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Biodiversity of aquatic insects in mining areas: Integrated approaches in the Rio Doce basin

Ana Dária Leite Viana
Doctor Scientiae

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Thesis submitted to the Ecology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Carlos Frankl Sperber

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“If there is magic on this planet, it is contained in water”
Loren Eiseley

ABSTRACT

VIANA, Ana Dária Leite, D.Sc., Universidade Federal de Viçosa, April, 2025. **Biodiversity of aquatic insects in mining areas: Integrated approaches in the Rio Doce basin**. Adviser: Carlos Frankl Sperber. Co-advisers: Frederico Falcao Salles and Tatiana Garabini Cornelissen.

Understanding biodiversity in aquatic ecosystems is key to effective conservation, especially in areas impacted by human activities. This thesis examines the effects of mining on aquatic insect communities, focusing on Ephemeroptera, Plecoptera, and Trichoptera (EPT) as bioindicators. Set in the Rio Doce Basin, severely affected by the Samarco-Vale/BHP Billiton disaster, the study integrates meta-analysis, fieldwork, and ecological modeling to assess biodiversity loss, species dispersal, and habitat changes. The findings show that mining intensifies environmental stress, leading to biodiversity decline and habitat fragmentation. Key ecological corridors crucial for maintaining species connectivity are identified, emphasizing their role in conservation. Climate projections indicate further habitat loss and species displacement, highlighting the combined risks of local and global environmental changes. This work refines biomonitoring strategies, improving cost-effectiveness and scalability while addressing taxonomic and biogeographic gaps in aquatic insect diversity. By combining multiple analytical approaches, it provides a comprehensive framework for freshwater conservation in degraded landscapes, offering insights to guide effective management and restoration efforts.

Keywords: meta-analysis; EPT; freshwater; macroinvertebrate; niche modeling

RESUMO

VIANA, Ana Dária Leite, D.Sc., Universidade Federal de Viçosa, abril de 2025. **Biodiversidade de insetos aquáticos em áreas de mineração: Abordagens integradas na bacia do Rio Doce**. Orientador: Carlos Frankl Sperber. Coorientadores: Frederico Falcao Salles e Tatiana Garabini Cornelissen.

A compreensão da biodiversidade em ecossistemas aquáticos é fundamental para a sua conservação efetiva, especialmente em áreas afetadas por atividades humanas. Esta tese examina os efeitos da mineração nas comunidades de insetos aquáticos, com foco em Ephemeroptera, Plecoptera e Trichoptera (EPT) como bioindicadores. Situado na Bacia do Rio Doce, gravemente afetada pelo desastre da Samarco-Vale/BHP Billiton, o estudo integra meta-análise, trabalho de campo e modelagem ecológica para avaliar a perda de biodiversidade, a dispersão de espécies e as mudanças de habitat. Os resultados mostram que a mineração intensifica o estresse ambiental, levando ao declínio da biodiversidade e à fragmentação do habitat. Os principais corredores ecológicos cruciais para manter a conectividade das espécies são identificados, enfatizando seu papel na conservação. As projeções climáticas indicam maior perda de habitat e deslocamento de espécies, destacando os riscos combinados das mudanças ambientais locais e globais. Este trabalho refina as estratégias de biomonitoramento, melhorando a relação custo-benefício, a escalabilidade e, ao mesmo tempo, abordando as lacunas taxonômicas e biogeográficas na diversidade de insetos aquáticos. Ao combinar várias abordagens analíticas, ele fornece uma estrutura abrangente para a conservação da água doce em paisagens degradadas, oferecendo percepções para orientar esforços eficazes de gerenciamento e restauração.

Palavras-chave: meta-análise; EPT; água doce; macroinvertebrados; modelagem de nicho

SUMMARY

GENERAL INTRODUCTION

Knowledge of the factors that shape biodiversity in aquatic ecosystems is essential for the development of conservation strategies, especially in regions affected by anthropogenic activities. This thesis was organized with the aim of integrating different analytical approaches and spatial scales to study the ecological processes that influence the biodiversity of continental lotic ecosystems, with a focus on aquatic insects, especially those of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT), which are known to be have species highly sensitive to environmental changes. The four chapters that comprise this work are interrelated and form a set that addresses EPT biodiversity in the context of one of Brazil's greatest environmental disasters, the Samarco-Vale/BHP Billiton mining tailings dam collapse that devastated the Rio Doce valley.

Almost ten years after the disaster, this work introduces a novel framework for assessing the resilience of aquatic communities by jointly considering habitat quality, landscape connectivity, and climate-driven shifts in species distributions. The interdisciplinary methodology and emphasis on practical applications-such as identifying ecological corridors and mismatches between future refugia and protected areas-contribute both conceptually and operationally to freshwater conservation. As one of the first comprehensive ecological studies using aquatic insects conducted in the aftermath of the Rio Doce disaster, this thesis not only enriches the scientific literature with original data and models, but also responds to an urgent socio-environmental challenge by providing tools for evidence-based ecological restoration.

The first chapter consists of a meta-analysis of the effects of mining on aquatic macroinvertebrate communities. The analysis shows that the response of taxonomic groups can vary significantly, with the group consisting of the orders Ephemeroptera, Plecoptera and Trichoptera being more sensitive and efficient at detecting environmental impacts than macroinvertebrates as a whole. This finding supports the methodological choice of focus groups in the following chapters and reinforces the importance of a more focused approach to assessing the environmental quality of aquatic ecosystems. In addition, the conclusions of this first chapter support a reduction in the costs associated with biomonitoring, as it reduces the volume of macroinvertebrate bioindicators to be collected and identified, and consequently, an expansion of this activity.

The second chapter introduces the context of the Rio Doce Basin, the site of the disaster caused by the collapse of the Samarco Dam, located in the city of Mariana, MG, Brazil. This chapter examines the interaction between abiotic factors, one of the three limiting filters of local biodiversity, in interaction with the presence of tailings, at three different spatial scales: within the water body, in the immediate vicinity of the rivers, and in the regional landscape. Using statistical models that incorporate these scales, the study shows that environmental degradation resulting from mining intensifies the effect of pre-existing environmental stressors, negatively affecting the richness and abundance of EPT in affected areas.

The third chapter complements the ecological perspective by addressing one of the other main factors regulating biodiversity: dispersal. Based on graph theory, it was possible to model the connectivity between Ephemeroptera communities in particular, and to identify the main connection points in the Rio Doce basin. These points act as ecological corridors and their conservation can guarantee gene flow and the stability of metacommunities throughout the Rio Doce basin. The results indicate that there is a significant disruption of dispersal routes, with fragmentation of communities. In addition, this study highlights key areas to ensure the sustainability of Ephemeroptera community populations throughout the basin.

The fourth chapter incorporates the temporal variable by projecting the potential distribution of four Ephemeroptera species (*Apobaetis irai*, *Campsurus cotaxe*, *Paramaka convexa*, and *Tricorythodes santarita*) under different current and future climate scenarios. Ecological niche modeling showed that as climate change progresses, the ranges of these species tend to shrink and migrate to higher elevations, with a reduction in suitable habitats. The study also shows that these refugia do not coincide with existing conservation units, highlighting critical gaps in current conservation strategies. The interaction between local anthropogenic impacts and global climate pressures places these species in a situation of vulnerability that requires integrated management and conservation actions.

Each chapter of this thesis contributes to the understanding of the processes that regulate the biodiversity of Ephemeroptera, Plecoptera and Trichoptera in impacted environments, using different methodological approaches and scales of analysis. The thesis provides a comprehensive understanding of the challenges facing aquatic insect communities in a scenario of increasing environmental impact.

The interrelationship between the chapters is also evident in the way each chapter addresses two of the three main vectors limiting local diversity - abiotic factors and dispersal - using EPTs as a model. Based on the meta-analysis of the sensitivity of this group in mining areas, the following chapters delve deeper into the Rio Doce Basin, evaluating how these vectors operate.

This thesis makes a significant contribution to the understanding and management of freshwater ecosystems in the Atlantic Forest by proposing a systemic vision that combines biomonitoring, ecological modeling, and spatial analysis. In addition to advancing scientific knowledge, the work can contribute to biodiversity conservation and impact mitigation in one of the most historically degraded regions of Brazil.

In addition to the four main chapters that structure this thesis, three additional articles have been included that complement and deepen the proposed objectives (Appendix). These articles deal with taxonomic and biogeographic aspects of specific groups of Ephemeroptera and Trichoptera, bringing unprecedented contributions on the diversity, distribution and morphological characteristics of species sensitive to environmental impacts in the Rio Doce Basin. Including these publications broadens the knowledge base needed to understand the regional biodiversity of aquatic insects, reduces taxonomic and geographical distribution gaps, and provides essential data to support the ecological analyses carried out in the central chapters. In this way, these studies strengthen the integrated approach of the thesis by linking taxonomy, biogeography, and applied ecology, fundamental elements for conservation in affected ecosystems.

CHAPTER 01

Trends and gaps in research on the effects of mining on aquatic macroinvertebrate communities

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Abstract

Mining, a severe danger to the environment worldwide, is causing significant disruptions to ecosystems, especially the vulnerable aquatic ones. Our systematic review and meta-analysis aimed to consolidate existing knowledge on the effects of mining on macroinvertebrate communities in lotic environments. The objectives were to evaluate research trends and gaps over recent decades and to quantify the impact of mining on variables associated with these communities. A comprehensive survey of articles indexed in Web of Science, Scopus, and Google Scholar, and the PRISMA flowchart was used to explain the inclusion and exclusion criteria for the studies, and a robust meta-analytic approach was used to analyze the data on the effects of mining on lotic macroinvertebrate community variables. This study confirms that mining activities have a negative impact on the richness and diversity of aquatic macroinvertebrate communities. This effect was more pronounced in the Ephemeroptera, Plecoptera and Trichoptera groups. It is recommended that future research, with its potential for progress and discovery, be extended to less explored geographical regions, as well as evaluating abiotic factors at broader spatial scales, to fill the gaps in our current understanding.

Keywords: Aquatic ecology, integrative review, meta-analysis, environmental impacts, aquatic insects

1. Introduction

Mining sites remain an environmental threat worldwide, causing disturbances to aerial, terrestrial, and aquatic ecosystems (Marquez et al., 2001). Recent ecological disasters involving mining companies in Brazil, such as the tailings dam collapses in the cities of Mariana (Nascimento et al., 2022) and Brumadinho (Thompson et al., 2020), have highlighted the environmental impacts resulting from the widespread growth of this industrial activity.

Aquatic ecosystems are particularly vulnerable to environmental changes triggered by mining activities (Cadmus et al., 2019). Benthic macroinvertebrates, organisms larger than 500 µm that spend at least part of their life cycle in aquatic environments, play a crucial role in this vulnerability. They are considered the most sensitive indicators of metal contamination (Rosenberg and Resh, 1993), underlining their significance in assessing the impact of mining on aquatic ecosystems.

The impact of mining on aquatic macroinvertebrate fauna is related to a range of effects on environmental characteristics, such as changes in the physical structure of the habitat, changes in the physicochemical characteristics of the water, and an increase in the concentration of heavy metals in the water (Smolders et al., 2003; Beck et al., 2020; Rico-Sánchez et al., 2022). These effects are reflected in biological communities, with consequences for resource availability and community structure (Bae et al., 2021).

Of all anthropogenic pressures, mining activities have the most prolonged lasting impacts on streams and rivers (Bae et al., 2021). Waterbodies near mined areas often show changes such as increased electrical conductivity, elevated concentrations of trace metals, acidification, decreases in organic matter, and sediment contamination (Pond et al., 2008; Bere et al., 2016).

Shifts in environmental conditions and resources cause abrupt changes in the composition of functional food groups and the structure of the macroinvertebrate community (Grubaugh et al., 1996). The number of sensitive species decreases while tolerant species dominate (Gerhardt et al., 2004). Several studies show that aquatic macroinvertebrate communities are less diverse and complex in streams near mining sites than reference sites (Matlou, 2017; Bae et al., 2021; Erasmus et al., 2021). However, differences between geographic regions, taxonomic groups, climatic zones of occurrence, and the type of diversity indices used in the assessment may reveal idiosyncrasies between studies.

The main question of this work is what effect mining will have on lotic aquatic macroinvertebrate communities. Based on this, the following hypotheses were raised: i) Mining activities will have a negative effect on the richness, abundance and diversity of aquatic macroinvertebrates; ii) The negative effect on parameters of lotic macroinvertebrate community structure will be greater for more sensitive taxonomic groups; and iii) The negative effect on the parameters of the lotic macroinvertebrate community structure will vary according to the latitudinal gradient.

2. Material e methods

A survey of articles indexed in Web of Science, Scopus, and Google Scholar was conducted on October 27, 2022, using the following combination of keywords: [Topic of Search TS= (mining OR "iron mining" OR "lithium mining" OR "mined land*" OR "mined area*") AND TS= ("benthic macroinvertebrate*"OR "aquatic insect*" OR “benthos” OR "aquatic invertebrate*")]. Filters were used by category and type of publication on each platform, excluding categories unrelated to the research topic, such as Pharmacology, Engineering, Social Science and Computer Science, and publications that were not articles. After the initial search, the first 300 articles from each search platform were downloaded to EndNote® to manage and organize the references according to the defined keywords.

2.1. Database and qualitative review

The PRISMA flowchart (*Preferred Reporting Items for Systematic Reviews and Meta-analyses*; Page et al. 2021) was used to explain the inclusion and exclusion criteria for the studies. The inclusion criterion in the database after the initial screening was articles in the English language. Of the 900 articles obtained, four were excluded because they were duplicates. After reading the titles, articles with the terms sea, ocean, marine, oceanic, lake, estuarian, ice, cold, alpine, and deep-sea, as well as articles with other classes of animals, were excluded, leaving 499 publications. Of these, 310 were excluded because they did not fit the theme, dealing with different topics such as heavy metal assessment, biotic indices, and studies in lentic environments; 129 articles were excluded because they were not about the effects of mining, lea ving 60 publications.

To create the database, the following information was extracted from the articles: a) author(s); b) title c) year of publication; d) country of the study; e) article source (e.g., journal name); f) target group g) mineral evaluated; e h) main question of the study (e.g., biomonitoring, taxonomic diversity).

2.2. Meta-analysis - Effects of mining on aquatic macroinvertebrate communities

Articles included in the qualitative review (n=60) were screened for inclusion or non-inclusion in the meta-analysis (Fig. 1) by applying the following criteria: i) studies that reported ecological variables of aquatic macroinvertebrates in areas with and without mining, ii) studies that reported mean values of macroinvertebrate community variables with some measure of variability around the mean and sample size explicitly reported in the publication.

For each study included in the meta-analysis, the following were recorded: 1) author(s) and year of publication, 2) journal in which the study was published, 3) country in which the study was carried out, 4) latitude and longitude coordinates of the study (if available from the author), 5) taxa used in the study, 6) mineral compound evaluated, 7) presence of hydrological, physicochemical, or landscape structure parameters in the study, 8) focus of the study (taxonomic diversity, functional diversity, ambiental recovery, biomonitoring e toxicology) 9) the mean data of the response variables in areas with and withoutmining, with the appropriate measure of variance and sample size.

For response variable means available only in figures, each figure was scanned for data extraction using ImageJ® software (Imagej.nih.gov/ij) after calibrating the figure using the values plotted on the y-axis as the numerical scale. When the same article provided more than one macroinvertebrate community response variable (e.g., macroinvertebrate richness and abundance), these were reported separately in the database, as was the case when the same article provided temporal data.

2.3. Data Analysis: Scientometrics

Mean, standard deviation and sample size information were extracted from the articles for scientometric analysis. The geographic coordinates of the studies and country of origin data were used to create a global distribution map. Analyses were performed using R software version 4.2.2 (R Core Team, 2023).

2.4. Data Analysis: Meta-analysis

A meta-analytic approach was used to analyze the data on the effects of mining on lotic macroinvertebrate community variables. Thus, statistical methods were used to combine the magnitude of results across different databases and related to the same question (Koricheva et al. 2013).

The metric used in the meta-analysis was the standardized mean difference (Hedge's d). This metric is suitable for estimating comparable effect sizes between studies comparing two or more groups, using the mean, standard deviation, and sample size values for each control or treatment group. To calculate Hedge's d, we used the mean of the control group(s) and the mean of the treatment group(s).

Thus, positive Hedge's d values indicate positive effects of mining on macroinvertebrate communities and negative values indicate negative effects of mining on macroinvertebrate communities. The significance of individual effects was indicated by evaluating confidence intervals (95%), with cumulative effects considered significant if the confidence intervals do not overlap zero (Hedges et al. 1999).

First, a general model was built including all variables reported for macroinvertebrates in mining areas. Variables with at least ten replicates of individual effect size (E++) were used to create the model. The effect of mining on each response variable was then tested separately using hierarchical multilevel models.

To test for differences between the levels used in the multilevel models, Q-heterogeneity statistics (Gurevitch and Hedges 1999) were used to partition the variance between groups (QB, between) and assess whether or not the categorical groups are homogeneous concerning effect sizes. Total heterogeneity (QT), within-group heterogeneity (QW), and between-group heterogeneity (QB) were calculated.

The presence of publication bias was assessed by visually inspecting a pattern of asymmetry in the funnel plot. Rosenthal's fail-safe numbers were also calculated, which indicate how many non-significant results, unpublished or missing studies, would have to be added to the sample to change the results from significant to non -significant (Rosenthal 1979, Rosenberg et al. 2000). All analyses were performed using the Metafor and Scales packages in the R program (R Core Team, 2023).

3. Results

3.1. Scientometrics

A total of 499 articles were thoroughly analyzed according to the established criteria. Of these, 439 were excluded according to the PRISMA criteria, which resulted in 60 studies for the systematic review (Fig. 1). These studies were published over the last 45 years (1977-2022), with an emphasis on the previous two decades (2002-2022), which accounted for more than 90% of the published studies (Fig. 2). The studies were conducted in 24 different countries. The United States led in the number of articles on mining and macroinvertebrates, with about 40% of the published studies (n=25). Countries such as Spain and Canada had more studies than the other countries (Fig. 3). Most studies were performed in the subtropical zone, followed by the temperate and tropical zones, with a small percentage of studies in the equatorial zone (Fig. 4).

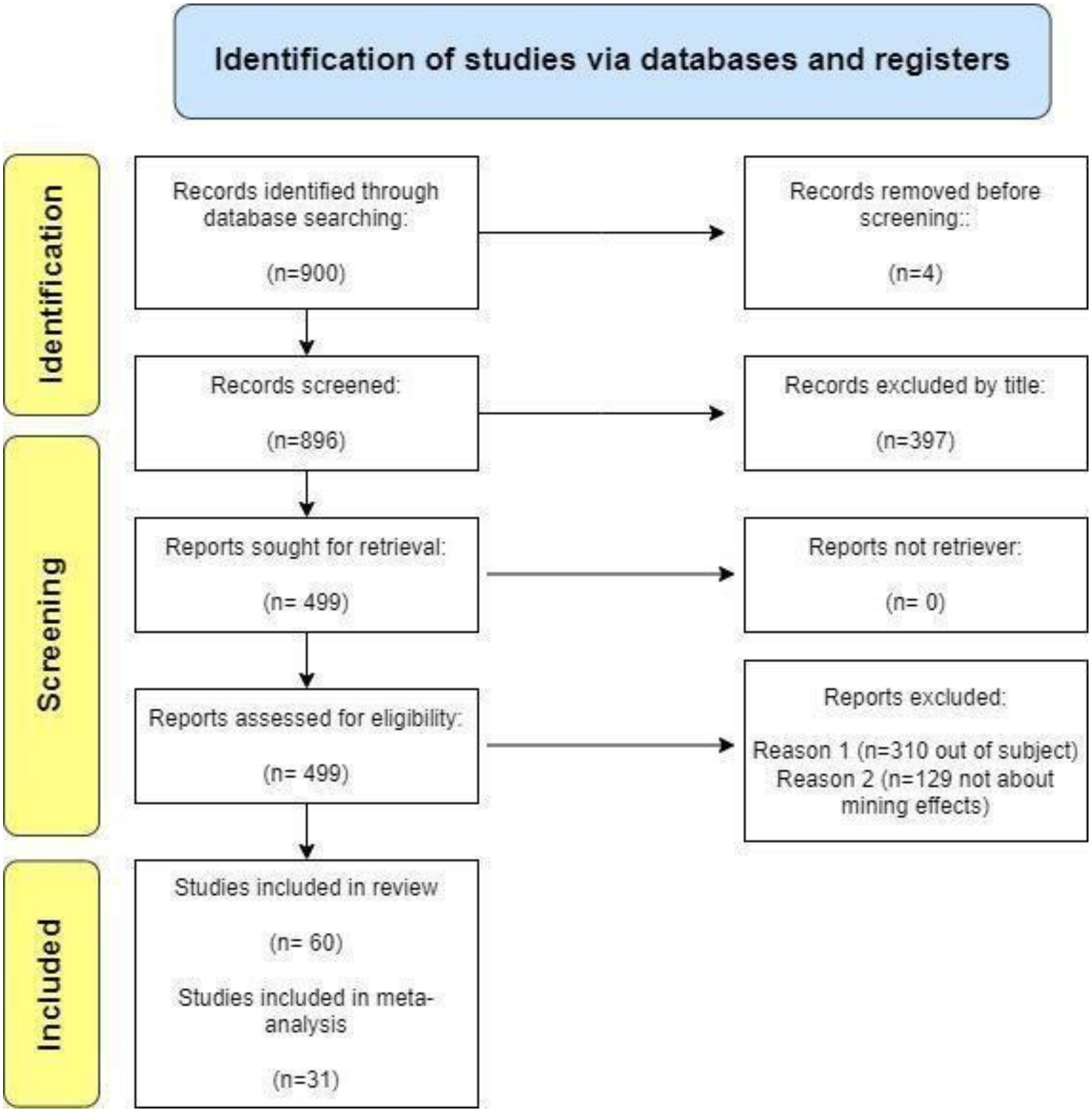

Fig 1. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) flowchart shows the different stages of searching and selecting the bibliographies used in this study.

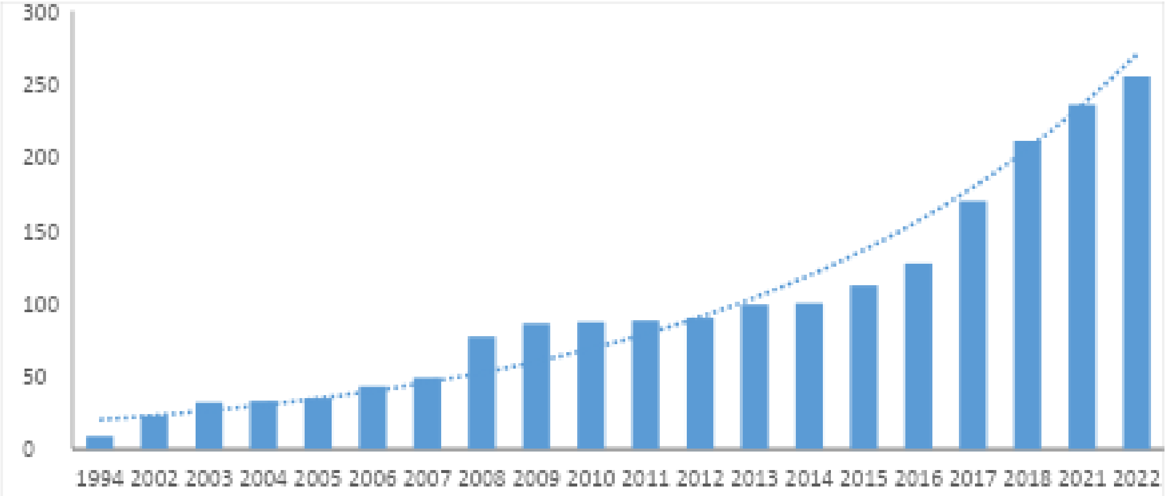

Fig 2. The cumulative number of articles published on macroinvertebrates and mining from 1994 to 2022. The bars indicate the cumulative number of studies per year, and the dotted line indicates the exponential growth of articles.

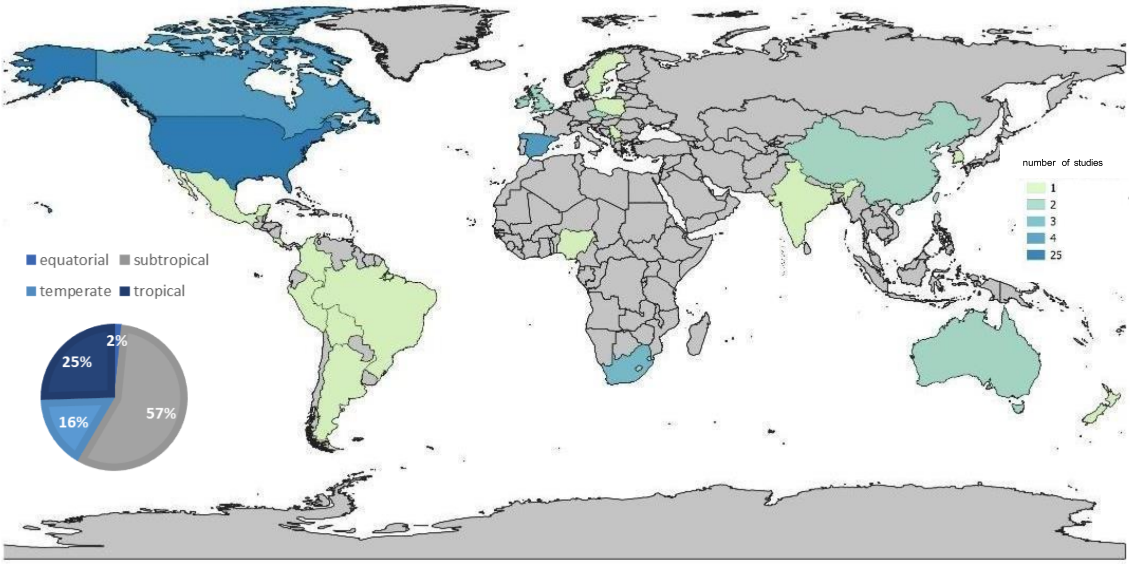

Fig 3. (A) Distribution of studies found among countries registered in the database, where darker colors indicate greater registrations. (B) Sector graph showing the percentage of studies conducted in equatorial, subtropical, temperate, and tropical regions.

The selected scientific articles were published in twenty-four different scientific journals, with three journals having the highest number of published articles: Environmental

Pollution, Journal of Freshwater Ecology, and Environmental Toxicology and Chemistry. Most of the journals presented only one article on the topic of this study. The main focus was on taxonomic diversity in 58% of the papers reviewed, with the remaining studies being related to toxicology (12.5%), functional diversity (10.6%), environmental recovery (10.2%) and biomonitoring (22%) (Fig. 4).

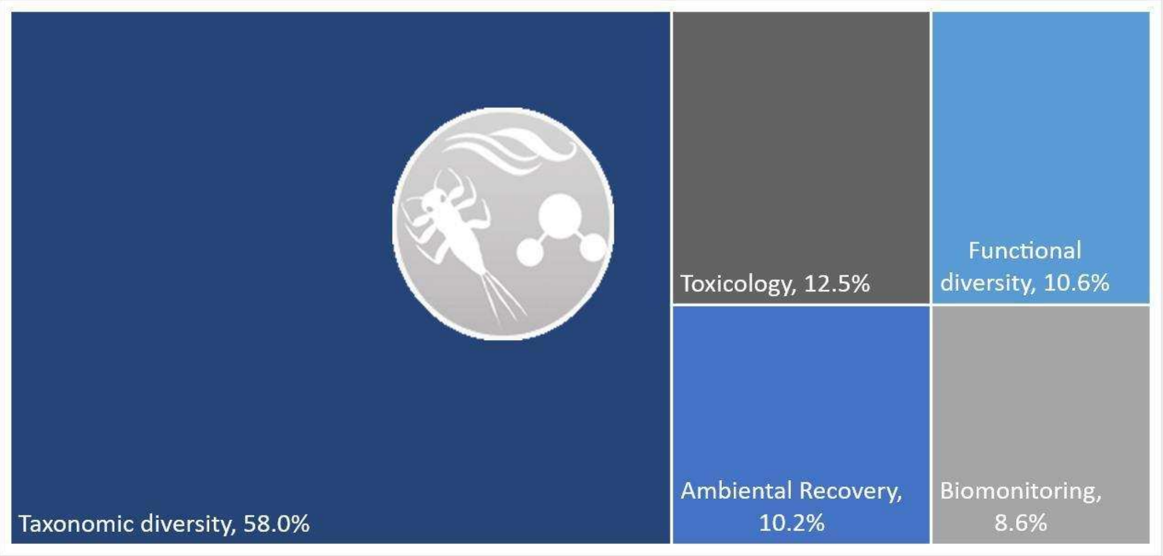

Fig 4. Distribution of records of the effect of mining on aquatic macroinvertebrates by study focus.

A total of 112 studies, or 44% of the database, focused on the general taxonomic group of aquatic macroinvertebrates, including mollusks, crustaceans, annelids, and insects. The other studies focused on insects of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) (98 studies), other insects (36 studies) and other invertebrates (9 studies) (Fig. 5). The studies assessed the structure of these communities using different parameters, the most commonly used being richness (48%), followed by abundance (38%) and diversity (11%).

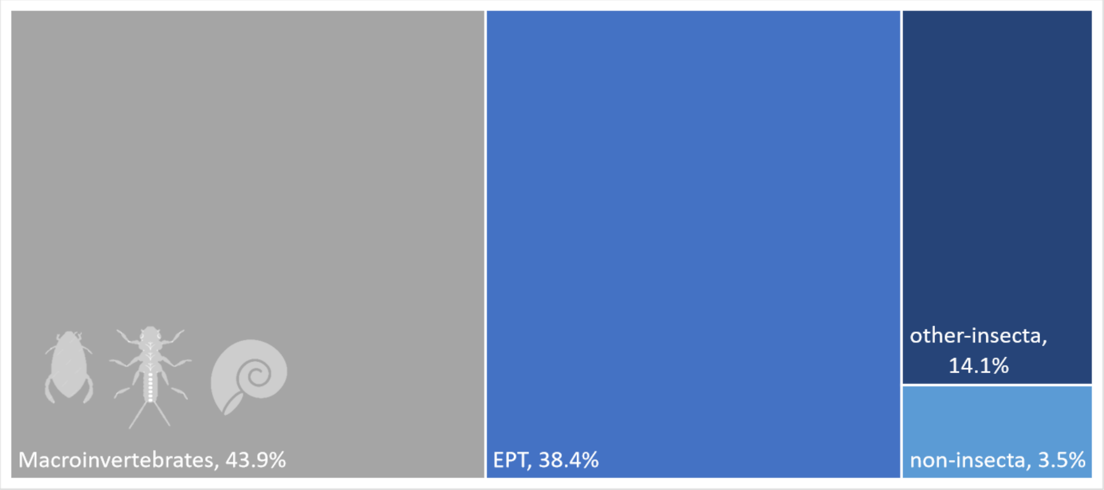

Fig 5. Distribution of records of mining effects on aquatic macroinvertebrates by taxonomic group used.

The selected studies had differentobjectives in assessing the effects of mining on aquatic macroinvertebrate communities, depending on the focus of the study. However, physicochemical parameters were the most widely measured abiotic parameters commonly considered in these assessments (74.9%). Other important abiotic characteristics, such as hydrological parameters related to stream width and flow velocity and landscape parameters related to vegetation type and land use, were measured to a lesser extent (Fig. 6).

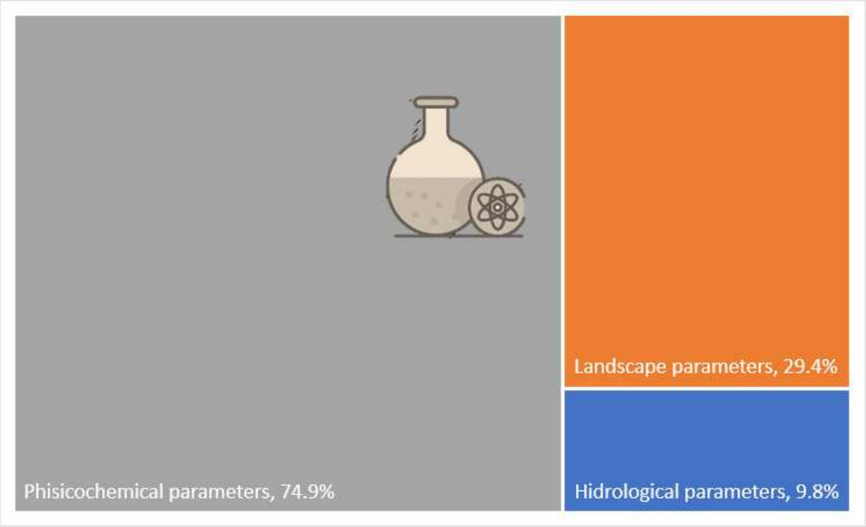

Fig 6. Distribution of parameters related to abiotic mechanisms in studies on the effect of mining on aquatic macroinvertebrates).

3.2. Meta-analysis: The effect of mining on aquatic macroinvertebrate communities

A total of 31 studies evaluating the effects of mining on aquatic macroinvertebrates published between 1994 and 2022 were identified. These met our inclusion criteria, resulting in 255 independent comparisons of the effects of mining on aquatic macroinvertebrate communities that were used to build the multilevel models.

A strong, negative and significant effect of mining on the structure of the aquatic macroinvertebrate community was observed (E++= -1.32, CI= -1.9622 to -0.6807), i.e., there was a significant reduction in the richness, abundance and diversity of aquatic macroinvertebrates in mined areas when compared to reference areas. The multilevel model indicated heterogeneity among the studies in the database ($Q = 713.3938$, $p < 0.0001$). Due to this heterogeneity, models incorporating moderators to investigate variation in effect size were constructed. The response variables used in the study and the climate zone were used as moderators.

Using the metrics as moderators, it can be seen that there is a significant reduction in macroinvertebrates in mined areas regardless of the metric used. The significance of the heterogeneity between the moderators indicates that the effect sizes are different, with the effect on the reduction of richness being the largest (E++= -1.53, CI= -2.16 to -0.89) compared to abundance (E++= -1.06, CI= -1.70 to -0.42) and diversity (E++=-1.31, CI= -2.01 to -0.61).

Heterogeneity was also examined separately within each response variable (richness, abundance and diversity). Considering only the studies reporting richness, there is a strong reduction of macroinvertebrates in mined areas compared to control areas (E++=-1.79, CI=-2.54 to -1.04), indicating heterogeneity in the data. To get around this, the taxonomic group was used as a moderator, which resulted in a difference according to the group studied; only for the non-insect taxonomic group was there no significance (Fig.7).

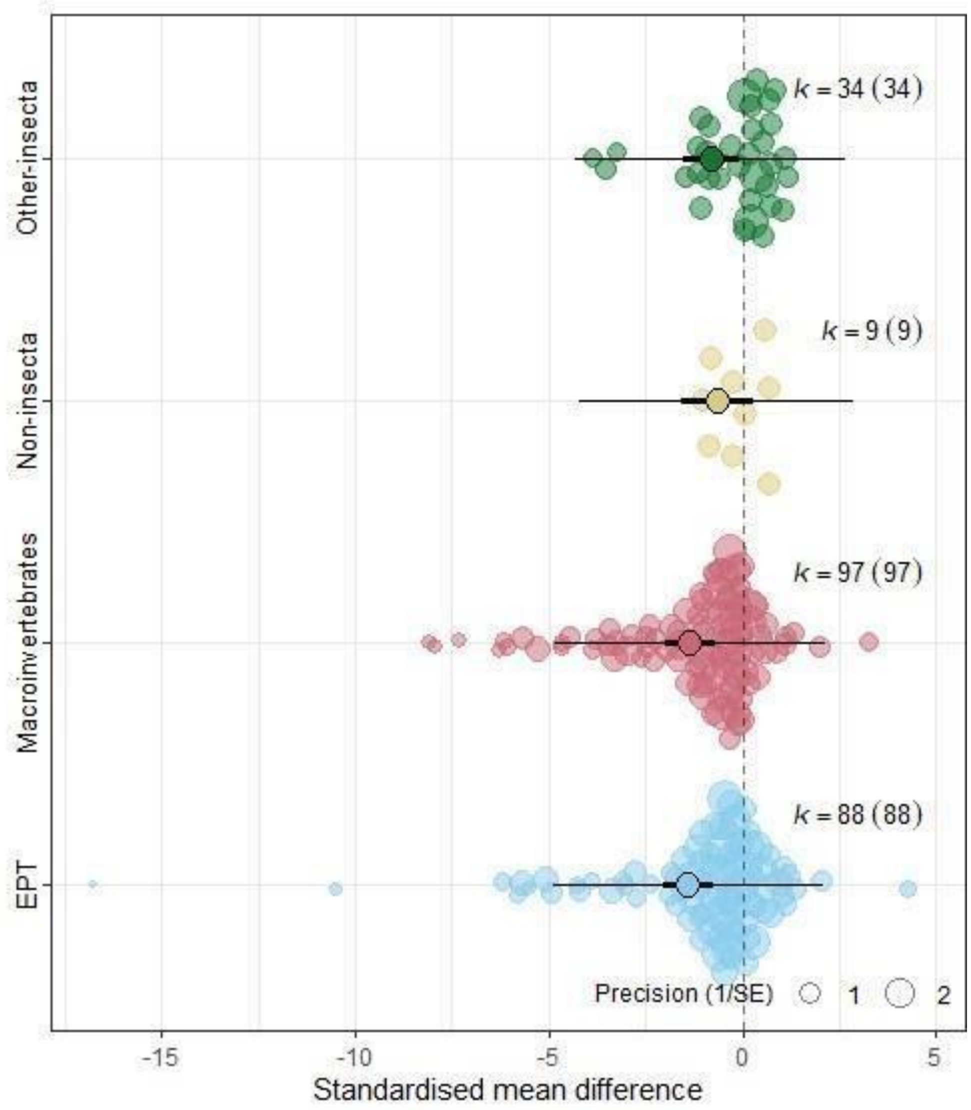

Fig 7. Standardized Mean Difference (SMD) effect sizes for different taxonomic groups of aquatic macroinvertebrates in areas impacted by mining. The global estimates for each taxonomic group are indicated by the black circles, with error bars showing the confidence intervals.

When the climate zone was used as a moderator, there was a difference in the effect according to the zone where the study was conducted, with the reduction being stronger in the subtropical area (E++=-2.22, CI=-3.27 to -1.14).

For abundance alone, mining has no significant effect. For diversity, there is a strong and negative effect (E++=-1.2862, CI=-2.32 to -0.24), especially when considering only the taxonomic group of macroinvertebrates (E++=-1.46, CI=-2.57 to -0.34). There is a significant reduction of macroinvertebrates in mined areas when the response variables Brillouin (E++=-2.31, CI= -4.08 to -0.17), Margalef (E++=-1.22, CI=-2.40 to -0.03) and Shannon (E++=-1.18, CI=-2.18 to -0.26) are considered as moderators. The heterogeneity between moderators is not significant, indicating thatthe effect size values of the three variables are equal.

There is a difference in effect when considering different regions around the world. The reduction in macroinvertebrate community structure parameters was greater in subtropical areas (E++=-1.70, CI= -2.54 to -0.86). The effect size in this case was only significant for the subtropical zone, indicating that the subtropical region is responsible for the pattern found.

