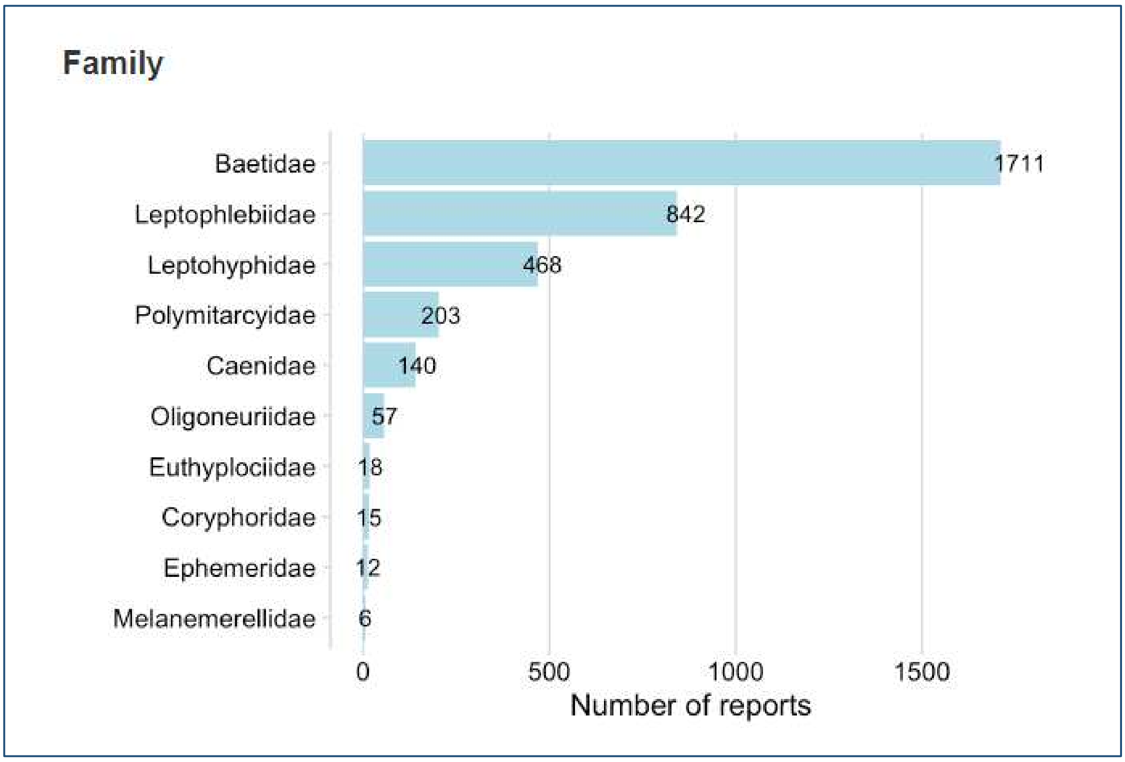


Figure 2.5. - Summary chart of records of Ephemeroptera from Brazil by family.



2.4. DISCUSSION

In the last published list of Brazilian mayflies, Salles *et al.* (2004) recorded 166 species distributed in 63 genera and 10 families. The updated list of Ephemeroptera from Brazil increases the number of genera to 82, and species to 414. Only two species that were present in the former list were considered invalid, here 19 additional species were not considered valid due to researchers' consensus or updated bibliography.

The database 'EphemBrazil', is unique and the first effort of this kind to compile and provide access of georeferenced and taxonomic information for the order Ephemeroptera in Brazil. Taxon-specific distribution data are essential for predicting geographical ranges and species responses to global change, which are important facets of conservation planning (Serra-Diaz & Franklin, 2019). We strongly believe that an open access, readily available and complete database may also facilitate more proactive conservation measures and improve scientific efforts towards those areas with inconspicuous knowledge. As more data become part of the network, it will allow for the identification of knowledge gaps with respect to specific taxa, and this in turn will help focusing resources to maximize the value of research regarding the order and diminish biodiversity shortfalls.

As an open-access database, as many others biodiversity databases around the world (Cayuela et al., 2012; Twardochleb et al., 2021; Lopez-Gonzalez et al., 2012), it can be used to investigate ecological communities, analyze species turnover along environmental gradients and population dynamics. Specifically for Ephemeroptera, complete datasets of species distribution are applied in the study of vicariance events (Molineri, et al., 2019) and delimitation of hypothetic biogeographic zones (Molineri et al., 2020). Additionally, our database can be used to map taxonomic diversity of mayflies and, when paired with environmental data, will provide a robust resource to quantify how the environment variables shape diversity patterns.

Moreover, the facility of integrating and accessing georeferenced species information provided by the website, simplifies the assessment of extinction risk, as one of the main IUCN criteria utilized for Ephemeroptera is the Extent of Occurrence (EOO) - which is defined by the IUCN as, 'the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all sites of present occurrence of a taxon (IUCN, 2019)' and requires the compilation of all occurrence records of each species. As the database is updated with more occurrence records of a given species, its extinction risk status may change as its extension of occurrence increases.

2.5. CONCLUSIONS AND FURURE DIRECTIONS

In the chapter we assembled a dataset containing basic taxonomic and distributional information of 414 species of the order Ephemeroptera from Brazil. This dataset is available through a dynamic, interactive and open access website, in which data can be visualized in dynamic maps or in downloadable grids. The database will be constantly updated as new data becomes available and we strongly recommend the submission of data to enrich it.

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3. Chapter Two – Knowledge assessment of Ephemeroptera from Brazil

3.1. INTRODUCTION

Mayflies can be found in freshwater ecosystems distributed throughout the world, with the highest diversity found in the Neotropics (Jacobus et al., 2019). The greatest advance in relation to the group's taxonomy in Brazil and, consequently in the Neotropical Region, begins in the first decade of the 21st century due to the increasingly increment on human resources (Domínguez & Dos Santos, 2014). In 2004, Brazil accounted with 166 species of mayflies (Salles et al., 2004), and the Neotropics occupied the third place in the world rank of species richness (Barber-James et al., 2008). As the knowledge of the order suffered an enormous expansion in Brazil with more than 400 species recorded in the Taxonomic Catalogue of the Brazilian Fauna (CTFB) (Salles & Boldrini, 2020), the realm assumed the first position in the rank with almost 900 species reported (Jacobus et al., 2019).

In spite of the generated knowledge about the order in this century, most of it is fragmented in the specialized literature. For example, the last scienciometric review of Ephemeroptera from Brazil was published by Shimano et al. (2014), since then, no knowledge assessment of Ephemeroptera has been done, which impairs the identification of gaps, bias and shortfalls on research, as it also hampers potential conservation actions for the order. According to Mingers and Leydesdorff (2015), scientometrics is the field of study that is most directly related to the evaluation and exploration of scientific research.

The aim of this chapter is to assess the knowledge of Ephemeroptera from Brazil by making a scienciometric review of the taxonomic and distributional information gathered in the previous chapter.

3.2. MATERIAL AND METHODS

Using the dataset presented in chapter one, we produced graphs to illustrate the current knowledge of Ephemeroptera in Brazil. Using the coordinates compiled we made two maps indicating occurrence distribution and species richness of Ephemeroptera in Brazil. The basis for this was a map of Brazil divided into a grid with one-degree side cells. In order to avoid duplicity of species inside a cell in the species richness map, we created a matrix of distances between occurrence points for all species. Using the centroid of each cell as a reference, we selected the closest occurrence point and excluded remaining points of a species inside each cell that had an occurrence point of that species. These maps were made using the software QGIS 3.10.

3.3. RESULTS

3.3.1. Taxonomic knowledge

The most representative families of Ephemeroptera from Brazil are Leptophlebiidae with 131 species and Baetidae with 130, together they sum 63% of total species richness for the country **(Figure 3.1**). Much less representative than Baetidae and Leptophlebiidae, Leptohyphidae occupies the third place containing 58 species, followed by Polymitarcyidae with 46 species. 'Other Families' (relictual or poorly diversified: Coryphoridae, Ephemeridae, Euthyplociidae and Melanemerellidae) sum nine species, representing only 2,2% of total richness. Additionally, Melanemerellidae is still represented by a single species, *Melanemerella brasiliana* Ulmer.

Leptophlebiidae is the most genera diverse family with 29, Baetidae accounts with 25 genera, Leptohyphidae eight, Polymitarcyidae seven, Oligoneuriidae five, and Caenidae four **(Figure 3.2).**

Figure 3.1. - Number and proportion of 414 species distributed across families of Ephemeroptera in Brazil.