The funnel plots showed no asymmetry in the residuals of the overall meta-analysis models (Fig. 8). The confidence levels were high for most comparisons in all models (Table 1). These results indicate the robustness of the effects found for the impacts of mining on the community outcomes assessed.

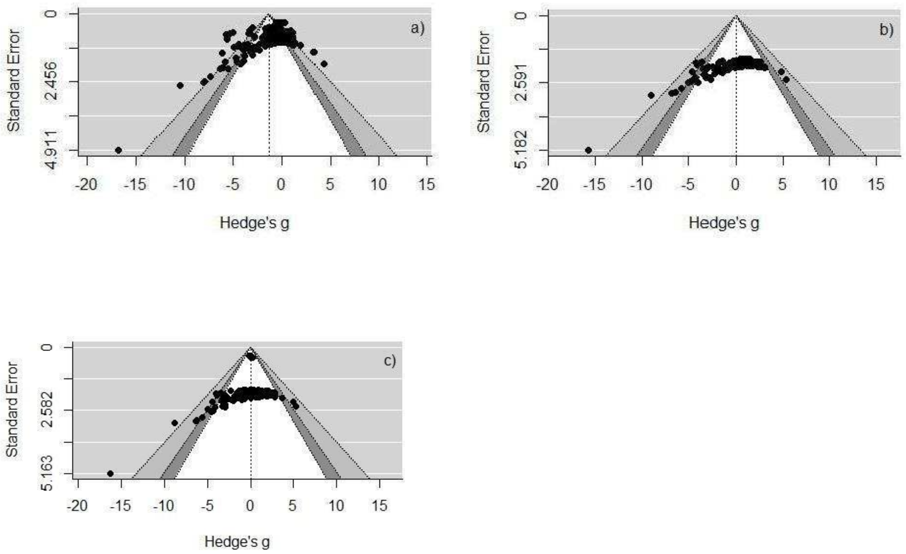

Fig 8. (a) Funnel plots for the model with the overall effect of mining on macroinvertebrates, (b) model including the response variable as a moderator, and (c) model including climate zone as a moderator.

Table 1. Safety numbers for the variables used in the random models (overall model) and multilevel models. NA indicates that the safety number was not calculated (not available) as the effect was not significant.

Variable	Model	Safety number (Rosenthal fail-safe number)	Number of comparisons used in the models
All variables grouped	Overall Random Model	18113	228
Richness	Multilevel	8671	112
Abundance	Multilevel	NA	89
Diversity	Multilevel	350	16

4. Discussion

Through an integrative and systematic review of 60 studies, a wide variety of approaches, study foci and metrics were mapped to investigate the effects of mining on macroinvertebrate biodiversity in lotic environments. The meta-analytic approach used confirmed our initial hypothesis that mining activity has a negative effect on the richness, abundance and diversity of aquatic macroinvertebrates. The meta-analysis also showed that the negative effect on the richness of the lotic macroinvertebrate community was more significant in the more sensitive taxonomic groups. This effect also varied with the latitudinal gradient, stronger in the subtropical zone.

The central knowledge gap revealed by the scientometric evaluation concerns the geographical distribution of studies evaluating the impact of mining on aquatic macroinvertebrates. The majority of studies were conducted in a single country (United States, 40% of the studies), with few studies in countries of the Southern Hemisphere, which, in addition to being major mineral producers (ANM, 2023), have an important share of the world's freshwater reservoirs (Pinto, 2017) and are highly biodiverse (Tydecks et al., 2018). The concentration of studies in a few countries is also reflected in their distribution across regions of the globe, with most studies conducted in the subtropical region. Thus, there is a biogeographical bias in studies of mining and aquatic macroinvertebrates.

Another guideline for future research is to extend the scale of assessment of abiotic factors to a landscape scale. Larger scales than those that only assess characteristics within the water body - the most common scale used in the reviewed studies (74.9%) - allow inferences aboutother environmental changes that mining areas are subject to and that may affect aquatic communities. In addition, understanding the mechanisms that influence community structure is essential for decision -making regarding the management of these degraded ecosystems.

The few studies focusing on biomonitoring and environmental recovery may be related to the keywords used in the search. Although the term "mining" was used, there were no terms associated explicitly with remediation of the effects of mining, as this was not the focus of this study. Therefore, this small number of studies does not necessarily mean a gap in studies on this topic.

The taxonomic groups "non-insecta" and "other insecta" were the least used in studies related to mined areas. This low proportion can be explained by the modest sensitivity of these groups to environmental stressors, as seen in the meta-analytic approach, where the effect size was higher for EPT and macroinvertebrates. This result could provide directions for future studies on the efficiency of using each of these taxonomic groups in environmental monitoring. In other words, perhaps using a taxonomic group with a smaller number of orders, such as EPT, is more efficient in terms of results than using a larger group that includes other insects.

Among the diversity structure parameters used, richness had the strongest effect in the meta-analytic approach. For abundance, there was no effect of mining. Still, this parameter may not be as appropriate since degraded areas may increase the number of generalist species due to a greater diversity of species that would occupy sites with more significant environmental heterogeneity.

5. Final remarks

The assessment of the impact of mining on macroinvertebrate communities in this study confirms the negative effect of mining activity on the richness and diversity of aquatic macroinvertebrates. This effect was more pronounced forthe Ephemeroptera, Plecoptera and Trichoptera groups. Guidelines for future research include the need to include more research in geographical regions that have not yet been explored, as well as the evaluation of abiotic factors related to the effects of mining at larger spatial scales.

An important insight brought by this study was the increased precision of using EPT in ecological studies, considering aquatic macroinvertebrates and mining areas. This information can help reduce sampling and identification time costs in studies with biomonitoring objectives, for example.

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CHAPTER 02*

Assessing the Ephemeroptera, Plecoptera and Trichoptera (EPT) community's response to the Rio Doce Mining Tailings Dam Disaster across three spatial scales

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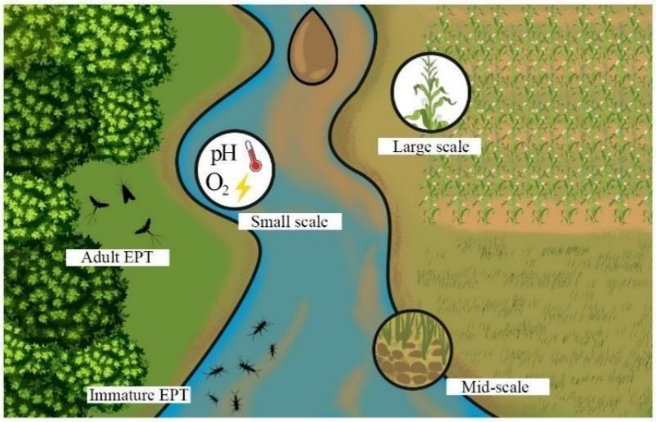
Highlights

- The collapse of the tailings dam caused changes in local abiotic conditions, an essential vector for limiting the biodiversity of biological communities.
- EPTs are organisms that spend an essential part of their life cycle in water, so their immature and adult stages can be affected by mining pollution.
- Stressors to aquatic insect communities can act at different spatial scales, from within the water to the local landscape.
- Mining tailings amplify the negative effects of stressors that act in places where tailings have not passed through.

Graphical Abstract



Abstract

The 2015 Mariana mining disaster in Minas Gerais, Brazil, profoundly altered the abiotic conditions of the Rio Doce basin, severely impacting aquatic fauna. Ephemeroptera, Plecoptera, and Trichoptera (EPT) insects, known for their sensitivity to environmental disturbances, are key indicators in biodiversity assessments. We selected forty sampling sites along the Rio Doce basin, twenty of which were in areas where mining tailings had passed through and twenty in areas that had not been affected. At these sites, we collected adult and immature EPT, as well as physical and chemical data on the water and characteristics of the River environment. With this work, we sought to answer two main questions: What is the response of the EPT community to the collapse of the mining tailings dam on the Rio Doce, What is the interaction between the effects of abiotic mechanisms, spatial scale and life stage on the response of the EPT community to the collapse of the mining tailings dam on the Rio Doce?, and How does climatic variation influence EPT community dynamics in the Rio Doce Basin? Seven years after the disaster, the EPT community responds negatively to the collapse of the mining tailings dam on the Rio Doce. The decrease in species richness, the dominance of generalist taxa, and the change in species composition in the areas where the tailings have passed confirm this response. It can also be seen that the presence of the tailings changed the environmental characteristics making the pre-disaster stressors even more deleterious to the EPT community.

Keywords: Aquatic insects, Ecological communities, Mine pollution, Water quality

*This chapter is formatted according to the instructions of the journal “Science of The Total Environment”.

1. Introduction

Mining has profound environmental consequences, particularly for aquatic ecosystems (Kotalik et al., 2019). On November 5, 2015, the Fundão dam collapse in Mariana released over 43.8 million cubic meters of mining tailings (Marta-Almeida et al., 2016), contaminating hundreds of kilometers of the Rio Doce basin before reaching the Atlantic Ocean (Lopes, 2016). This disaster, one of Brazil’s most severe environmental catastrophes, caused extensive socio-environmental damage throughout the basin (Guerra et al., 2017).

Heavy metal and chemical contamination from tailings (Cordeiro et al., 2019) has significantly altered the watershed's abiotic conditions. The impacts include shifts in soil and water chemistry, increased sedimentation and turbidity, geomorphological changes, bank erosion, and riparian vegetation loss (Santos et al., 2019). Nearly a decade later, these effects persist, underscoring the ongoing challenges of ecosystem restoration (Pereira et al., 2024).

Macroinvertebrates play a key role in the dynamics of continental aquatic ecosystems, as they are a link between basal resources and other organisms (Nogueira et al., 2011). As well as being an important food resource, they act in the physical and chemical alteration of sediments, known as bioturbation (Chakraborty et al., 2022). They also provide other important ecosystem services, whether cultural, supportive or regulatory, such as removing toxic substances from the water when they emerge into the terrestrial environment (Jacobus et al., 2019).

Aquatic macroinvertebrates respond sensitively to environmental changes, allowing for causal analysis, change prediction, and ecological assessments (Dale & Beyeler, 2001). Among them, Ephemeroptera, Plecoptera, and Trichoptera (EPT) are particularly sensitive (Wallace et al., 1996) and widely used in environmental monitoring (Albutra, 2017). Their abundance, habitat diversity, varied feeding strategies, low pollution tolerance, and ease of sampling in both immature and adult stages make them valuable for biodiversity studies (Sabha, 2022; Hamada et al., 2014; Qazi et al., 2012).

Most ecological studies and protocols for monitoring aquatic environments with EPT focus on life stages that occur directly in side the water (Lowe et al., 2013; Buss et al., 2015; Keci et al., 2022; Rimcheska & Vidinova, 2020). This approach focuses on collecting immatures of different orders of aquatic insects, as well as adults of Hemiptera and Coleoptera, and is habitat- and season-dependent, often making collection of these insects a real challenge.

Although immature organisms are widely used in ecological assessments, their identification is usually limited to the genus and family level (Jones, 2008) due to a lack of knowledge about the immature stage, the Haeckelian deficit (Faria Jr. et al. 2021). Studies also show that there is some correspondence between the responses of adult and immature individuals (Mendoza-Penagos et al., 2021). EPT adults may provide more refined responses to a wide range of environmental changes, but both stages are good models for understanding the effects of anthropogenic actions in aquatic environments (Gomes et al., 2022).

Ecosystem structure changes are influenced by three vectors that limit local biodiversity: biotic, abiotic, and dispersal mechanisms (Mittelbach & McGill, 2019). The abiotic mechanism is the first to change in situations of environmental impact. In aquatic environments, it affects species adaptation, including water temperature, oxygen, pH, and bank stability for the immature stage. For adults, factors such as light intensity, vegetation structure, and land use also have an influence (Martins et al., 2017)

In studies of anthropogenic impacts on biological communities, it is important to consider the different habitats in which species establish themselves throughout their life cycle, as well as abiotic mechanisms at multiple scales. In this study, smaller scales include the physicochemical characteristics of the water, while larger scales include the River environment and landscape features related to hydrography and land u se and settlement.

Water quality parameters such as temperature, dissolved oxygen, and alkalinity influence the distribution of aquatic insects (Prommi & Payakka, 2015). While tolerance to pH variation varies among species, low pH levels can disrupt physiological processes, including gill function (Bell, 1971). Temperature directly affects macroinvertebrate distribution and interacts with factors like dissolved oxygen, whose higher availability improves aquatic organism survival (Webb et al., 2008; Nebecker, 1972). Electrical conductivity, shaped by temperature, pH, and organic matter decomposition, indicates ion concentration and is often linked to declining water quality and altered conditions for aquatic insects (EPA, 2012).

Assessing habitat quality provides a more comprehensive view in addition to assessing water quality parameters for the aquatic insect community (Galdean et al., 2000). Qualitative characteristics of watershed reaches, such as vegetation cover, substrate type, and bank erosion, can help contextualize habitats (Callisto et al., 2002). Habitat assessment protocols are an important tool for environmental monitoring and provide qualitative and quantitative answers about the habitat diversity of a site.

Changes in land use, particularly the conversion of native vegetation into agricultural or urban areas, lead to significant biodiversity loss (Castro et al., 2016). In river ecosystems, these transformations affectwater quality by destabilizing banks, increasing sediment deposition, and homogenizing headwaters. Additionally, the removal of riparian vegetation raises light exposure and water temperature, altering habitat conditions for aquatic species (Martins et al., 2017). Recognizing these impacts is essential for designing effective conservation and management strategies to sustain ecosystem health.

According to Sabo and Hagen (2012), the distribution of aquatic insects is strongly related to topography, sub-basin width, channel sinuosity, and drainage density. In addition, other factors, such as annual climatic variation, can shape the geometry of the stream network (Sangireddy et al., 2016) and explain regional differences in the ecological structure of aquatic communities.

With this work, we sought to answer the following questions: i) What is the response of EPT communities to the failure of the mining tailings dam on the Rio Doce? ii) What is the interaction between the effects of abiotic mechanisms, spatial scale and life stage on the response of EPT communities to the failure of the mining tailings dam on the Rio Doce? iii) How does climatic variation influence EPTcommunity dynamics in the Rio Doce Basin?

We expect greater diversity, richness and abundance of EPT in areas unaffected by tailings. We also expect abiotic mechanisms to vary between spatial scales according to life stage, such that immatures would respond to stressors internal to the aquatic ecosystem (smaller scales), while adults would be influenced by factors at larger scales. In addition, we expect the composition of the EPT community to vary annually, with greater abundance and diversity in the dry season.

This is the first study of EPT ecology in the Rio Doce basin following the 2015 disaster. Understanding the processes that limit the diversity of aquatic macroinvertebrates, especially EPT, in terms of species composition, abundance, richness, and distribution at different spatial scales in the face of a major environmental disturbance could be the first step toward proposing specific mitigation measures for the Rio Doce.

2. Material and Methods

2.1. Site Description

Covering 86,000 km² across Minas Gerais and Espírito Santo, the Rio Doce Basin is one of the largest in southeastern Brazil. It lies within the Atlantic Forest, a biome that has lost over 77% of its original area (Vancine et al., 2024). The basin also includes the Iron Quadrangle, a region with some of the world's largest iron ore reserves (Wanderlay et al., 2016).

Along the Rio Doce basin, 40 sampling points were selected, including the main channel of the river and its main tributaries (Table 1). In the dry season, we collected from the 40 points, 20 of which were in areas where mining tailings had passed through and 20 in unaffected areas (Fig 1). In the rainy season, we collected data from 16 sampling points, 9 in reference areas and 7 in areas affected by tailings. Sampling took place between January, August and September 2022.

At three of the 40 sites, no adults were sampled during the dry season, two light traps stopped working (Point 14 and Point 29) and one was stolen (Point 26). During the dry season we did not sample point 24, also because the light trap stopped working.All these points were not included in the statistical analysis

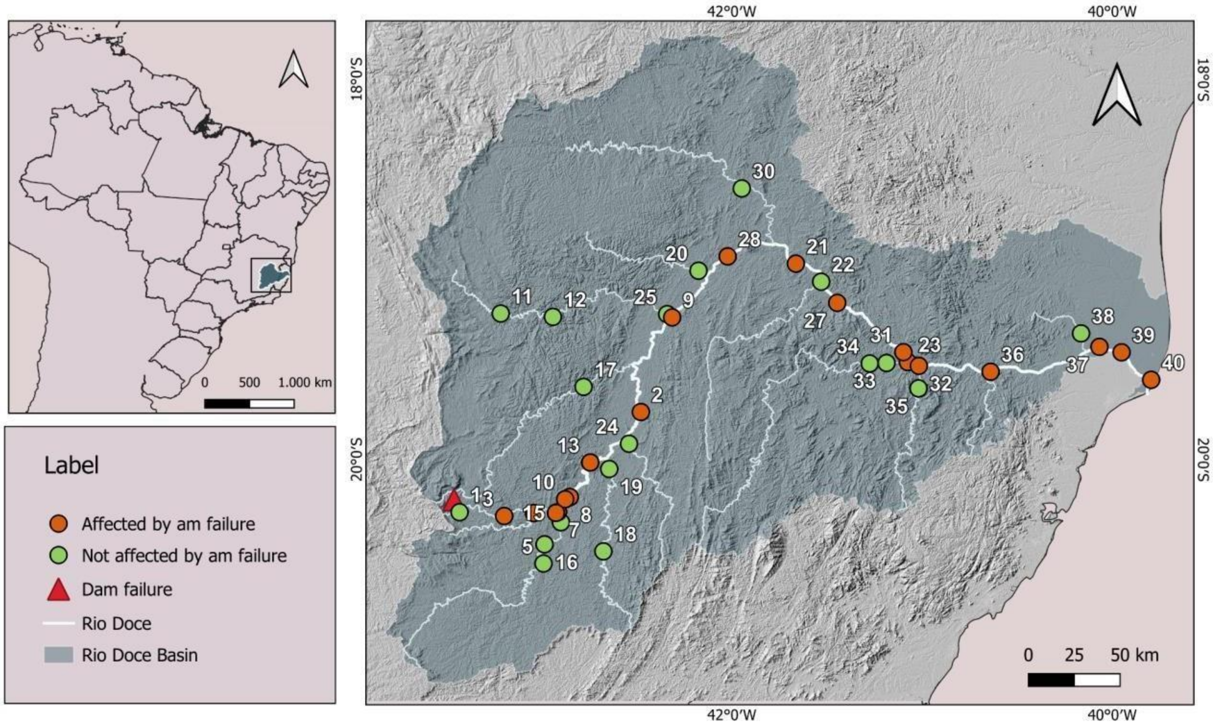


Fig. 1. Map of the Rio Doce Basin with 40 sampling points. Points through which tailings have passed are marked in orange, and reference points are marked in white.

Table 1. Geographical coordinates of the 40 selected sampling points in the Rio Doce basin and their classification as affected by mining tailings. All reference points not affected by tailings are located on tributaries of the Rio Doce.

Point	Coordinates (UTM)			River	Locality	Tailing	Season of collection
	east	west					
1	663787	7757153	23k	Gualaxo do Norte	Mariana, MG	no	rainy, dry
2	764251	7814314	23k	Doce	Parque Estadual do Rio Doce, MG	yes	rainy, dry
3	688284	7754717	23k	Gualaxo do Norte	Paracatu de baixo, , MG	yes	dry
4	704609	7756231	23k	Carmo	Barra Longa, MG	yes	dry
5	709518	7726784	23k	Piranga	Guaraciaba, MG	no	rainy, dry
6	719392	7750689	23k	Piranga	Rio Doce, MG	no	dry
7	717886	7756690	23k	Doce	Rio Doce, MG	yes	dry
8	724451	7765328	23k	Doce	Rio Doce, MG	yes	dry
9	782354	7869156	23k	Doce	Naque, MG	yes	rainy, dry
10	721890	7764082	23k	Doce	Rio Doce, UHE Risoleta neves, MG	yes	rainy, dry
11	687610	7872625	23k	Santo Antônio	Santo Antônio do Rior abaixo, MG	no	rainy, dry
12	716429	7870370	23k	Santo Antônio	Ferros, MG	no	dry
13	735965	7785306	23k	Doce	São José do Goiabal, MG	yes	rainy, dry
14	738866	7879318	23k	Santo Antônio	Santo Antônio da Fortaleza, MG	no	dry

15	716721	7756192	23k	Carmo	Rio Doce, MG	yes	dry
16	710222	7737948	23k	Piranga	UHE Brito, MG	no	dry
17	732869	7829391	23k	Piracicaba	Jaguaraçu, MG	no	rainy, dry
18	742458	7733441	23k	Casca	Jequiri, MG	no	dry
19	746357	7781360	23k	Casca	São José do Goiabal, MG	no	dry
20	797357	7896137	23k	Corrente	Periquito, MG	no	dry
21	219512	7900508	24k	Doce	Tumiritinga, MG	yes	rainy, dry
22	233687	7890184	24k	Caratinga	Barra do Cuieté, MG	no	dry
23	282205	7844032	24k	Doce	Aimorés, MG	yes	rainy, dry
24	757449	7795917	23k	Matipó	Raul Soares	no	dry
25	779627	7871089	23k	River Santo Antônio	Naque, MG	no	dry
26	263294	7861088	24k	Doce	Resplendor, MG	yes	dry
27	242767	7877983	24k	Doce	Conselheiro Pena, MG	yes	dry
28	813646	7904202	23k	Doce	Governador Valadares, MG	yes	rainy, dry
29	202153	7937309	24k	Suaçuí Grande	Matias Lobato, MG	no	dry
30	188844	7943845	24k	Suaçuí Grande	Matias Lobato, MG	no	dry
31	279689	7849766	24k	Doce	Aimorés, MG	yes	dry
32	288311	7841895	24k	Doce	Baixo Guandu, ES	yes	rainy, dry
33	270455	7843334	24k	Manhuaçu	Aimorés, MG	no	dry
34	261172	7842938	24k	Manhuaçu	Aimorés, MG	no	rainy, dry
35	288291	7828762	24k	Guandu	Baixo Guandu, ES	no	dry
36	327949	7838898	24k	Doce	Colatina, ES	yes	rainy, dry
37	387897	7853854	24k	Doce	Linhares, ES	yes	dry
38	377681	7861617	24k	Doce	Linhares, Lagoa Juparanã Mirim, ES	no	dry
39	400131	7850752	24k	Doce	Linhares e Povoação, ES	yes	dry
40	416523	7834814	24k	Doce	Povoação, Foz do River Doce, ES	yes	dry

2.2. Field Sampling

2.2.1. Physical-chemical parameters

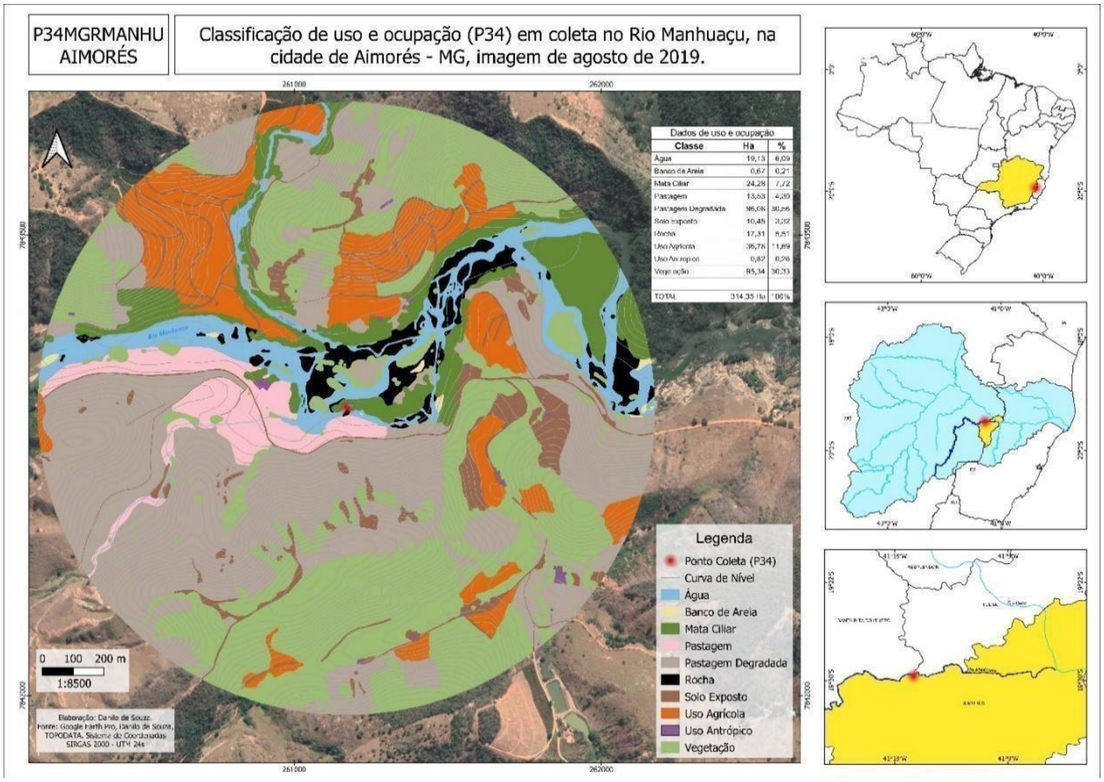
To assess changes in water quality, we used a multi-parameter meter (ASKO AK88) to measure the following physicochemical water parameters: hydrogen potential (pH), dissolved oxygen (%), electrical conductivity (S/cm), and water temperature (°C). The water sample for measuring the parameters was collected in a 0.5 l plastic container, in which the parameters were measured only once. Measurements were made in the field at the same time as the immature individuals were collected (Table 4).

2.2.2. River Characterization

The Rapid Habitat Diversity Assessment Protocol developed by Callisto et al. (2002) is an adaptation of the Ohio Environmental Protection Agency (EPA, 1987) protocol that classifies habitat quality for aquatic macroinvertebrates. The protocol assesses various structural and functional aspects of the stream environment, considering factors such as the integrity of riparian vegetation, the presence of suitable substrates for colonization, the complexity of water flow, and the degree of anthropogenic disturbance (Fig. 16, Supplementary material). In this study, for each sampling point a score was assigned based on the condition of the habitat, with values ordered upward as environmental integrity increases. The sum of these scores classifies the score ranges into three categories: natural, altered, and impacted. This method allows for a standardized and comparable assessment across areas. In building the models, we used the protocol score as a continuous variable to test the hypotheses.

2.2.3. Land use and occupation

To characterize the changes in the landscape associated with the dynamics of land use, the database was used to determine the proportion of each type of land use around the 40 points sampled, with a bufferof 1 km from the center of the sampling point (Neves, 2022), which corresponds to the average measure of dispersal capacity of EPTs (Arce et al., 2021). The types of land used are riparian forest, vegetation, pasture, rock, anthropogenic use, urban area, and agricultural use (Fig. 2).



Dados de uso e ocupação

Classe	Ha	%
Água	19,13	6,09
Banco de Areia	0,67	0,21
Mata Ciliar	24,28	7,72
Pastagem	13,53	4,30
Pastagem Degradada	96,06	30,56
Solo Exposto	10,45	3,32
Rocha	17,31	5,51
Uso Agrícola	36,78	11,69
Uso Antrópico	0,82	0,26
Vegetação	95,34	30,33
TOTAL	314,35 Ha	100%

Fig. 2. Example of the graphic classification of land use and occupation of sampling point number 34 carried out by Neves (2022) in the River Doce basin.

2.2.4. Hydrography

Hydrographic parameters refer to the spatial configuration of the drainage network, including features such as drainage density, stream order, and the presence of dams. Upstream and downstream distances and drainage density were calculated in Qgis (QGIS Development Team, 2024) using geoprocessing tools to measure the length of watercourses within the watershed and their corresponding areas (Table5, Supplementary material). These variables describe the structure of the hydrographic network and can influence the quality and availability of habitat for aquatic organisms.

2.2.5. Aquatic insects sampling

The target groups of aquatic invertebrates were collected using two methods: i) sampling of the substrates available in the water body, with the aim of collecting immatures using a D-type netwith a mesh size of 0.5 mm, and ii) the use of Pennsylvania-type light traps (Frost, 1957) to collect adults, with one trap per sampling point set before dusk and collected the following morning. For the collection of immatures, at each sampling point, a 100 m stretch was defined, divided into five sections parallel to the river and separated by 20 m (adapted from de Lima et. al, 2022), with the aim of collecting from the differenttypes of substrate available in the water body. The samples were sorted, and the organisms collected were fixed in a solution of 80% ethyl alcohol.

2.3. Laboratory procedure

All the material sampled was checked using a stereoscopic microscope, and the individuals of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT), which were used in the analysis, were identified to the taxonomic level of genus, with the exception of the adults of the Hydroptilidae family (Trichoptera), identified until family level, due to the large numberof individuals. Identification manuals (Olifiers et al., 2004; Salles, 2006; Dominguez et al., 2006; Dominguez et al., 2009; Hamada et al., 2014, Hamada et al., 2018), slides and photographs of the specimens collected were used to identify the taxa. The individuals from the two methodologies are housed in the Entomology Museum of the Federal University of Viçosa (UFV).

2.4. Data analysis

To ensure comparable species richness between impacted and non-impacted areas, rarefaction curves were generated using EPT individuals. This method standardizes sampling effort by subsampling without replacement, allowing fair comparisons. The analysis was conducted with the "iNEXT" package in R (Chao et al., 2014).

A nonparametric multivariate analysis (PERMANOVA) was used to analyze species composition between seasons and between areas affected and unaffected by tailings, testing the null hypothesis that the dispersion of observations is equivalent in all groups formed. The dissimilarity metric used was the Bray-Curtis distance, taking into account the presence/absence of species. Stratification was performed by sampling point to minimize the effects of pseudoreplication.

We applied multivariate dispersion homogeneity analysis (PERMDISP) to test whether the possible differences observed in community composition between the dry and wet seasons and between areas affected or not by tailings could be explained by variations in data dispersion. The results were plotted using non-metric multidimensional scaling (NMDS) (Clarke, 1993) with three dimensions (k = 3). The fit of the model was assessed using the stress value to ensure that the representation was adequate. Next, the Indicator Species Analysis (IndVal) was performed to verify the occurrence of indicator species for sites with and without tailings and seasons.

We standardized the continuous variables of physicochemical parameters and land use using the scale function in R to ensure comparability between different magnitudes. Due to a large number of zeros, which makes it difficult to see the graphical results, we categorized the land use variables as "low", "medium", and "high" based on the 25% and 75% quartiles. To assess multicollinearity at different scales, we generated a correlation matrix using the corrgram package. Graphical analysis of the pairs highlighted correlation patterns between variables, with emphasis on the influence of the "Reject" variable.

To test the determinant vector hypotheses, we fitted generalized linear mixed models (GLMMs) with a distribution appropriate to the type of response variable, correcting for overdispersion when necessary. The normality and heterodasticity of the data were also assessed. The significance of the terms in the adjusted model was assessed by deleting them, based on the p-values, until we reached the minimum appropriate model for each response variable. When significant interactions between variables and tailings were detected (e.g., interaction between pH and presence of tailings), we performed additional tests to examine the effects within each of the levels of the categorical variable tailings (yes and no).

The models included fixed and random effects to provide greater flexibility in the choice of error distributions and model structure. For example, the variable "sampling point" was included as a random effect to control for pseudoreplication since we used data from both seasons. We used a correlation matrix to detect possible multicollinear relationships amongthe explanatory variables of each hypothesis. Our dependent variables were species richness, abundance and diversity. To assess diversity, we used Hill's numbers, which allow a unified interpretation by incorporating different emphases on species abundance. Calculations were performed for species richness, Shannon-Hill diversity and Simpson-Hill diversity. All statistical analyses were performed using R software (R Development Core Team, 2025).

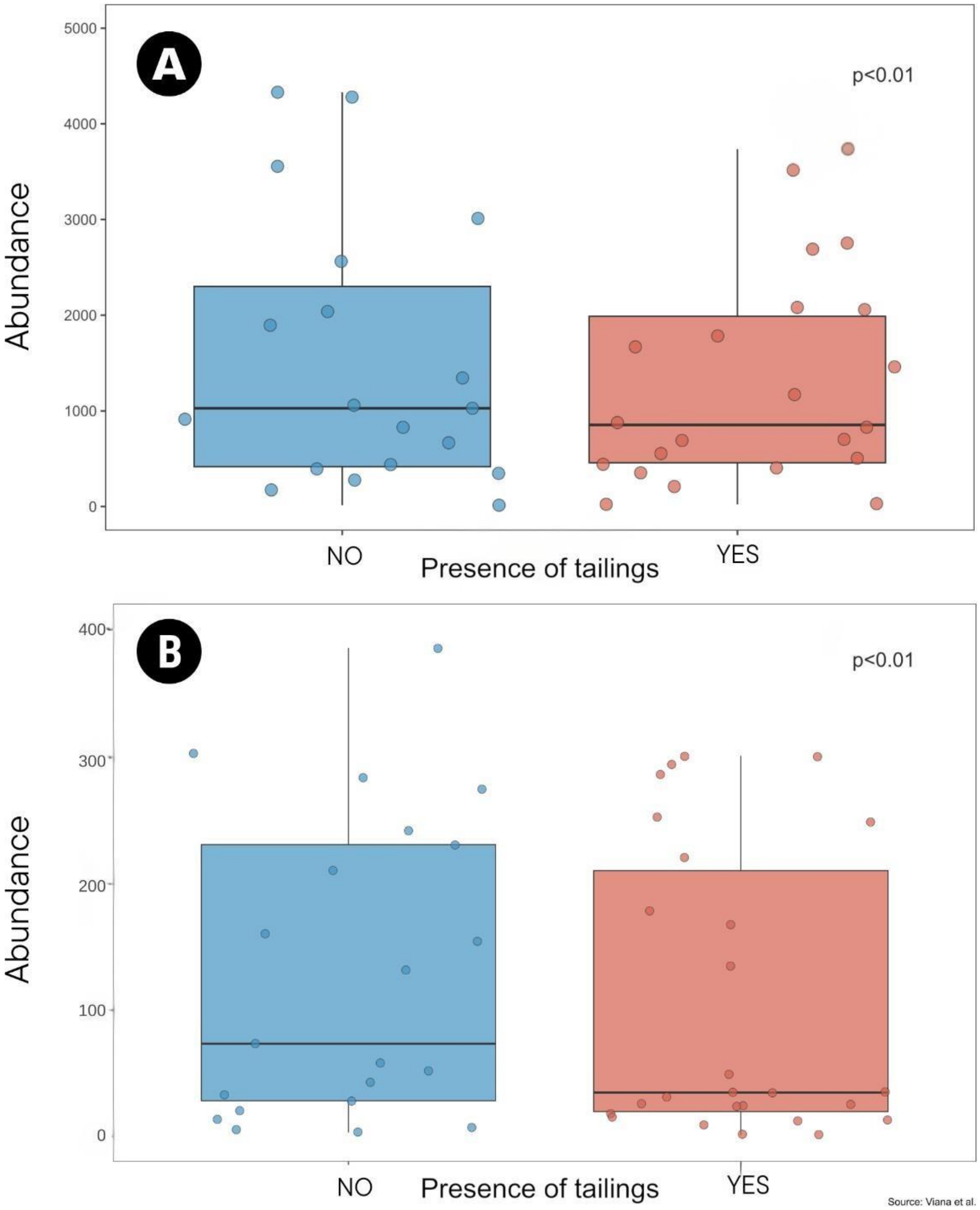
3. Results

3.1. EPT community structure

We collected a total of 10,600 immature individuals, distributed in 19 families and 46 genera. Among the immatures, the family Baetidae (Ephemeroptera) had the highest abundance and richness of genera, with *Cloeodes* Traver, 1938 (23.7%), *Americabaetis* Kluge, 1992 (20.8%) and *Paracloeodes* Day, 1955 (17.7%) being the most abundant genera. Most of the collected immature individuals belonged to the order Ephemeroptera (9146 individuals), followed by Trichoptera (1384 individuals) and Plecoptera (70 individuals). The set of reference areas where the tailings did not pass represented 69.4% of the total number of immature individuals, with a richness of 41 genera. In areas where the tailings had passed through, richness was 29 genera (Table 2, Supplementary material).

We collected 457.625 adult individuals belonging to 18 families and 66 genera. Among the adult individuals, Trichoptera was the most abundant, with 89.6% of the total sample, followed by Ephemeroptera (10.29%) and Plecoptera (0.1%). The family Hydroptilidae (Trichoptera) was the most abundant, with 65.8% of the total abundance, while Baetidae (Ephemeroptera) showed the greatest richness with 13 genera sampled, among which Paracloedes was the most abundant. The abundance of adults was higher in the reference areas where no tailings had passed (32.7%) (Table 3, Supplementary material).

In order to assess the community structure, we analyzed the parameters of abundance and richness and Hill's diversity with the tailings as an explanatory variable. We obtained a significant result in the model for the abundance of immature EPT (Chi=350.93, p<0.01) and adult EPT (Chi=22156, p<0.01) as a function of the presence of tailings. We detected higher abundance values in immatures (Fig. 3A) and adult (Fig. 3B) of EPT communities in areas where there was no tailings passage.



Source: Viana et al.

Fig. 3 . (A) Effect of the presence of mining tailings on the richness of immature EPT and (B) adults sampled at 40 points along sites affected and not affected by the passage of mining tailings. In both graphs, the red boxes represent the areas where the tailings passed through and the blue areas where they didn't. The lines inside the box represent the median, the central value of the data.

Both the immature and the adult rarefaction curves indicate that the number of species is higher in areas with tailings at the same abundance. However, in both plots, the curves overlap, indicating that there are no significant differences in species diversity between areas.

The composition of immatures (Chi=0.1741, p=0.956) and adults (Chi=0.8615, p=0.555) did not differ between areas with and without tailings. For immatures, the composition of immature EPT genera varied by season (Chi=10.399, p=0.001). The low stress value (0.1199) indicates that the NMDS model fits the data well (Fig. 5). When calculating the value indicator index (IndVal) considering the two seasons, we found that the genera *Farrodes* Peters, 1971 (IndVal=0.5286, p<0.001) and *Traverella* Edmunds, 1948 (IndVal=0.1825, p=0.02) have a moderate association with the rainy season (Fig. 6).