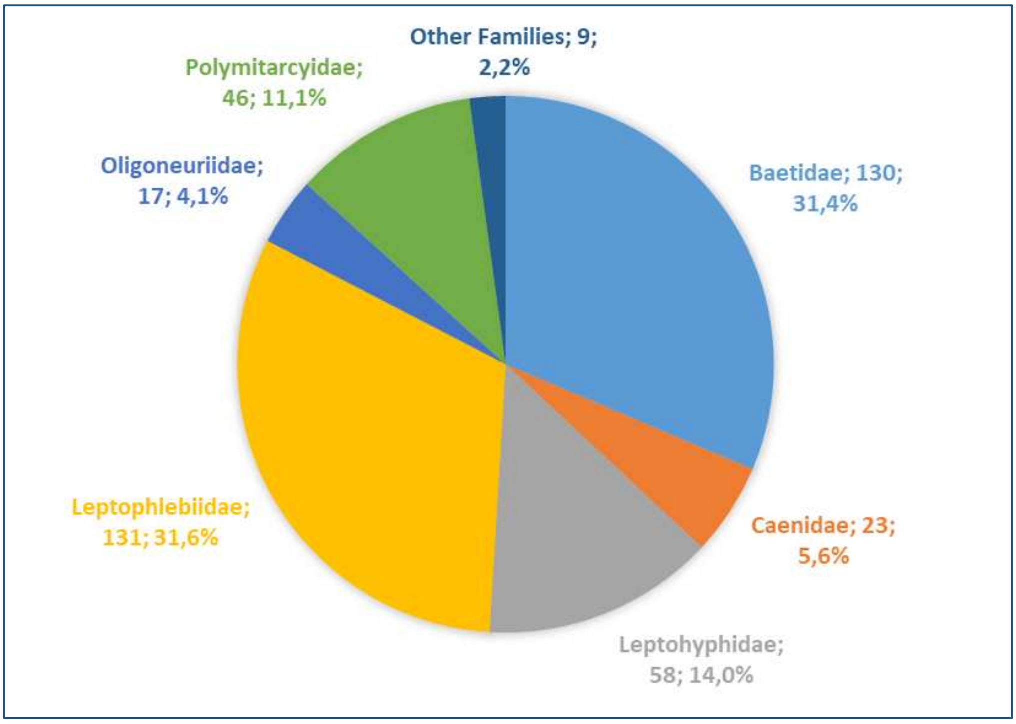
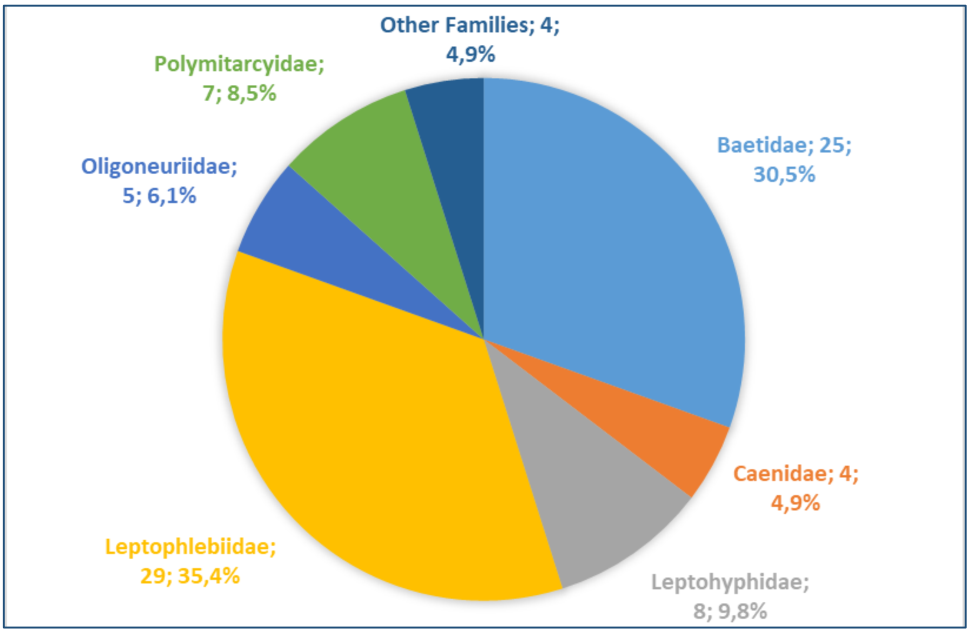


Figure 3.2. - Number and proportion of the 82 genera distributed across families of Ephemeroptera in Brazil.

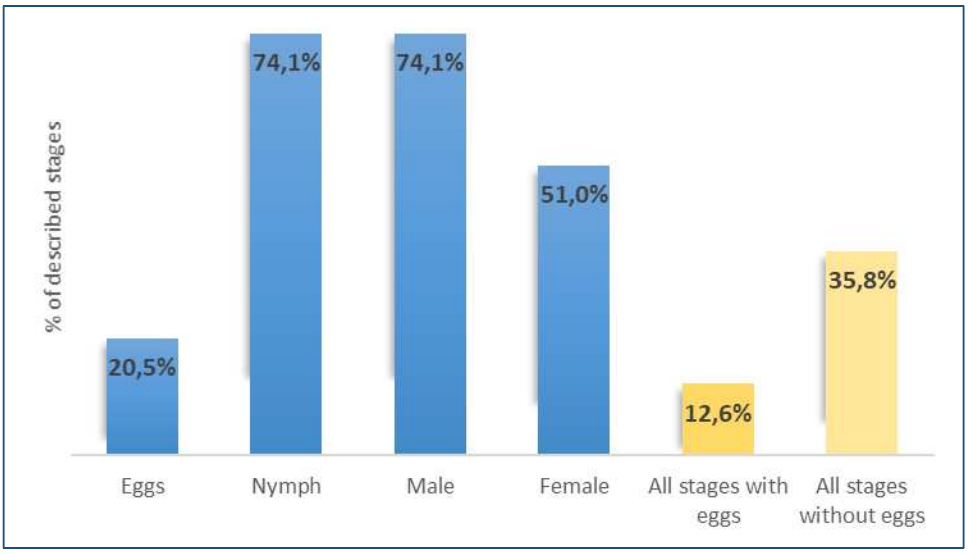


In regard of described semaphoronts, Baetidae and Leptohyphidae present a predominance of species descriptions based on nymphs, yet in Leptophlebiidae and Polymitarcyidae male adult is the predominant stage described. Adult female is the most described stage of Oligoneuriidae **(Table 3.1)**. Of the 414 species of mayflies in Brazil, only 51% have had the adult female stage described, and 74,1% both adult male and nymphs **(Figure 3.3)**. In overall, only 12,6% have all life stages known by science. If the egg stage is excluded, then the number increases to 35,8%.

Table 3.1. - Number of described life-stages for each family of Ephemeroptera.

		Life Stage			
Family	**Species Number**	Egg	Nymph	Adult Male	Adult Female
Baetidae	130	0	128	65	45
Caenidae	23	14	17	22	15
Coryphoridae	2	1	1	2	1
Ephemeridae	1	1	1	1	1
Euthyplociidae	5	4	3	5	5
Leptohyphidae	58	17	49	37	26
Leptophlebiidae	131	17	80	117	70
Melanemerellidae	1	1	1	0	1
Oligoneuriidae	17	2	11	12	15
Polymitarcyidae	46	28	16	46	32
Total	**414**	**85**	**307**	**307**	**211**

Figure 3.3. - Proportions of described life stages of Ephemeroptera including or excluding the egg stage.



3.3.2. Distributional knowledge

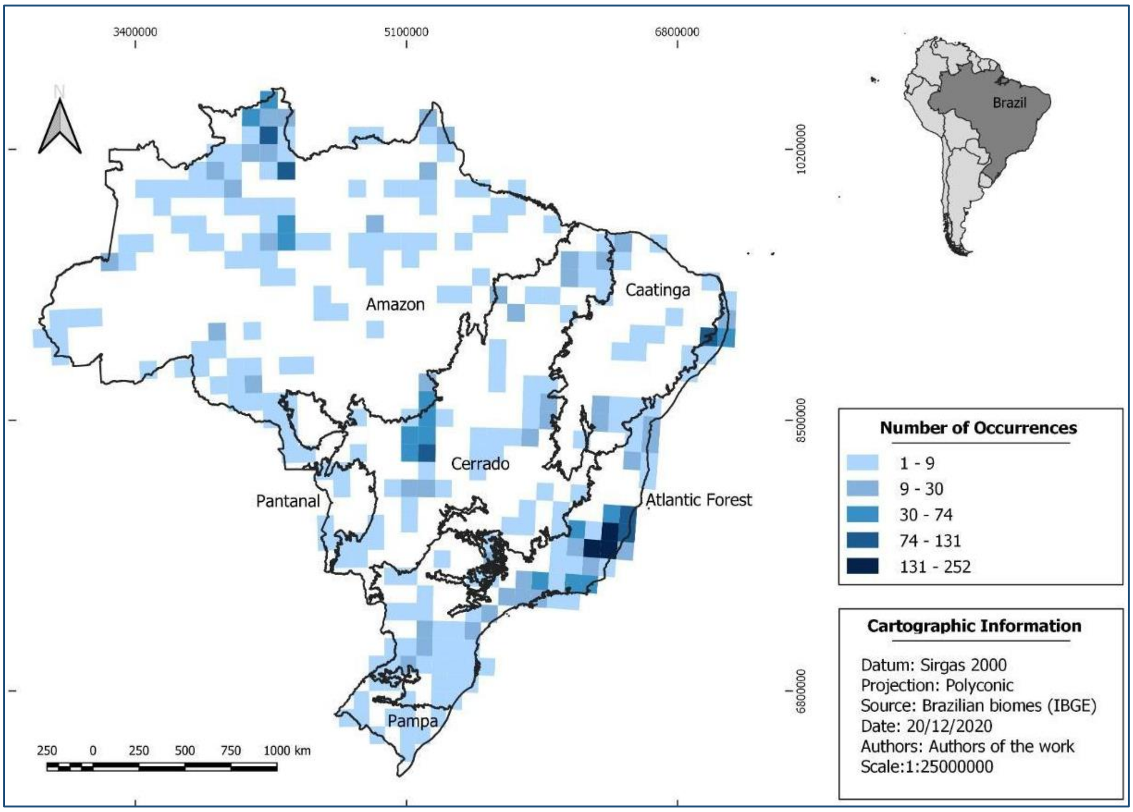
The records of the order Ephemeroptera are unevenly distributed all over Brazil. **Figure 3.4** includes all coordinates compiled and distributed across the six Brazilian biomes, with different proportions of records for each, Amazon 27,7%, Caatinga 3,16%, Cerrado 17,25%, Atlantic Forest 50,5%, Pampa 0,37% and Pantanal 0,97%. The map of Brazil has 851 cells with 1 degree on each side, in which only 284 has any occurrence record of mayflies, meaning that 3472 records are distributed in only 33% of the territory. In addition, Pantanal, Pampa and Caatinga biomes present a very small number of occurrences, and the most interesting fact is that Pantanal has 56% of its land covered by water.

Species richness (**Figure 3.5**) is concentrated mainly in specific areas across the biomes, such as the central and north areas of Amazon and Atlantic Forest and in isolated points like southwest of Cerrado. Interestingly, the distribution of species records is very similar with distribution of species richness, evidencing that there might be a pattern on sampling effort in Brazil.

Atlantic Forest and Amazon biomes host the greater number of species, 236 and 187, respectively, followed by Cerrado with 140 and Caatinga with 61 species.

The less representative biomes are Pampa and Pantanal hosting 20 and 11 species, respectively (**Figure 3.6)**.

Figure 3.4. - Map of Brazil showing the distribution of the 3472 occurrence records of 410 species of Ephemeroptera.

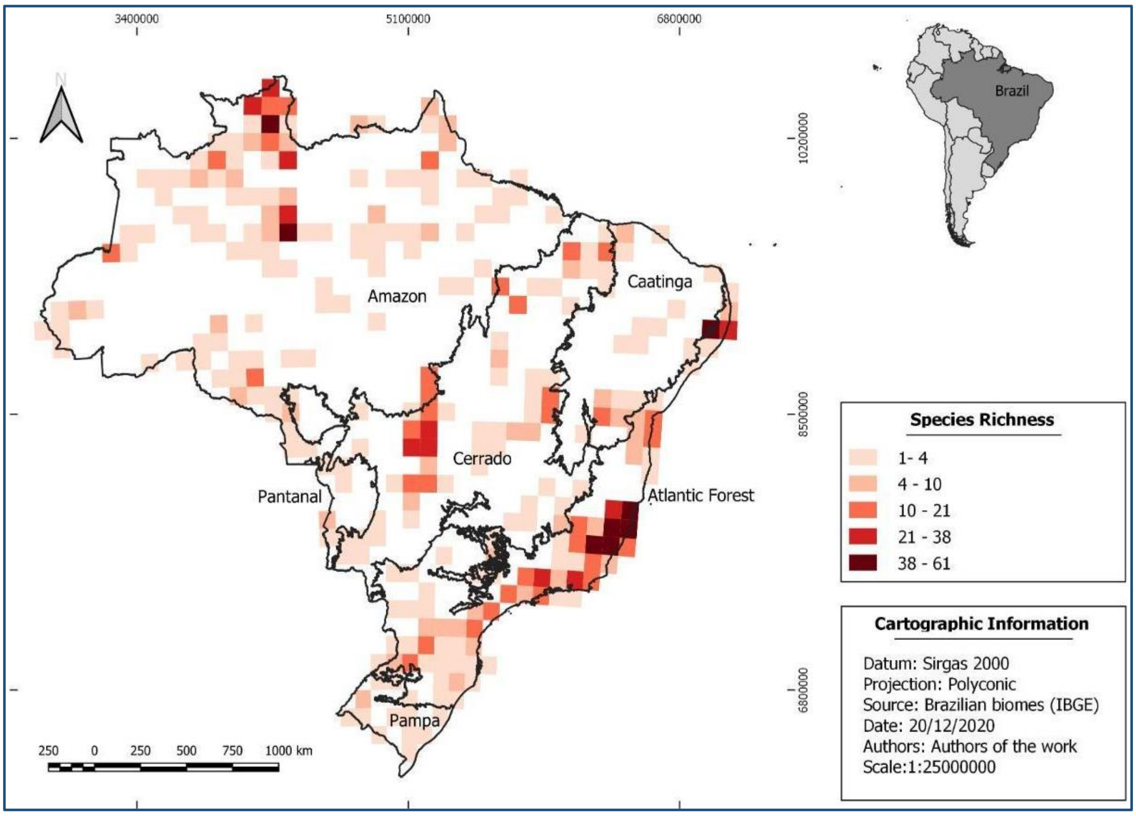


The latitude on the map ranges from -33º to 6º and longitude varies from -73º to -34º.

Approximately 59% of all species present in Brazil are endemic to the country. The proportions of species endemism of each biome is subdivided in “Endemic of biome” which means species occurrence is restricted to that biome, and “Endemic of Type-locality in the biome” meaning that the species is endemic to the biome and occur only on its type-locality from original description.

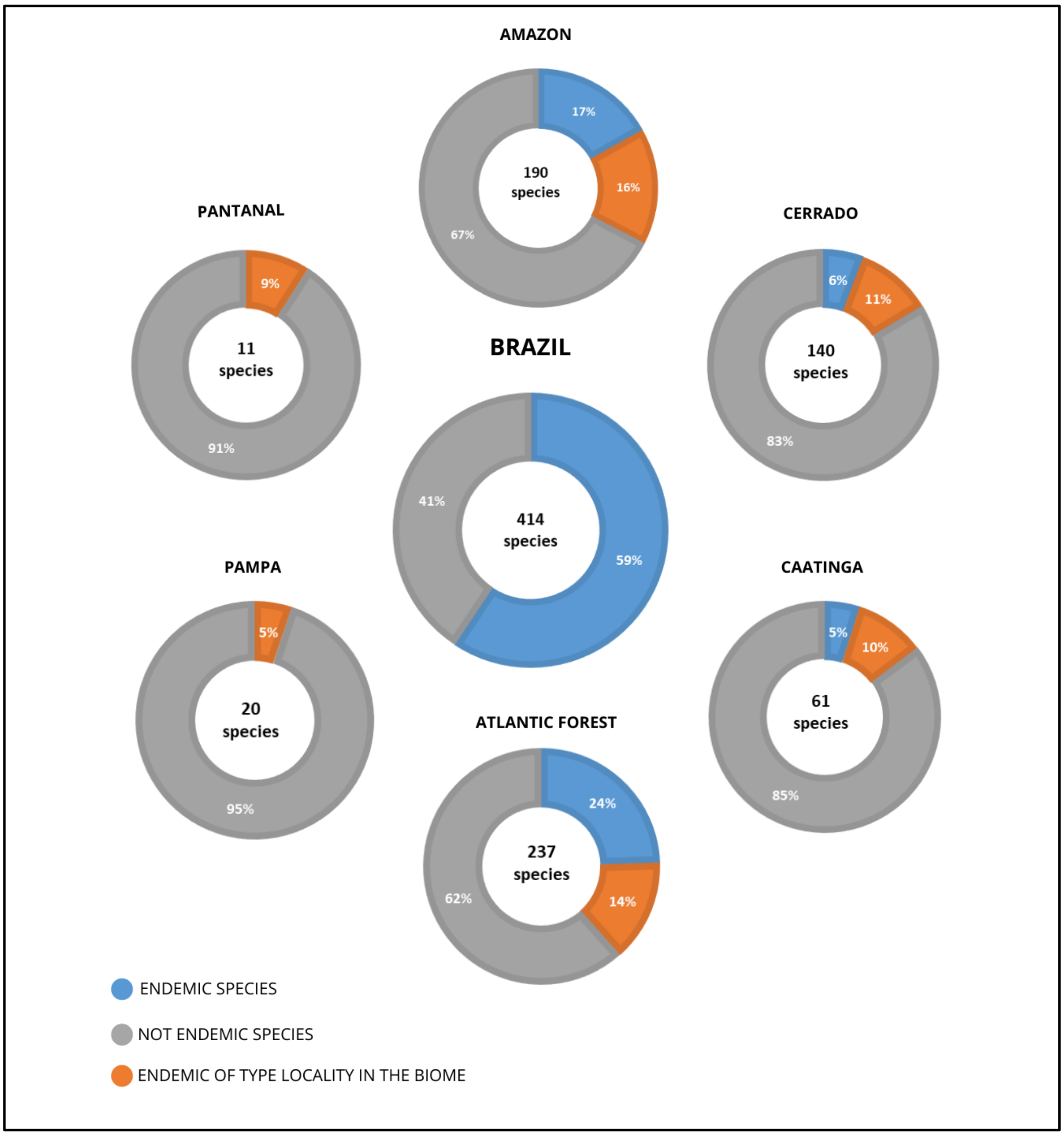
Atlantic Forest hosts the highest endemism level of all biomes (46%), followed by Amazon (41%) and Cerrado (25%). In total, 91 (22%) species of Brazilian mayflies are only known from its type-locality.