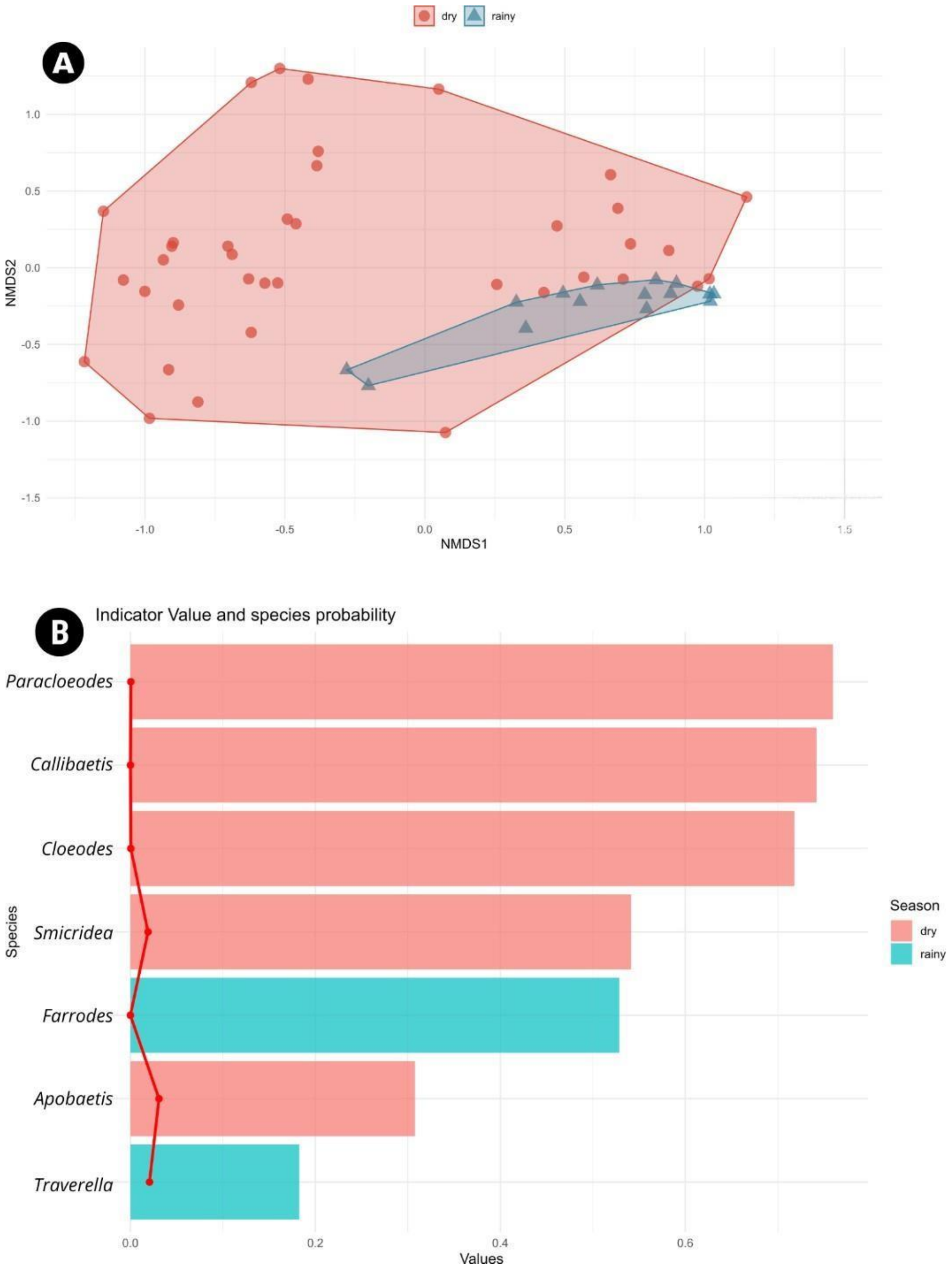


Fig. 4. (A) NMDS ordination for the composition of immature EPT between the dry and wet seasons in the Rio Doce Basin, the graphic shows the variation among rainy season (blue) and dry season (red); (B) Indicator values of genera in relation to the dry and wet seasons. The bars show the indicator values (IndVal), and the red line represents the probability of association, highlighting the genera of immature EPT indicators for each season.

For adults, the composition of immature EPT genera also varies according to the season (Chi=11.919, p=0.001). The NMDS model also fits the data well (Stress= 0.1199) (Fig 5). The Indicator of Value Index (IndVal), considering the two seasons for the adult community, revealed that the species *Americabaetis labiosus* Lugo-Ortiz & McCafferty, 1996 (IndVal = 0.3549, p=0.00816) and *Campsurus truncatus* Ulmer, 1920 (IndVal= 0.4673, p= 0.00037) have an affinity with the rainy season (Fig. 6)

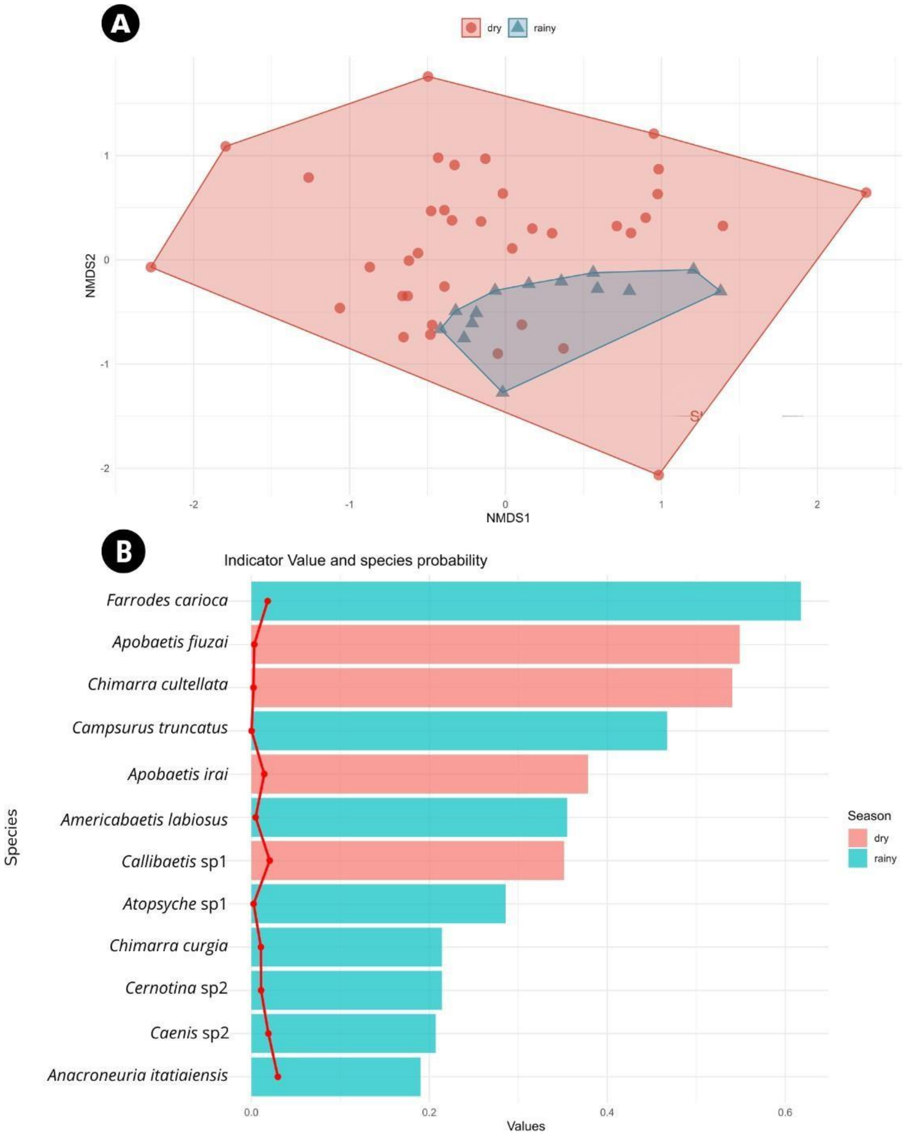


Fig. 5. (A) NMDS ordination for the composition of adult EPT between the dry and wet seasons in the Rio Doce Basin. The graphic shows the variation among rainy season (blue) and dry season (red); (B) indicator values of species in relation to the dry and wet seasons. The bars show the indicator values (IndVal), and the red line represents the

probability of association, highlighting the species of adult EPT indicators for each season.

3.2. Drivers of EPT diversity

3.2.1. Small scale

The measured physicochemical water variables covered a wide range of pH (7.05-8.52), dissolved oxygen (49.5-82.2%), temperature (20.3-28.6°) and electrical conductivity (9.25-97.4) (Table 4, Supplementary Material).

To test the hypotheses raised, the first abiotic mechanism evaluated was the smallest scale related to the physico-chemical characteristics of the water, i.e., within the river. We detected an effect of two of the abiotic stressors interacting with the tailings on the abundance of immatures: pH (Chi=6.022, $p=0.0141$) and electrical conductivity (Chi=16.014, $p<0.001$). This effect varies according to climatic seasonality (Fig. 6).

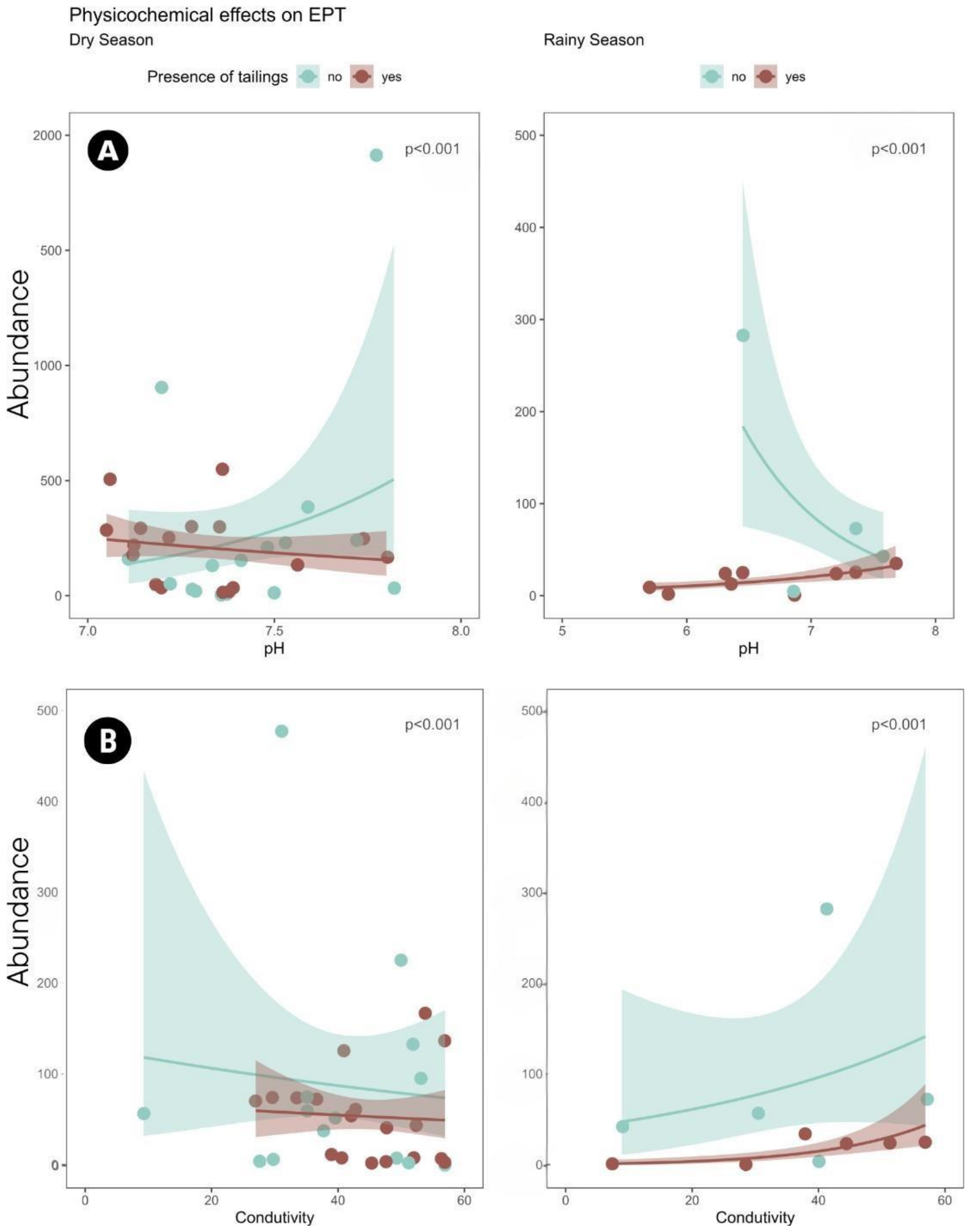


Fig. 6. Effect of the interaction among pH anf tailings(A) and electrical conductivity and tailings (B) on the abundance of immature EPT sampled at 40 points along sites affected and unaffected by the passage of mine tailings during the dry (left) and rainy (right) seasons. In all four plots, the line represents the fit of a generalized linear mixed

model (GLMM), incorporating the "Collecting point" as random effect, with a negative binomial distribution, estimating the general trend of abundance as a function of the interaction among tailings and pH (A); electrical conductivity (B). Around the line, the shaded band corresponds to the 80% confidence interval.

For Hill's Diversity, in immature EPT, we detected an effect of the interaction between tailings passage and the following abiotic stressors: pH, with an effect on Richness-Hill (Chi=6.9947, p=0.007) (Fig. 7, A) and Simpson Hill (Chi=6.3949, p<0.01) (Fig. 7, B); and conductivity with an effect on the Shannon Hill index (Chi=9.8363, p=0.001)(Fig. 7, C).

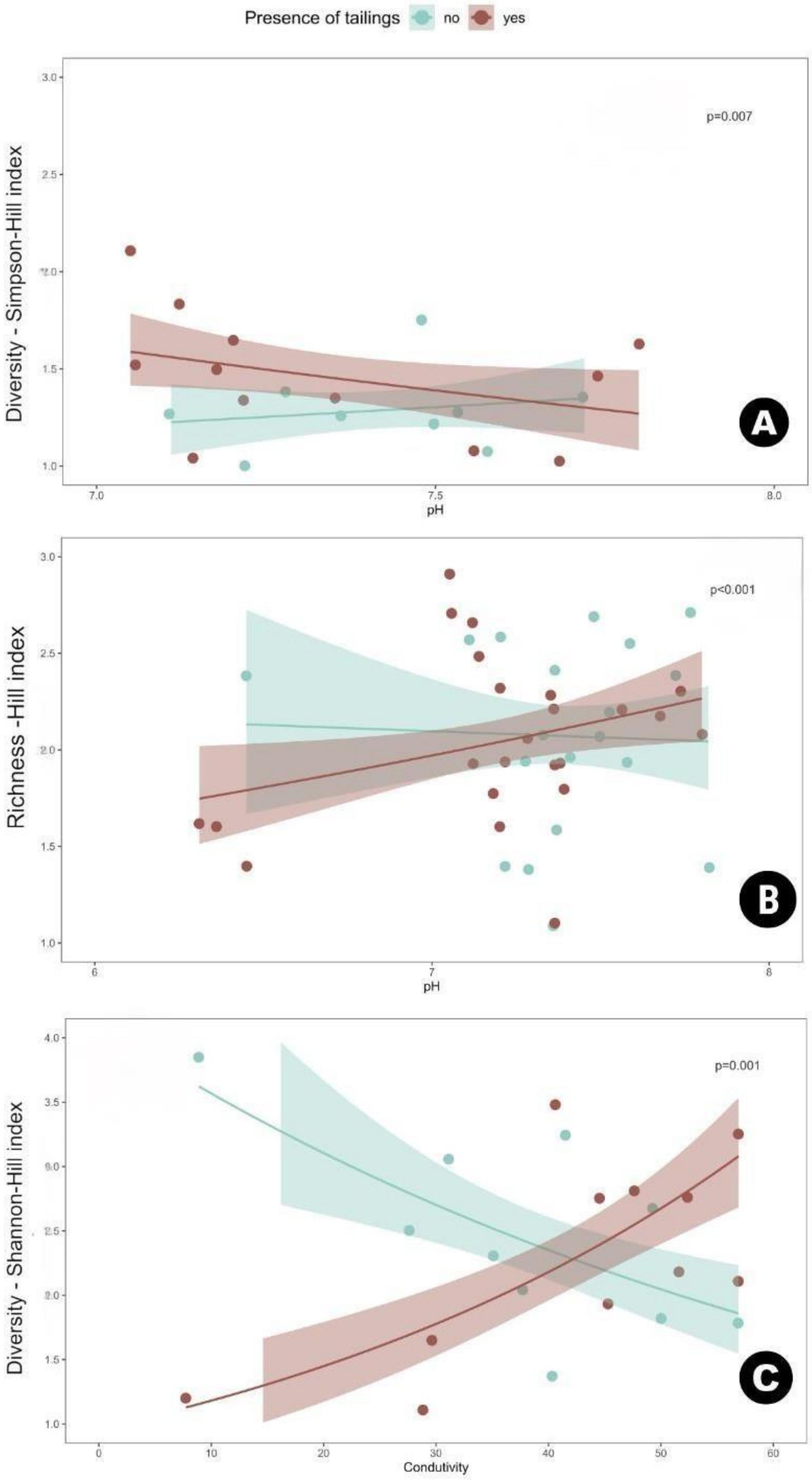

Presence of tailings
no
yes
p=0.007
Diversity - Simpson-Hill index
3.0
2.5
2.0
1.5
1.0
7.0
7.5
8.0
pH
A
p<0.001
Richness -Hill index
3.0
2.5
2.0
1.5
1.0
6
7
8
pH
B
p=0.001
Diversity - Shannon-Hill index
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
10
20
30
40
50
60
Condutivity
C

Fig. 7. (A) Effect of the interetaion among pH and tailings on the diversity of immature EPT measured by the Simpson -Hill index; (B) Effect of the interetaion among pH and tailings on the diversity of immature EPT measured by the Richness-Hill index; and (C) Effect of interetaion among eletrical condutivity and tailings on the diversity of immature EPT measured by the Shannon-Hill index. In all three plots, the line represents the fit of a generalized linear mixed model (GLMM), incorporating the "Collecting point" as random effect, with a negative binomial distribution, estimating the general trend of diversity as a function of pH (A and B) and electrical conductivity (B). Around the line, the shaded band corresponds to the 80% confidence interval.

When evaluating the adult community, there is an effect of the interaction between tailings and temperature (Chi=16.014, p<0.001) (Fig. 8A). There is also a significative effect of pH (Chi=4.2291, p=0.0397) (Fig. 8B) on the richness of adult EPT measured by the Hill index .

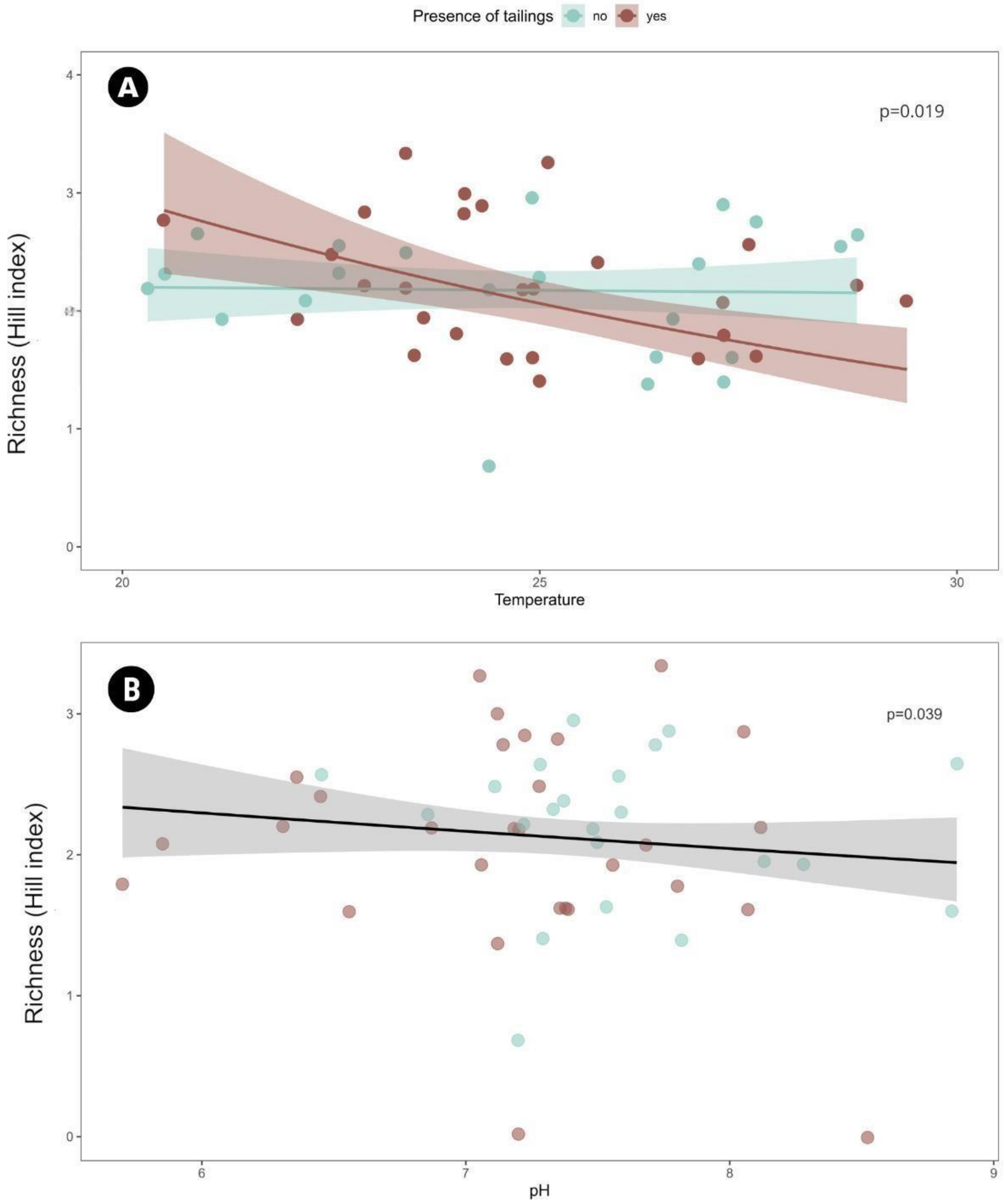


Fig. 8. (A) Effect of the interetaion among temperature and tailings on the diversity of adult EPT measured by the Richness-Hill index; (B) Effectof the interetaion among pH and tailings on the diversity of immature EPT measured by the Richness-Hill index; and (C) Effect of pH on the diversity of immature EPT measured by the Richness-Hill index. In both plots, the line represents the fit of a generalized linear mixed model (GLMM), incorporating the "Collecting point" as random effect, with a negative

3.2.2. Mid-Scale

We detected environmental stressors at an intermediate spatial scale, as assessed by "habitat quality" parameters for immature abundance. The result of the Rapid Habitat Diversity Assessment Protocol applied to the 40 sampling points showed a predominance of degraded environments according to the score. Of these, the majority were sites where no tailings had passed, indicating the state of environmental degradation throughout the Rio Doce Basin, including the reference areas where no tailings had passed. We observed an effect of habitat quality on the abundance of immature EPT, with variation for each of the seasons. In both seasons, we observed an increase in abundance in the reference areas as the score increased, with higher scores indicating better habitatquality (Fig. 9).

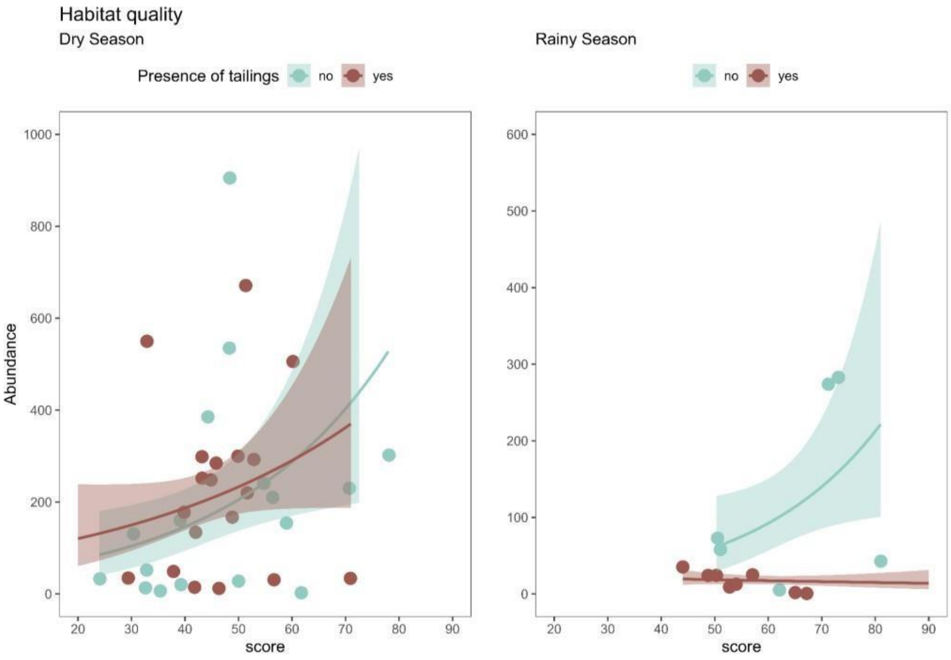
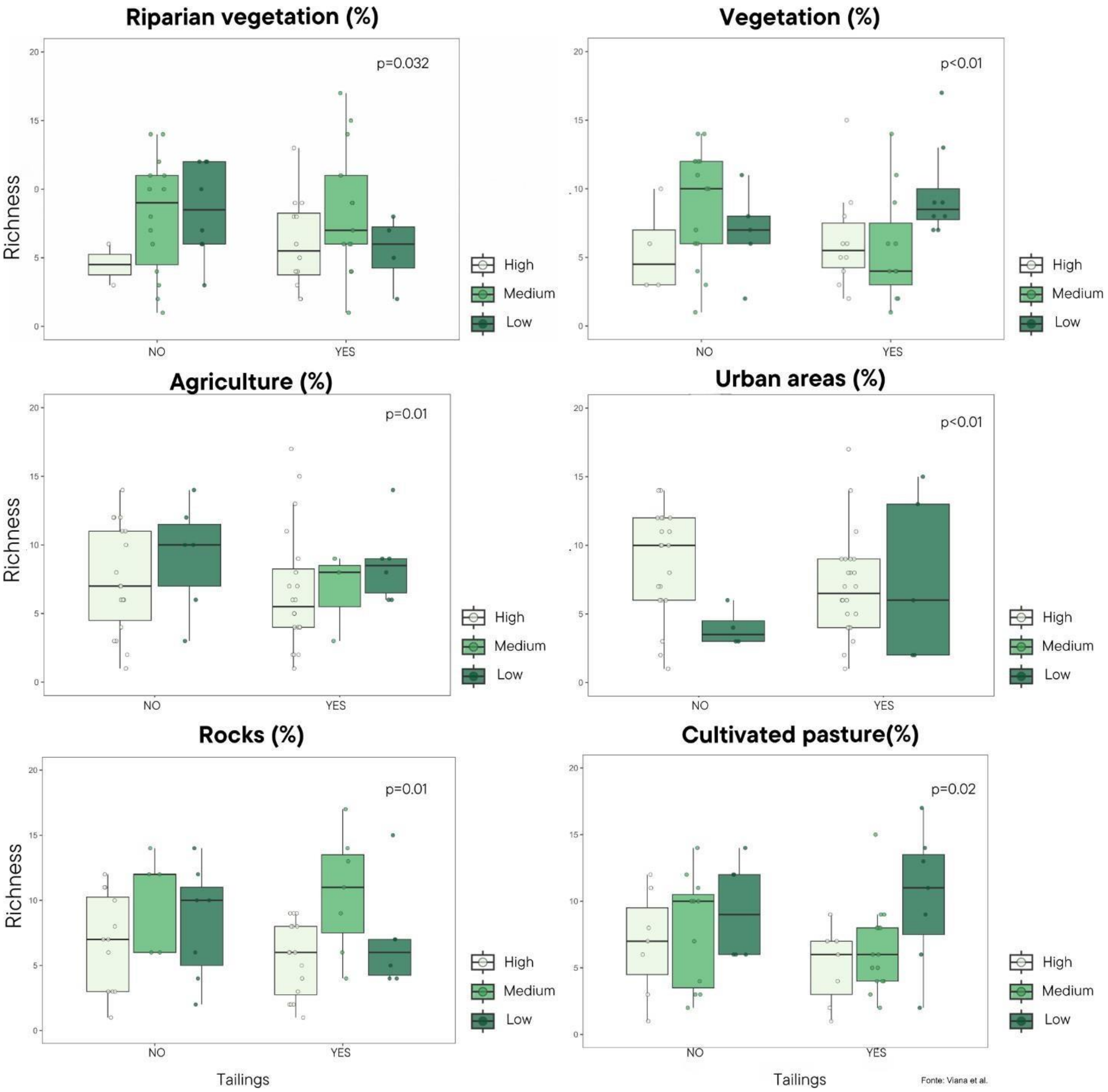


Fig.9. (A) Effectof the interetaion among pH and tailings on the diversity of immature EPT measured by the Simpson -Hill index; (B) Effect of the interetaion among pH and tailings on the diversity of immature EPT measured by the Richness-Hill index; and (C) Effect of interetaion among eletrical condutivity and tailings on the diversity of immature EPT measured by the Shannon-Hill index. In all three plots, the line represents the fit of a generalized linearmixed model (GLMM), incorporating the "Collecting point" as random effect, with a negative binomial distribution, estimating the general trend of diversity as a function of pH (A and B) and electrical conductivity (B). Around the line, the shaded band corresponds to the 80% confidence interval.

We found no significant effect of habitat quality on the richness, abundance or diversity of adult EPT in the Rio Doce Basin.

3.2.3. Large Scale

The different factors related to the larger scale had an effect on all the parameters evaluated for immature EPT (Fig 10). For richness, we observed the effect of the percentage of urban area, riparian forest, vegetation, rock, agricultural use and pasture. When evaluating the graphical representations (Fig. 10), we observed that areas where the tailings did not pass through and have a low percentage of urban area show greater richness. Unaffected areas show an increase in richness proportional to the incre ase in the percentage of riparian forest; this pattern is reversed in areas with tailings, where richness decreases even in areas with a high percentage of riparian forest.



Fonte: Viana et al.

Fig. 10. Effect of the interaction between tailings and different factors related to the larger scale on the richness of immature EPT genera. The boxplots show how the presence of mining tailings and differentlevels of land use (% cover) jointly influence EPT richness. Box colors represent land use levels (light to dark green for high to low). The central line in each box indicates the median, with individual points showing observed values. All the significant interactions shown of the figure were detected via a GLMM with negative binomial distribution and sampling point as a random effect, indicates that the impact of tailings on richness depends on surrounding land degradation.

For the abundance of immature EPT, there was an effect of the interaction between tailings and the percentages of riparian forest, vegetation, rock, and urban area. In areas where no tailings have passed through, abundance is higher for low percentages of urbanized areas; on the other hand, areas with tailings have higherabundance values for high percentages of urbanized area (Fig. 11).

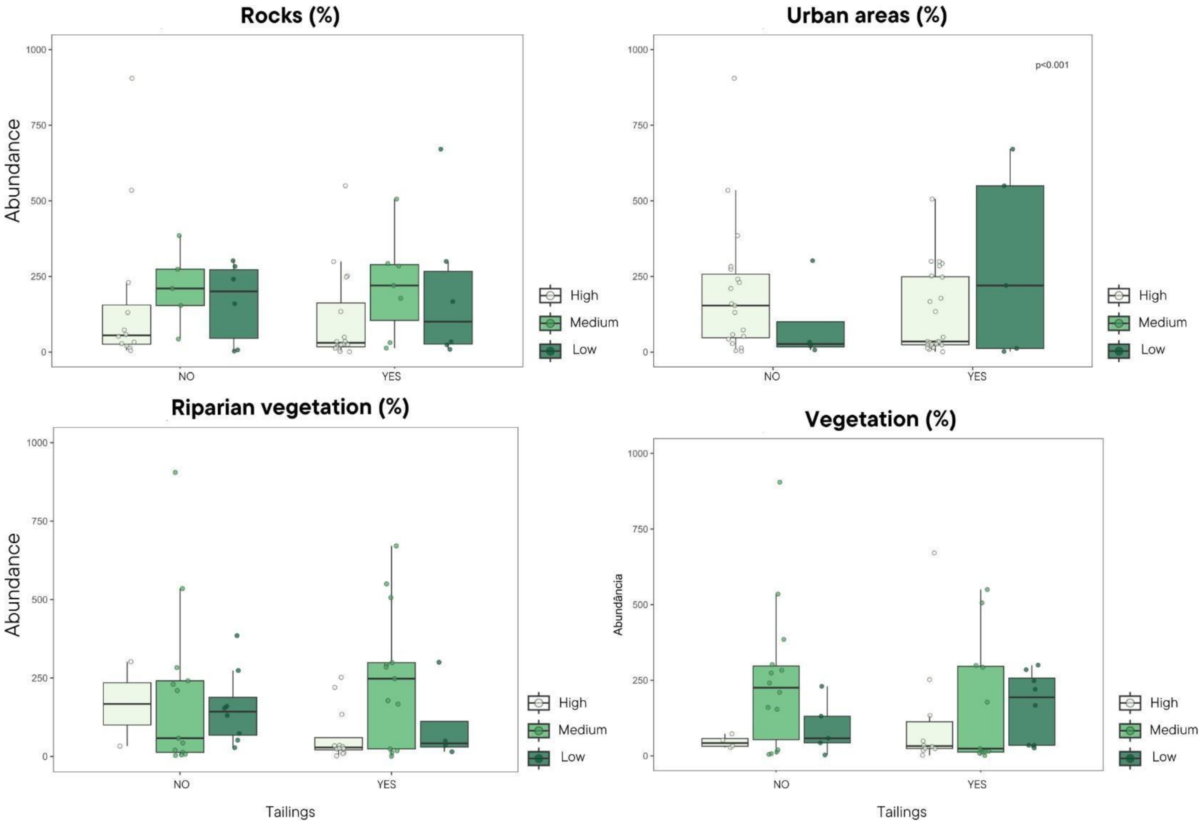


Fig. 11. Effect of the interaction between tailings and different factors related to the larger scale on the abundance of immature EPT genera. The boxplots show how the presence of mining tailings and differentlevels of land use (% cover) jointly influence EPT abundance. Box colors represent land use levels (light to dark green for high to low). The central line in each box indicates the median, with individual points showing observed values. All the significant interactions shown of the figure were detected via a GLMM with negative binomial distribution and sampling point as a random effect.

In relation to Hill's diversity numbers, for immature EPT richness, we obtained an effect of the percentage of vegetation, riparian vegetation, rock and urban area. The response of richness by Hill's index for immatures is similar to that previously evaluated for riparian forest, with greater richness in areas with a higher percentage of riparian forest, as well as for the percentage of urban area which, in areas without tailings when at a low percentage, shows greater richness. For the Shannon-Hill index, which provides a robust and interpretable measure combining species richness and evenness, the average proportions in reference areas reflect a higherindex; in affected areas, this value was only higherin areas with a high percentage of vegetation. Simpson-Hill also suffered the effect of the vegetation variable, with a pattern similar to that found for Shannon-Hill (Fig. 12).

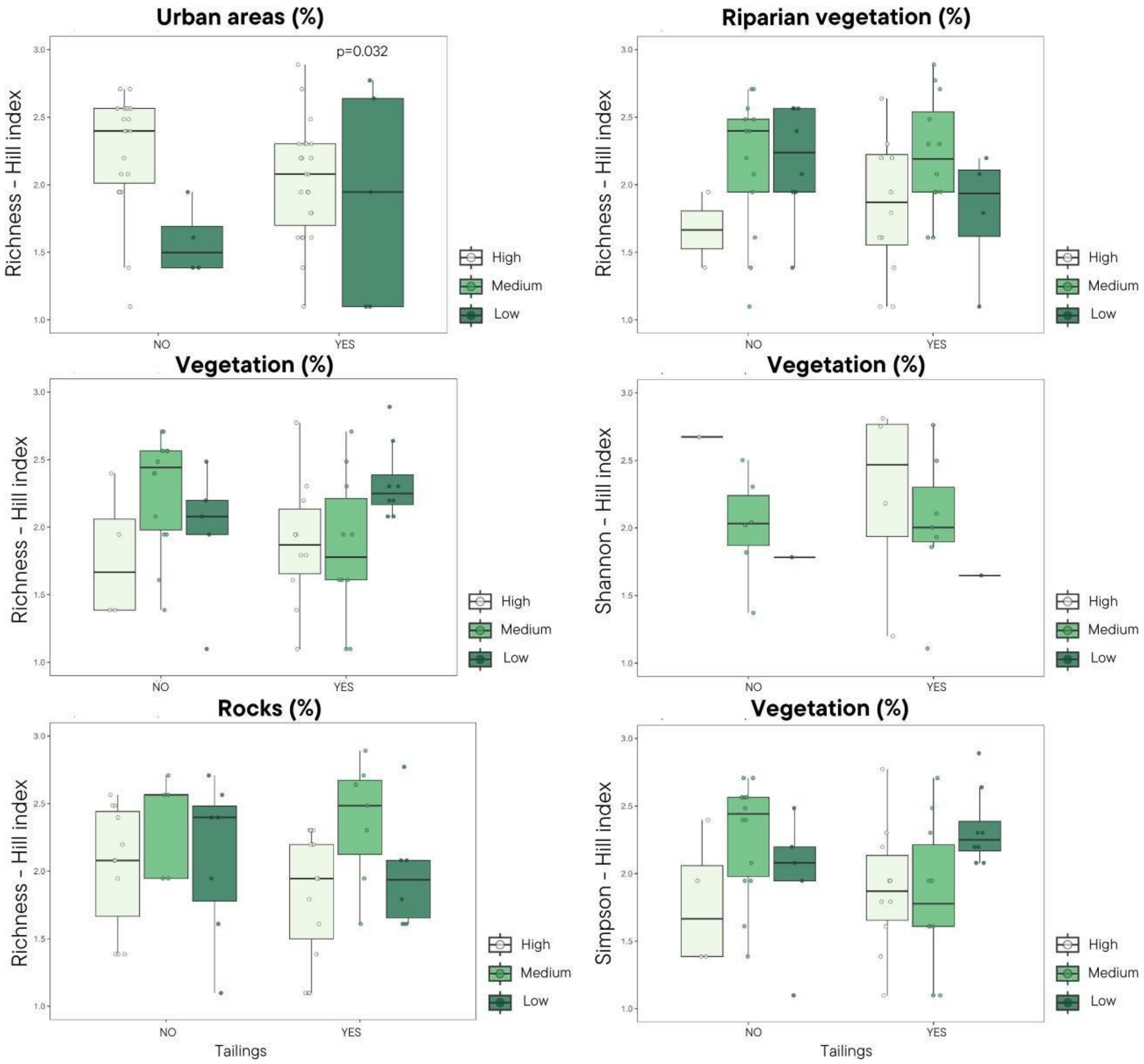


Fig. 12. Effect of the interaction between tailings and differentfactors related to the larger scale on the diversity of immature EPT genera, measured by the Hill numbers. The boxplots show how the presence of mining tailings and different levels of land use (%

cover) jointly influence EPT diversity. Box colors represent land use levels (light to dark green for high to low). The central line in each box indicates the median, with individual points showing observed values. All the significant interactions shown of the figure were detected via a GLMM with negative binomial distribution and sampling point as a random effect.

Adult EPT abundance was higherin areas with high riparian forest cover, especially where there was no tailings passage. However, this effect was attenuated in impacted sites, suggesting thatdegradation limits the benefits of vegetation. Abundance varied with land use, being higher in areas with greater pasture cover and agricultural use but with high variability between sampled sites (Fig. 13).