Figure 3.5. - Map of species richness distribution of 410 species of Ephemeroptera in Brazil.



The latitude on the map ranges from -33º to 6º and longitude varies from -73º to -34º.

Figure 3.6. - Endemicity proportions and species number of Ephemeroptera in Brazil and across its six biomes.



3.4. DISCUSSION

3.4.1. Taxonomic knowledge

Compared with the Salles et al. (2004), the Brazilian fauna of mayflies expanded from 166 species to 414 in the last 16 years. Total number of species of Leptophlebiidae and Baetidae families almost tripled, from 90 to 261, being the most specious families. Species belonging to ‘other families’ (relictual and poorly diversified) increased from four to nine, and Melanemerellidae is still endemic for Brazil and represented by only one species.

Polymitarcyidae genera jumped from three to seven with the description of *Hubbardipes* Molineri, Salles & Peters, 2015; *Priasthenopus* Molineri, Salles & Peters, 2015 and *Tortopsis* Molineri, 2010, and the record of *Asthenopodes* Ulmer, 1924a. Oligoneuriidae genera decreased as the former genus *Oligoneurioides* was designated by Salles et al., (2013) to subgenus of *Oligoneuria.* Also, Baetidae genera expanded from 20 to 25, and Leptophlebiidae from 20 to 29 genera, two of them described in 2020 *Diamantina* Salles, Domínguez & Nascimento and *Macuxi* Cruz, Salles, Hamada & Falcão.

Semaphoront descriptions varied largely across families. One of the main disparities found is the reduced number of descriptions of nymphs compared with those available for adults. Cardoso et al. (2015) suggested this predominance of descriptions of adults may be related to the relative simplicity of identifying and describing the adults in comparison with the nymphs in most mayfly species, given to a reduced number of morphological characters in adults compared to nymphs. Despite this, families like Baetidae and Leptohyphidae present the opposite disproportional description of life stages, as the former counts with almost the totality of nymphs described. Six years ago, Oligoneuriidae species were mostly known by nymphs (Cardoso et al., 2015); now, female adult stage predominates.

Overall, only 12,6% of Brazilian mayflies have all life stages known by science, if the egg stage is excluded, then the number increases to 35,8%. Given the fundamental need for an adequate classification of organisms (Marques and Lamas, 2006), missing data on the characteristics of one life stage or one of the sexes, in the case of the adults, may often lead to misidentifications of specimens. In some extreme cases, new taxa may be falsely described due to the lack of data on one other life stage (Falcão et al., 2011).

Around just 20% of the species have their eggs described. If the Haeckelian shortfall were to be overcame, phylogenetic analyses, tree resolution and node support could be improved based on egg characters. For example, Dominguez & Cuezzo (2002), found in egg characters extra support for phylogenetic relationships of the genus *Traverella* Edmunds. Inversely, the addition of egg characters produced changes in the topology of *Hermanella* Needham & Murphy. Thus, remarkable taxonomic improvement could be done using egg characters for recognition and association of distinct semaphoronts. Lopez et al. (2021), used egg characters to associate nymphs and adults of two different species of *Terpides* Demoulin, indicating that the eggs are informative tools for the association of life stages.

It is undeniable that the taxonomy of Ephemeroptera in Brazil advanced tremendously, it is essentially due to the second generation of PhD's graduated in the 2000's, and the pupils they acquired and supervised as professors in universities and institutes. In addition, as pointed by Domínguez & Dos Santos (2014), Brazil invested heavily in science and technology between early 2000's and late 2010's, contributing to the debut of the country in world mainstream science, achieving very fruitful collaborations with researchers from all over the world, benefiting inventories and descriptions of new species.

Despite the notorious increment of taxonomic studies on Ephemeroptera in the last two decades, the order follows the corollary of invertebrate biodiversity knowledge: neglected and far from being a conservation priority. Traditional taxonomy is on the verge of extinction, facing ever scarcer resources (Cardoso et al., 2011). Conversely, the worldwide declining insect abundance, biomass, and range driven by anthropogenic actions, presses for credible taxonomic inventory of the planet before some species are vanished without being properly named (Montgomery et al., 2020).

Specifically for Ephemeroptera, estimates of species to be formally described (i. e., the Linnean shortfall) range from one thousand species for the world (Sartori & Brittain, 2015) and ten thousand only for Brazil (Cardoso et al., 2015). Even though these estimates do not converge, descriptions of new mayflies from Brazil have not reached an asymptote; seventeen species and four genera have been described or recorded for the country between July 2019 and November 2020, an average of one species per month. The ongoing rate of discovery of new taxa of Ephemeroptera in Brazil signals the potential biodiversity knowledge awaiting to be revealed by future studies.

The discovery of new species necessarily affects studies in systematics, biogeography and population biology for the order, as it represents the basic units of studies in ecology and evolution (Hortal et al., 2015; McNeely, 2002). For biodiversity conservation, taxonomy is important primarily because in order to protect a taxon it is essential to know it first, and secondly, because no conservation action can protect undescribed species (Ely et al., 2017). Moreover, Red lists are the first step in conservation efforts, and obviously, an organism cannot be assessed if it is not known to science.

3.4.2. Distributional knowledge

Mayflies can be found in almost all realms across the world, except in Antarctica (Barber-James et al., 2008). In Brazil, they occur in all biomes, but only 33% of the territory presents data about Ephemeroptera richness, of these, only 23 cells presented more than 30 occurrence records, which represents less than 3% of the area of Brazil.

The cells presented here as of high richness must be seen with caution, as we believe the discrepancy found on species distribution is primarily due to bias on sampling effort, with the areas where researchers are present being usually more surveyed (red squares on Figure 4 are related to areas with universities and research institutes, such as INPA, UFRR, UFES, UFPE and UNEMAT). De Marco Jr & Vianna (2005) found patterns on distribution of Odonata and linked to bias on sampling effort towards regions where researchers are more active; this may be the most studied bias in biodiversity data and involves several syndromes caused by the behavior of researchers (Sastre & Lobo, 2009).

This bias is ambivalent in a way that some regions like central of Atlantic Forest were extensively sampled, having lists of species and conservation related studies (Mariano & Polegatto, 2011; Massariol et al., 2014; Salles et al., 2004, 2010); whereas Pantanal, which is the sixth biggest wetland of the planet has never received a dedicated research on its mayfly fauna, representing one of the main knowledge shortage of Ephemeroptera in Brazil. Pantanal is home to a high number of aquatic species, with almost 300 species of macrophytes and 325 species of fish (ICMBio, 2021), the alternation between flood and drought in a short period allows the rapid nutrient cycling, explaining in part, the large concentration of organisms (BPBES, 2020). With that said and considering the varied niches that some mayfly species can occupy (see Salles & Ferreira, 2014), it is reasonable to expect a less inconspicuous presence of these organisms in the biome.