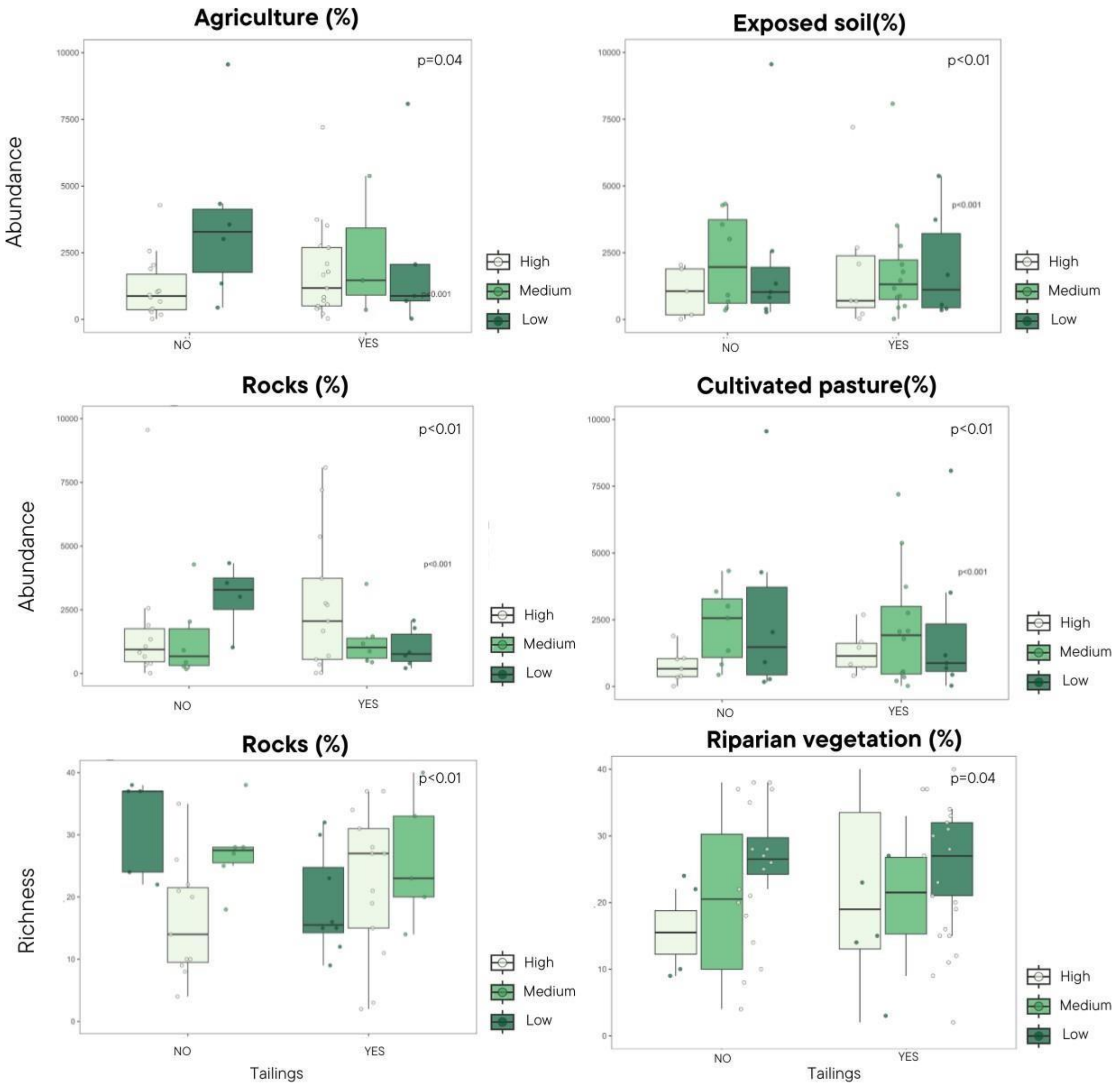


Fig. 13. Effect of the interaction between tailings and different factors related to the larger scale on the abundance and richness of adult EPT species. The boxplots show how the presence of mining tailings and different levels of land use (% cover) jointly influence EPTabundance and richness. Box colors represent land use levels (light to dark green for high to low). The central line in each box indicates the median, with individual points showing observed values. All the significant interactions shown of the figure were detected via a GLMM with negative binomial distribution and sampling point as a random effect.

The Hill Diversity Indices confirm these patterns, indicating greater diversity in areas with preserved vegetation, while tailings impacts reduced community equitability. The Hill

2 index, which corresponds to Simpson's dominance index, was higher in riparian and vegetated areas but decreased in its tailings-affected areas, reinforcing the negative influence of disturbance on the structure of the adult EPT community (Fig. 14).

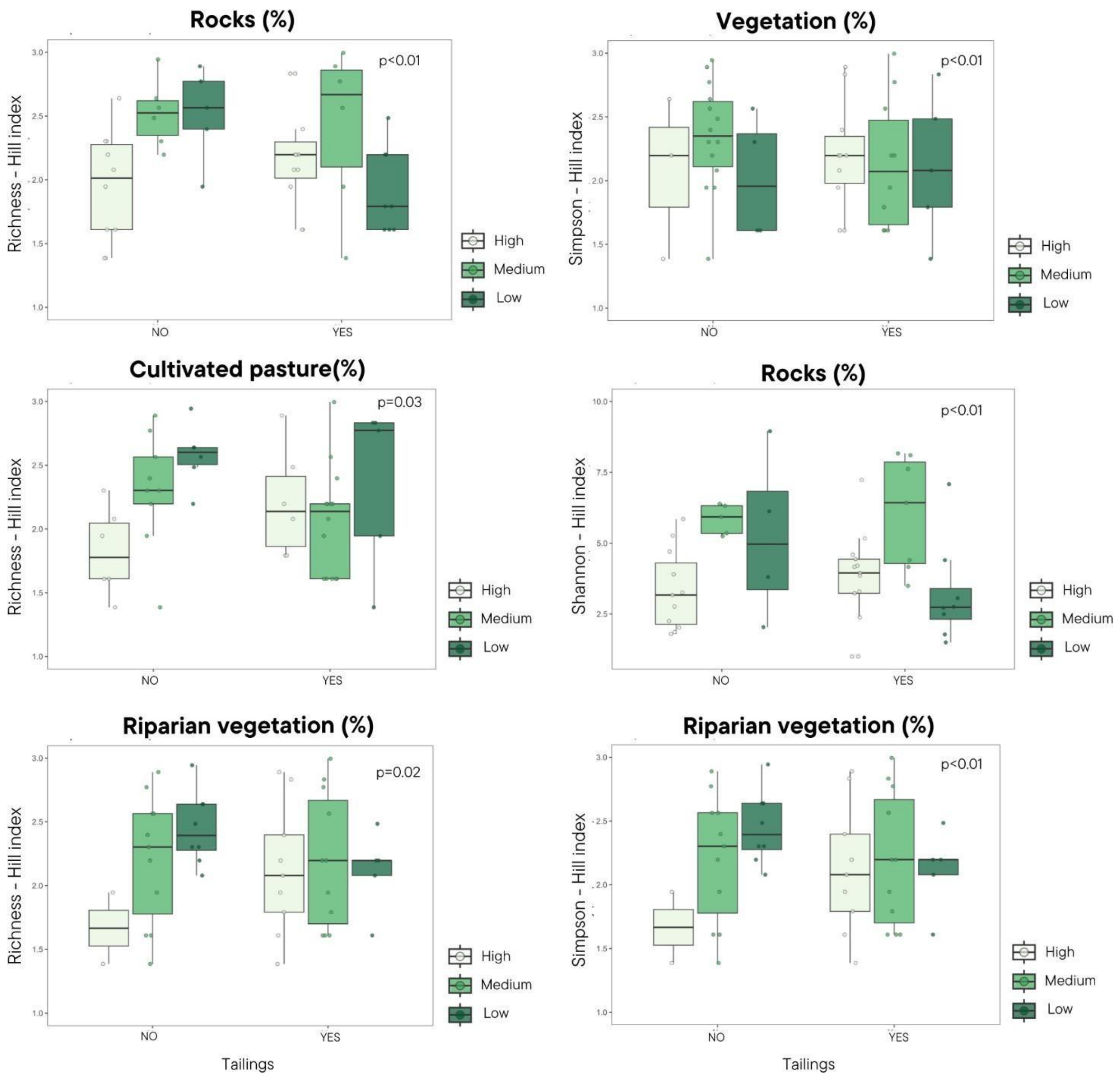


Fig. 14. Effect of the interaction between tailings and different factors related to the larger scale on the divertity of adult EPT species, measured by the Hill numbers. The boxplots show how the presence of mining tailings and different levels of land use (% cover) jointly influence EPT diversity. Box colors represent land use levels (light to dark green for high to low). The central line in each box indicates the median, with individual points showing observed valu es. All the significant interactions shown of the figure were detected via a GLMM with negative binomial distribution and sampling point as a random effect.

EPT richness showed a negative relationship with drainage density, indicating that sites with higherdrainage density tended to have less diverse communities (Fig. 15). The abundance of adult insects was strongly influenced by the hydrographs studied, suggesting that this factor should be considered when modulating the structure of aquatic communities.

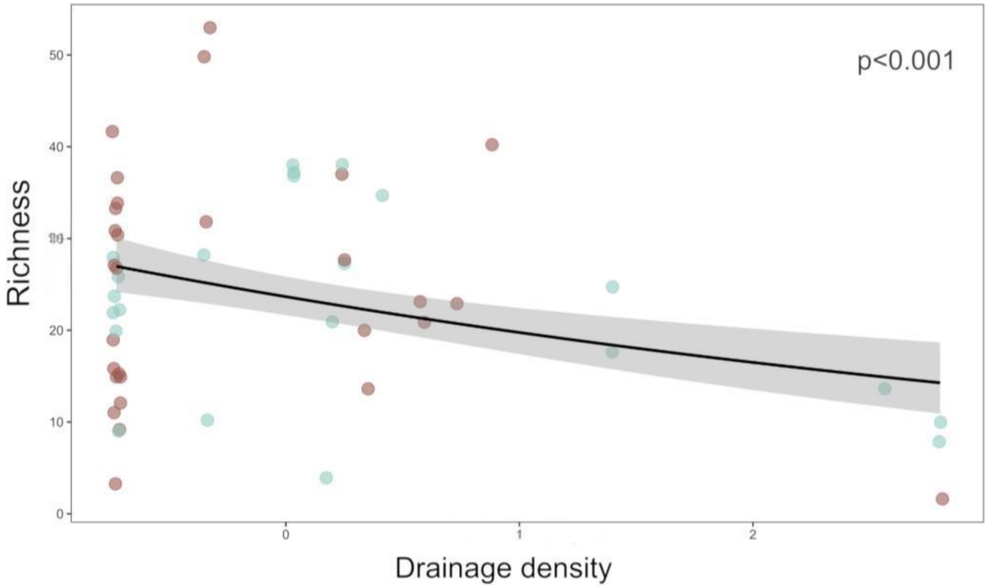


Fig. 15. Effect of the interaction between drainage density and tailings on the richness of adult EPT species. The plot shows the relationship between drainage density (x-axis) and species richness (y-axis), with point colors indicating the presence (red) or absence (green) of tailings. The black line represents the fit of a generalized linearmixed model (GLMM) with a negative binomial distribution and sampling point as a random effect, estimating the overall trend of richness across drainage density values. The shaded band around the line corresponds to the 80% confidence interval. The significant result ($p < 0.001$) suggests that drainage density significantly influences richness, modulated by the presence of tailings.

4. Discussion

Our results show that EPT diversity in the Rio Doce basin is influenced by multiple abiotic factors operating at different spatial scales. The interaction between the impact of the tailings with physico-chemical water stressors, habitat quality, hydrography and land use plays a central role in structuring the community. These drivers act across small (phisicochemical conditions, such as pH, conductivity, and temperature), medium(habitat quality in the riparian zone), and large spatial scales (land use and hydrological features like drainage density and dam presence), and highlight the importance of a multiscale approach to understand and manage aquatic biodiversity.

The abundance and richness of immature EPT genera were subtly higher in the unaffected rivers. Despite the presence of tailings, species richness was maintained in the Rio Doce, which can be explained by a possible replacement of more sensitive taxa by groups more tolerant of the altered environmental conditions. Among the immature individuals, the two most abundant species were *Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996 and *Cloeodes irvingi* Waltz & McCafferty, 1987, both of which have many species with generalist habits (Buss and Salles, 2007; Merritt et al., 2009). Souza et al. (2011) state that these genera may be found in conserved environments, but they are not restricted to them. In addition, the most abundant genera mentioned above have a wide distribution in Brazil (Salles, 2024) and are commonly found in aquatic entomofauna surveys (Righi-Cavallaro et al., 2010; Amaral et al., 2015).

Species composition remained similar between impacted and unimpacted areas, varying only between seasons for both adults and immatures. The lack of difference in composition between areas with and without tailings may be related to the large scale of the basin, where multiple environmental and ecological factors interact, diluting the direct effect of tailings on species composition (Sitati et al., 2024). Dispersal processes over time may also have contributed to community restructuring, smoothing out local differences (Heino and Mickra, 2006).

Among EPT adults, species richness was affected by the presence of mining tailings, with fewer species found in areas where tailings had passed through. The trajectory of mining tailings left a trail of destruction in the watercourses through which they passed, resulting in sediment accumulation, increased water turbidity, changes in geographic configuration, bank erosion, and loss of riparian vegetation (Lopes, 2016), negatively affecting the three spatial scales considered in this work. These changes directly affect the aquatic insect community, which has a fundamental part of its life cycle in the water and depends on favorable characteristics in the environment for its development.

Our results show that seasonality is the main driver of changes in EPT community composition, with significant differences between the dry and rainy seasons. This indicates that temporal environmental fluctuations have a strong impact on community structure. During the rainy season, increased water flow mobilizes sediments, causing the re-suspension of tailings deposited on the riverbed, which can alter habitat quality and resource availability. These changes tend to favor generalist taxa over specialists, contributing to shifts in community composition. IndVal analysis supports this, highlighting taxa that respond to seasonal hydrological and chemical changes. These findings underscore the need to account for seasonal variability in ecological assessments, especially in rivers affected by past contamination.

Considering the first spatial scale of abiotic factors, the parameters of electrical conductivity and pH interact with the presence of tailings, directly influencing the structure of the immature EPT community in the Rio Doce Basin. The effect of these factors on the diversity of Hill's reinforces this trend, indicating that variations in pH and conductivity affect the composition and structure of species in areas affected by tailings. In the small-scale assessment of adults, there is also a variation in richness as a function of the interaction between temperature and tailings.

Electrical conductivity is a key indicator of water quality, reflecting the concentration of dissolved ions, particularly salts, and serving as an indirect measure of contamination levels (Esteves, 1988). Elevated values often signal anthropogenic impacts, especially in mining-affected areas, where metallic ions and other by-products of metal processing increase ionic loads in aquatic systems. In the study sites, the maximum electrical conductivity recorded was 97.4 S/cm, indicating extremely high ionic con centrations consistent with severe contamination, likely associated with mining activities. In the Rio Doce basin, such elevated conductivity levels have been strongly linked to chemical degradation and the loss of ecological integrity in affected water bodies (Viola et al., 2016). Moreover, increased conductivity can disrupt osmoregulatory processes in aquatic insects, potentially reducing species richness and altering community composition.

In this study, sites with higher electrical conductivity values had a higher number of individuals during the rainy season. However, electrical conductivity is typically much lower during the rainy season compared to the dry season, due to dilution effects from increased water volume. The observed high abundance, characterized by the prominent presence of generalist organisms such as those belonging to the genera *Americabaetis* (Ephemeroptera: Baetidae) and Smicridea (Trichoptera: Hydropsychidae), suggests a shift in the composition of the aquatic insect community, with a loss of specialist species. In this context, organisms with adaptive traits to tolerate adverse conditions dominate the environment, leading to functional homogenization (Clavel et al., 2011).

Still at the micro spatial scale, there was an effect of the interaction between tailings and pH on the abundance of immatures (Fig. 8A) and of the pH variable on the richness of adults (Fig. 8B). Even though the pH range is within the tolerance limits for the occurrence of different EPT taxa (Timm, 1997; Lock and Goethals, 2011; Hamid and Rawi, 2017), in environments with tailings, higher pHs represented a decrease in the numberof genera sampled. In reference environments, the variation in richness remained more stable and varied slightly with the variation in pH, indicating that the presence of tailings and their consequences can reduce the tolerance of organisms to adverse conditions and affect the structure of the EPT community.

Water temperature plays a crucial role in various stages of aquatic invertebrate life cycles, influencing embryonic development, larval growth, emergence, metabolism, and survival (Haidekker & Hering, 2008). Figure 8 shows a decline in adult invertebrate richness as temperature rises in areas affected by tailings. This pattern is linked to riparian forests, which provide shade and help regulate water temperature, maintaining favorable conditions for aquatic ecosystems (Bunn et al., 1999). Where the tailings have passed through, this vegetation has been completely suppressed, with the temperature increase being more widespread and determining the non-occurrence of certain species in these places. This argument becomes more consistent when we look at the results of the large-scale analysis, which showed that species richness, abundance and diversity are greater in sites with a higher percentage of vegetation and riparian forest.

Considering the medium scale of abiotic conditions, there are the qualitative characteristics of the environmental attributes of the river and its adjacent surroundings, such as the vegetation cover of the bed and the type of substrate, which help to contextualize the environment in which the aquatic insect community is inserted (Nessimmian et al., 2008). In the Rio Doce Basin, the interaction between tailings and the attributes measured by the habitat quality protocol affects the structure of the immature EPT community. In the rainy season, the increase in habitat quality does not represent an increase in abundance in sites affected by tailings (Fig. 9), a pattern that is the opposite of that which occurs in the reference areas and in the dry season, indicating that even in better habitat quality there is no increase in the abundance of immature EPT in areas with tailings in the rainy season.

The predominance of degraded environments, even in reference areas, highlights the state of widespread degradation in the Rio Doce Basin. The increase in abundance in better-quality reference areas suggests that habitat heterogeneity can mitigate the negative impacts of tailings and other environmental stressors. However, for adults, habitat quality had no significant effect on richness, abundance or diversity. In other words, although local conditions influence the immature phase, other factors, such as dispersal, may be more decisive when we look at the adult EPT community.

According to Rivers and Bailey (2006), there is a strong relationship between land use and the structure of aquatic macroinvertebrates. The interaction between tailings and the different types of land use had an effect on the richness and abundance of immature EPT. In sites without tailings, richness was higher in areas with less urbanization and greater vegetation cover and riparian forest. However, in affected areas, this relationship was reversed, with reduced richness even in sites with structured vegetation, suggesting that contamination compromises community recovery, as was also observed when evaluating the smaller scales. The presence of rocks and agricultural use also affected richness, butthe impacts of tailings appear to override these effects, as occurs with abiotic factors at other scales, homogenizing the composition of the species.

Abundance followed a similar pattern, with a greater number of individuals in preserved sites but increasing in urbanized areas affected by tailings, possibly due to the dominance of tolerant species, which was evident when we performed a more exploratory analysis of the data. These results highlight that, although vegetation and habitat quality influence biodiversity, contamination compromises community recovery, reinforcing the need for combined restoration and decontamination actions.

Hill diversity indices demonstrated the influence of the interaction between tailings and land use on the community structure of immature EPT. In areas without tailings, Hill richness (Hill-0) was higherin locations with greater vegetation cover, riparian forests and less urbanization, reinforcing the role of these habitats in maintaining diversity. However, in impacted areas, the relationship was less evident, indicating that the presence of tailings may neutralize the benefits of vegetation. The Shannon-Hill (Hill-1) and Simpson-Hill (Hill-2) indices, which consider species equitability and dominance, followed similar patterns. In reference areas, habitats with greater vegetation cover and less urbanization supported more balanced communities, while in locations with tail ings, diversity was reduced, regardless of habitat structure. These results may suggest that contamination affects not only species richness but also community stability, reinforcing the need for combined actions to restore the environment and mitigate the effects of tailings. For adults, we observed a pattern that was distinct from that of immatures. Adult richness is mainly influenced by the presence of riparian vegetation, where areas with greater cover have greater richness, especially in regions not impacted by the tailings. However, this effect is attenuated in areas affected by the tailings, suggesting that degradation resulting from the impact of the disaster may limit the benefits of riparian vegetation.

Regarding abundance, a more variable effect is observed, where areas with greater pasture cover and agricultural use have higher mean values but with great dispersion in the data. Hill's diversity indices indicate that adult diversity responds in a more complex way to the interaction between land use and tailings. The Shannon-Hill and Simpson-Hill indices suggest that diversity is greater in areas with greater vegetation cover and the presence of riparian vegetation, although this effect is more evident in regions without tailings. For the Hill 2 index, which emphasizes dominance (Rosewell et al., 2021), the values are higher in areas with greater agricultural coverage and vegetation; however, areas that have undergone the passage of tailings have a reduction in this divers ity parameter. These results reinforce the importance of considering different spatial scales and environmental variables when assessing impacts on aquatic communities.

The drainage density variable is highly related to the hydrographic order of the rivers; lower values of richness and diversity are expected in passages of higher order, where there is greater flow and less heterogeneity of microhabitats (Abebe et al., 2021). The negative relationship between drainage density and EPT diversity reflects its impact on the structure of the communities. Higher-order rivers, with greater water volume and less structural complexity, can limit the presence of more specialized organisms, favoring more generalist and resistant species(Wegscheider et al., 2023). Furthermore, the influence of dams may be more studies as it could play an important role of these structures in altering the ecological dynamics of rivers, possibly creating barriers to dispersal and modifying resource availability (Wang et al., 2021).

More than seven years after the disaster, the deleterious effects of the passage of the tailings can still be recognized since they are reflected in changes in the structure and composition of the aquatic invertebrate community. Other studies carried out years after the disaster also report the persistent toxic effects of the passage of the tailings on aquatic communities (Weber et al., 2020; Macêdo et al., 2020). The dissimilarity in the composition of EPT adults between areas with and without the passage of the tailings can provide insights into the health and dynamics of ecosystems, as well as the factors that influence these differences. Taking into account the context of the disaster and the assessment of abiotic characteristics at three scales, it can be inferred that even if aquatic insects are able to disperse to the environments that were affected by the tailings, the abiotic characteristics are still not suitable for the colonization of these organisms.

5. Final remarks

According to the parameters evaluated, the EPT community, in 2022, seven years after the disaster, responded negatively to the collapse of the mining tailings dam in the Rio Doce. The decrease in richness, the dominance of generalist taxa, and the change in the species composition of the areas where mining tailings passed corroborate this response. Therefore, areas affected by the passage of tailings present EPT diversity patterns that are different from areas not affected by the tailings. It is also seen that the presence of the tailings altered the environmental characteristics in such a way that the stressors preceding the disaster had an even more deleterious effect on the EPT community.

EPT diversity in the Rio Doce basin is influenced by abiotic factors at different scales, with distinct effects depending on the life stage of the aquatic insects. While immature insects responded to both local stressors, such as pH, and landscape factors, such as land use, adults showed less sensitivity. These results reinforce the importance of multiscale approaches in assessing environmental impacts and planning the ecological recovery of the basin. Although adults responded to stressors on a larger spatial scale, as expected, there was also a response on a smaller spatial scale within the aquatic ecosystem. Since adults emerged from the water bodies around where they were collected, they disperse little and have a short life cycle. In addition to being consistent with the life history of EPT, this response also indicates the suitability of the concomitant use of adults and immatures in studies of the diversity of insects of the orders Ephemeroptera, Plecoptera and Trichoptera.

4. Supplementary material

Table 2. List of the abundance of immature EPT genera surveyed at the 40 sampling stations in the Rio Doce basin.

Order	Family	Genera	n
Ephemeroptera	**Baetidae**	*Americabaetis* Kluge, 1992	2205
		Apobaetis Day, 1955	75
		Aturbina Lugo-Ortiz & McCafferty, 1996	221
		Baetodes Needham & Murphy, 1924	30
		Callibaetis Eaton, 1881	1175
		Camelobaetidius Demoulin, 1966	314
		Cloeodes Traver, 1938	2522
		Cloeon Leach, 1815	3
		Cryptonympha Lugo-Ortiz & McCafferty, 1998	53
		Paracloeodes Day, 1955	1883
		Rivudiva Lugo-Ortiz & McCafferty, 1998	65
		Waltzoyphius McCafferty & Lugo-Ortiz, 1995	3
	Caenidae	*Caenis* Stephens, 1835	25
	Leptohyphidae	*Leptohyphes* Eaton, 1882	5
		Traverhyphes Molineri, 2001	355
		Tricorythodes Ulmer, 1920	89
		Tricorythopsis Traver, 1958	9
	Leptophlebiidae	*Farrodes* Peters, 1971	*87*
		Hagenulopsis Ulmer, 1920	*5*
		Terpides Demoulin, 1966	2
		Traverella Edmunds, 1948	10
		Ulmeritus Traver, 1956	1
		Simothraulopsis Demoulin, 1966	5

	Oligoneuriidae	*Lachlania* Hagen, 1868	*3*
	Polymitarcyidae		1
Plecoptera	**Perlidae**	*Anacroneuria* Klapálek, 1909	*70*
Trichoptera	**Calamoceratidae**	*Phylloicus* Müller, 1880	1
	Glosossomatidae	*Mortoniella* Ulmer, 1906	1
	Heliopsychidae	*Helicopsyche* von Siebold, 1856	9
	Hydrobiosidae	*Atopsyche* Banks, 1905	4
	Hydropsychidae	*Leptonema* Guérin-Méneville, 1843	43
		Macronema Pictet, 1836	1
		Smicridea McLachlan, 1871	1075
	Hydroptilidae	*Alisotrichia* Flint, 1964	2
		Hydroptila Dalman, 1819	26
		Neotrichia Morton 1905	1
		Oxyethira Eaton, 1873	1
	Leptoceridae	*Nectopsyche* Müller, 1879	50
		Oecetis McLachlan, 1877	3
		Triplectides Kolenati, 1859	1
	Odontoceridae	*Marilia* Müller 1880	5
	Philopatamidae	*Chimarra* Stephens, 1829	148
	Polycentropodidae	*Cernotina* Ross, 1938	2
		Cyrnellus Banks, 1913	9
		Polyplectropus Ulmer, 1905	1
		Protoptila Banks, 1904	1
		Total	10600

Table 3. List of the abundance of adult EPT genera surveyed at the 40 sampling stations in the Rio Doce basin.

Order	Family	Species	n
Ephemeroptera	**Baetidae**	*Americabaetis alphus* Lugo-Ortiz & McCafferty, 1996	810
		Americabaetis labiosus Lugo-Ortiz & McCafferty, 1996	48
		Americabaetis longetron Lugo-Ortiz & McCafferty, 1996	56
		Americabaetis sp.	134
		Americabaetis sp1	1
		Apobaetis fiuzai Salles & Lugo-Ortiz, 2002	236
		Apobaetis irai De Lima, Massariol, Cruz & Hamada, 2022	93
		Apobaetis sp.	1
		Aturbina beatrixae Gillies, 2001	48
		Aturbina georgei Lugo-Ortiz & McCafferty, 1996	108
		Baetidae - fêmeas	2710
		Baetodes sp.	1
		Callibaetis sp.	3
		Callibaetis sp1	84
		Callibaetis sp2	4
		Callibaetis sp3	1
		Camelobaetidius anubis (Traver & Edmunds, 1968)	5

	Camelobaetidius billi Thomas & Dominique, 2000	159
	Camelobaetidius sp.	24
	Camelobaetidius sp1	3
	Camelobaetidius sp2	50
	Cloeodes auwe Salles & Batista, 2004	48
	Cloeodes aymore Massariol & Salles, 2011	3
	Cloeodes irvingi Waltz & McCafferty, 1987	353
	Cloeodes sp.	5
	Cloeodes sp1	1
	Cloeon smaeleni Lestage, 1924	2
	Cryptonympha dasilvai Salles & Francischetti, 2004	13
	Cryptonympha sp1	8
	Cryptonympha sp2	2
	Cryptonympha sp3	2
	Harpagobaetis sp1	1
	Paracloeodes ibicui Lugo-Ortiz & McCafferty, 1996	14
	Paracloeodes sp.	882
	Paracloeodes sp2	364
	Paracloeodes sp3	30
	Paracloeodes sp4	51

	Paracloeodes sp5	2
	Paracloeodes waimiri Nieto & Salles, 2006	363
	Rivudiva sp1	2
	Rivudiva sp2	1
	Waltzoyphius fasciatus Lugo-Ortiz & McCafferty, 1995	5
Caenidae	*Brachycercinae*	36
	Brasilocaenis elidioi (Lima, Molineri, Pinheiro & Salles, 2016)	92
	Caenis sp1	20
	Caenis sp2	12
	Caenis sp3	9
Leptohyphidae	*Leptohyphes plaumanni* Allen, 1967	22
	Macunahyphes australis (Banks, 1913)	1039
	Traverhyphes (Mocohyphes) yuati Molineri, 2004	5952
	Traverhyphes (Traverhyphes) pirai Molineri, 2001	1006
	Tricorythodes bullus Allen, 1967	203
	Tricorythodes santarita Traver, 1959	293
	Tricorythodes sp.	43
	Tricorythodes tragoedia Souto, Angeli & Salles, 2017	145
	Tricorythopsis baptistai (Dias & Salles, 2005)	730
	Tricorythopsis minimus (Allen, 1973)	977

	Tricorythopsis sp.	2
	Tricorythopsis sp1	1
	Tricorythopsis yacutinga Molineri, 2001	155
Leptophlebiidae	*Askola paprockii* Dominguez, Molineri & Mariano, 2009	20
	Farrodes carioca Domínguez, Molineri & Peters, 1996	1336
	Hermanella amere Nascimento & Salles, 2013	4
	Hermanella nigra Nascimento & Salles, 2013	1
	Paramaka convexa (Spieth, 1943)	38
	Paramaka sp.	9
	Simothraulopsis caliginosus Nascimento, Salles & Hamada, 2017	71
	Simothraulopsis diamantiensis Mariano, 2010	91
	Thraulodes alegre	4
	Thraulodes luisae Souto, Da-Silva & Nessimian, 2014	38
	Thraulodes sp1	24
	Thraulodes sp2	20
	Tikuna bilineata (Needham & Murphy, 1924)	6
	Traverella excelsior Nascimento & Lima, 2020	4303
	Traverella sp.	3
	Ulmeritoides nigribullae Salles & Domínguez, 2012	19
Oligoneuridae	*Lachlania* sp.	4

		Oligoneuria amandae Salles et al., 2013	4
	Polymitarcyidae	*Campsurus cotaxe* Molineri & Salles, 2017	1374
		Campsurus latipennis (Walker, 1853)	480
		Campsurus mirim Pantoja, Viana & Salles, 2023	27
		Campsurus truncatus Ulmer, 1920	32
		Tortopsis canum Gonçalves, Da-Silva & Nessimian, 2011	66
		Tortopus harrisi Traver, 1950	2
Plecoptera	**Perlidae**	*Anacroneuria mineira* Novaes & Bispo, 2014	14
		Anacroneuria atrifrons Klapálek, 1922	14
		Anacroneuria itatiaiensis Baldin, Bispo & Novaes, 2013	16
		Anacroneuria piranga Castillo-Velásquez, Gonçalves & Salles, 2023	1
		Anacroneuria sp.	1
Trichoptera	**Ecnomidae**	*Austrotinodes* sp.	2
	Glossosomatidae	*Mortoniella parauna* Blahnik & Holzenthal 2011	790
		Mortoniella pusilla Blahnik & Holzenthal 2011	4350
		Mortoniella sp.	1003
		Protoptila sp.	694
		Protoptila sp2	20
	Helicopsychidae	*Cochlyopsyche sp*	3093
		Cochlyopsyche sp1	2227
		Cochlyopsyche sp2	2732

	Feropsyche	17
	Feropsyche sp.	2
	Helicopsyche sp.	3631
	Helicopsyche sp2	29
Hydrobiosidae	*Atopsyche (Atopsyche) chirihuana* Schmid, 1989	2
	Atopsyche (Atopsyche) erigia Ross 1947	8
	Atopsyche sp.	92
	Atopsyche (Atopsyche) urumarca Schmid, 1989	6
Hydropsychidae	*Blepharophus* sp.	62
	Blepharopus diaphanus Kolenati, 1859	496
	Leptonema columbianum Ulmer, 1905	780
	Leptonema crassum Ulmer, 1905	986
	Leptonema sp.	8
	Leptonema sp1	2
	Leptonema sp4	38
	Leptonema cf. *crassum*	38
	Leptonema sparsum Ulmer, 1905	454
	Leptonema viridianum Navás, 1916	22
	Macronema immaculatum Mosely, 1934	28
	Macronema sp.	12
	Smicridea (Rhyacophylax) atrobasis Flint, 1983	308

	Smicridea (Rhyacophylax) coronata Flint, 1980	3756
	Smicridea (Rhyacophylax) iguazu Flint, 1983	4822
	Smicridea (Rhyacophylax) jundiai Almeida & Flint, 2002	2900
	Smicridea (Rhyacophylax) palmar Sganga, 2005	27
	Smicridea (Rhyacophylax) radula Flint, 1974	1609
	Smicridea (Rhyacophylax) sp. nov	2843
	Smicridea (Rhyacophylax) sp8	180
	Smicridea (Rhyacophylax) vilela Flint, 1978	2960
	Smicridea (Smicridea) bivittata (Hagen, 1861)	1554
	Smicridea (Rhyacophylax) appendiculata Flint 1972	200
	Smicridea (Rhyacophylax) flinti	1728
	Smicridea (Rhyacophylax) palmar Sganga, 2005	355
	Smicridea (Rhyacophylax) piraya Flint, 1983 Flint, 1983	4
	Smicridea (Rhyacophylax) radula Flint, 1974	12175
	Smicridea (Rhyacophylax) scalprum Vilarino, Dias & Calor, 2019	2
	Smicridea sp.	19978
	Smicridea (Rhyacophylax) sp.	17878
	Smicridea (Rhyacophylax) sp12	4
	Smicridea (Rhyacophylax) sp14	2

	Smicridea (Rhyacophylax) sp8	702
	Synoestropsis grisoli Navás, 1924	2
	Synoestropsis sp1	2
Hydroptilidae		325244
Leptoceridae	*Achoropsyche* sp.	338
	Grumichella sp.	10
	Nectopsyche cf. maculipennis	20
	Nectopsyche fuscomaculata Flint, 1983	172
	Nectopsyche ortizi Holzenthal, 1995	6
	Nectopsyche punctata (Ulmer, 1905)	362
	Nectopsyche sp.	1006
	Nectopsyche sp.nov	1620
	Nectopsyche sp1	18
	Nectopsyche sp2	2
	Nectopsyche sp5	108
	Nectopsyche sp6	24
	Nectopsyche sp7	2
	Nectopsyche splendida (Navás, 1917)	68
	Oecetis excisa Ulmer, 1907	4
	Oecetis martinae Quinteiro & Calor, 2015	4
	Oecetis punctipennis (Ulmer, 1905)	6

	Oecetis sp.	196
Odontoceridae	*Marilia aranan* Bonfá-Neto, Salles & Vilarino, 2023	14
	Marilia guaira Flint, 1983	40
	Marilia krenak Bonfá-Neto, Salles & Vilarino, 2023	4
	Marilia maxakali Bonfá-Neto, Salles & Vilarino, 2023	2
	Marilia sp.	36
Philopotamidae	*Chimarra (Curgia)* sp.	11
	Chimarra (Curgia) cipoensis Flint, 1998	2
	Chimarra (Curgia) cultellata Flint 1983	560
	Chimarra (Curgia) scopuloides Flint 1974	38
	Chimarra (Curgia) hyoeides Flint 1983	334
	Chimarra usitatissima Flint, 1971	84
	Chimarra (Curgia) scopuloides Flint, 1974	436
	Chimarra sp.	11
	Chimarra sp4	4
Polycentropodidae	*Cernotina antonina* Holzenthal & Almeida, 2003	30
	Cernotina cacha Flint, 1971	2
	Cernotina longispina Barcelos-Silva, Camargos & Pes, 2013	10
	Cernotina perpendicularis Flint, 1971	24
	Cernotina sp.	504

	Cernotina sp1	2
	Cernotina sp2	102
	Cernotina sp3	6
	Cyrnellus fraternus (Banks, 1915)	18
	Cyrnellus mammillatus Flint, 1971	36
	Cyrnellus cf. *minimus*	54
	Cyrnellus risi (Ulmer, 1907)	870
	Cyrnellus sp.	3756
	Cyrnellus sp2	8
	Cyrnellus sp3	2
	Nyctiophylax neotropicalis Flint, 1971	30
	Nyctiophylax sp.	78
	Polyplectropus elongatus (Yamamoto) 1966	156
	Polyplectropus sp.	44
	Polyplectropus ulmeriana Flint 1983	2
Total		**466652**

Table 4. Data on the abiotic characteristics of the 40 points sampled in the River Doce basin. T = Temperture, DO = Dissolved oxigen, EC = Eletrical condutivity, pH = Hydrogen potential, RVEG = Riparian vegetation, ES = Exposed soil, VEG = Vegetation, AR = Sand, PAS = Pasture, RO = Rocks, ANT = Anthropic use, AGR=Agriculture, and UA = Urban area.