Pantanal is not an exception; all Brazilian biomes present in different scales some level of Wallacean shortfall, that is, a lack of knowledge about the geographical distribution of species (Lomolino, 2004). Amazon occupies 49% Brazil's area (IBGE, 2021), and is responsible for 15–20 % of global river flow (Salati & Vose, 1984). As depicted in **Figure 3.5** and by the number of species recorded for the biome, most of Amazon lacks survey effort and the number of species recorded is lower than that of Atlantic Forest, which covers only 13% of Brazil. One main factor is the continental proportions of Amazon, in consequence, many inland areas of Amazonas and Pará states are inaccessible by land or air, hampering investigative studies due to difficult logistics of travelling to such areas. In addition, the number of researchers to cover the immense area of the biome is short, limiting fieldwork and taxonomic studies of Ephemeroptera to regions in the vicinity of where researchers are allocated.

The similarity between maps of occurrence records **(Figure 3.4)** and species richness **(Figure 3.5)** stems from all bias and limitations discussed above, as spatial variation in survey effort dictates the evenness of species descriptions through time, causing the Linnean shortfall to be spatially, temporally, and taxonomically structured for most taxa (e.g. Diniz-Filho et al., 2005; Rodrigues et al., 2010). This symmetry of distributional and taxonomic studies can partially explain the high proportion of endemism of Ephemeroptera in Brazil and in less sampled biomes, such as Caatinga and Amazon, as biodiversity inventories tend to be concentrated near locations that offer convenient access and logistics. Consequently, there is a reduction in the range of sampling area and, thus, the occurrence area of a given taxon (Hortal et al., 2007; Kadmon et al., 2004).

Such symmetry and geographic bias in overall, can affect estimates of conservation threat status. One of the main criteria used to assess extinction risk of mayfly species is the EOO, incomplete distributional data causes the EOO of many species to be underestimated unless corrections are made (Cardoso et al., 2011). Such corrections are essential for many species described from a single or few locations, which is the case of 91 species of Ephemeroptera in Brazil known only from their type locality. Endangerment status classifications based on incomplete sampling may overestimate the real risk of extinction, and certain taxa and/or regions may be unduly prioritized for conservation action (Riddle et al., 2011).

3.5. CONCLUSIONS AND FUTURE DIRECTIONS

The assessment performed in this chapter unveiled several aspects of the taxonomic and distributional knowledge available for Ephemeroptera from Brazil:

- The Brazilian fauna of mayflies expanded from 166 species to 414 in the last 16 years;
- Leptophlebiidae and Baetidae are the most diverse families;
- Only 12,6% of the species have all life stages described;
- All occurrence records of Ephemeroptera in Brazil are distributed in only 33% of the country and are biased towards the vicinities of institutions where researches are hosted;
- Each biome presents different proportions of occurrence of mayflies (Amazon 27,7%, Caatinga 3,16%, Cerrado 17,25%, Atlantic Forest 50,5%, Pampa 0,37% and Pantanal 0,97%);
- Despite being a wetland, Pantanal is under-studied in regard to Ephemeroptera;
- The Atlantic Forest and Amazon host the great diversity of species;
- Of the 414 valid species, 91 are only known from their type locality;
- Around 59% of the species are endemic of Brazil.

The uneven distribution of studies conducted across Brazil, despite their great extension and scientific importance, is strongly biased and generates relevant knowledge gaps related to the fauna of Ephemeroptera. Such negligent knowledge limits our capability of evaluating the true status of the species in the Brazilian territory, hampering conservation actions for the order as a whole. The current knowledge available stresses the need to conduct focal studies filling the gaps of unsurveyed areas and suggests an increased focus in finding the unknown semaphoronts of the species.

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4. Chapter Three – Environmental threats to mayfly communities in Brazil

4.1. INTRODUCTION

The term 'Anthropocene' is an important consequence of the recognition of the increasing intensity of the degree to which humans are driving patterns in global biogeochemistry and biodiversity (Gibbard & Walker, 2014). Despite our limited knowledge of world biodiversity and its rate of extinction, some authors argue that such alterations on the environment are driving a decline in many forms of life and that a sixth mass extinction is underway (Wake & Vredenburg, 2008; Barnosky et al., 2011; Montgomery et al., 2020). Of the supposed 3-100 million existing species, it is estimated that at least 3000 of them are lost each year (Wilson, 2003; González-oreja, 2008). A global meta-analysis of insect abundances revealed a 45% decline in insect abundance across two-thirds of the taxa evaluated (Dirzo et al., 2014).

For aquatic insects, several studies have directed their efforts to identify potential causes that decrease their abundance and increase the risk of their extinction worldwide (Brasil et al., 2020; Callisto et al., 2012; Contador et al., 2012; Uieda, 2017). Specifically for Ephemeroptera, known threats include water contamination and toxicity by heavy metals (Mebane et al., 2020), deforestation and sewage (Couceiro et al., 2007), mining (Espinosa et al., 2020), sediment input (Akamagwuna & Odume, 2020), pesticides (Egler et al., 2012), land cover changes (Siegloch et al., 2017; Wong et al., 2020), and drought events (Aspin et al., 2019).

Together with other groups of benthonic macroinvertebrates, Ephemeroptera species are widely endorsed as bioindicators of water quality and ecological integrity (Barbour et al., 1999; Bauernfeind & Moog, 2000; Menetrey et al., 2008; Brasil et al., 2020), as they are sensitive to the effect of human activities on streams (Beketov, 2004; Hubbard & Peters, 1978; Akamagwuna et al., 2021; Dedieu et al., 2015). The nymphal stage of mayflies is the dominant life history stage, which allow them to integrate environmental conditions over long periods of time (Mathuriau et al. 2011). In this sense, Ephemeroptera assemblages are a good picture of local environmental conditions and, therefore, are prone to be affected by all environmental disturbances caused by the threats mentioned above.

In the face of these myriad of threats that challenge the survival of aquatic insects, the accelerated decline in abundance, biomass and distribution of insects worldwide (Montgomery et al., 2020), and the presence of nine species of Ephemeroptera in the Red List of Endangered Species of Brazilian Fauna (ICMBio., 2018), it is extremely important to identify environmental threats present in the national territory and determine, among them, those that are more detrimental to a species regarding its biology, life history and distribution range.

However, all the knowledge we have in hand for Ephemeroptera from Brazil is derived from taxonomic studies, as research on any ecological aspect of Ephemeroptera or aquatic insects at the species level are extremely rare. Therefore, we are at a moment when we rely exclusively on taxonomic works, such as species descriptions and checklists, but these works lack information regarding environmental conditions of the habitat where the species were collected. Interestingly, most of the time, the authors of such publications have some knowledge of the environmental threats occurring were the specimens were collected, as they usually are the ones that go on field trips.

In this context*, Best Professional Judgment* (BPJ) happens to be a useful tool to collect and assess information regarding environmental threats occurring countrywide. BPJ, is based on the integration of knowledge of several scientists from a study area in order to gather and align their knowledge, opinions, criteria and experiences in the evaluation of a set of information or events through the application of questionnaires. For example, in aquatic environments, BPJ has been used to assess and compare benthic indexes (Borja et al., 2014; Dauvin et al., 2012; Ranasinghe et al., 2009; Teixeira et al., 2012), and to determine the level of agreement in identifying the degree of disturbance in benthic macro-infaunal communities (Murray et al., 2016; Teixeira et al., 2010; Thompson et al., 2012; Weisberg et al., 2008).

In this chapter, we aim to gather information regarding ongoing ecosystem alterations detrimental to mayflies' persistence across the six Brazilian biomes.

4.2. MATERIAL AND METHODS

4.2.1. List of threats and evaluation

In order to compose a list of environmental threats, we conducted a search for threats cited in scientific literature (pollution by fertilizers and sewage, deforestation, urbanization) known to be detrimental to invertebrates in aquatic environments (Dudgeon, 2020). Afterwards, we elected the corresponding threats cited in assessments of the insect orders that have aquatic representatives and that are included in the Brazilian red list (ICMBio., 2018), Ephemeroptera and Odonata, which are the only two groups of aquatic insects included in the assessments of national extinction risk in Brazil (see **Box 4.1**).

After listing these threats, experts with taxonomic and field experience were asked to grade the weight of nine environmental threats to extinction risk of Ephemeroptera considering the occurrence of each threat at six Brazilian biomes: Amazon, Cerrado, Caatinga, Atlantic Forest, Pampa, and Pantanal. A short *Qualtrics* survey (see **Box 4.1**) was sent by email to 23 researchers in July 2020, they were asked to answer the survey separately for each biome.

Box 4.1. - Perception of environmental threats across Brazilian biomes
1- In which biome your work is/was concentrated?
2- In your biome, what is the weight of each of the following environmental threats to the risk of extinction of mayflies' populations? (Threats were presented in randomized order and with the options **low, medium or high**). ● Agriculture - silting ● Agriculture - Deforestation of riparian forest/ removal of riparian vegetation/ conversion to land use ● Agriculture- Water pollution by chemicals and organic matter ● Aquaculture ● Urbanization ● Constructions (hydroelectric, thermal, mining) ● Water pollution by domestic and industrial effluents ● Climatic events - drought ● Climatic events - flood
3- Is there any other threat present in your biome that was not listed in the previous question? If so, please describe it.

4.3. RESULTS

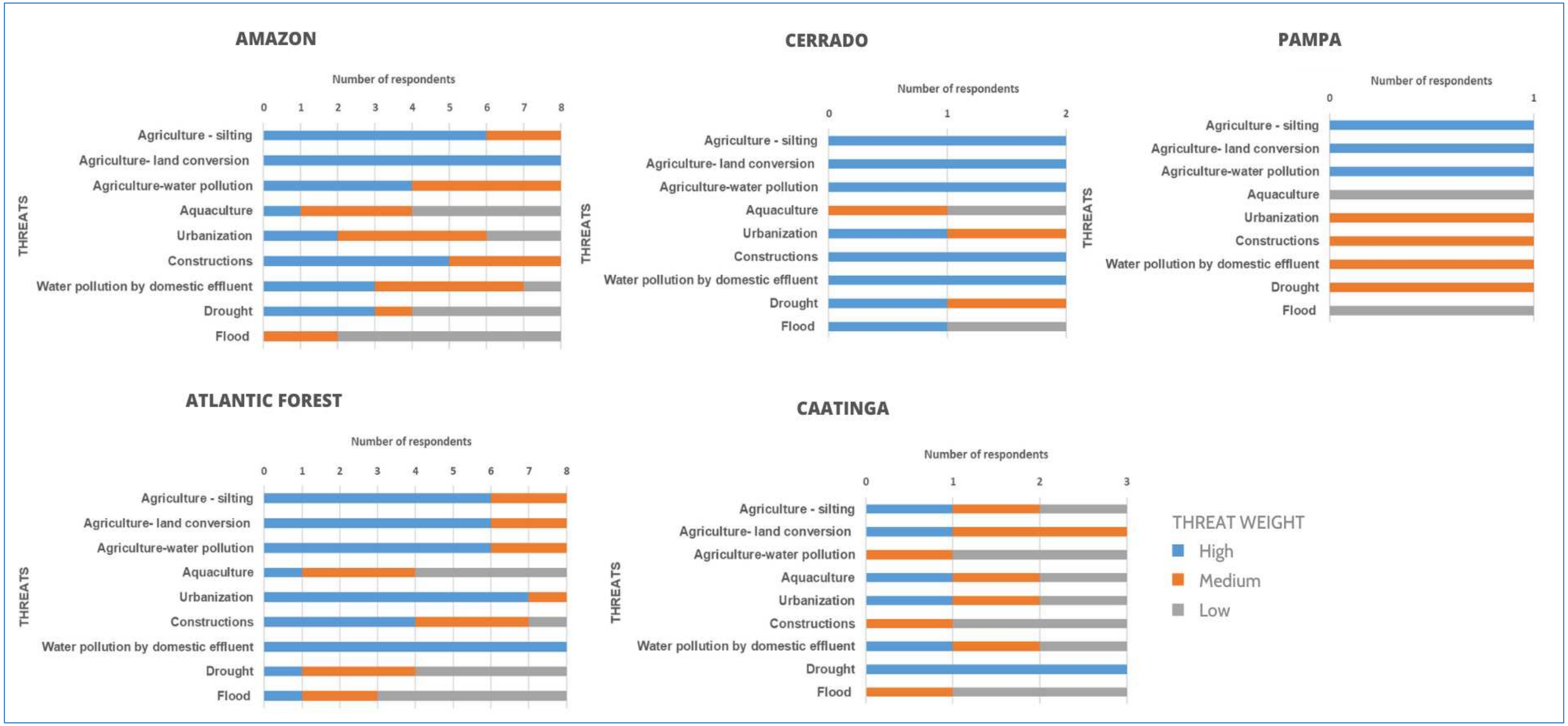
4.3.1. Environmental threats survey

We received 22 answers in the “Perception of environmental threats across Brazilian biomes” survey. Amazon and Atlantic Forest received 8 answers each; Caatinga 3; Cerrado 2; Pampa 1 and Pantanal 0. Using only those respondents who provided scores for all threats, we present survey results as proportions of answers given to each weight for each threat across the biomes **(Figure 4.1).**

Results can be interpreted by accessing, if possible, the consensus or not among researchers about the weight of each threat in a given biome. Pampa received only one answer, which unfortunately will limit broad discussions about environmental threats occurring in there, or we would be risking to make biased assumptions by considering only a single opinion.

All respondents considered agriculture related threats to have a high weight on the risk of extinction of mayflies in Cerrado and Pampa. Cerrado also achieved consensus for constructions and water pollution by domestic effluent. Atlantic forest achieved consensus only for water pollution by domestic effluent, and 75% of respondents considered agriculture related threats and 87,5% considered urbanization to have a high weight on the risk of extinction of mayflies. For Caatinga, only drought achieved consensus among experts.

Figure 4.2. - Answers of the survey "Perception of environmental threats across Brazilian biomes". Bars show the number of respondents by threat weight for each environmental threat across biomes. Pantanal had no respondents.



4.4. DISCUSSION

Threats to aquatic ecosystems vary enormously in number and importance according to the different regions of Brazil, human population density, land use, and the predominant socioeconomy (Agostinho et al., 2005). The experts' perception truly reflects that affirmation by showing that mayflies suffer with different weights and sets of environmental threats in each Brazilian biome.

4.4.1. Agriculture related threats: Land cover changes

Agriculture related threats (silting, land conversion and water pollution) have been pointed as the main sources of threat in Amazon, Cerrado and Pampa, and Brazil is one of the world's producers of agricultural commodities and it has been a major contributor to land use and land cover changes (Sousa-Neto et al., 2018). The main land-cover change that affects aquatic insects' assemblages is the removal of riparian vegetation (Souza et al., 2020). The riparian zone represents a link between the terrestrial and aquatic ecosystems, as organic matter (leaves, twigs and reproductive parts of plants) enter in streams and serve as main source of energy input, especially in forested headwater streams (França et al., 2009). This material increases nutrient supply and organic matter to streams (Balian & Naiman, 2005) and may also serve as a habitat to be colonized, a refuge from predators or water flow and oviposition sites for fauna (Salles & Ferreira-Junior, 2014). Additionally, by removing the vegetation, light incidence in the stream increases, favoring the proliferation of algae and accelerating organic matter breakdown, which depletes the food source of filterers (Richardson & Béraud, 2014).

Souza et al., (2020) found a relationship between land cover levels and the composition of EPT species, proving land cover to be more influential than the interference from other anthropic impacts. Moreover, losses of minimum percentage of 6,5% of the vegetation in buffer zones of 50m width are enough to trigger abrupt changes in EPT communities, resulting in declines of biodiversity. Notwithstanding, there is an inverse relationship between buffer zone width and threshold for the loss of aquatic insects diversity (Dala-Corte et al., 2020).

Currently, the biomes undergoing more pressure on the original land cover are the largest ones, the Amazon (419 Mha, i.e., 49% of the country) and Cerrado (203 Mha, i.e., 23% of the country). Souza-Junior et al. (2020) discovered that between 1985 and 2017, 33% of Brazil`s area suffered vegetation loss, which includes forest and non-forest formations, and greater rates occurred in the Amazon (41,8%) and Cerrado (33,8%) biomes. In Cerrado, 17 Mha of its area have been cleared and replaced with soy plantations and 60 Mha was used as pasture (INPE, 2015). In Pampa, a research estimated a replacement of at least 50% of its original vegetation (Overbeck et al., 2013), and in a time series analysis from 1975 to 2005, natural pastures area decreased almost 3 million hectares with the conversion of Pampa's grasslands into other agricultural activities (Oliveira et al., 2017).

In Amazon, since 2013, official deforestation rates have been on an upward trend. In 2019, 10,129 Km^2 of forest was clear-cut, an increase of 34% compared to 2018 (7,536 Km^2) (Silva Junior et al., 2021). Human interference in the forest is centuries old, either for exploiting wood or non-wooden products, or for the practice of traditional slash-and-burn agriculture and intensive cattle-raising, with the last being responsible for about 80% of all deforested areas in Legal Amazonia (Vieira et al., 2008).