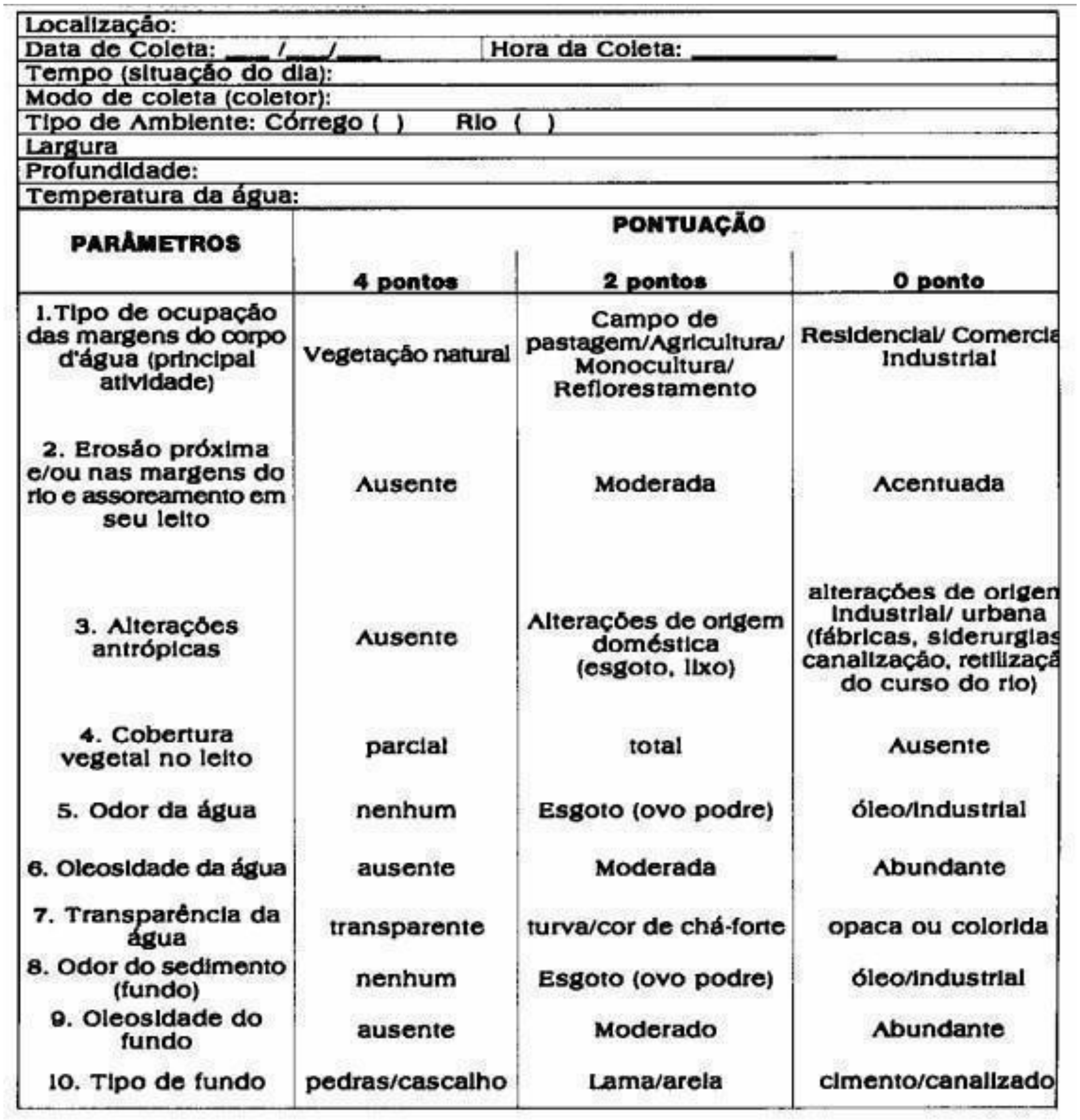
	Tailings	T (ºC)	DO (%)	EC S/cm	pH	RVEG (%)	ES (%)	VEG (%)	AR (%)	PAS (%)	RO (%)	ANT (%)	AGR (%)	UA (%)
1	No	26.4	58.1	9.25	7.53	8.9	3.02	87.44	0	0	0	0	0	0
2	Yes	23.5	71.2	40.6	7.39	0	0	0	1.46	1.14	1.71	0.03	0	0
3	Yes	26.2	59.5	52	7.2	3.41	3.63	49.14	0	12.2	0	0.06	0.73	0
4	Yes	25	80.3	42.1	7.12	3.31	2.15	35.93	0	43.01	0.03	0.57	0	11.87
5	No	24.9	72.3	37.7	7.41	21.82	0.58	29.12	0	42.42	0.41	0.11	0	0
6	No	24.1	56.6	84.9	7.22	2.58	4.04	11.39	0.05	73.44	0	0.17	0.79	0
7	Yes	23.4	57.2	93.6	7.11	15.95	1.6	24.88	0.22	47.07	1.28	0.48	0	0
8	Yes	20.5	72.4	36.6	7.14	7.14	4.22	26.7	0	55.41	0.52	4.46	0	0
9	Yes	22.1	63.9	62	7.56	0	10.52	15.97	4.42	23.27	0	0.31	0.12	0
10	Yes	20.5	66	97.4	7.33	10.11	5.45	35.78	2.86	31.81	0	1.08	0	0
11	No	25.1	49.5	27	7.05	6.11	3	35.79	0	43.77	0.74	0.22	0	0
12	No	22.5	65.3	29.6	7.28	15.82	1.14	58.42	0.57	0	3.39	0.05	0	0
13	Yes	22.2	57.5	51.2	7.5	9.78	11.73	29.06	0.5	0	0	0.83	0	0
15	Yes	23.6	58.6	40.9	7.06	9.11	2.85	25.56	0.31	55.09	0.74	0.4	0.39	0
16	No	22.9	71.1	33.5	7.35	6.54	1.34	33.66	0	49.6	0	0.62	2.34	0
17	No	24.4	78.8	50	7.2	8.58	0.71	26.96	0.92	0	0	10.43	0	0
18	No	20.3	61.8	39.6	7.48	6.8	5.3	20.74	0.08	64.21	0.11	0.52	0	0
19	No	22.6	62.5	53.1	7.59	43.87	2.15	22.85	0	26.04	0.47	0.09	1.83	0
20	No	20.9	53.4	29.8	7.28	27.54	21.04	4.94	0.04	41.05	0	0.06	2.4	0
21	Yes	21.2	72.4	51.9	8.28	4.29	1.85	22.26	0.25	1.7	0	1.37	0	0
22	No	22.9	71.4	53.8	8.12	4.8	1.68	13.02	4.65	1.43	1.36	0.59	0	10.94
23	Yes	26.6	82.2	35.1	8.13	0.59	25.61	31.14	12.86	0	11.78	0	0	0.48
24	No	23.4	65.4	39	7.18	31.74	4.34	10.06	0	7.55	0	0	0	0
25	No	26.3	61.1	27.6	7.29	4.9	11.91	16.2	2.49	17.49	0	0.73	0	14.79
27	Yes	24	73.2	47.7	7.8	6.65	3.44	48.52	3.15	0	2.46	0.75	0	0
28	Yes	24.1	54.4	52.4	7.12	7.57	1.84	24.48	0	6.63	0.6	1.2	0	0
30	No	23.4	65.5	42.8	7.74	7.27	0.4	72.31	0	15.44	0	0.08	0	0
31	Yes	24.6	66.7	48.5	8.07	6.79	2.52	23.63	0	4.16	11.9	0.13	0.32	0
32	Yes	26.9	70.1	61.3	7.37	4.99	1.59	23.23	0	0.76	4.81	0	0	47.94
33	No	27.6	79.8	35.1	7.72	3.83	4.09	21.77	0	1.81	3.19	0.13	0.89	0
34	No	27.2	65.8	31.1	7.77	7.72	3.32	30.33	0.21	4.3	5.51	0.26	11.69	0
35	No	24.3	71.9	56.4	8.05	2.05	2.91	9.65	0.18	0	0.06	0.11	0.09	0
36	Yes	27.2	73	49.3	7.82	2.07	0	14.13	11.8	0	0	0	0	43.78
37	Yes	26.9	59.7	47.6	7.38	7.3	1.69	10.95	0.53	5.19	0	0	37.2	0
38	No	24.4	54.1	65.2	7.22	11.24	21.57	4.21	0	8.81	0	0.43	24.75	0
39	Yes	NA	NA	NA	NA	21.76	0.27	23.83	9.72	0.87	0	0.11	0	0
40	Yes	28.6	74.1	45.3	8.52	2.15	1.07	19.77	8.07	37.75	0	0.27	0	9.87

Table 5. Data on the abiotic characteristics of the 40 points sampled in the River Doce basin. Upstream dam distance, Downstream dam and Distance from the tailings dam collapse are measured in kilometers (Km). Drainage density is measured in Km/Km².

Collecting point	Tailings	River Order	Drainage density	Upstream dam distance	Downstream dam	Distance from the tailings dam collapse
1	no	2	0,152899824	9,00	117,05	14,9
2	yes	6	0,006879941	101,00	125,00	220,0
3	yes	3	0,152899824	56,00	70,26	48,9
4	yes	5	0,051426102	NA	38,80	80,0
5	no	5	0,046944858	8,00	30,10	185,4
6	yes	5	0,046944858	40,50	31,18	116,4
7	no	6	0,006879941	55,00	16,86	102,3
8	yes	6	0,006879941	2,50	223,30	121,8
9	yes	6	0,006879941	186,80	39,20	307,0
10	no	6	0,006879941	69,00	3,00	116,2
11	yes	3	0,022278863	NA	96,50	481,8
12	yes	4	0,022278863	NA	40,36	425,6
13	no	6	0,006879941	44,85	180,90	164,0
15	yes	5	0,051426102	NA	18,10	101,0
16	yes	5	0,046944858	7,00	65,17	150,4
17	no	4	0,044692737	12,29	130,08	304,3
18	no	3	0,095107632	63,20	58,80	306,7
19	no	3	0,095107632	54,30	178,88	193,2
20	no	3	0,053818757	65,50	12,20	353,0
21	no	6	0,006879941	67,88	96,17	413,0
22	yes	3	0,068043742	157,00	75,00	435,7
23	no	3	0,006879941	5,00	17,00	514,1

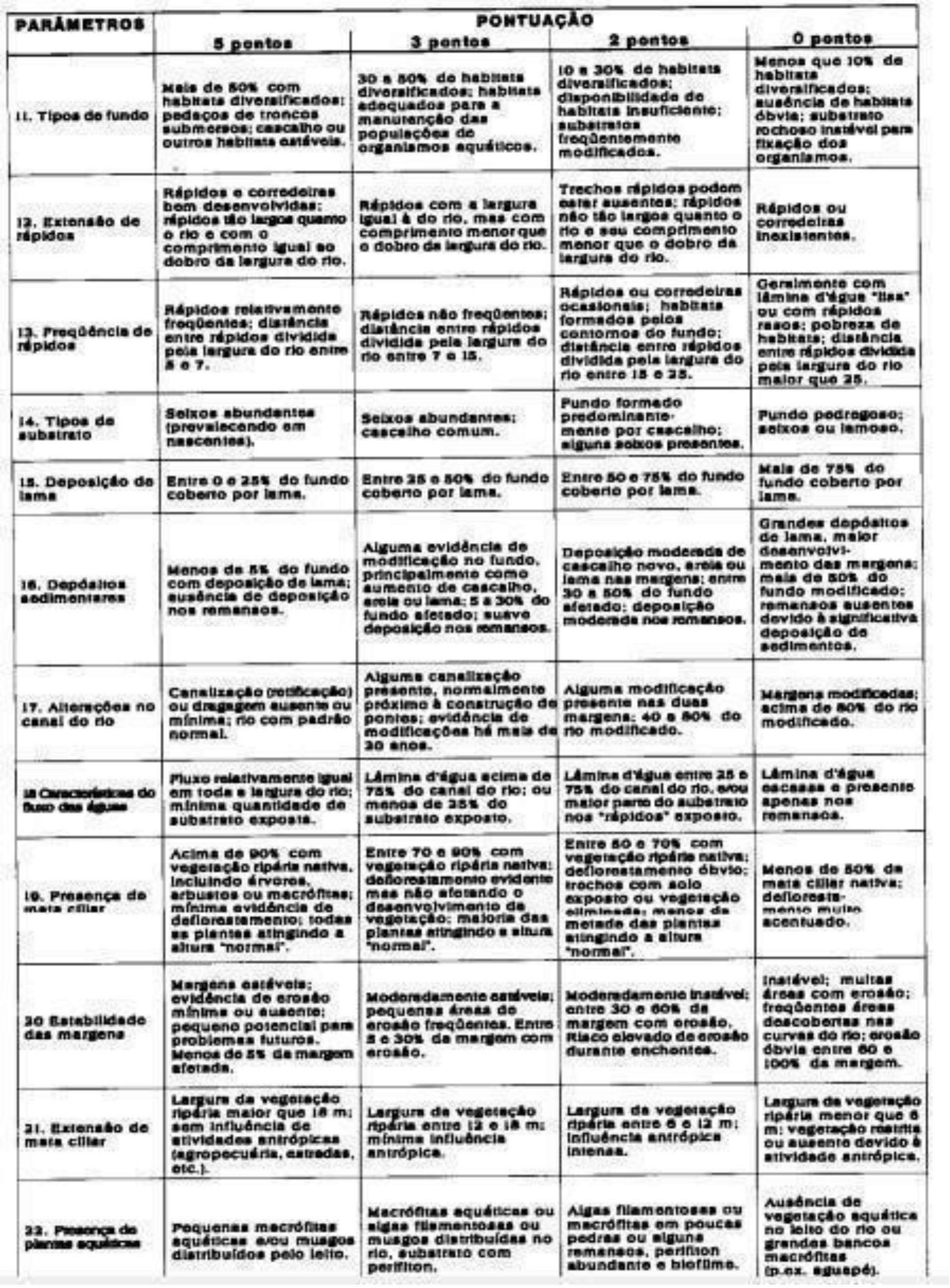
24	yes	6	0,060816012	58,54	154,34	206,8
25	no	4	0,022278863	46,40	38,91	311,2
27	yes	6	0,006879941	106,17	57,89	451,2
28	yes	6	0,006879941	17,00	147,07	362,0
30	yes	4	0,024276451	NA	13,30	462,8
31	yes	6	0,006879941	161,70	3,00	506,7
32	no	6	0,006879941	11,40	11,00	520,5
33	no	4	0,038828489	80,55	30,64	523,7
34	no	4	0,038828489	68,50	42,38	535,7
35	yes	3	0,073905109	NA	26,74	539,1
36	no	6	0,006879941	34,77	NA	565,7
37	yes	6	0,006879941	102,12	NA	633,5
38	no	NA	0,142512077	NA	NA	642,2
39	yes	6	0,006879941	115,62	NA	647,0
40	yes	6	0,006879941	140,00	NA	672,0

Fig. 16. Rapid Habitat Diversity Assessment Protocol (Callisto et al., 2002) for stretches of river basin used to characterize the habitat at the 40 collection points in the Rio Doce basin.



Localização:
Data de Coleta: ___ /___/___ **Hora da Coleta: ________**
Tempo (situação do dia):
Modo de coleta (coletor):
Tipo de Ambiente: Córrego ( ) Rio ( )
Largura
Profundidade:
Temperatura da água:

PARÂMETROS	PONTUAÇÃO		
	4 pontos	2 pontos	0 ponto
1. Tipo de ocupação das margens do corpo d'água (principal atividade)	Vegetação natural	Campo de pastagem/Agricultura/ Monocultura/ Reflorestamento	Residencial/ Comercia Industrial
2. Erosão próxima e/ou nas margens do rio e assoreamento em seu leito	Ausente	Moderada	Acentuada
3. Alterações antrópicas	Ausente	Alterações de origem doméstica (esgoto, lixo)	alterações de origen industrial/ urbana (fábricas, siderurgias canalização, retilizaçã do curso do rio)
4. Cobertura vegetal no leito	parcial	total	Ausente
5. Odor da água	nenhum	Esgoto (ovo podre)	óleo/industrial
6. Oleosidade da água	ausente	Moderada	Abundante
7. Transparência da água	transparente	turva/cor de chá-forte	opaca ou colorida
8. Odor do sedimento (fundo)	nenhum	Esgoto (ovo podre)	óleo/industrial
9. Oleosidade do fundo	ausente	Moderado	Abundante
10. Tipo de fundo	pedras/cascalho	Lama/areia	cimento/canalizado



PARÂMETROS	PONTUAÇÃO			
	5 pontos	3 pontos	2 pontos	0 pontos
11. Tipos de fundo	Mais de 50% com habitats diversificados; pedaços de troncos submersos; cascalho ou outros habitats estáveis.	30 a 50% de habitats diversificados; habitats adequados para a manutenção das populações de organismos aquáticos.	10 a 30% de habitats diversificados; disponibilidade de habitats insuficiente; substratos freqüentemente modificados.	Menos que 10% de habitats diversificados; ausência de habitats óbvia; substrato rochoso instável para fixação dos organismos.
12. Extensão de rápidos	Rápidos e corredeiras bem desenvolvidas; rápidos tão largos quanto o rio e com o comprimento igual ao dobro da largura do rio.	Rápidos com a largura igual à do rio, mas com comprimento menor que o dobro da largura do rio.	Trechos rápidos podem estar ausentes; rápidos não tão largos quanto o rio e seu comprimento menor que o dobro da largura do rio.	Rápidos ou corredeiras inexistentes.
13. Freqüência de rápidos	Rápidos relativamente freqüentes; distância entre rápidos dividida pela largura do rio entre 5 e 7.	Rápidos não freqüentes; distância entre rápidos dividida pela largura do rio entre 7 e 15.	Rápidos ou corredeiras ocasionais; habitats formados pelos contornos do fundo; distância entre rápidos dividida pela largura do rio entre 15 e 25.	Geralmente com lâmina d'água "lisa" ou com rápidos rasos; pobreza de habitats; distância entre rápidos dividida pela largura do rio maior que 25.
14. Tipos de substrato	Seixos abundantes (prevalecendo em nascentes).	Seixos abundantes; cascalho comum.	Fundo formado predominantemente por cascalho; alguns seixos presentes.	Fundo pedregoso; seixos ou lamoso.
15. Deposição de lama	Entre 0 e 25% do fundo coberto por lama.	Entre 25 e 50% do fundo coberto por lama.	Entre 50 e 75% do fundo coberto por lama.	Mais de 75% do fundo coberto por lama.
16. Depósitos sedimentares	Menos de 5% do fundo com deposição de lama; ausência de deposição nos remansos.	Alguma evidência de modificação no fundo, principalmente como aumento de cascalho, areia ou lama; 5 a 30% do fundo afetado; suave deposição nos remansos.	Deposição moderada de cascalho novo, areia ou lama nas margens; entre 30 a 50% do fundo afetado; deposição moderada nos remansos.	Grandes depósitos de lama, maior desenvolvimento das margens; mais de 50% do fundo modificado; remansos ausentes devido à significativa deposição de sedimentos.
17. Alterações no canal do rio	Canalização (retificação) ou dragagem ausente ou mínima; rio com padrão normal.	Alguma canalização presente, normalmente próximo à construção de pontes; evidência de modificações há mais de 20 anos.	Alguma modificação presente nas duas margens; 40 a 80% do rio modificado.	Margens modificadas; acima de 80% do rio modificado.
18 Características do fluxo das águas	Fluxo relativamente igual em toda a largura do rio; mínima quantidade de substrato exposta.	Lâmina d'água acima de 75% do canal do rio; ou menos de 25% do substrato exposto.	Lâmina d'água entre 25 e 75% do canal do rio, e/ou maior parte do substrato nos "rápidos" exposto.	Lâmina d'água escassa e presente apenas nos remansos.
19. Presença de mata ciliar	Acima de 90% com vegetação ripária nativa, incluindo árvores, arbustos ou macrófitas; mínima evidência de deflorestamento; todas as plantas atingindo a altura "normal".	Entre 70 e 90% com vegetação ripária nativa; deflorestamento evidente mas não afetando o desenvolvimento da vegetação; maioria das plantas atingindo a altura "normal".	Entre 50 e 70% com vegetação ripária nativa; deflorestamento óbvio; trechos com solo exposto ou vegetação eliminada; menos da metade das plantas atingindo a altura "normal".	Menos de 50% da mata ciliar nativa; deflorestamento muito acentuado.
20 Estabilidade das margens	Margens estáveis; evidência de erosão mínima ou ausente; pequeno potencial para problemas futuros. Menos de 5% da margem afetada.	Moderadamente estáveis; pequenas áreas de erosão freqüentes. Entre 5 e 30% da margem com erosão.	Moderadamente instável; entre 30 e 60% da margem com erosão. Risco elevado de erosão durante enchentes.	Instável; muitas áreas com erosão; freqüentes áreas descobertas nas curvas do rio; erosão óbvia entre 60 e 100% da margem.
21. Extensão de mata ciliar	Largura da vegetação ripária maior que 18 m; sem influência de atividades antrópicas (agropecuária, estradas, etc.).	Largura da vegetação ripária entre 12 e 18 m; mínima influência antrópica.	Largura da vegetação ripária entre 6 e 12 m; influência antrópica intensa.	Largura da vegetação ripária menor que 6 m; vegetação restrita ou ausente devido à atividade antrópica.
22. Presença de plantas aquáticas	Pequenas macrófitas aquáticas e/ou musgos distribuídos pelo leito.	Macrófitas aquáticas ou algas filamentosas ou musgos distribuídas no rio, substrato com perifiton.	Algas filamentosas ou macrófitas em poucas pedras ou alguns remansos, perifiton abundante e biofilme.	Ausência de vegetação aquática no leito do rio ou grandes bancos macrófitas (p.ex. aguapé).

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CHAPTER 03

An approximation approach to estimate the Ephemeroptera (Insecta) dispersal in the Rio Doce Basin

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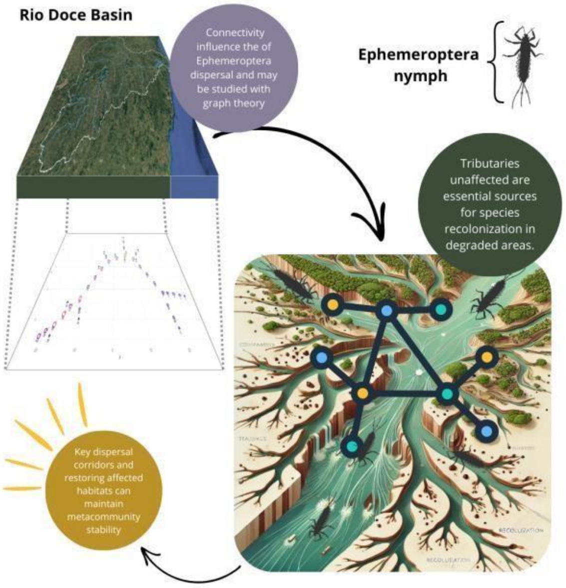
Highlights

- Local and regional connectivity constraints influence the dispersal of Ephemeroptera in the Rio Doce Basin.
- The collapse of the tailings dam has altered the spatial structure of aquatic insect communities, affecting their dispersal corridors.
- The betweenness metric reveals that a few key sites act as critical connectors in the dispersal network.
- Tributaries unaffected by mining tailings are essential sources for species recolonization in degraded areas.
- The modular structure of the dispersal network highlights the importance of targeted conservation actions for maintaining metacommunity stability.

Graphical Abstract



Abstract

Understanding the dispersal patterns of aquatic organisms is crucial for biodiversity conservation, especially in impacted riverine ecosystems. Ephemeroptera comprises highly sensitive aquatic insects whose dispersal dynamics are influenced by hydrological connectivity and environmental disturbances. In this study, we used graph theory to model the metacommunity connectivity of Ephemeroptera in the Rio Doce Basin, southeastern Brazil, a region severely affected by a large-scale mining disaster. We sampled 40 sites across the basin, including impacted and non-impacted areas, and analyzed species co-occurrence networks to assess connectivity patterns. Our results indicate that dispersal is highly structured, with a few key nodes (sampling sites) acting as critical corridors for species movement. Betweenness centrality revealed that sites in the middle Rio Doce function as major connectivity hubs, facilitating species exchange between impacted and non-impacted areas. Conversely, isolated sites, particularly in tributaries, exhibited limited integration within the network. These findings emphasize the importance of preserving key dispersal corridors and restoring affected habitats to maintain metacommunity stability. This study provides valuable insights for aquatic biodiversity conservation in anthropogenically disturbed landscapes by integrating metapopulation theory and network analysis.

Keywords: mayflies, dispersal, graph theory, Rio Doce, metacommunity, mining.

1. Introduction

The metacommunity concept describes groups of local communities connected by species dispersal and interactions (Leibold et al., 2004). Its integration into ecology over the past two decades has significantly advanced knowledge of community structuring (Holyoak et al., 2005). Variations in the influence of local and regional factors in metacommunities often stem from dispersal network structures, where organism movement is shaped by available landscape pathways (Ricklefs, 2008), such as ocean currents or continental aquatic systems (Smith et al., 2009).

Dispersal dynamics are particularly relevant for certain groups of organisms, such as aquatic insects, which are highly dependent on environmental characteristics for their mobility and reproductive success (Manna and Rezende, 2021). The order Ephemeroptera, whose name alludes to the short duration of the winged reproductive phase, comprises aquatic insects highly sensitive to environmental perturbations (Shah et al., 2021). This characteristic allows for causal inference, prediction of environmental changes, and diagnosis of ecological conditions at a given site (Chakravarty and Gupta, 2024).

The immature stage of Ephemeroptera is aquatic and disperses downstream, which is highly dependent on water flow patterns (Sabha, 2022). Considering only their active displacement, adults tend to move upstream, and their short life cycle limits their dispersal (Hamada et al., 2014).

Adults (imagoes) of Ephemeroptera fly upstream in swarms that take them some distance from the water body from which they emerged (Gerber et al., 2024). However, although adults can disperse, egg deposition occurs in water bodies, limiting larval dispersal to these environments (Brittain, 1982).

Ephemeroptera nymphs disperse primarily by current drift, a passive process in which individuals are transported downstream (Sweeney, 1984). Nymphal drift facilitates the colonization of new habitats and maintains gene flow between populations. Some nymphs also move actively within water bodies, but to a lesser extent (Brittain, 1990). Nymphal drift occurs mainly at night and is influenced by currents, making it an essential mechanism for colonizing new habitats (Kriska, 2023).

A key aspect of conserving sensitive groups such as Ephemeroptera is identifying areas where species have the greatest chance of success. Understanding population connectivity and implementing interventions that consider the spatial limitations of communities at local and regional scales can guide actions to mitigate landscape-scale disturbances and improve biological responses to conservation actions (Wahl et al., 2024).

Identifying areas connected by metacommunity networks requires estimates of dispersal that account for segments with different levels of permeability (Grant et al., 2007; Campbell et al., 2015). In addition, dispersal patterns between sites can affect colonization and extinction rates, affecting local populations' persistence (Holland and Hastings, 2008). Knowledge of dispersal patterns in a metacommunity provides valuable information formaintaining spatial connectivity and allows for more effective conservation funding allocation (Saunders et al., 2020).

Graph theory, a mathematical approach that examines relationships within a set through graph structures (Gross et al., 2018), has been applied to identify potential dispersal corridors for threatened species (Hofman et al., 2018). A graph is defined by two sets: vertices, which represent the objects, and edges, which represent the connections between them (Lesne, 2006). Depending on the application, these connections can be directed or undirected and have associated weights (Diestel, 2024). Combining the metapopulation concept with graph theory offers a valuable tool for ecologists to estimate connectivity using physical network data (Diniz et al., 2020).

In this work, we modeled the connectivity of the Ephemeroptera metapopulation of a river basin subjected to a series of anthropogenic disturbances, the Rio Doce Basin, located between Espírito Santo and Minas Gerais, Brazil. The basin was severely affected by the collapse of an ore tailings dam along the entire Doce River (Milanez et al., 2016). The dam, owned by mining companies Vale and BHP Billiton and located in the city of Mariana- MG, Brazil, released more than 43.8 million cubic meters of mining tailings on November 15, 2015. Considered one of the largest environmental disasters in Brazil, this event caused serious socio-environmental problems beyond the site of the spill (Guerra et al., 2017).

Thus, our main question is: What are the dispersal flows that connect the Ephemeroptera metacommunity in the Doce River Basin, and how are they structured? Identifying these structural patterns of dispersal can help inform decisions about mitigation efforts, with the goal of maintaining spatial connectivity and community structure.

2. Material Methods

2.1. Study area and sampling design

The Doce River Basin, part of the Atlantic Forest Domain, is located in southeastern Brazil and has a drainage area of 86,000 km², making it one of the largest river basins in the region (Cupolillo, 2008). Precipitation varies from 150 mm in the dry season (October to March) to 1300 mm in the rainy season (April to September) (ANA, 2025).

We collected data at 40 sampling points, including the river's main channel and its main tributaries. Of the selected points, 20 were in areas crossed by mining tailings, and 20 were in unaffected areas. Samples were collected between August and September 2022. At three of the 40 sites, no adults were sampled during the dry season, two light traps stopped working (Point 14 and Point 29) and one was stolen (Point 26). All these points were not included in the statistical analysis

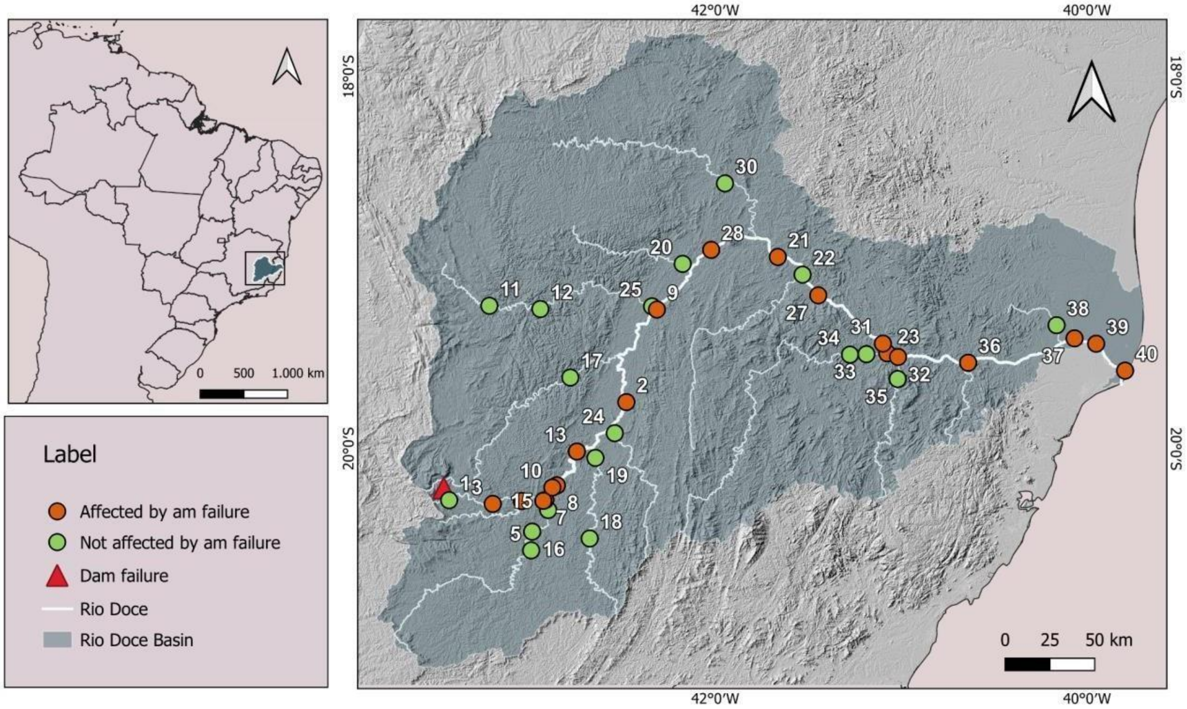


Fig 1. Map of the Rio Doce Basin with 40 sampling points. Points through which tailings have passed are marked in orange, and reference points are marked in white. The thicker white line represents the Rio Doce. The location of the dam failure is indicated with a red triangle.

Table 1. Geographical coordinates of the 40 selected sampling points in the Doce River basin and their classification as affected by mining tailings. All reference points not affected by tailings are located on tributaries of the Doce River.

Point	**Coordinates (UTM)**			**River**	**Locality**	**Tailing**
	east	**west**				
1	663787	7757153	23k	Gualaxo do Norte	Mariana, MG	no

2	764251	7814314	23k	Doce	Parque Estadual do Rio Doce, MG	yes
3	688284	7754717	23k	Gualaxo do Norte	Paracatu de baixo, , MG	yes
4	704609	7756231	23k	Carmo	Barra Longa, MG	yes
5	709518	7726784	23k	Piranga	Guaraciaba, MG	no
6	719392	7750689	23k	Piranga	Rio Doce, MG	no
7	717886	7756690	23k	Doce	Rio Doce, MG	yes
8	724451	7765328	23k	Doce	Rio Doce, MG	yes
9	782354	7869156	23k	Doce	Naque, MG	yes
10	721890	7764082	23k	Doce	Rio Doce, UHE Risoleta neves, MG	yes
11	687610	7872625	23k	Santo Antônio	Santo Antônio do Rior abaixo, MG	no
12	716429	7870370	23k	Santo Antônio	Ferros, MG	no
13	735965	7785306	23k	Doce	São José do Goiabal, MG	yes
14	738866	7879318	23k	Santo Antônio	Santo Antônio da Fortaleza, MG	no
15	716721	7756192	23k	Carmo	Rio Doce, MG	yes
16	710222	7737948	23k	Piranga	UHE Brito, MG	no
17	732869	7829391	23k	Piracicaba	Jaguaraçu, MG	no
18	742458	7733441	23k	Casca	Jequiri, MG	no
19	746357	7781360	23k	Casca	São José do Goiabal, MG	no
20	797357	7896137	23k	Corrente	Periquito, MG	no
21	219512	7900508	24k	Doce	Tumiritinga, MG	yes
22	233687	7890184	24k	Caratinga	Barra do Cuieté, MG	no
23	282205	7844032	24k	Doce	Aimorés, MG	yes
24	757449	7795917	23k	Matipó	Raul Soares	no
25	779627	7871089	23k	River Santo Antônio	Naque, MG	no
26	263294	7861088	24k	Doce	Resplendor, MG	yes
27	242767	7877983	24k	Doce	Conselheiro Pena, MG	yes
28	813646	7904202	23k	Doce	Governador Valadares, MG	yes
29	202153	7937309	24k	Suaçuí Grande	Matias Lobato, MG	no
30	188844	7943845	24k	Suaçuí Grande	Matias Lobato, MG	no
31	279689	7849766	24k	Doce	Aimorés, MG	yes
32	288311	7841895	24k	Doce	Baixo Guandu, ES	yes
33	270455	7843334	24k	Manhuaçu	Aimorés, MG	no
34	261172	7842938	24k	Manhuaçu	Aimorés, MG	no
35	288291	7828762	24k	Guandu	Baixo Guandu, ES	no
36	327949	7838898	24k	Doce	Colatina, ES	yes
37	387897	7853854	24k	Doce	Linhares, ES	yes
38	377681	7861617	24k	Doce	Linhares, Lagoa Juparanã Mirim, ES	no
39	400131	7850752	24k	Doce	Linhares e Povoação, ES	yes
40	416523	7834814	24k	Doce	Povoação, Foz do Rior Doce, ES	yes

2.2. Sampling

Ephemeroptera nymphs were collected from the available substrates in the water body using a D-type net with a mesh size of 0.5 mm. At each sampling point, a 100 m stretch was defined, divided into five sections parallel to the river of 20 m each, with the aim of collecting from the differenttypes of substrate available in the water body (adapted from Lima et al., 2022). The samples were sorted, and the collected organisms were fixed in an 80% ethyl alcohol solution.

The specimens were identified to the taxonomic level of genus using identification manuals (Salles, 2006; Dominguez et al., 2006; Dominguez et al., 2009; Hamada et al., 2014; Hamada et al., 2018), as well as slides and photographs. The specimens will be deposited at the Museum of Entomology of the Federal University of Viçosa (UFV).

2.3. Data analysis

A complex graph theory analysis was performed to investigate the co-occurrence structure and connectivity between sampling points. First, a co-occurrence matrix was constructed from the species abundance matrix. Each row of this matrix represents a sampling point, while the columns correspond to the genera of Ephemeroptera. We then transformed this matrix into a graph, where the vertices represent the species and the edges indicate the intensity of co-occurrences between species, weighted by the abundance of each species at the different sampling points (Fig. 2).

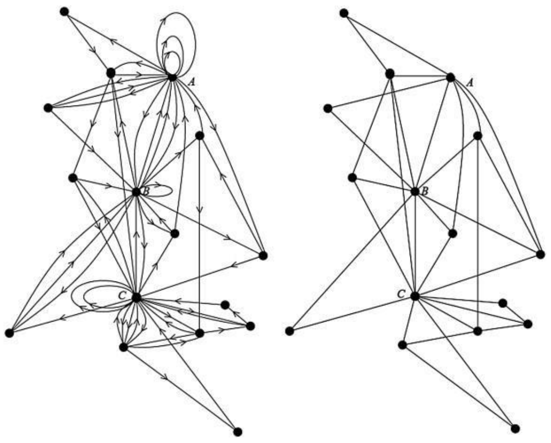


Fig. 2 - Examples of a directional graph on the left and a non-directional graph on the right. Each vertex represents a biological community, and each edge represents a connection between them (adapted from Phillips et al., 2015).

Aquatic insect abundance data were used to construct presence-absence and co-occurrence matrices. Networks of interactions between sampling sites were modeled using an adjacency matrix based on Bray-Curtis dissimilarity, with a cut-off threshold to establish connections between nodes. In addition, a directional network was structured considering the hydrographic hierarchy of the Doce River Basin, with the sub -basins connected to the main river.

Centrality metrics were calculated for the generated networks, including *degree* (degree of connectivity), *closeness*, and *betweenness* (betweenness centrality). The robustness of the network was analyzed by removing the nodes with the highest betweenness and checking the effect on the connectivity metrics and the number of components. In biological terms, the **degree** can represent the number of direct connections a species has with others in the network. In a dispersal analysis, species with high degree interact with many areas, indicating a central role in habitat connectivity; **closeness** can be interpreted as the ease with which a species can reach others within the network, with species with high closeness having greater accessibility to different habitats, indicating efficiency in dispersal and colonization of new areas; and **betweenness** measures how much a species acts as a bridge between different parts of the network, so that high betweenness connects isolated areas, facilitating gene flow and colonization of new environments (Mason and Verwoerd, 2007)

To explore the relationship between spatial characteristics and network metrics, centrality metrics were associated with the geographic coordinates of the collection points, extracted from shapefiles with the coordinates of the sampling points, and visualized on maps. The influence of geographic location was assessed by correlating spatial distances with network metrics. Finally, linear regression analyses explored relationships between betweenness and proximity. All analyses were performed using the *vegan* and *igraph* packages in the R software (R Development Core Team, 2023).

3. Results

We sampled a total of 8295 Ephemeroptera nymphs, distributed in four families and 12 genera. The most abundantgenus was *Americabaetis* Kluge, 1992 (15%), followed by *Callibaetis* Eaton, 1881 (14%) and *Paracloeodes* Day, 1955 (13%) (Table 1). The areas with and without tailings had a similar richness of genera (16 genera). Still, the area's abundance was slightly higher (55% of the total individuals) without tailings passage.

There was an effect of *betweenness* on *closeness* (p<0.01), indicating that nodes with higher centrality (*betweenness*) tend to be more connected (*closeness*) in the network. In the rainy season, there was no relationship between betweenness and *closeness*. From the visualization of the graph, we can infer that the growth in *betweenness* centrality (the number of times a vertex acts as a path between other vertices) is related to the increase in connectivity (Fig. 3).

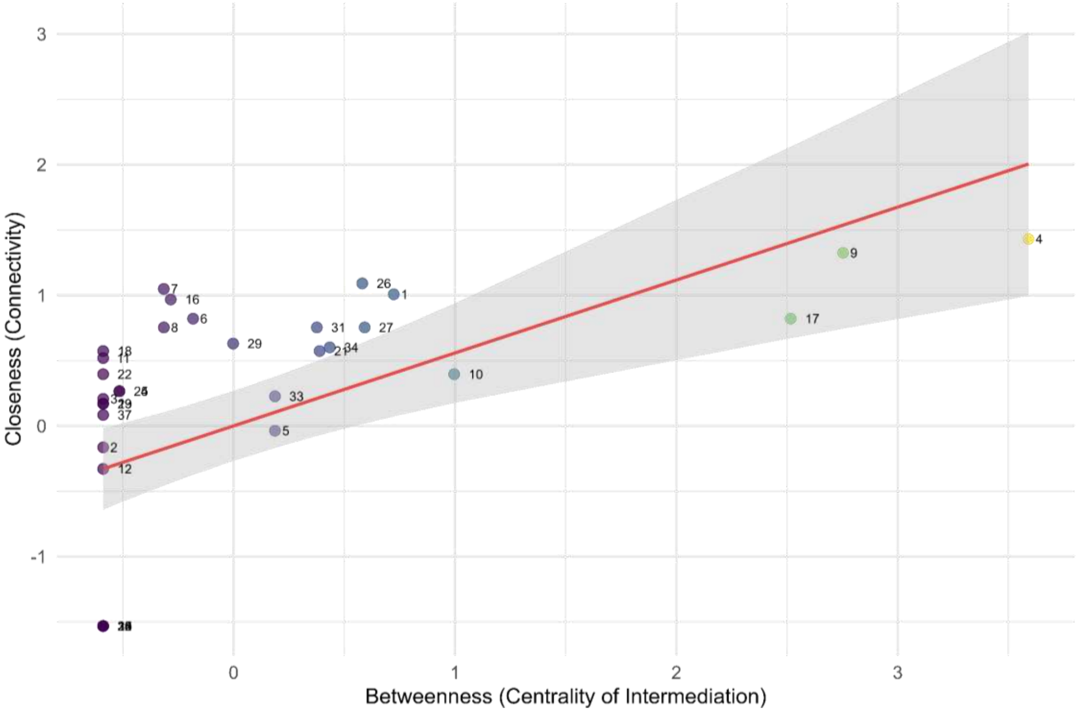


Fig 3. Gaprich representation of the regression of predicted connectivity (*closeness*) and centrality of intermediation (*betweenness*) for stream segments.

When we analyze the *betweenness* distribution, we see that many nodes have values close to zero, indicating that they are isolated or have little relevance as intermediaries in the network. This asymmetry in the distribution shows that few nodes play a central role in connecting different parts of the network. This pattern suggests that local connectivity is more important than global connectivity. The nodes with the highest *betweenness* are those that most often act as intermediaries in the shortest paths between other nodes.

The complex network highlighting *betweenness* shows the nodes that are the critical points for connecting different segments of the network, represented by higher *betweenness* values (larger circles) (Figure 4). High betweenness values, such as nodes 9, 28, 21, and 27, are essential for connecting differentnetwork parts located in the central Rio Doce region. The nodes with values close to zero, such as points 1, 5, 34, and 40, have little or no influence on the *betweenness* of the sample points. When analyzing the graph, we also observed the influence of nodes with high *betweenness* on connecting isolated regions.

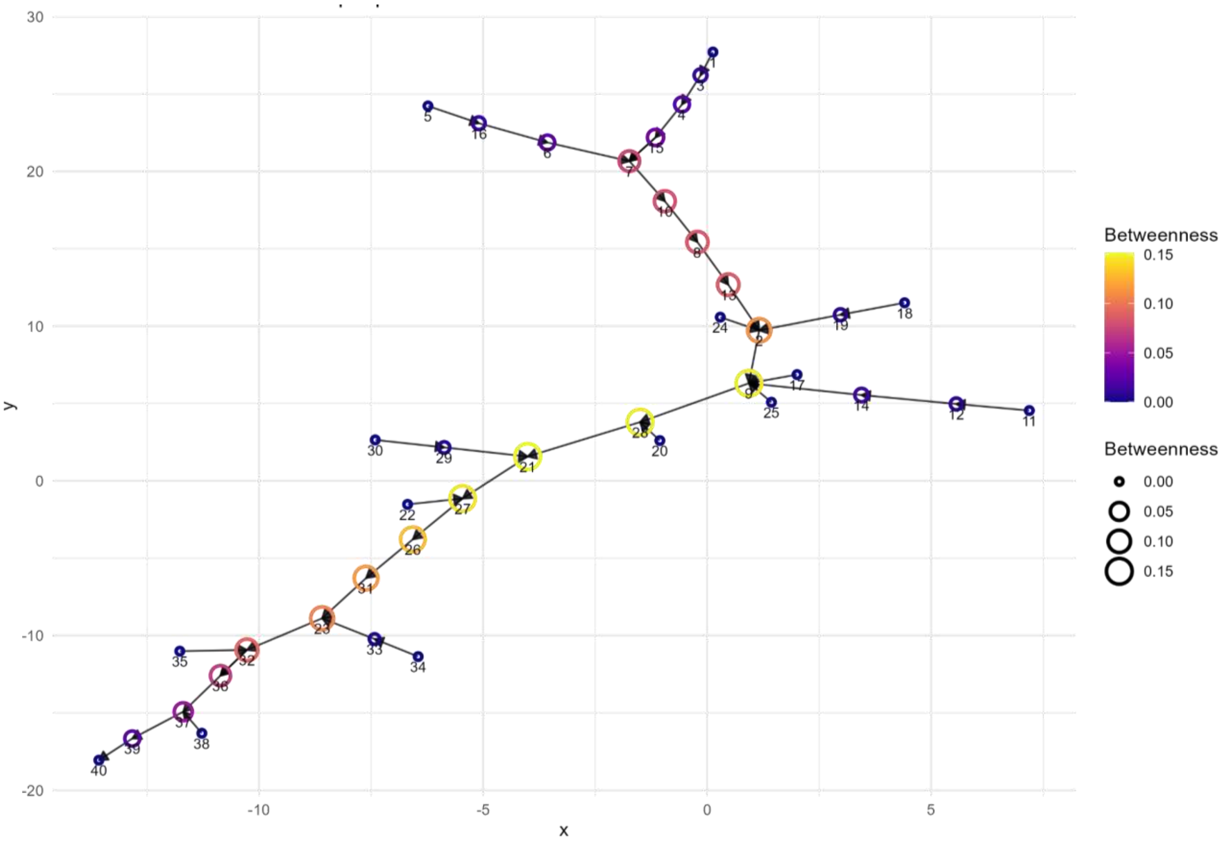


Fig 4. Representation of the directional network with *betweenness* highlights, where warmer values (yellow) and larger circles indicate higher *betweenness* values.

The species co-occurrence network is determined by the intensity of connectivity and the position of nodes in the communities (Fig. 5). The proximity of nodes in the layout suggests that they belong to the same cluster, as seen in the graph's central region. Thus, we can identify nodes 4, 9, 26, and 7 as highly connected.

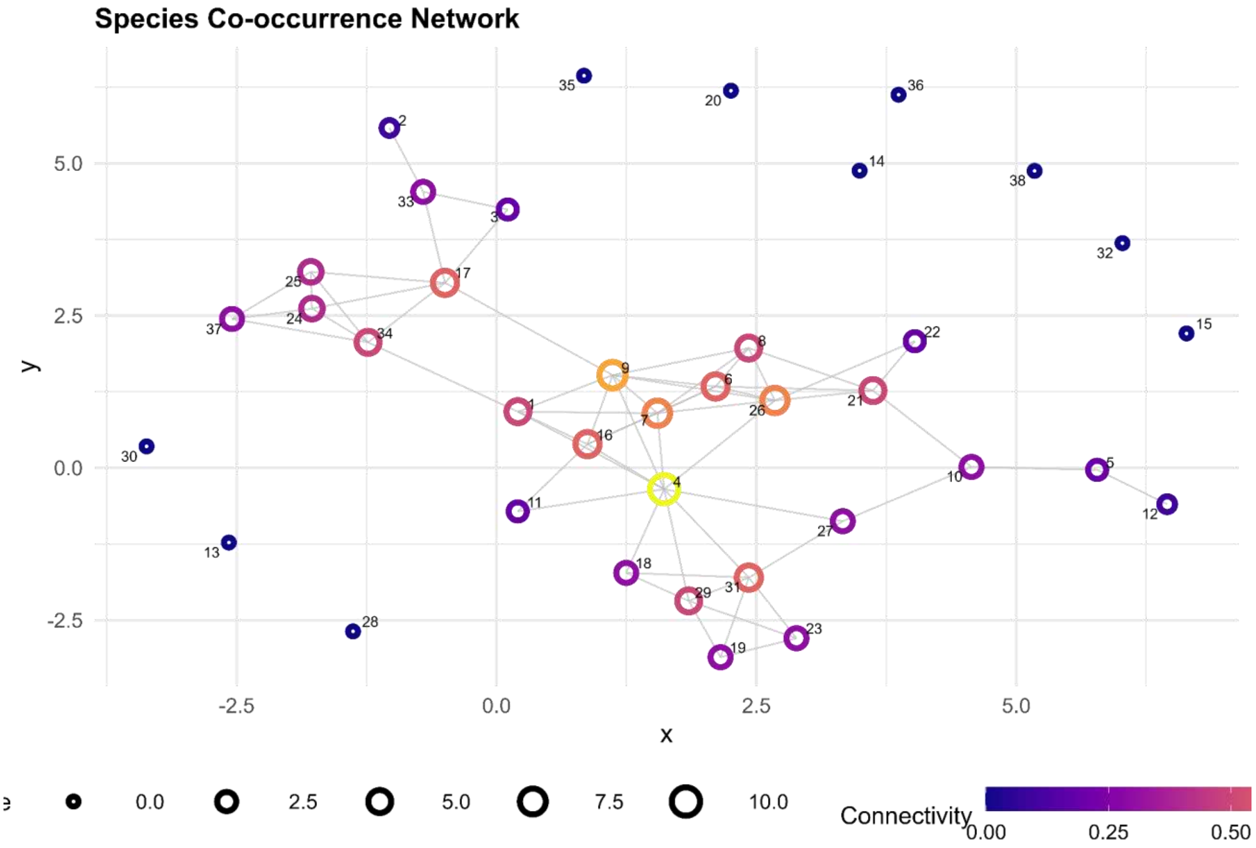
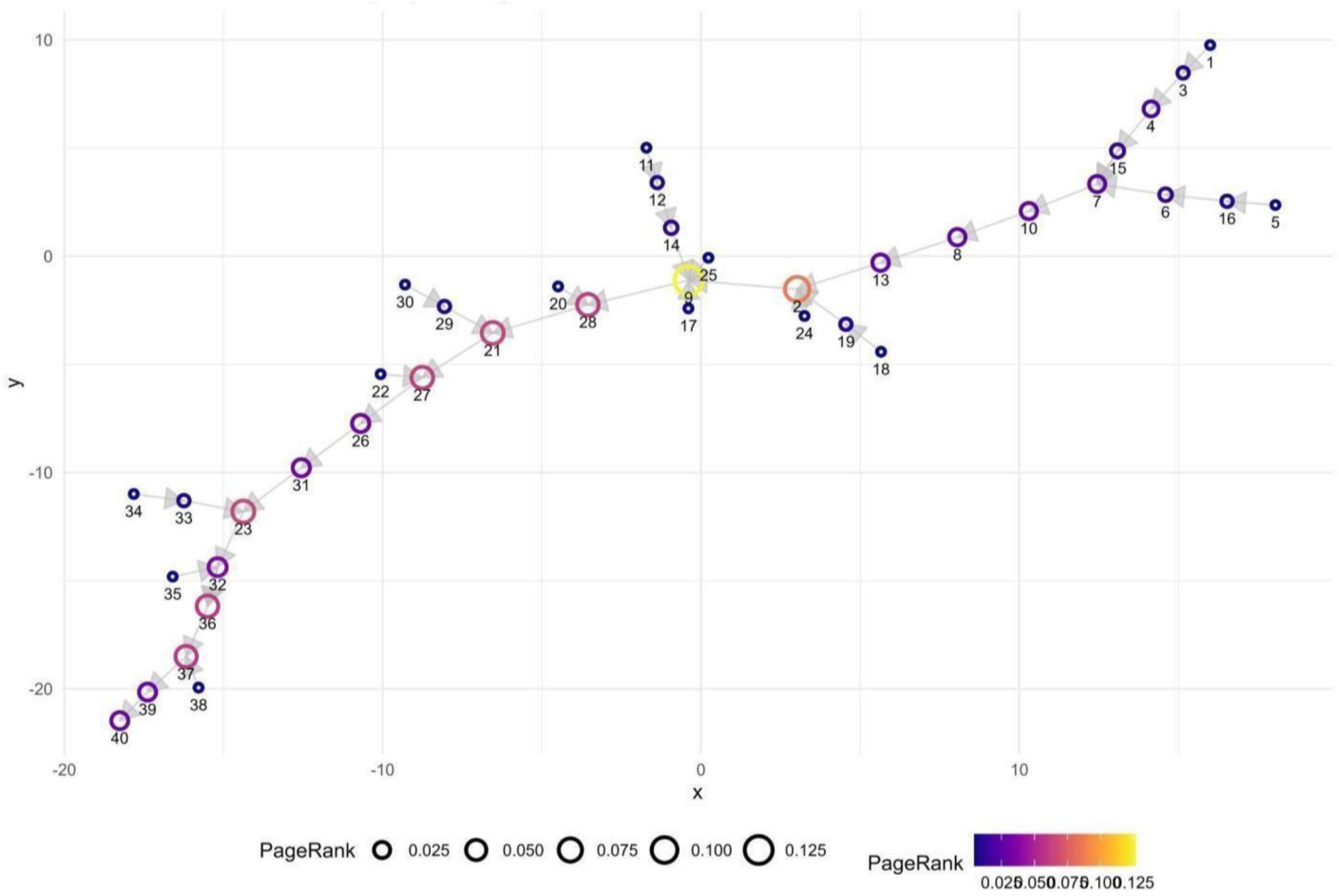


Fig 5. Graphic representation of the species co-occurrence network in the Doce River Basin. Warmer colors and larger circles represent greater connectivity.

The *PageRank* metric assesses a vertex's importance by considering both the quantity and quality of the connections it receives. In the network analyzed, we observed that the dispersion of organisms is centralized in a few highly influential nodes, such as vertices 2 and 25. In contrast, most peripheral nodes have less structural impact (Fig. 6).

Fig 6. Graphical representation of the directed network of collection points in the Doce River Basin, with PageRank highlighted. Warmer colors and larger circles represent higher PageRank values.



We used the Louvain algorithm to identify the communities in the network, and eight groups were found (Fig. 7). The distribution of *betweenness* by community (Fig. 8) shows that community 5 has the highest values, indicating critical nodes for connectivity. In contrast, communities 3 and 4 have low values, indicating less influence in mediating flows. In addition, the variability within each community shows that some groups have nodes with very different *betweenness*, such as community 7. In contrast, others, such as those belonging to community 4, are more homogeneous.

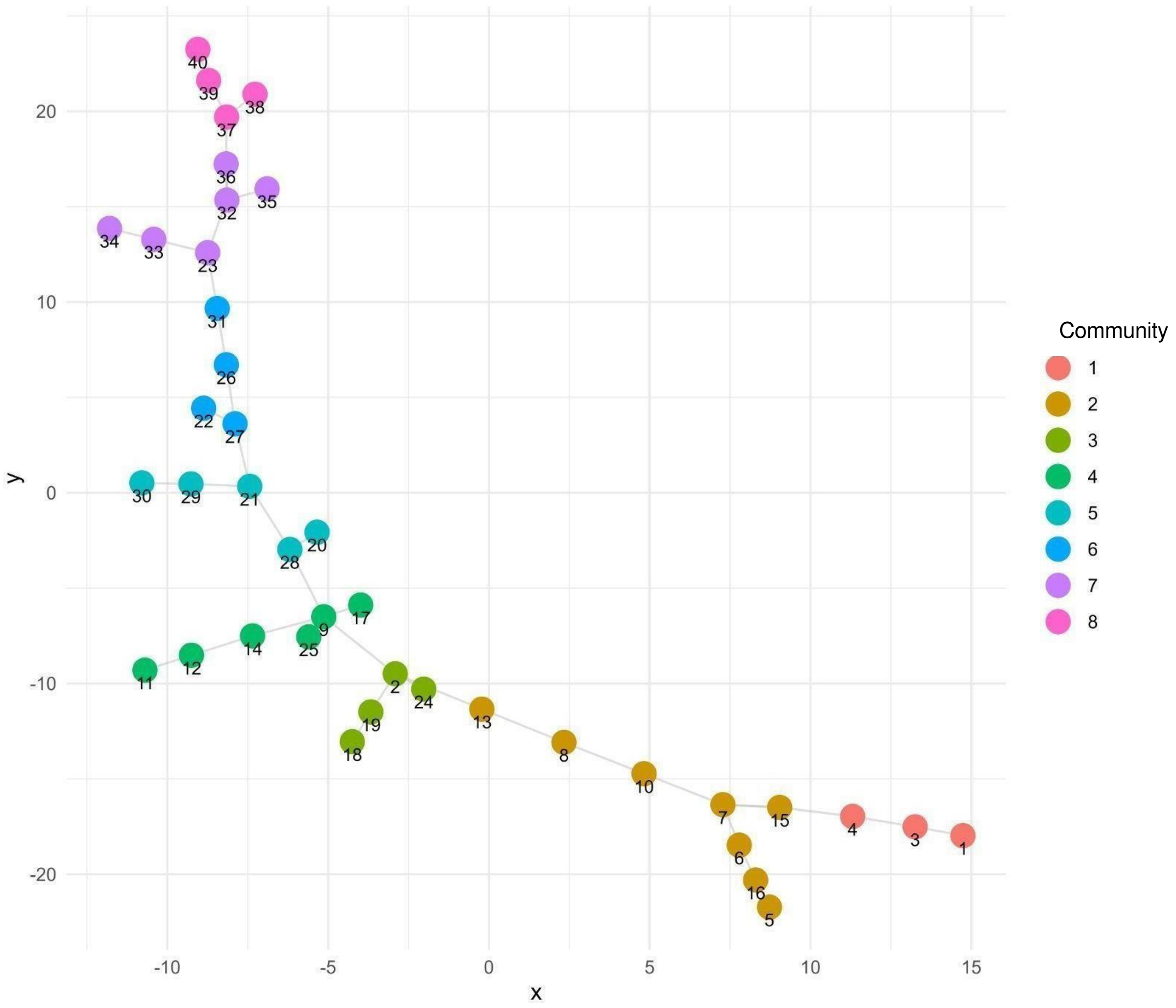


Fig 7. Graphical representation of the Rio Doce Basin communities identified through the directional graph. Each of the eight identified communities is represented by a different color.

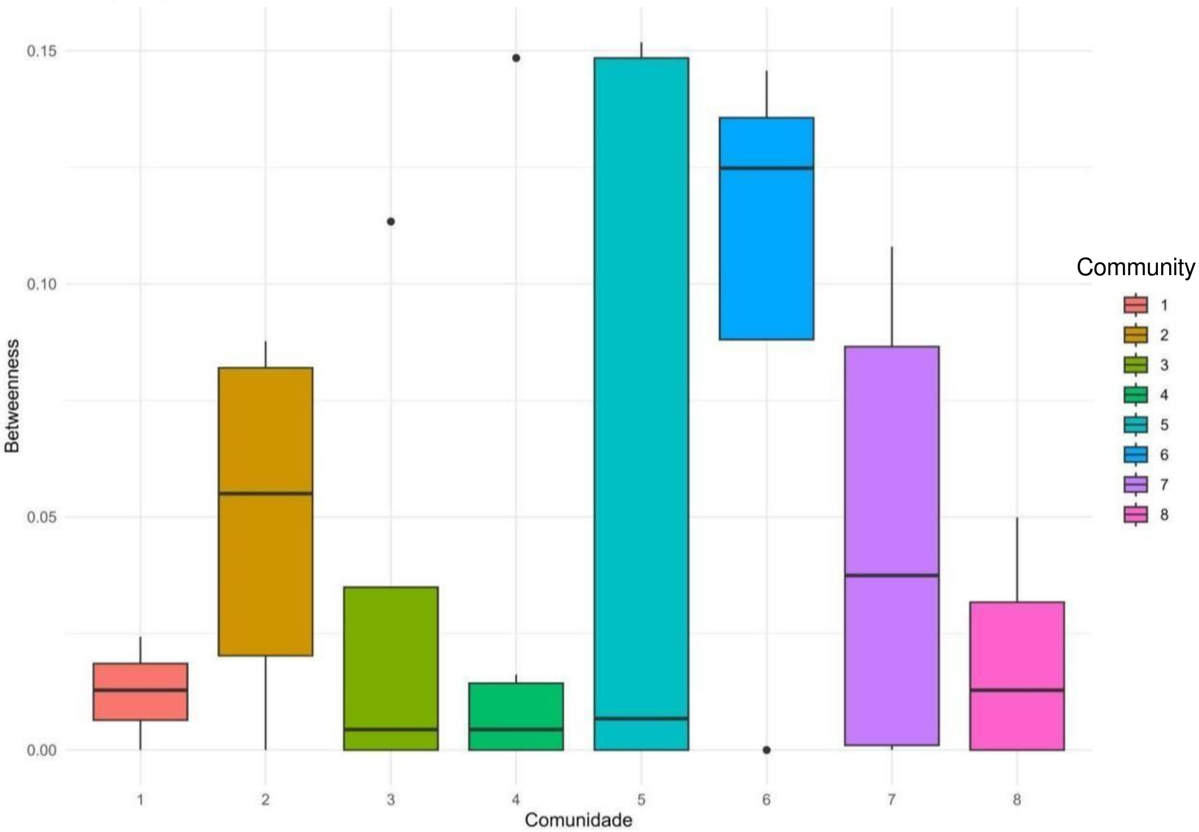


Fig 8. Bar graphic comparing the directional graph. Each of the communities identified is represented by a different color, as identified in the right side.

4. Discussion

The analysis of the *betweenness* metric, which measures how often a node acts as an intermediary between others (Bunn et al., 2000), reveals that only a few nodes play a crucial role in the network's intermediation. Most nodes present values close to zero, indicating a smaller influence on global connectivity. This pattern reinforces the idea that the network structure is modular, with certain specific nodes functioning as critical points for the interconnection of different segments.

In addition, we found a significant relationship between the *betweenness* and *closeness* metrics (fig. 3). This suggests that intermediate nodes facilitate the connection between streams or segments of the network and are also more locally connected. These results highlight the importance of regional connectivity in the dispersal dynamics of organisms. Brown and Swan (2010), when evaluating three different river basins, also highlighted the hierarchical structure of rivers as a gradient generator, highlighting the importance of the factors that structure communities so that regional effects and dispersal play a more significant role in the main channels.

The most relevant sampling points for connecting different parts of the Rio Doce watershed are located in Naque-MG (point 9), Tumiritinga-MG (point 21), Conselheiro Pena (point 17), and Governador Valadares-MG (point 28). These cities, located in the middle Rio Doce region, act as main corridors for the dispersal of aquatic insects. Thus, they are essential for maintaining the dispersal flows of these organisms throughout the basin, especially concerning the downstream dispersal dynamics.

Many sampling points showed low or no integration during the dry season. We observed this pattern in the Piranga (Guaraciaba-MG, point 5), Gualaxo do Norte (Mariana-MG, point 1), and Doce rivers (Linhares-ES, point 40). The isolation of point 40 is easily explained since it represents the mouth of the main river, as does point 1, which represents the most upstream sampling point in our sampling design. Point 5 may be isolated due to the presence of the Brito Hydroelectric Dam, located in Ponte Nova-MG, which may be limiting its functional connection with adjacent points despite its geographical proximity.

The analysis of Figure 2 reveals that the points located in the Rio Doce, especially in the middle portion, play a more significant role in the dispersal routes compared to the points in the tributary rivers. For aquatic macroinvertebrates, which depend on water flow to disperse, the assessment of dispersal centrality is essential (Brizuela et al., 2024). Thus, preserving points with high centrality, such as point 4, in the Rio Carmo, is critical to ensure species dispersal.

The assessment of the species co-occurrence network highlights the Rio Carmo (Barra Longa-MG) as the most important in terms of connectivity and relationships established with other rivers, such as the Rio Doce and the Rio Gualaxo do Norte. This result is especially relevant considering the impact of mining on the basin since this sampling point was affected by the passage of tailings. Therefore, the adequate managementof this area is essential forthe recovery of the basin and for the maintenance of the dispersal flow of the Ephemeroptera species.

The high connectivity between points 4 and 9 highlights their importance in mitigating the impacts of the dam collapse. It also highlights the crucial role of the connections between the tributary rivers and the Rio Doce for the stability of the community. The tributaries not affected by the tailings serve as sources of replenishment, while the Rio Doce acts as a sink. The unsuitability of these locations can compromise species' survival in degraded environments, such as those that have suffered the passage of mining tailings.

Dispersal is one of the three processes that limit the biodiversity of local communities (Mittelbach and McGill, 2019). After passing through the dispersion filter, the abiotic process is crucial in the establishment of species in the location (Vellend, 2010). Even though these processes work simultaneously, after passing through the dispersion filter, the non-adequacy related to abiotic processes, such as substrate availability and water temperature, for example, can be an impediment to the establishment of species at the site in the long term (Brizuela et al., 2024).

If we consider genera that are rare in the Doce River Basin, such as *Rivudiva* Lugo-Ortiz & McCafferty, 1998, the presence of ecological corridors and intermediate habitats is essential to maintain viable populations (Walz, 2011). Points with a high degree of intermediation in the network, where there are several possible dispersal pathways, such as points 16 and 18, the only ones where the genus *Waltzoyphius* McCafferty & Lugo-Ortiz, 1995 was collected, may have low connectivity between species, due to limitations related to the abiotic characteristics of the site.

Points 18 and 19, located in the Casca River, presented a high *betweenness*, suggesting they connect different river segments. However, the low proximity of these points concerning all others in the network (low closeness) may explain the exclusive occurrence of genera such as *Waltzoyphius* and *Criptonympha* Lugo-Ortiz & McCafferty, 1998. However, it should also be taken into account that not all Ephemeroptera species will be good dispersers, unlike other groups of aquatic insects that have a high dispersal potential, such as those of the order Trichoptera (Lancaster et al., 2024).

In the PageRank graph, points 2 in the Rio Doce and 25 in the Rio Santo Antônio stand out for presenting the highest values, indicating that they are critical areas for conservation, as they influence the overall connectivity of the network. Disturbing points close to these areas can result in significant impacts on connectivity. The study by Brizuela and collaborators (2024) demonstrated that restoration focused on this aspect increased the importance of downstream areas for the connectivity of aquatic macroinvertebrates. Thus, the most relevant point for population flow is not only the already preserved pointbut also the one that serves as a path between several preserved points since most peripheral nodes have a smaller structural impact. The results show that the structure of the dispersal network of the Ephemeroptera metacommunity in the Rio Doce Basin has points that play a critical role in regional connectivity. Sullivan and Manning (2019) found that removing sensitive functional groups of aquatic insects can significantly reduce ecosystem connectivity, with cascading effects on the food chain. Identifying areas of low integration, such as the Gualaxo do Norte River and the Piranga River, represent structural barriers that can limit connectivity. The great influence of the Carmo River on connectivity reinforces the need for management strategies aimed at recovering areas impacted by mining.

5. Final remarks

The dispersal flows of the Ephemeroptera metacommunity in the Rio Doce Basin are structured modularly, with some points playing a critical role in regional connectivity. Locations such as Naque-MG (point 9), Tumiritinga-MG (point 21), Conselheiro Pena (point 17), and Governador Valadares-MG (point 28) act as main corridors, especially in the middle portion of the basin, facilitating downstream dispersal.

In contrast, stretches such as the Piranga (point 5) and Gualaxo do Norte (point 1) rivers present low integration. The network structure highlights the importance of regional connectivity for maintaining biodiversity. The Carmo River (point 4) is essential for the connection between rivers affected and unaffected by tailings, functioning as a source of replacement for impacted species. In addition, the dispersal of species can also be limited by regional factors, such as abiotic factors that can restrict their establishment even after crossing physical barriers.

Suggestions forfuture studies include the construction of complex networks that consider dams and other structures along the river that may be impacting structural connectivity; the use of a multilayer approach to assess seasonal changes in structural connectivity; and sampling in different locations of the subbasins and new subbasins. In addition, the assessment of the dispersal of adult mayflies can act as a complementary assessment since the direction of dispersal is the opposite of that of immatures. We conclude that it is essential to consider landscape connectivity, the dispersal of organisms, and the variation in the intensity of environmental selection. These practices can improve the effectiveness of management practices, as well as ensure the stability of the mayflies' community in the Rio Doce Basin.

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CHAPTER 04

Ecological Niche and Distribution Projections of Four Mayfly Species in the Rio Doce Basin Under Climate Change

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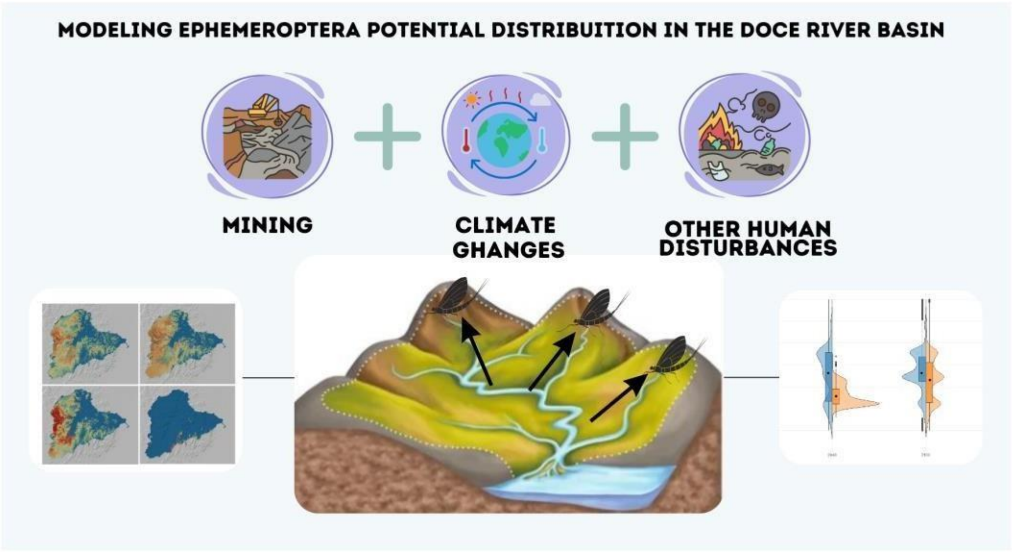
Highlights

- Four Ephemeroptera species (*Apobaetis irai, Campsurus cotaxe, Paramaka convexa* and *Tricorythodes santarita*) were modeled under four future climate scenarios.
- The projections indicate that the species' future distribution areas do not coincide with conservation units, increasing the vulnerability of these populations.
- The Rio Doce Basin, already severely affected by the passage of mining tailings, will face even more challenges due to the intensification of climate impacts in interaction with human activities.
- Species tend to migrate to higher altitudes, seeking climatic refuges.
- The Rio Santo Antônio sub-basin is potentially home to three of the four species modeled, but faces intense pressure from mining and hydroelectric dams, requiring urgent conservation.
- Protecting these four species means preserving the Basin's ecosystem services and aquatic biodiversity, reinforcing the need for conservation measures and continuous monitoring.

Graphical abstract



Abstract

Rio Doce basin hosts a wide variety of Ephemeroptera, aquatic insects known for their sensitivity to water quality. The region suffers from pollution caused by mining activities and other anthropogenic impacts. The present study used ecological niche modeling to project the current and future distribution of four species (*Apobaetis irai, Campsurus cotaxe, Paramaka convexa* and *Tricorythodes santarita*) under four different climate scenarios. The results show that these species tend to migrate to higher elevations but face habitat loss and niche reduction by 2100. Future ranges do not coincide with protected areas, highlighting a conservation gap for these species. The Rio Santo Antônio sub-basin stands out as a priority area for conservation, as it is home to three of the four species studied, but is under heavy pressure from mining and hydroelectric dams. In order to maintain the biodiversity and ecological functions of the Rio Doce Basin, the conservation of these species is essential. Effective action will ensure the species conservation and the ecological stability of the region.

Keywords: habitat loss, aquatic insects, environmental management, Ephemeroptera

1. Introduction

Ephemeroptera is an order of cosmopolitan aquatic insects whose presence in different aquatic environments highlights their ecological and functional importance. In Brazil, the order comprises ten families, 81 genera, and 428 species (Salles et al., 2024). Among the most diverse families, Baetidae and Leptophlebiidae stand out, representing more than half of the Brazilian species (Salles et al., 2024). Leptohyphidae follows them (Dominguez, 2006), and Polymitarcyidae has a great representative family among the group of bigger mayflies, like Euthyplociidae (Miller et al., 2018).

Because of their resilience and life cycle flexibility, mayflies have successfully colonized diverse freshwater habitats (Niedrist & Füreder, 2023). Ephemeroptera are an important bioindicator of water quality due to species-specific responses to environmental degradation and also play an indispensable role in ecosystem services, such as filtering toxins from water before transitioning to terrestrial ecosystems (Jacobus et al., 2019).

Freshwater ecosystems, on which Ephemeroptera depend for part of their life cycle, are particularly vulnerable to anthropogenic changes. They have higher extinction rates and population decline than other natural environments (Almond et al., 2020). The communities that live in aquatic ecosystems are susceptible to these alterations (Jacobsen et al., 2008), which may lead to substantial losses in their biodiversity.

Aquatic ecosystems in the Atlantic Forest exhibit exceptional diversity and high endemism (Padial et al., 2021). Beyond ongoing threats from human activities and a history of degradation, this biome suffered Brazil's worst environmental disaster—the 2015 mining tailings spill that polluted the Rio Doce Basin (Milanez & Losekann, 2016). This is especially important for the groups we're studying, such as Leptohyphidae and Polymitariidae, in which nymphs live on the bottom substrate. This devastating event has created an alarming scenario for biological communities, especially when combined with the consequences of climate change (Poff et al., 2002).

When selective pressures outweigh species interactions, environmental changes can reshape communities by replacing specialized species for more tolerant ones, or by driving species loss (Vellend, 2010). Climate change intensifies other stressors in freshwater ecosystems, including land-use change, overexploitation, and biological invasions (Sage, 2020; Souza et al., 2024). In tropical freshwaters, human impacts combined with climate change create new challenges for conservation. (Urban, 2015; Stella and Bendix, 2019).

Climate change scenarios drive major shifts in species physiology, behavior, and freshwater ecosystem diversity and function (Woodward et al., 2010). Species respond to rising temperatures by shifting distribution, changing phenology, or reducing body size (Gallegos-Sanchez et al., 2022). Despite their vulnerability, freshwater ecosystems-especially in the tropics-remain understudied in climate impact research (Pires et al., 2018; Tollefson, 2022). By the end of the current century, projections for the Atlantic Rainforest indicate a temperature rise of 4.8-5.6°C, with precipitation decreasing in north while increasing in south and southeast (Vale et al., 2021).

Ecological Niche Modeling is a widely used tool for understanding how climatic and environmental factors influence species distribution and community richness dynamics (Elith & Leathwick, 2009; Elith et al., 2011). Beyond predicting species distributions, it allows for biodiversity estimation under different scenarios, supporting the identification of conservation priority areas (Zhang et al., 2012).

Applying current and future distribution models in climate change scenarios can enhances understanding of Ephemeroptera species and geographic distribution. Additionally, it helps assess conservation strategies for other species inhabiting tropical streams impacted by mining. In this study, using ecological niche modeling (ENM), we aim to answer the following questions: What is the current distribution of Ephemeroptera in the Rio Doce Basin? How will climate change impact the distribution range and species richness? What will be the implications for the ecological dynamics of Ephemeroptera and biomonitoring using Ephemeroptera?

2. Material and methods

2.1. Study Area

Situated in southeastern Brazil, the Rio Doce Basin spans 86,000 km² across the states of Minas Gerais and Espírito Santo, making it one of the region's largest basins. It is entirely within the Atlantic Forest biome, a forest ecosystem that has been reduced to less than 23% of its original extent (Vancine et al., 2024). The Rio Doce Basin is divided into three regions: Upper, Middle, and Lower. The Upper Rio Doce extends from its sources to the confluence with the Rio Piracicaba, near Ipatinga (MG). The Middle stretches from this confluence to the Minas Gerais–Espírito Santo border, and the Lower runs from this border to the river's mouth in the Atlantic Ocean.

2.2. Occurrence data

A total of 509 occurrence records of Ephemeroptera species were compiled from field sampling carried out at 40 sites throughout the Rio Doce (Fig. 1); specimens deposited in the collection of the UFV Entomology Museum; and data from the Global Biodiversity Information Facility platform (GBIF, 2024), filtered from the polygon between coordinates 20º 19' S and 20º 37' S and 41º 43' W and 41º 53' W, which delimit the Rio Doce Basin. To minimize spatial autocorrelation between occurrences, records were spatially filtered based on a 5 km buffer (Cobos et al. 2019).

Four species, with more than six records, were selected for distribution modeling of the sixty-nine species recorded. The species to be modeled were selected from Brazil's four most diverse families: Baetidae, Leptophebiidae, Leptohyphidae and Polymitarcyidae. The species of Baetidae was the most recently described for the area, *Apobaetis irai* De Lima, Massariol, Cruz & Hamada, 2024 (9 occurrences), whose descriptive article hypothesizes that it is endangered (De Lima et al., 2024). For Leptophlebiidae, the choice of *Paramaka convexa* (Spieth, 1943) (6 occurrences) was based on the lack of previous records of this species in the Atlantic Forest until the present work, besides its inclusion on the list of threatened species in the Bacia di Rio Doce (Drummond et al., 2021). *Tricorythodes santarita* Traver, 1959 (21 occurrences) was selected from the family Leptohyphidae, also widely distributed in the Basin. Within Polymitarcyidae, the criterion used for the selection was the species with the widest distribution, *Campsurus cotaxe* Molineri & Salles, 2017 (8 occurrences).

The species were also selected based on their natural history. We have two filter-feeding species, one that lives on the surface of the substrate, *Paramaka convexa*, and *Campsurus cotaxe,* that lives burrowed into the substrate. *Apobaetis irai* lives in sand, in backwater areas, and *Tricorythodes santarita* has a scraping feeding habit and is predominant in watercourses with little canopy cover (Baptista et al., 2006).

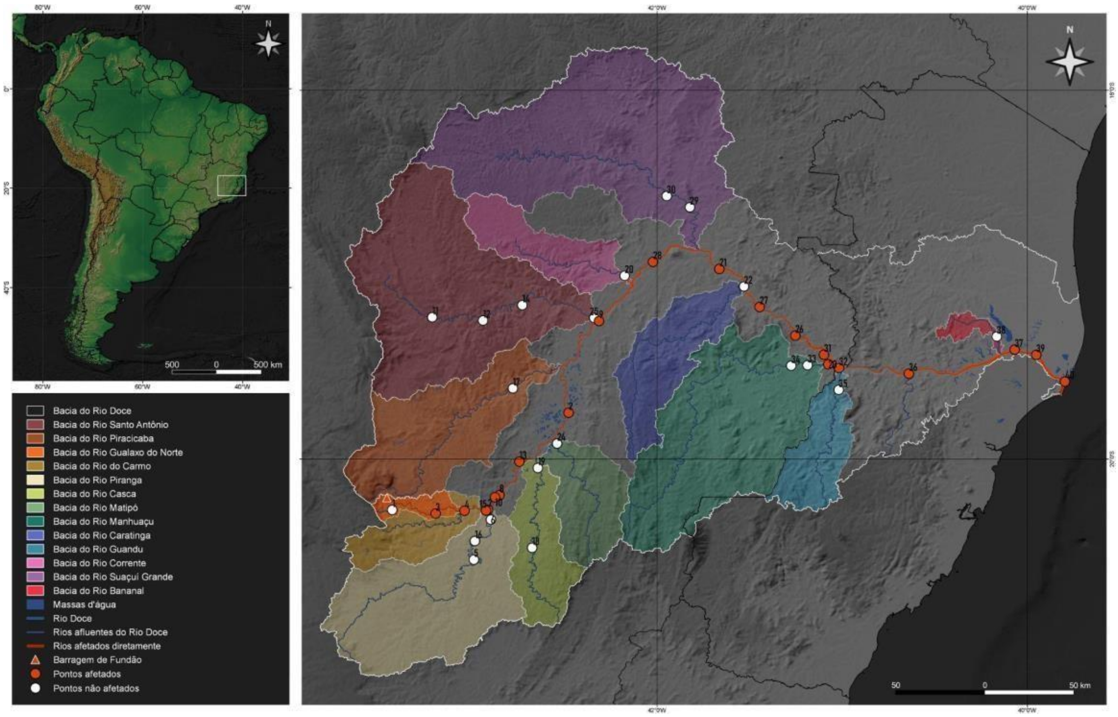


Fig. 1. Map of the Rio Doce Basin with the location of the sampling points.