4.4.2. Agriculture related threats: Silting

Silting (i.e. fine sediment input in aquatic environments) produced by soil and gully erosion associated with cattle grazing and crop cultivation has a negative impact on stream biota (Nakamura & Yamada, 2005). A major impact of anthropogenic silt is the simplification of faunal communities due to the siltation of stream bottoms with consequent reduction of habitat complexity, heterogeneity and stability (e.g., Couceiro et al., 2011; Palmer et al., 2000). Nymphs of some Ephemeroptera species, such as of *Paramaka convexa* Spieth are filterers of particulate matter suspended in water and have fragile respiratory structures (gills) exposed to the current. With the excessive suspension of sediments, the species feeding and breathing processes can be negatively affected by decreasing the availability of habitat and food resources, and by clogging respiratory structures (Bond & Downes, 2003; Buss et al., 2004; Chambers et al., 2006).

In a study in Amazon, Couceiro et al. (2010) found that anthropogenic suspended silt has had a significantly negative impact on pH, electrical conductivity and both indices were associated with reduction in the richness and density of macroinvertebrates and with changes in community composition. In Cerrado, the expansion of economic activities has been pointed as a major driver of the suppression of green areas, erosion processes and consequent reduction of biodiversity in water basins (Masullo et al., 2019).

4.4.3. Agriculture related threats: Water pollution

Aquatic organisms are exposed to toxicants dissolved in water or compounds bound to food, particulate or dissolved organic matter (Antwi & Reddy, 2015). These toxicants come from agrochemicals such as fertilizers, herbicides or pesticides (Dudgeon, 2020). When pesticides are applied to protect crops from pests and diseases, only around 15% of the preparation hits the target and the rest is distributed into the soil, water and air; and finally reaching the nearby water bodies through run-off (Armbrust & Peeler, 2002; Hilz & Vermeer, 2011). Some representatives of the orders Ephemeroptera and Odonata are very sensitive to pesticides. Although, in a laboratory study, mayfly nymphs of *Cloeon dipterum* and *Caenis miliaria* were most sensitive to insecticides when compared to a crustacean and a damselfly (Beketov, 2004).

Brazil is the world's largest consumer of pesticides, accounting for approximately 20% of the total world use, and insecticides are the second most used class of pesticides in the country (IBAMA, 2014). In a review of occurrence data on pesticide residues in Brazilian freshwaters, Albuquerque et al. (2016) found fipronil, an insecticide commonly used in rice fields and in household products, in 54% of the water samples analyzed in fractions of Pampa and Atlantic Forest biomes. Moreover, across the studies reviewed, they calculated potential risk to aquatic life for 59% of the pesticides with the occurrence data in Brazil. In another study, carbofuran caused a general reduction in the macroinvertebrate assemblage in an experimental floodplain rice area, with the most critical time for these assemblages seen after the 10th day of application (Baumart et al., 2011). Across 35 sampling sites of surface and groundwater in a small area in Cerrado, herbicides residues frequency and concentrations were higher after agriculture application season, indicating the higher vulnerability of both systems to contamination during the application period (Dores et al., 2008).

Additionally, one respondent of our survey pointed to the use of insecticides for mosquito control as an environmental threat present in Atlantic Forest. In a soil, water and fish tissue sampling analysis of seven sites in a state park in the Atlantic Forest, 27 different pesticide residues were found, among pyrethroids, chloroacetanilides, organophosphates, organochlorines and others. Organochlorines use is allowed only for health authorities and are frequently used for control of vector-borne diseases, but watercourses and soil end up contaminated by the remnants leached from surfaces (Moraes et al., 2003).

4.4.4. Agriculture related threats: Fires

A respondent pointed uncontrolled fires for land conversion in pastures and for agriculture use in Cerrado as a threat for mayflies in the biome. Brazil is a key region because it is one of the areas of the globe most affected by fires (Bowman et al., 2011), with fires occurring naturally in Cerrado for thousands of years (Pessenda et al., 2005). Since the 1960s, fire regime has been altered by anthropic activities, mostly land cleaning for agricultural purposes (Sano et al., 2010). By 2015, 41% of native Cerrado vegetation (86 Mha) had been cleared for agricultural use (INPE, 2015). The loss of deep-rooted shrubs and trees alters carbon storage and evapotranspiration (Spera et al., 2016), which may contribute to a climate transition to hotter temperatures and lower rainfall period in up to one month in dry season (Costa & Pires, 2010), which may ultimately cause drought events (discussed in a topic below) and alterations in aquatic ecosystems. Additionally, uncontrolled fires might reach riparian zones, diminish or extirpate them, and cause major disturbances as already discussed above.

In 2020, apocalyptic fires ravaged the Brazilian Pantanal (Mega, 2020). Since 2019, the biome is suffering a persistent drought with the reduction of 57% of rainfall in the wet season of 2020. This combination of hot, dry conditions pushed flammability thresholds to their highest since 1980 (Libonati et al., 2020). Uncontrolled fires have engulfed hundreds of thousands of hectares across Pantanal, mostly related to anthropogenic activities, such as deforestation, cleaning, and reforming pastures

(Marengo et al., 2021). Until November 2020, 27%, an area equivalent to 4,117 hectares have already been consumed by fire, but the total loss will take months to calculate (Chaves et al., 2020). The impacts of the fires are long lasting. Charcoal and ash contaminate rivers, increase organic matter concentration, and promote the multiplication of harmful bacteria that will consume all oxygen in the water and kill fishes and invertebrates (Chaves et al., 2020; Libonati et al., 2020). Additionally, eroded soils are flushed downstream when the rain arrives and stream levels rise, leading to ecosystem alterations already discussed above in “Silting” (Campo et al., 2006).

4.4.5. Urbanization

Urbanization has been pointed to have high weight on the risk of extinction of mayflies in the Atlantic Forest. Most Brazilian cities have experienced uncontrolled development and increased occupation of river margins and their floodplains (Pompeu et al., 2005). In developing countries, urban population growth has often not been accompanied by efficient public policies (Couceiro & Hamada, 2011). The need for eliminating liquid waste produced in urban areas, the draining of the rainwater that accumulates on impervious surfaces, and the lack of awareness by the population that throws solid waste into the water bodies, increasing the pollution load, contributed to the degradation of streams located in urban areas (King et al., 2011).

Martins et al. (2017) recorded an increased impact of urbanization on streams in Amazon, mainly due to the increase of total impervious area (TIA) and domestic sewage input, causing an elevation in abundance of tolerant taxa and reduction of sensitive groups. Several studies have shown that TIA is an excellent surrogate variable that accumulates (or summarizes) the effect of variables associated with the consequences of urbanization on streams (King & Baker, 2010). The efficiency of TIA in summarizing the urbanization impacts is due to its strong correlation with density of human population, which causes chemical changes in the water of urban streams, and with drainage system, which generates great oscillations in temperature and flow of the streams (Fogaça et al., 2013).

A study conducted in a river basin in Atlantic Forest using TIA as a measure of urbanization impact, showed a clear reduction of EPT richness with increasing TIA. Of 27 genera found in the basin, only 15 were present in streams highly impacted by urbanization whereas 24 occurred in low impacted streams (Fogaça et al., 2013).

Ephemeroptera genera *Cloeodes* Traver and *Caenis* Stephens were only present in streams with low percentage of TIA, believed to be due to factors as reduction of litter input from riparian vegetation causing a reduction of particulate organic matter available for filterers and collectors; higher intensity and frequency of flood peaks causing severe stress on the biota by reducing refuge areas and trapping individuals in upper areas to die by desiccation and low oxygenation (Walsh et al., 2005); and reduction of habitat availability to the biota, mostly due to sharp decreases of stream basal flow (Sung & Li, 2010).

4.4.6. Water pollution by domestic effluent

Threat posed by water pollution by domestic effluent was considered to have a high weight on the extinction risk of Ephemeroptera in Cerrado and Atlantic Forest. The pollution of aquatic systems results from diffuse sources in the watershed through land use activities, as well as from direct discharge of pollutants, such as industrial and domestic sewage dump (Aguiar et al., 2011). Organic pollution caused by domestic sewage results, especially, in water eutrophication (Couceiro et al., 2007), which can cause toxic algal blooms, increase in water turbidity, diminish dissolved O^2, and reduce macrophyte cover (Pan et al., 2015). Historically, Ephemeroptera, Plecoptera, and Trichoptera (EPT) are considered intolerant of eutrophication (Wiederholm, 1984). Ometo et al. (2000) compared two streams with opposite levels of pollution in Atlantic Forest and discovered a reduction of macroinvertebrate richness in more polluted streams, mostly associated with a reduced amount of dissolved oxygen and an increment in dissolved organic carbon.