2.3. Ecological Niche Modeling

Each of the three occurrence databases was used for niche differentiation tests. The data were split into 75% for training and 25% for testing the ecological niche model. A similar method was applied to distribution modeling, with a calibration area deliberately defined along the Rio Doce Basin. The geographic distribution of Ephemeroptera species was estimated using ecological niche modeling (ENM), which correlates species presence records with environmental variables to assess habitat suitability (Soberon & Peterson, 2005).

To define abiotic components, 19 bioclimatic variables from WorldClim (Hijmans et al. 2005) were used at a 30-second resolution. The environmental layers were clipped to the calibration area, and highly correlated variables (r ≥ 0.80) were filtered using the "ntbox" package in R (Osorio-Olvera et al. 2020). Bioclimatic variables selected through correlation analysis were used for species modeling. Future climate projections were incorporated from the EC-Earth and CMCC-ESM2 models in WorldClim v2.1The selected bioclimatic variables were averaged for each model within SSP1-2.6 and SSP5-8.5 scenarios, enhancing the robustness of species distribution estimates under different climate futures.

SSP1-2.6 and SSP5-8.5 represent contrasting climate trajectories. SSP1-2.6, an optimistic scenario, projects reduced emissions and a shift to renewable energy, with temperature increases of 1.3°C by 2040 and 1.8°C by 2100. SSP5-8.5, a high-emission scenario reliant on fossil fuels, could raise temperatures by 2.0°C by 2040 and over 4.4°C by 2100, intensifying extreme events.

The best ENMs were calibrated and selected using the maximum entropy method in Maxent v3.4.4 (Phillips et al. 2006) with the R package "kuenm" (Cobos et al. 2019). Candidate models were generated by combining four regularization multipliers (0, 0.5, 1, 2) with all possible combinations of four feature classes (linear, quadratic, product, and hinge).

Candidate models were evaluated based on significance (partial ROC with 100 iterations and 50% bootstrapped data), omission rates (E = 5%), and complexity (AICc). The best models met two criteria: (1) significance and (2) omission rates ≤ 5%. From these, final models were those with delta AICc ≤ 2.

The final model was created using five bootstrap replications with logistic outputs, incorporating all occurrences and selected parameterizations. The relative contribution of each environmental factor was assessed through percentage contribution, permutation importance, and jackknife regularized training gain.

The final model was applied to the calibration area under future climate scenarios RCP 2.6 and RCP 8.5 for 2041–2060 and 2081–2100. To account for projection uncertainties, two climate models (MIROC6 and HadGEM3-GC31-LL) were used independently, and their rasterized results were averaged for each scenario. Climate layers from the Community Climate System Model (CCSM6) were also utilized.

A MESS (Multivariate Environmental Similarity Surface) analysis (Elith et al. 2010) was conducted to detect areas at risk of extrapolation in model transfers. This method identifies regions with significant environmental differences where predictions may be unreliable. To prevent over-interpretation, we excluded model fits in strictly extrapolated areas (Cobos et al. 2019).

Species distribution was characterized by quantifying its proportion across different altitudes in current and future scenarios. An elevation layer was obtained from a 1 km resolution global DEM, derived from 30 arc-second SRTM30 data (USGS) produced by NASA’s Shuttle Radar Topography Mission (SRTM).

Niche overlap reflects species interactions, competition, and distribution patterns, helping infer coexistence and responses to environmental changes. It also highlights how environmental factors shape current and future distributions. Using the Wallace package in R, niche overlap was assessed with Schoener’s D and Warren et al.'s I indices.

Additionally, environmental ordination and occurrence density grids visualized species distribution in environmental space. Principal Component Analysis (PCA-env) further illustrated niche overlap, enabling direct species comparisons.

3. Results

3.1. Ecological niche modeling estimates and Habitat suitability

3.1.1. *Campsurus cotaxe* Molineri & Salles, 2017

Correlation analysis allowed the selection of the least correlated bioclimatic variables for niche modeling, ensuring a reduction of redundancy in the environmental data. With a correlation threshold of 0.85, the variables selected for modeling were BIO1 (mean annual temperature), BIO4 (temperature seasonality), BIO5 (maximum temperature of the hottest month), BIO6 (minimum temperature of the coldest month), and BIO13 (precipitation of the wettest month).

Modeling the current potential distribution of *Campsurus cotaxe* in the Rio Doce Basin showed an average habitat suitability of 0.26 (±0.19). Only 3,89% of the total area showed suitability values higherthan 0.7, indicating thatthe basin's highly favorable areas for *C. cotaxe* are limited. Our results indicate that the species finds more outstanding environmental suitability in the Upper and Middle Rio Doce, especially in the Upper Rio Doce (deep red region), where the proportion of the occupied area is more significant (Fig. 2).

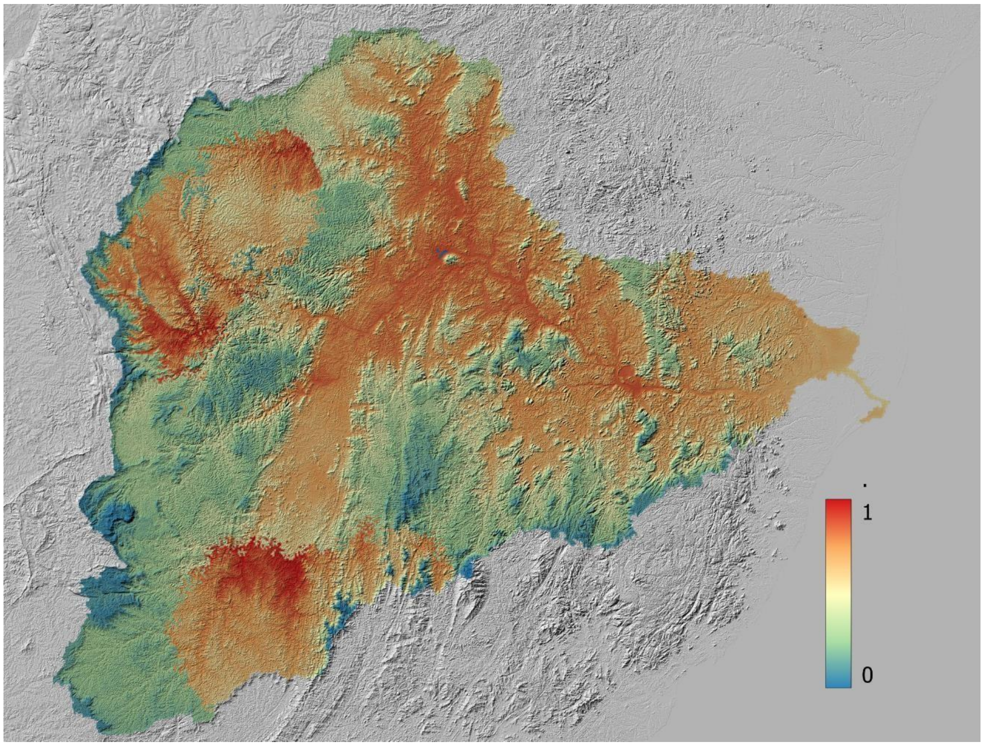


Fig.2. Map of the potential distribution of *Campsurus cotaxe* in the Rio Doce Basin, obtained by ecological niche modeling. The color scale represents environmental suitability, ranging from low (blue) to high (red).

In the optimistic scenario, the species maintains a wide distribution in the basin in 2040, with areas of high environmental suitability concentrated mainly in higherelevation regions (Fig. 3A). There is a slight reduction in suitable areas in 2100. However, the species maintains a significant niche within the basin (Fig. 3B), suggesting some resilience to less severe climate change. In this optimistic scenario, *Campsurus cotaxe* expands its presence at low and high altitudes, while intermediate areas become less favorable over time. The persistence of losses between 500-1000m indicates that these regions may undergo environmental changes that make it difficult for the species to occupy them (Fig. 4A).

In the pessimistic scenario, there is a more significant loss of suitable habitat in 2040, with areas of high suitability already reduced compared to the optimistic scenario (Fig. 3C). By 2100, the distribution of the species is drastically reduced, with almost the entire basin becoming unsuitable (predominantly blue), indicating a high risk of local extinction (Fig. 3D). *Campsurus cotaxe* progressively loses suitability at middle and high altitudes and is pushed to low altitudes (0-300m). In 2100, a severe reduction in distribution could increase the species' vulnerability, making it more susceptible to extreme environmental events and habitat degradation (Fig. 4B).

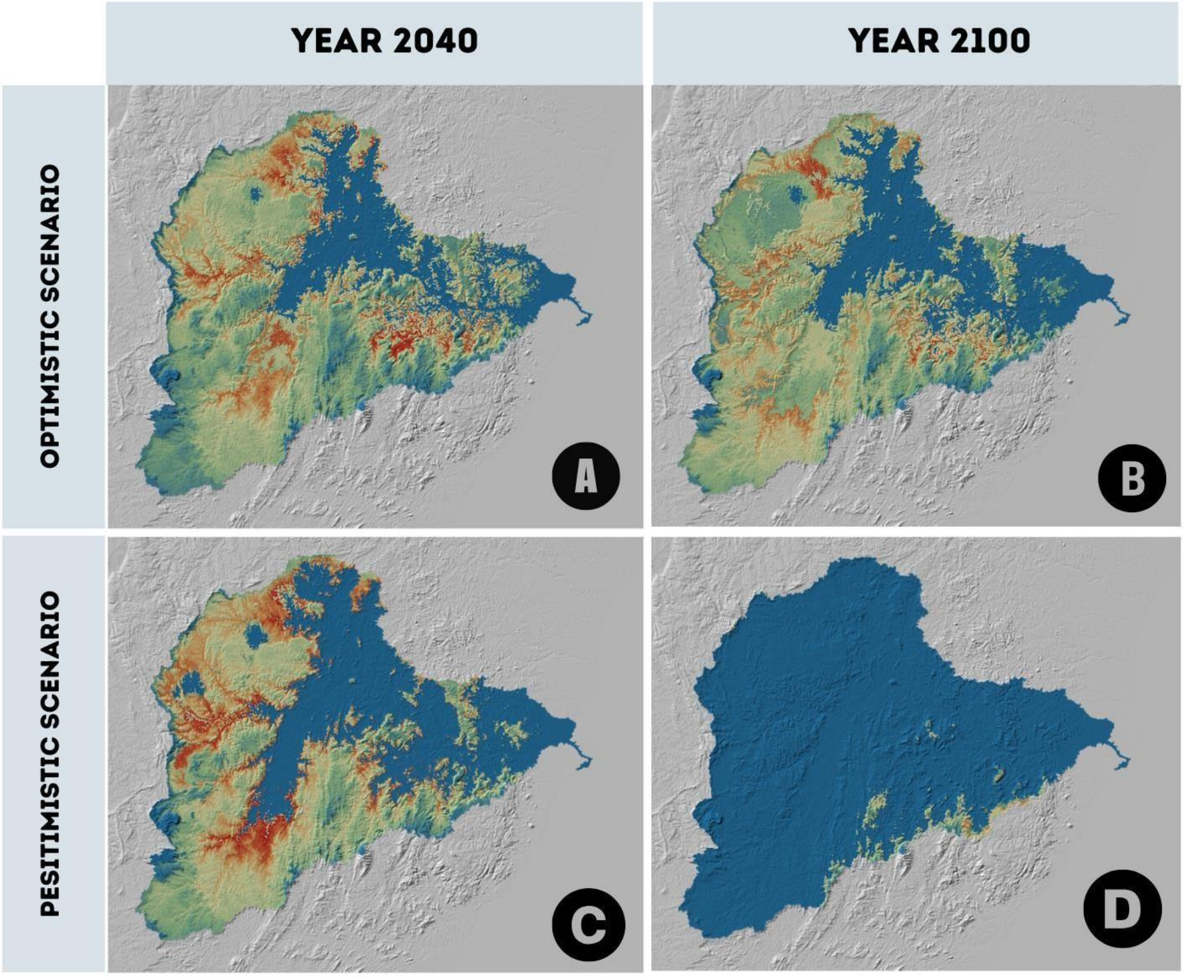


Fig 3. Projection of the geographic distribution of *Campsurus cotaxe* under different climate scenarios and future time periods. Colors indicate environmental suitability for the species, with warmer regions representing a higher probability of occurrence.

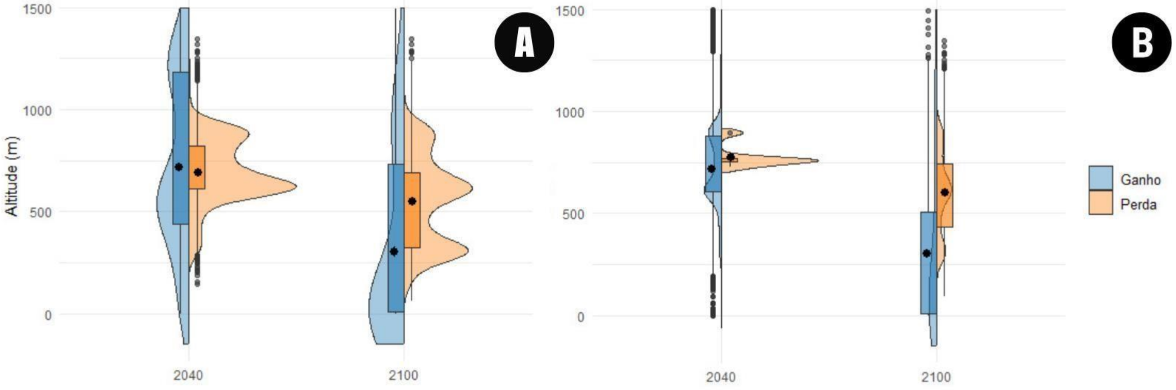


Fig 4. Violin graphs showing the altitudinal variation of areas of gain (blue) and loss (orange) for *Campsurus cotaxe* in the optimistic (A) and pessimistic (B) scenarios for the years 2040 and 2100. The vertical axis indicates elevation (m), while the distribution of colors represents regions where environmental suitability has expanded or contracted over time.

3.1.2. Apobaetis irai **De Lima, Massariol, Cruz & Hamada, 2022**

Correlation analysis was used to model the Apobaetis irai species and select the least correlated bioclimatic variables to minimize redundancy in the environmental data. The variables selected were BIO1 (average annual temperature), BIO2 (average daily thermal amplitude), BIO3 (isothermality), BIO5 (maximum temperature of the warmest month), BIO6 (minimum temperature of the coldest month), BIO10 (average temperature of the warmest quarter), BIO12 (annual precipitation), BIO13 (precipitation of the wettest month), BIO15 (seasonality of precipitation), BIO18 (precipitation of the warmest quarter), and BIO19 (precipitation of the coldest quarter).

Only 11,5% of the total area had values higher than 0.7, indicating that highly favorable habitats for the species are scarce in the basin. The Upper and Middle Rio Doce represent the main habitats favorable to the species in the Basin (Fig. 5).

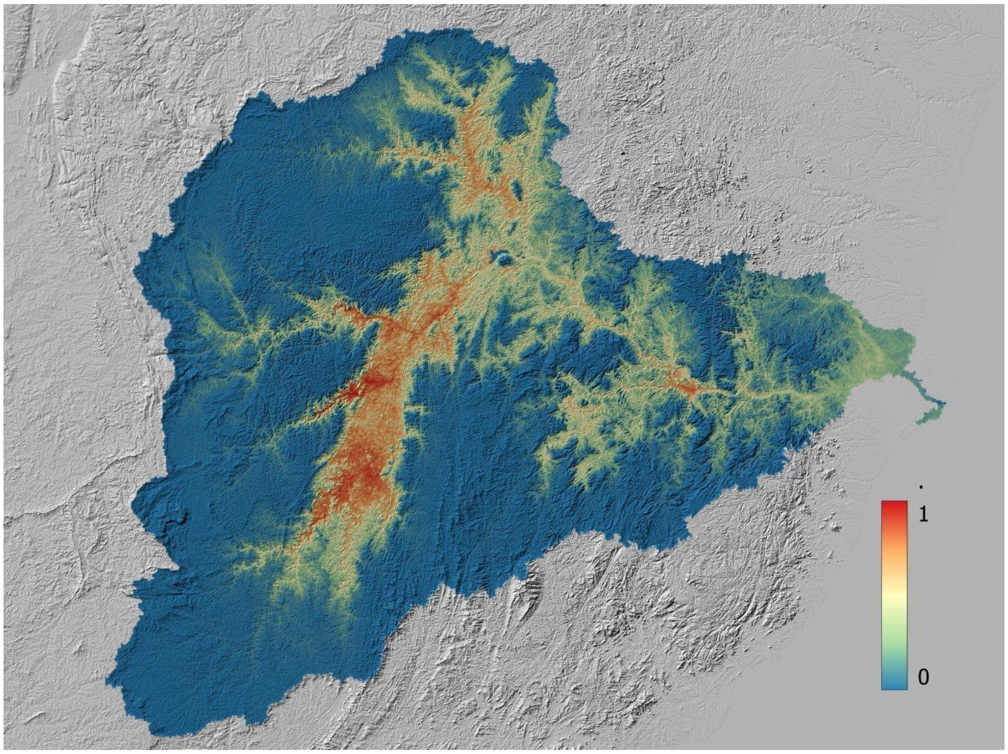


Fig 5. Map of the potential distribution of *Apobaetis irai* in the Rio Doce Basin, obtained by ecological niche modeling. The color scale represents environmental suitability, ranging from low (blue) to high (red). White dots indicate the occurrence records used in the modeling.

When we look at the optimistic scenario, the species maintains a relatively wide distribution in 2040, with areas of high suitability concentrated mainly in the central and higher regions of the basin (Fig. 6A). By 2100, environmental suitability decreases slightly, but the species still maintains some favorable areas, suggesting a moderate reduction in suitable habitat. The optimistic 2040 scenario shows gains mainly at intermediate altitudes (up to 500m), suggesting expansion into these areas. In 2100, there was a redistribution, with gains distributed more at higher altitudes (between 1000 and 1500m), suggesting that the species may find new suitable areas at higher altitudes. Losses occur mainly at intermediate altitudes; this pattern continues in 2100 (Fig. 7A).

There is a more pronounced reduction in environmental suitability, with a significant loss of highly suitable areas, especially at intermediate altitudes, in the pessimistic scenario of 2040 (Fig. 6C). The reduction in suitable habitat is drastic in the pessimistic scenario of 2100, with most of the basin becoming unsuitable (predominantly blue), indicating a high risk of local extinction of species (Fig. 6D). In the pessimistic scenario, *Apobaetis irai* progressively loses suitability at middle and high altitudes and is pushed to low altitude areas (0-300m) (Fig. 7B). The drastic decline in habitat suggests that the species may become highly vulnerable to environmental degradation as its distribution becomes increasingly restricted to limited areas.

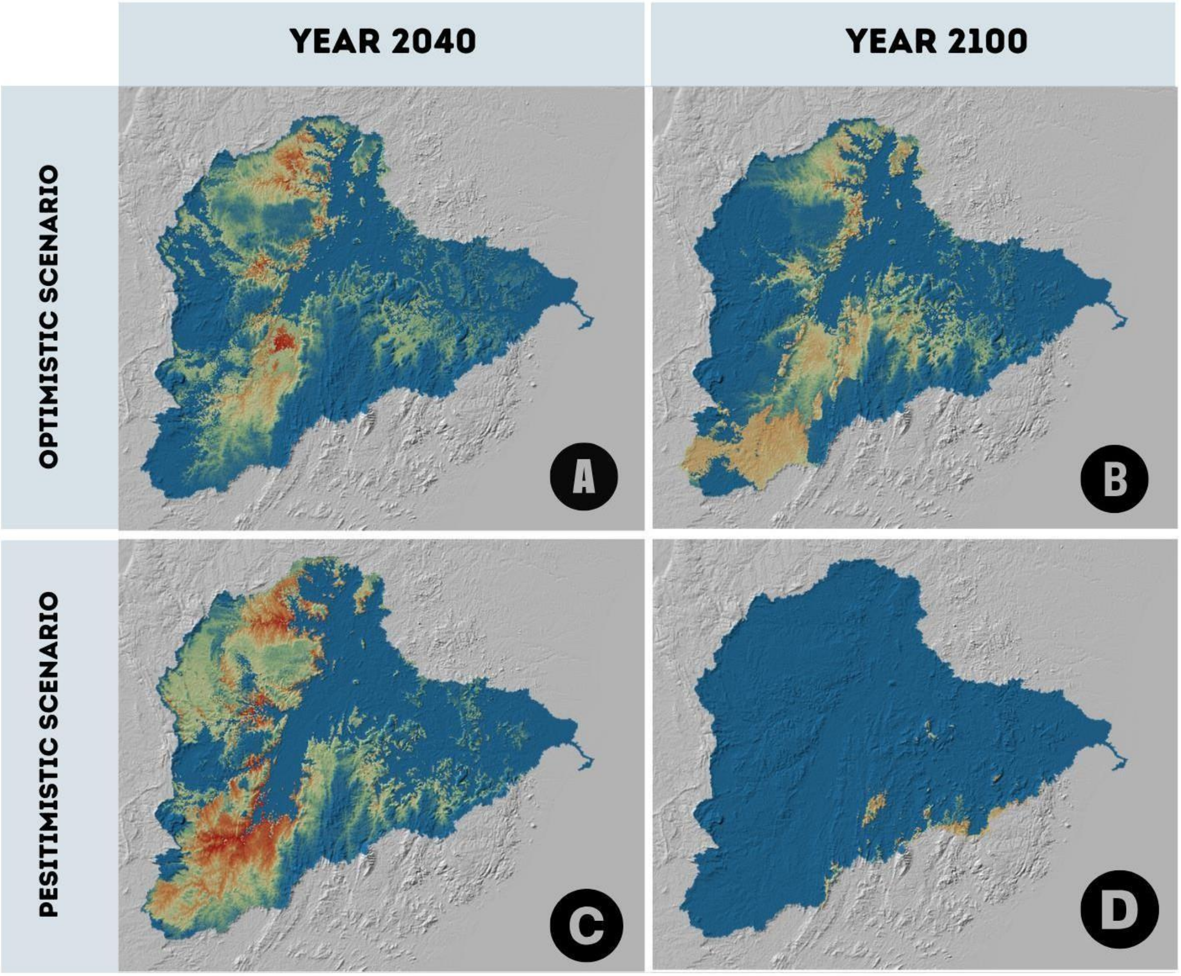


Fig. 6. Projection of the geographic distribution of *Apobaetis irai* under different climate scenarios and future time periods. Colors indicate environmental suitability for the species, with warmer regions representing a higher probability of occurrence.

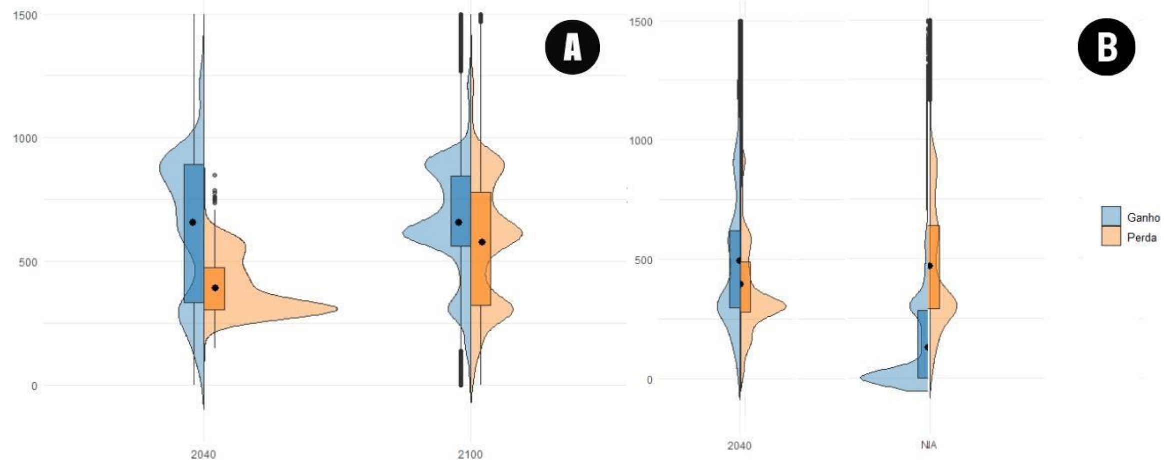


Fig. 7. Violin graphs showing the altitudinal variation of areas of gain (blue) and loss

(orange) for *Apobaetis irai* in the optimistic (A) and pessimistic (B) scenarios for the years 2040 and 2100. The vertical axis indicates elevation (m), while the distribution of colors represents regions where environmental suitability has expanded or contracted over time.

3.1.3. ***Paramaka convexa*** **(Spieth, 1943)**

Correlation analysis was used to model the Apobaetis irai species and select the least correlated bioclimatic variables, minimizing redundancies in the environmental data. The variables chosen were BIO1 (average annual temperature), BIO2 (average daily thermal amplitude), BIO3 (isothermality), BIO5 (maximum temperature of the warmest month), BIO6 (minimum temperature of the coldest month), BIO10 (average temperature of the warmest quarter), BIO12 (annual precipitation), BIO13 (precipitation of the wettest month), BIO15 (seasonality of precipitation), BIO18 (precipitation of the warmest quarter), and BIO19 (precipitation of the coldest quarter).

The species *Paramaka convexa* finds the best environmental conditions in the Middle and partially in the Upper Rio Doce, while the Lower Rio Doce shows negligible suitability for its presence. Only 3.89% of the total area had values higher than 0.7, indicating that the highly favorable habitats for the species are limited within the basin (Fig. 8).

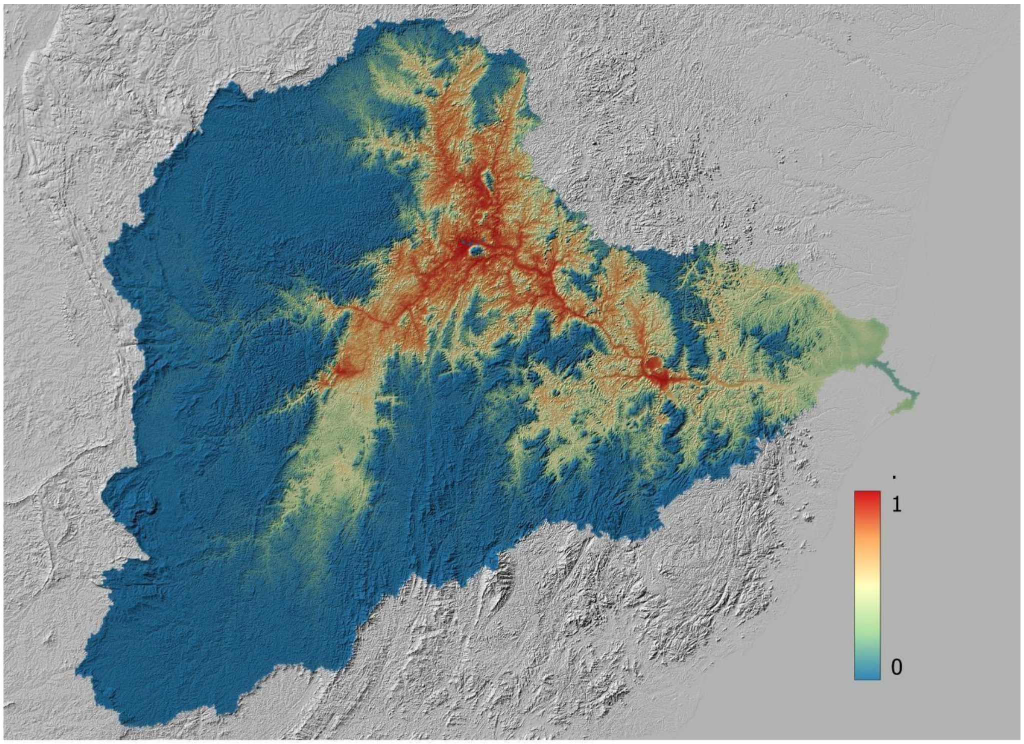


Fig.8. Map of the potential distribution of *Paramaka convexa* in the Rio Doce Basin, obtained by ecological niche modeling. The color scale represents environmental suitability, ranging from low (blue) to high (red). White dots indicate the occurrence records used in the modeling.

In the optimistic scenario, *Paramaka convexa* maintains a relatively wide distribution in 2040, with areas of high suitability (red and yellow) concentrated mainly in the higher regions of the basin (Fig. 9A). There is a slight decrease in environmental suitability in 2100. However, the species still maintains some favorable areas (Fig. 9B). *Paramaka convexa* expands its presence at intermediate altitudes (500m) and maintains a stable distribution until 2100 (Fig. 10A). Although there are localized losses in these ranges, the gains compensate for this reduction.

The pessimistic scenario shows a more significant reduction in highly suitable areas by 2040 (Fig. 9C). The loss of suitable habitat is severe by 2100, with a large part of the basin becoming unsuitable for the species (predominantly blue), indicating a high risk of local extinction (Fig. 9D). *Paramaka convexa* loses environmental suitability at intermediate altitudes. By 2100, environmental suitability is restricted to lower altitudes (300-500m), suggesting niche contraction (Fig. 10B).

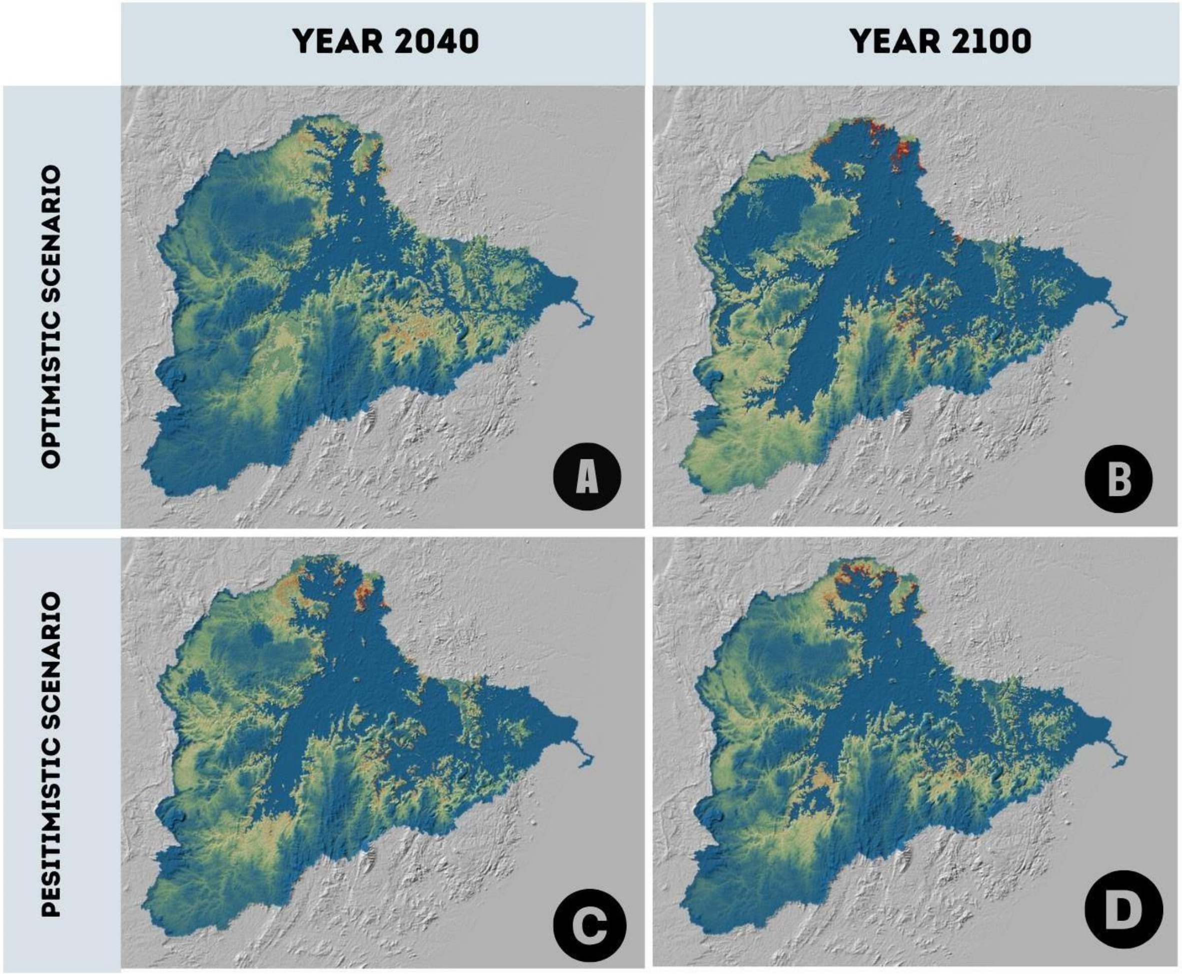


Fig. 9. Projection of the geographic distribution of *Paramaka convexa* under different climate scenarios and future time periods. Colors indicate environmental suitability forthe species, with warmer regions representing a higher probability of occurrence

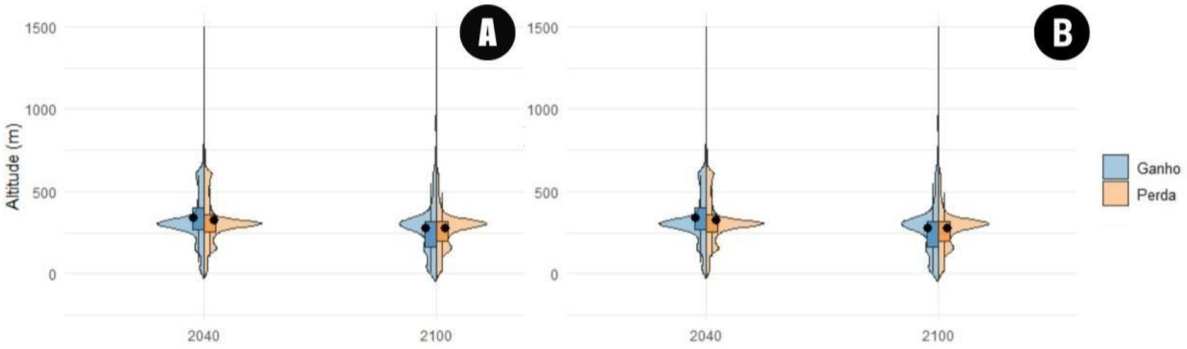


Fig. 10. Violin graphs showing the altitudinal variation of areas of gain (blue) and loss (orange) for *Paramaka convexa* in the optimistic (A) and pessimistic (B) scenarios for the

years 2040 and 2100. The vertical axis indicates elevation (m), while the distribution of colors represents regions where environmental suitability has expanded or contracted over time.

3.1.4. Tricorythodes santarita Traver, 1959

To model the Tricorythodes santarita species, the correlation analysis of climatic variables was applied to ensure that the model was representative. The variables chosen were BIO1 (average annual temperature), BIO5 (maximum temperature of the warmest month), BIO6 (minimum temperature of the coldest month), BIO12 (annual precipitation), and BIO17 (precipitation of the driest quarter).

Modeling the potential distribution of *Tricorythodes santarita* in the Rio Doce Basin revealed an average habitat suitability of 0.057 (±0.13), with values ranging from 0.00 to 0.97. The distribution of values showed a highly asymmetric pattern, with most areas having low suitability (Fig. 11).

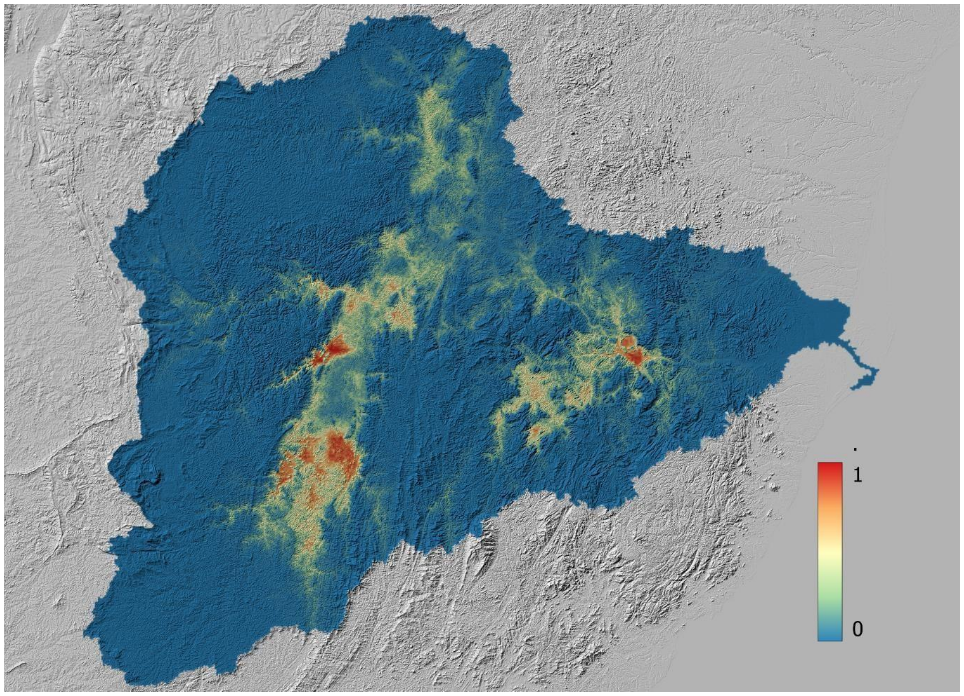


Fig.11. Map of the potential distribution of *Tricorythodes santarita* in the Rio Doce Basin, obtained by ecological niche modeling. The color scale represents environmental suitability, ranging from low (blue) to high (red). White dots indicate the occurrence records used in the modeling.

In the optimistic scenario, *Tricorythodes santarita* suffers a moderate reduction in habitat over time. In 2040, the basin still has areas with suitable conditions (Fig. 12A), but this area decreases by 10-15% by 2100, resulting in a more fragmented distribution (Fig. 12B). Despite the gradual loss, the species maintains viable populations in some regions. *Tricorythodes santarita* maintains its environmental suitability at low altitudes (0-400m). However, there is a loss of habitat at intermediate altitudes (500-700m), suggesting a progressive restriction of the species to lower elevations (Fig. 13A). The fact that there are no significant gains at higheraltitudes indicates that the species is not expanding into new niches.

In the pessimistic scenario, environmental suitability decreases dramatically. By 2040, occupancy is reduced to 20-30% of the basin (Fig. 12C), and by 2100, less than 10% of the area remains suitable (Fig. 12D). The predominance of the inhospitable regions suggests a high risk of local extinctions, with species restricted to small, isolated fragments. In this scenario, the species is firmly restricted to low elevations, while middle and high elevations become inhospitable for survival by 2100. Unlike the optimistic scenario, where the species maintains a relatively stable niche, there is a clear pattern of progressive loss in the pessimistic scenario (Fig. 13B).