An additional effect of sewage input is the nutrient enrichment in streams (e.g. nitrogen and phosphorous), which was shown to cause shifts in trophic position of aquatic invertebrates, with a decrease in the trophic positions of omnivorous taxa and a generalist predator along an eutrophication gradient presumably due to changes in their diet. For omnivores, this dietary shift is likely to be related to the increased nutritional quality of primary producers in more eutrophic parts of the landscape. These alterations will affect species interactions and food web structure in aquatic ecosystems (Lee et al., 2021).

4.4.7. Drought

Drought events has been the only environmental threat that reached consensus among experts in Caatinga. According to the Intergovernmental Panel on Climate Change (IPCC, 2013), many arid and semiarid regions are likely to become warmer and drier by the end of this century due to anthropogenic climate change. For instance, the semiarid region in Northeast Brazil comprises the biome Caatinga, in which climate models predict a temperature increase of about 4°C and rainfall reduction of about 40%, and increases in the intensity or duration of drought by the end of the 21st century (Marengo et al., 2009; 2010). This region is dominated by reservoirs that have been turning eutrophic due to nutrient inputs from land and concentration of the nutrients in the water because of high evaporation rates and long drought periods (Barbosa et al., 2012; Brasil et al., 2016; da Costa et al., 2016).

Additionally to the harmful effects of eutrophication discussed in the previous topic, a reduction in water flow and flooded area implies a reduction in energy and matter flow from the watershed to aquatic ecosystems, slowing biogeochemical processes and making biogeochemical cycles more closed in these aquatic ecosystems. Moreover, a reduction in water flow and flooded area may decrease the length of vertical and horizontal gradients of physical conditions, resources and organisms, spatially homogenizing the biogeochemical processes in the aquatic ecosystems (Roland et al., 2012).

Connections among aquatic ecosystems are the main route of dispersal of different populations of mayfly nymphs by passive dispersal in stream flow (drift) (Heino et al., 2015; Lancaster & Briers, 2008). A general decrease in precipitation in a region could result in a decrease in the connectivity of aquatic ecosystems, causing reduction of dispersal and gene flow among populations due to habitat fragmentation (Fahrig, 2003). Moreover, the increase in temperature combined with the reduction of rainfall in the semi-arid region may reduce the number of lakes, ponds and temporary streams causing a decrease in habitat size and availability for species that live in those environments (Roland et al., 2012).

4.4.8. Constructions: Dams

Natural dynamics of Brazilian rivers have been profoundly impacted by the construction of reservoirs (BPBES, 2020). Brazil already has a larger area than Belgium submerged by dams (Fearnside & Pueyo, 2012). Dams affect aquatic wildlife by blocking migrations (Duponchelle et al., 2016; Pelicice et al., 2014) and fragmenting populations in smaller and isolated subpopulations (Agostinho et al., 2008). Almeida et al. (2009) found a reduction in abundance of species of the genera *Baetodes* Needham & Murphy, *Hagenulopsis* Ulmer, and *Leptohyphes* Eaton in areas after the impoundment compared with areas above the impoundment. They associated this reduction with the loss of connectivity between the two areas, which impair drift behavior of organisms, as species abundance equaled after the reconnection of water flow.

Additionally, damming turns free-flowing environments into lentic ones, which eliminates unique habitats, such as rapids and rocky areas (Lees et al., 2016; Winemiller, 2016). The submersion of such habitats might extirpate mayfly species from genera like *Baetodes* Needham & Murphy, *Camelobaetidius* Demoulin, *Oligoneuria* Pictet, and *Lachlania* Hagen, which rely on water flow for breathing processes, and displace others like *Leentvaaria* Demoulin and *Rondophlebia* Salles & Boldrini that live in rocky substrates. Moreover, collectors-filtering taxa were absent from sites below dams in dry season when water flow is reduced, believed to be due impairment of species' feeding behaviors that rely on water movement to happen (Almeida et al., 2009).

Dams retain sediment and nutrients, a process that is intensified by the conversion of rivers into reservoirs, as is planned for many Amazon rivers (Fearnside, 2015; 2016). For example, the Santo Antônio and of Jirau, on Madeira River, annual average surface concentration of suspended sediments decreased by 20% in the river below these dams (Latrubesse et al., 2017) and the concentrations of P-total in the Paraná River decreased by about 65% after the construction of Porto Primavera dam (Roberto et al., 2009). Mendoza-Lera et al. (2012) found a reduction of 20% in leaf-litter breakdown rates below dams, and associated this finding to a lower abundance of shredders macroinvertebrates, as in impaired freshwater systems, consumption of leaf litter by shredders may be altered through changes in food palatability, in shredder abundance and species composition (Maltby, 1994) and biomass. As a result of the reduction of nutrient inputs, flood pulses cause "oligotrophization" of floodplains located downstream of dams (González et al., 2013), affecting primary productivity and leading to a compromise of the sedimentary balance, the main vector of coastal erosion (BPBES, 2020).

4.4.9. Constructions: Mining

The impacts of mining on ecosystems and conservation are global and of major concern because of their synergy with other drivers of ecosystem changes (Fernandes & Ribeiro, 2017). Areas degraded by mining combine vegetation suppression, habitat fragmentation, compaction of exposed material, low infiltration rates and water storage capacity, groundwater retraction, soil nutrient leaching, disruption of nutrient cycling, erosion, pollution of soil and water resources (with solvents, oils, greases and heavy metals), increased air and water particles, oxygen deficiency in water bodies, light and noise pollution, intentional introduction of non-native species during restoration, landscape alterations, and the destruction of local communities (e.g., Costa-Milanez et al., 2017; Murguía et al., 2016; Parise, 2016; Selinus et al., 2013).

Disasters caused by mining occur all over the planet, and most of them have anthropogenic fingerprints. In recent years, two disasters occurred in Brazil. On November 5th, 2015, a dam breach released 55–62 million m3 of iron ore tailings slurry directly into the Doce River watershed (GFT, 2015). The mine slurry filled hydrologic networks along 663.2 km of the Doce River through the states of Minas Gerais, and Espírito Santo before reaching its estuary (INPE, 2015). Most recently, on January 25th, 2019 another dam collapsed in Minas Gerais, discharging 12 million m3 of iron ore tailings slurry directly into Paraopeba river, with extensive environmental damage reported over some 600 km of the Paraopeba river and its tributaries (Cambridge & Shaw, 2019). Cost estimates for restoration of the threatened Brazilian Atlantic Forest ecosystems are around 20 billion dollars (GFT, 2015), as the destruction of riparian, freshwater and marine ecosystems eliminated irreplaceable natural resources and ecological processes that support traditional livelihoods, disrupting fisheries, agriculture, tourism and provisioning of fresh water (Fernandes et al., 2016).

In regard of the occurring Ephemeroptera species in both areas, several of them were probably affected by the breach, actively by being buried under the wave of slurry, or passively by the suspension of sediments, which can clog in gills and impair respiratory processes. Additionally, researchers working in the assessment of environmental impacts of the breach in Brumadinho are investigating the loss of connectivity between the tributaries of Ferro-Carvão river, which, if true, may have caused a major disturbance and poses future threats to the maintenance of mayfly populations.

In Doce River, a study on water chemistry by the Minas Gerais State Water Agency (IGAM) found high levels of arsenic, cadmium, copper, chromium, nickel and mercury in water samples following the burst (IGAM, 2015). Once in the aquatic ecosystem, most heavy metals persist in sediments, from where they are slowly released into the overlying water and ultimately reach the body of aquatic organisms (Bouchelouche & Arab, 2020). Metal contamination can reduce benthic macroinvertebrate species richness (Clements et al., 2000, 2013), as well as their density, growth, and reproduction (Gray & Delaney, 2008).

4.5. CONCLUSIONS AND FUTURE DIRECTIONS

This chapter provides a national perspective concerning environmental threats that may cause mayfly population declines and that may increase extinction risk. Our survey results reveal what knowledgeable respondents judged to be the most detrimental threats to mayfly species persistence, revealing differences among the Brazilian biomes. Even though such opinions cannot be used to evaluate the extent of such threats, our results are, in part, supported by several studies in the literature. We expect that the perspective provided by researchers, coupled with the comprehensive review provided for most important threats, will be useful for future studies aiming to understand drivers of mayfly diversity and abundance in different scales across the country. Additionally, in the context of the evidences of major disturbances in aquatic environments, it is paramount to direct narrower efforts in unveiling the vulnerability of the diverse taxa of Ephemeroptera from Brazil to the different threats and in different sites.

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