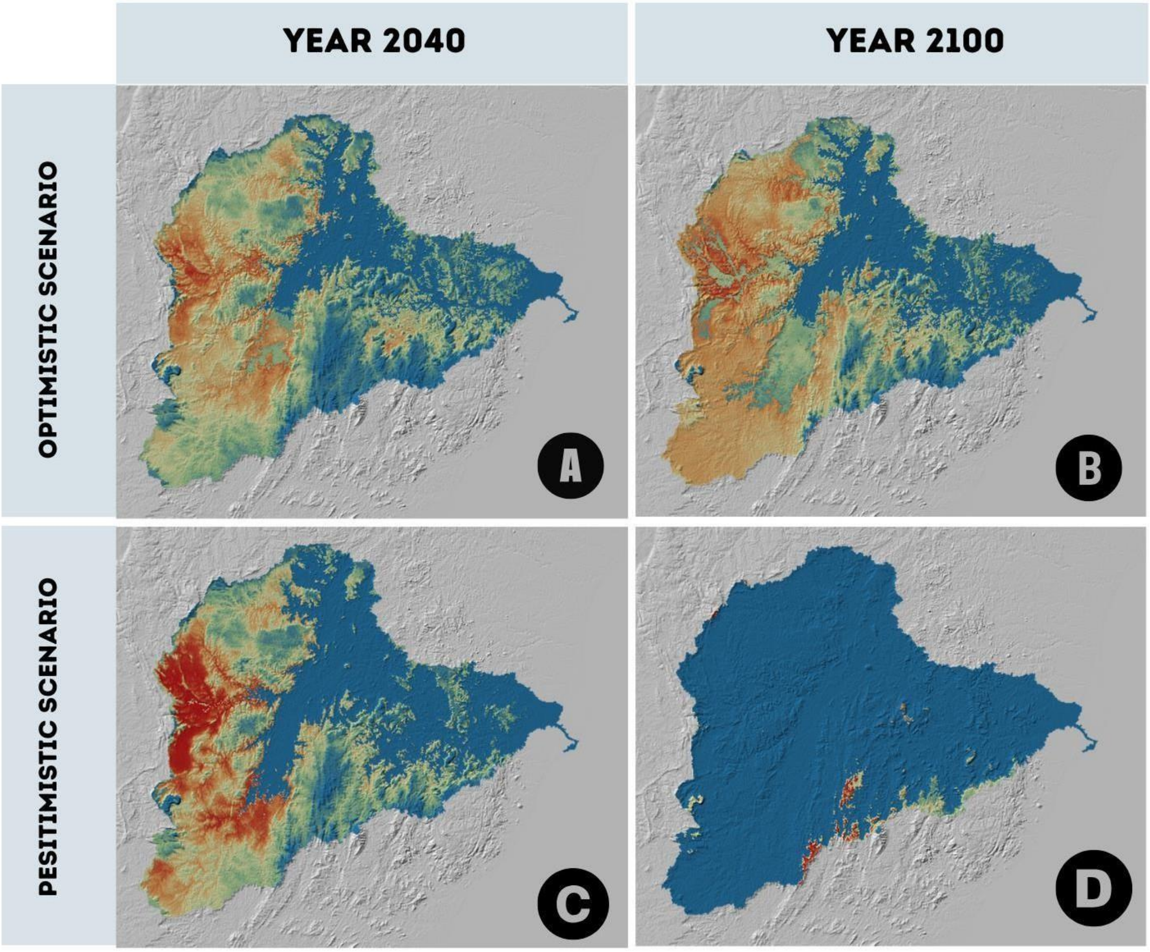


Fig. 12*.* Projection of the geographic distribution of *Tricoythodes santarita* under different climate scenarios and future time periods. Colors indicate environmental suitability for the species, with warmer regions representing a higher probability of occurrence.

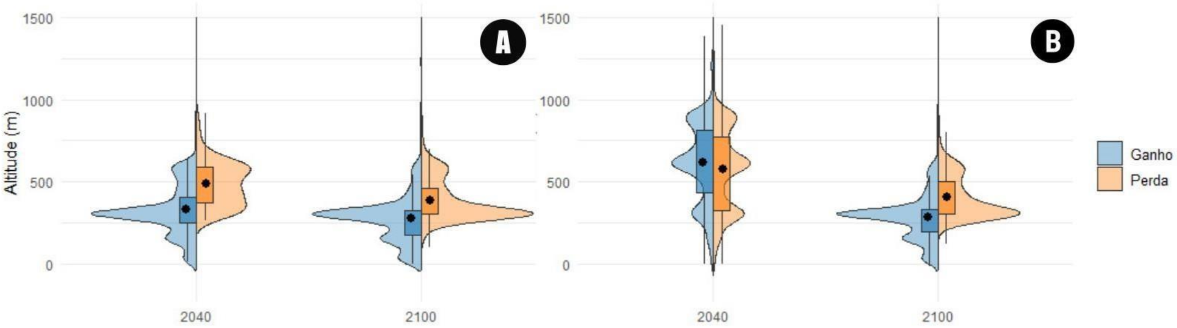


Fig. 13. Violin graphs showing the altitudinal variation of areas of gain (blue) and loss (orange) for *Tricorythodes santarita* in the optimistic (A) and pessimistic (B) scenarios for the years 2040 and 2100. The vertical axis indicates elevation (m), while the distribution of colors represents regions where environmental suitability has expanded or contracted over time.

3.2. Niche overlap

3.2.1. *Apobaetis irai* and *Campsurus cotaxe*

The species share a significant environmental niche, as indicated by the ellipses' overlap (Fig 14. D) and the index D = 0.42. However, *Campsurus cotaxe* has a broader niche, occupying a greater diversity of environmental conditions than *Apobaetis irai*. The similarity between the niches is not random (p = 0.0396), indicating thatecological factors may influence the overlap.

The difference in the size of the ellipses suggests that *Campsurus cotaxe* may be a more generalist species, while *Apobaetis irai* may have a more restricted distribution.

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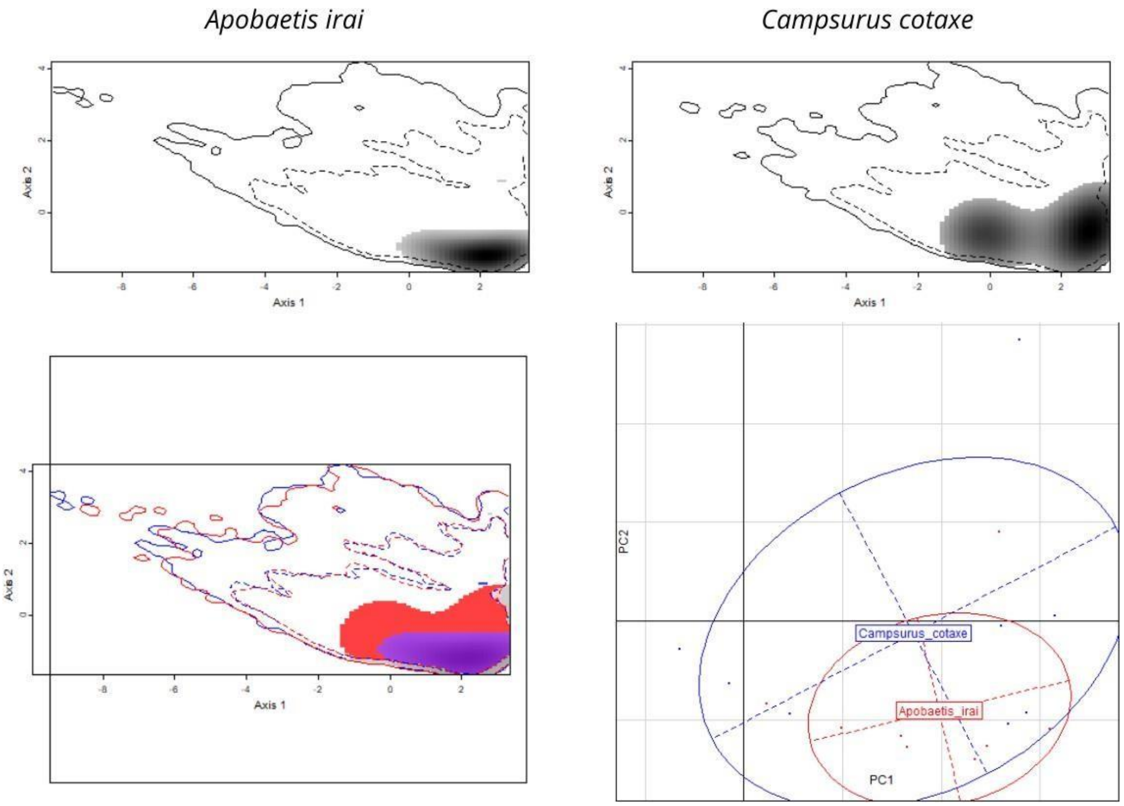

Fig. 14. Niche overlap between *Apobaetis irai* and *Campsurus cotaxe*. Density of occurrence of *Apobaetis irai* (A) and *Campsurus cotaxe* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

3.2.2. *Apobaetis irai* and *Paramaka convexa*

The species share a similar environmental niche, as evidenced by the extensive overlap of ellipses in the PCA and the substantial overlap in niche distribution. The overlap appears to be greater than in the case of *Apobaetis irai* vs. *Campsurus cotaxe*, indicating that *Paramaka convexa* has a niche closer to that of *Apobaetis irai*. The species *Apobaetis irai* has a slightly broader niche, while *Paramaka convexa* has a more restricted distribution in the environmental space.

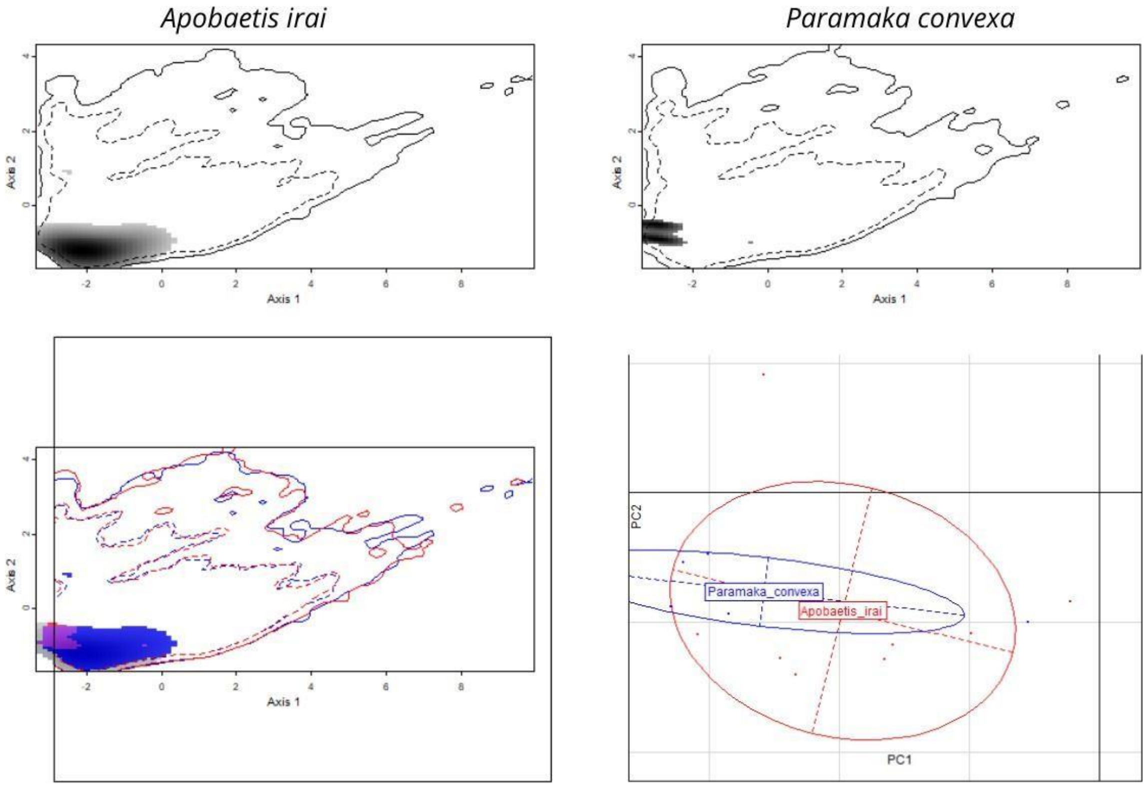

Fig. 15. Niche overlap between *Apobaetis irai* and *Paramaka convexa*. Density of occurrence of *Apobaetis irai* (A) and *Paramaka convexa* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

3.2.3. *Apobaetis irai* and *Tricorythodes santarita*

The niche overlap between *Apobaetis irai* and *Tricorythodes santarita* is very high, as shown by the large intersection in the density graphs and the strong purple component in the overlap graph. The environmental niches of the two species are almost identical, differing only slightly at the edges of their distributions. The PCA analysis confirms the high similarity, with the confidence ellipses of the species strongly overlapping.

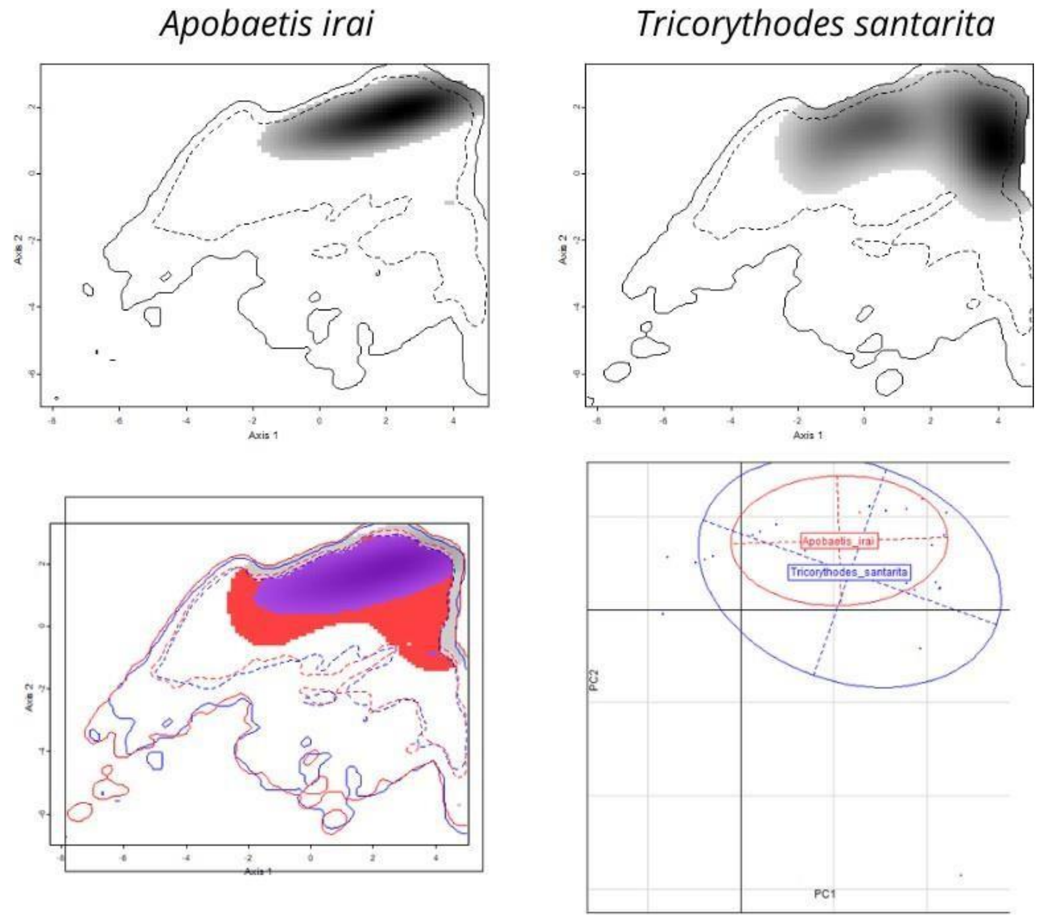

Fig. 16. Niche overlap between *Apobaetis irai* and *Tricoythodes santarita*. Density of occurrence of *Apobaetis irai* (A) and *Tricorythodes santarita* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

3.2.4. *Campsurus cotaxe* and *Paramaka convexa*

The niche overlap between *Campsurus cotaxe* and *Paramaka convexa* is small, as indicated by the slight overlap in the density plots and the tiny purple component in the overlap plot. The species have different environmental requirements, with *Campsurus cotaxe* being more generalist, while *Paramaka convexa* occupies a more restricted and specialized niche. The PCA confirms this separation, showing ellipses with little overlap and distributed in different regions of environmental space.

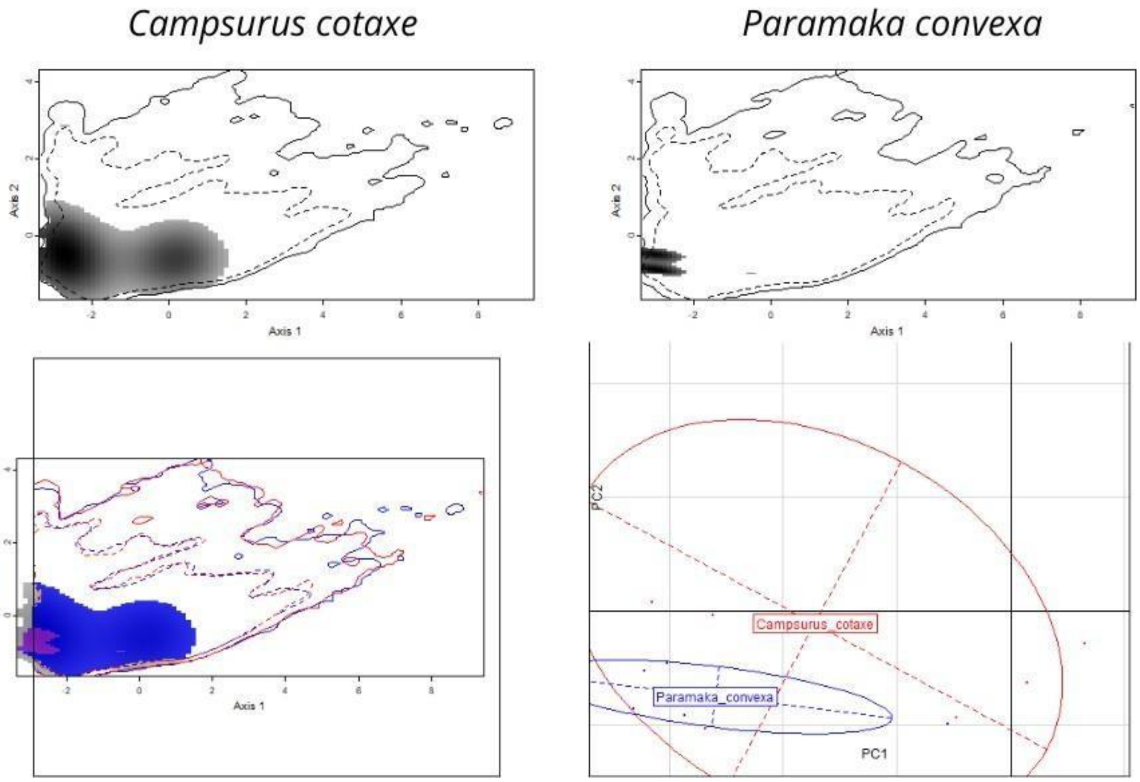

Fig. 17. Niche overlap between *Campsurus cotaxe* and *Paramaka convexa*. Density of occurrence of *Campsurus cotaxe* (A) and *Paramaka convexa* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

3.2.5. *Campsurus cotaxe* x *Tricorythodes santarita*

The niche overlap between *Campsurus cotaxe* and *Tricorythodes santarita* is high, as indicated by the large intersection in the density plots and the strong purple component in the overlap plot. The environmental niches of the two species are broadly shared, and the PCA confirms this high similarity by showing strongly overlapping confidence ellipses.

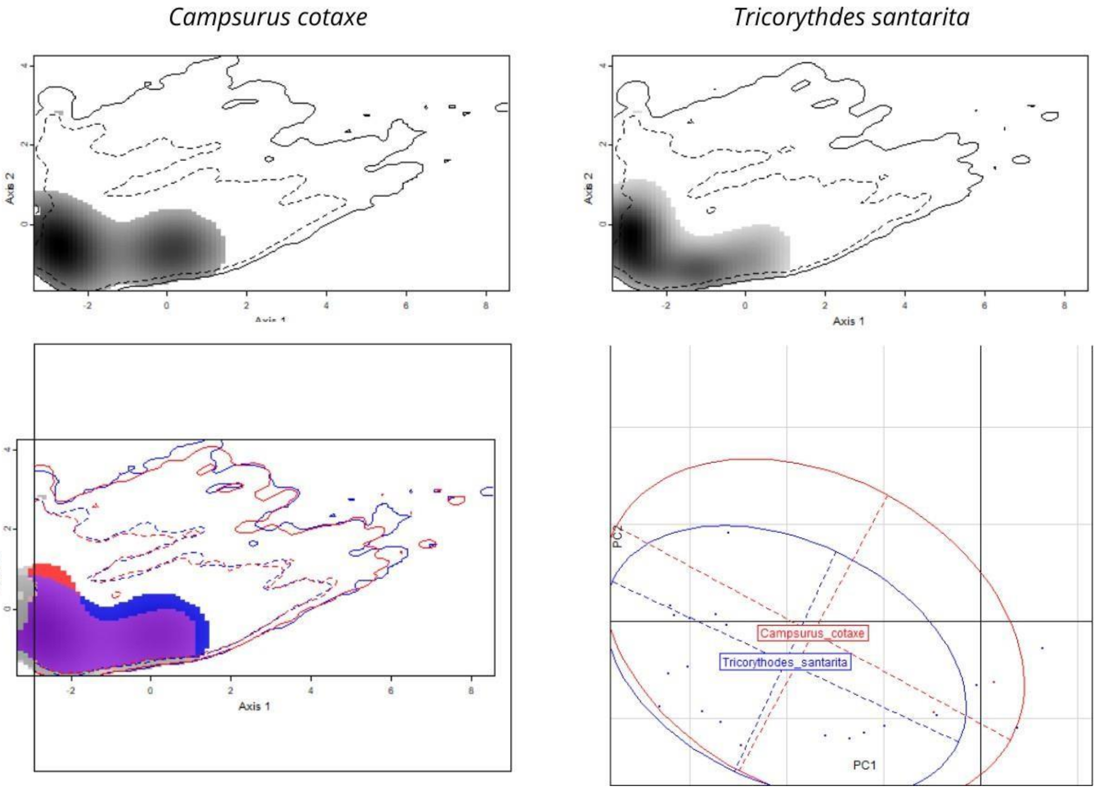

Fig. 18. Niche overlap between *Campsurus cotaxe* and *Tricorythodes santarita*. Density of occurrence of *Campsurus cotaxe* (A) and *Tricorythodes santarita* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

3.2.6. *Paramaka convexa* and *Tricorythodes santarita*

The niche overlap between *Paramaka convexa* and *Tricorythodes santarita* is small, as indicated by the small intersection in the overlap graph and the tiny purple component. The species occupy different niches, with Tricorythodes santarita having a broader niche and *Paramaka convexa* having a highly restricted niche. The PCA confirms this separation, showing ellipses with little overlap and distributed in different regions of the environmental space.

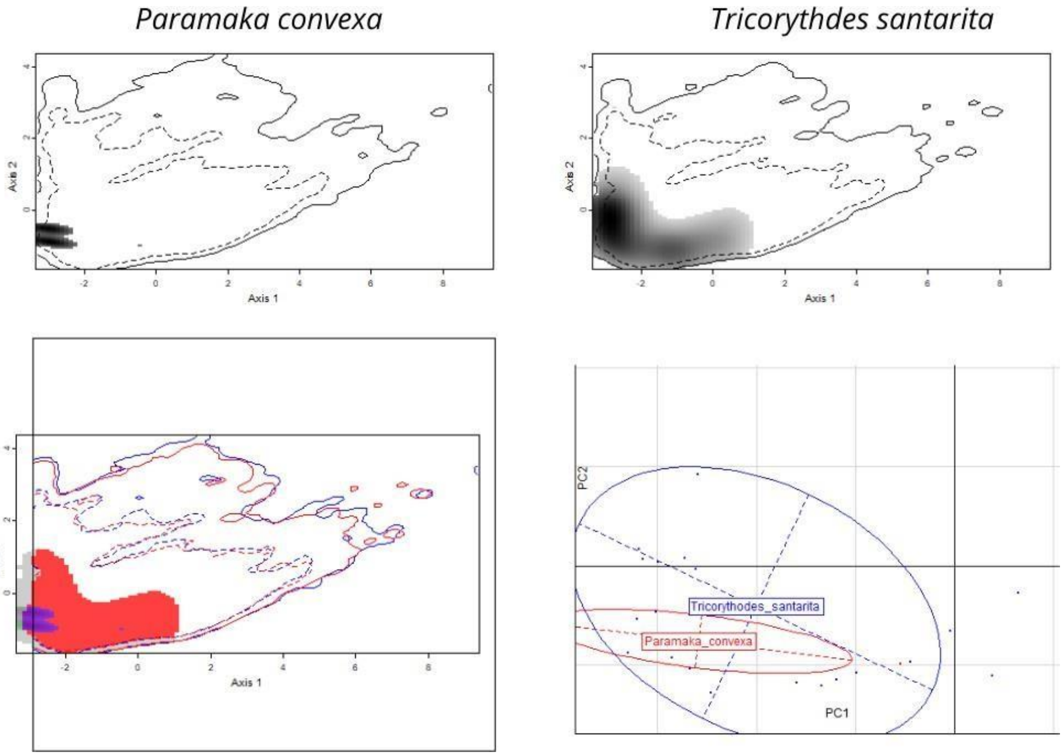

Fig. 19. Niche overlap between *Paramaka convexa* and *Tricorythodes santarita.* Density of occurrence of *Paramaka convexa* (A) and *Tricorythodes santarita* (B). Overlap between the niches of the two species (C), with the purple region indicating a strong intersection between the species' preferred habitats. PCA analysis confirming the similarity of the environmental niches, with the confidence ellipses of the species strongly overlapping (D).

4. Discussion

Modeling the distribution of Ephemeroptera in the Rio Doce Basin showed that the species studied have different environmental niches, with more outstanding suitability in the Upper and Middle Rio Doce regions. Even in these areas, the low proportion of highly suitable areas (suitability values greater than 0.7) suggests that favorable habitats within the basin are limited. This current distribution reflects both the environmental heterogeneity of the region and the ecological factors that determine the presence of these species.

Future climate change scenarios indicate different patterns of impacts on species distribution and richness. In the optimistic scenario, some species' environmental suitability, such as *Apobaetis irai*, is relatively maintained, with possible altitudinal shifts indicating some resilience. However, the pessimistic scenario predicts a drastic reduction in habitat suitability over time, with significant loss of suitable areas and, in most cases, a high risk of local extinction by 2100. The trend for species to move to lower altitudes could make them more vulnerable to extreme environmental events and habitat degradation.

These changes have significant implications for the ecological dynamics of Ephemeroptera. Habitat contraction can reduce population connectivity, minimize gene flow, and increase species' vulnerability to additional environmental stresses (Monaghan et al., 2002), such as pollution caused by dam failures in the Rio Doce Basin. In addition, the risk of niche overlap between some species can increase competition, especially in remaining areas of high suitability.

We observed that several hotspots, which are areas of current and future occurrence of the species evaluated, overlap with conservation gaps, i.e. these areas have high potential richness of Ephemeroptera but are unprotected. For example, *Paramaka convexa* has the potential to occur in areas of the Middle Rio Doce that, in addition to having very few protected areas (De Oliveira et al., 2021), are subject to the effects of pollution from mining and other anthropogenic activities (Drummond et al., 2021). Therefore, these areas, which include the mouth of the Rio Matipó to the border between Minas Gerais and Espírito Santo, must be the focus of future studies.

The region mentioned above represents a critical area for the conservation of Ephemeroptera. In addition to the lack of protection, the region suffers from a history of anthropogenic impacts, such as contamination by mining tailings. The tendency for species to migrate to marginal areas of the basin, often unprotected, increases the vulnerability of these communities. Investment in conservation strategies to protect aquatic ecosystems is essential to ensure the preservation of aquatic biodiversity and to mitigate the effects of climate change combined with the effects of anthropogenic pressures.

The establishment of protected areas is usually based on the need to conserve terrestrial vertebrate species, but these proposed conservation initiatives protect only 20% of freshwater species (Duponchelle, 2021). According to Cardoso et al. (2023), in order to conserve the fauna of Ephemeroptera, 50% to 70% of the priority areas for species of this order must be within protected areas.

Although the largestnumberof records for most species is in the eastern part of the Basin, in the State of Espírito Santo, we have values in this region for future scenarios that indicate low or zero values, with most cells having no information at all. This output showed cold pixels (blue) despite the collection effort and the large number of records in the region.

Here, we analyzed the responses of four species belonging to four different families of Ephemeroptera. Still, it is possible that analyses for other species belonging to the same functional food groups and exploiting the same niches may reveal similar responses. Below is a more detailed discussion of the current and future suitability of each species in the Rio Doce Basin.

4.1. Campsurus cotaxe

The distribution of *Campsurus cotaxe* in the Rio Doce Basin is concentrated in the upper and middle Rio Doce, especially in the higher areas (Fig. 4). However, its environmental suitability is limited, suggesting a dependence on specific conditions, such as water quality and hydrology, which are often altered by anthropogenic activities, such as mining. Compared to the current potential distribution, both scenarios show a distribution gap in the central region of the basin, coinciding with the Rio Doce, affected by the collapse of the tailings dam. This reduction in the species' potential range in the Rio Doce may reflect an even more vulnerable state for the species, as there may be an interaction between the disaster event and climate change.

In the optimistic scenario, the species maintains a wide distribution, extending to the sub-basins of Santo Antonio, Suaçui Grande, Machuaçú, and the mouths of Rio Matipó and Rio Casca rivers, corresponding to the boundaries between the Upper and Middle Rio Doce. There is a slight decrease until 2100, indicating a resilience to moderate variations, as long as environmental degradation does not intensify. In the pessimistic scenario, there is a sharp decline in environmental suitability, resulting in a sharp reduction in distribution and a possible risk of local extinction. In 2100, the relevant occurrence of *C. cotaxe* is limited to the towns of Afonso Cláudio and Brejetuba, in Espírito Santo. Moving to lower altitudes could increase its vulnerability to extreme events, compromising its persistence in the Basin.

4.2. Apobaetis irai

The modeling shows that *Apobaetis irai* has a potential distribution with favorable areas in the central regions of the Upper and Middle Rio Doce, which have been affected by mining tailings. In the optimistic scenario, the species maintains a wide distribution until 2040 and moves to higher altitudes (1000-1500m) until 2100, making this an area of climatic refuge.

In the pessimistic scenario, there is a significant loss of habitat, forcing the species to remain at lower altitudes (Fig. 7), which on the map correspond, for example, to the municipality of Barra Longa, among other towns that were the first to be severely affected by mining tailings in the Carmo and Gualaxo do Norte sub-basins. By 2100, the pattern of occurrence will be restricted to the municipalities of Simonesia, Manhuaçu and the highlands of the Serra do Brigadeiro in Minas Gerais. This niche shrinkage may compromise its ecological function, and conservation strategies will be essential for its persistence in the area.

4.3. Paramaka convexa

Paramaka convexa is a species with a distribution limited to the Neotropical region (Salles et al., 2023). In the Rio Doce Basin, its distribution is reduced to the Middle Rio Doce, with less favorable conditions in the Lower Rio Doce, in the sub-basins of the Rio Suaçuí Grande, and fragments of the Rio Machuaçú sub-basin. This limited and fragmented occurrence indicates a strong dependence on environmental quality and a high vulnerability to hydrological impacts in these areas. In the optimistic scenario, the species remains stable until 2040, with possible expansion to intermediate altitudes (500 m), suggesting some adaptive capacity. By 2100, the disappearance of the species in the Middle Rio Doce region, where its potential distribution currently occurs, is remarkable. In both scenarios, P. convexa moves, albeit in small proportions, to higher geographical regions representing the upper end of the Rio Doce Basin, such as the Ribeirão Laranjeiras sub-basin.

In the pessimistic scenario, there is a strong contraction of the niche, restricting the species to low altitudes (300-500 m) by 2100, which could increase the vulnerability of *Paramaka convexa* to environmental changes. These areas correspond mainly to the municipalities of Franciscópolis, Malacacheta, and Água Boa, located north of the Rio Suaçuí Grande sub-basin. This limitation could hinder its dispersal, intensify competition with other species, and affect the structure of the aquatic community as a whole.

4.4. Tricorythodes santarita

Tricorythodes santarita shows low environmental suitability in the Rio Doce basin, with a fragmented distribution and low mean values, indicating ecological challenges already underway. In the most optimistic scenario, its restriction to low altitudes (0-400 m) could jeopardize its long-term survival. In this case, the species maintains its distribution in the upper reaches of the Rio Santo Antoniobasin in 2040. In 2100, its distribution is projected to be limited to the sub-basins of the Rio Suaçui Grande, Rio Suaçui Pequeno and Rio Corrente, in the regions close to the edge of the Serra do Espinhaço.

In the pessimistic scenario, the species' occupation falls to 20-30% of the Basin by 2040 and less than 10% by 2100, increasing the risk of local extinction. The species' distribution is concentrated in the areas corresponding to the municipalities of Conselheiro Pena, Alto Caparaó and Manhumirim, with the most prominent area covering the municipalities of Manhuaçu and Simonésia, in Minas Gerais. To a lesser extent, the species also appeared in 2100 in the convergence areas corresponding to the Sete Salies Cave, Serra do Espinhaço and Brigadeiro State Park. Without expansion to higher altitudes (Fig. 13), *T. santarita* may remain vulnerable, reinforcing the need for conservation actions to prevent its disappearance and mitigate the impacts of climate change on aquatic biodiversity.

All species evaluated show a future projection of shifting occurrences from more central areas of the basin to more peripheral regions. However, few of the species' potential distributions in future scenarios correspond to protected areas, although the states of Minas Gerais and Espírito Santo have a large number of conservation units in other locations (ICMBio, 2025).

For future studies, it is essential to investigate the Rio Manhuaçu sub-basin to understand what characteristics influence the occurrence of these species. In addition, more collections at the site could help to identify the possibility of a similar pattern for other invertebrate species.

Another sensitive point is the great importance of the Rio Santo Antonio sub-basin as a potential area of great relevance for the occurrence of at least 3 of the species studied (*C. cotaxe, P. convexa* and *T. santarita*). This sub-basin is subject to the increasing implementation of mining activities and the installation of hydroelectric dams, with severe impacts on the fauna of local aquatic organisms (PIRH SANTO ANTÔNIO, 2019; Vilan et al., 2024).

All the species evaluated show a future projection of shifting occurrences from more central areas of the basin to marginal areas. However, few of the species' potential distributions in future scenarios correspond to environmental protected areas, even though the states of Minas Gerais and Espírito Santo have a large number of protected areas in other locations (ICMBio, 2025).

This sub-basin has long been impacted by large-scale iron ore mining and remains central to discussions on the limits of extractive expansion. It contains 2,153 mines, covering 49.03% of its total area. In 2021, local municipalities received around US$115,000 in mining royalties (Vilan et al., 2024).

The potential distribution of these species is restricted to small areas vulnerable to threats like tailings dam failures (Fig. 5). Expanding Ephemeroptera inventories in knowledge-gap regions is crucial for understanding species diversity and validating these models. Biological collections need more investments and organization of data that can be available to other scientists. These actions will further support scientific progress in Brazil (Cardoso et al., 2023).

For effective aquatic insect conservation, Chowdhury et al. (2022) recommend integrating insects into protected area management, establishing dedicated reserves, extending conservation efforts to both threatened and widespread species, and strengthening monitoring programs.

Another important issue to consider when considering future scenarios is that if the most sensitive species disappear from certain regions due to climate change combined with severe anthropogenic disturbance, the interpretation of biological indices for biomonitoring could be compromised, requiring adjustments to monitoring protocols and a re-evaluation of the metrics used.

Given climate change and anthropogenic activities impacts, Ephemeroptera species conservation of the studied in this work in the Rio Doce Basin requires integrated actions. The low overlap between future distribution areas and conservation units reinforces the need for more effective protection. The sub-basin of the Rio Santo Antônio is a critical point, both in terms of biodiversity and environmental pressures. Faunal surveys, expansion of biological collections and improved monitoring are essential for stabilizing the region's aquatic ecosystems.

Ensuring the conservation of these four species goes beyond the protection of the individual species; it means the conservation of the entire community of aquatic insects, which are of fundamental importance for nutrient cycling and water quality. Habitat degradation compromises ecosystem resilience and threatens biodiversity. Therefore, it is essential to combine scientific and political efforts to implement effective conservation measures to prevent the total extinction of these species and to safeguard important ecosystem services for the Rio Doce Basin region.

5. Final remarks

The current distribution of Ephemeroptera in the Rio Doce Basin is mainly concentrated in the Upper and Middle Rio Doce, with low environmental suitability in the Lower Rio Doce. The modeling showed that the species analyzed have a fragmented distribution, with areas of high suitability representing a small portion of the Basin, and most of the species studied finding more favorable conditions in higher regions.

In the optimistic scenario, some species may find refuge at higher altitudes and maintain a wide distribution by 2040, despite local losses at intermediate altitudes. In the pessimistic scenario, there is a substantial reduction in environmental suitability, resulting in a shift to lower altitudes and a high risk of local extinction for species with a more restricted niche, such as *Paramaka convexa* and *Tricorythodes santarita*. Species richness tends to decrease, with specialized species replaced by more tolerant ones as climate change renders some areas unsuitable.

These changes have implications for ecological dynamics and biomonitoring using aquatic insects. Habitat contraction can reduce population connectivity and increase the vulnerability of species, as well as increase competition for resources, leading to competitive exclusion. In biomonitoring, the extinction of sensitive species can make biological indices less effective, requiring adjustments to monitoring protocols. In addition, the inadequacy of protected areas to ensure the survival of these species reinforces the need for new conservation strategies to mitigate the effects of climate change.

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CONCLUSIONS

The results obtained throughout this thesis reiterate that mining imposes severe pressures on aquatic communities, with particularly strong effects on sensitive groups such as Ephemeroptera, Plecoptera and Trichoptera. The robustness of the patterns identified indicates that these organisms are effective indicators of environmental degradation and their use can optimize ecological monitoring, making it more sensitive, efficient and financially viable.

The integrated analysis of the data showed that the impacts of mining go beyond direct contamination: tailings alter existing environmental filters, amplifying physical and chemical stressors and modifying the structure and composition of biological communities. This amplification of environmental effects demonstrates that tailings contamination does not act in isolation, but interacts in complex ways with the local and regional environmental context. Spatial patterns of biological connectivity have also been profoundly affected, with fragmentation of dispersal pathways and compromised integrity of metacommunities. The identification of key areas for gene flow and biodiversity conservation provides an important rationale for conservation actions that focus on landscape functionality, rather than just occasional habitat conservation.

Climate change projections reveal a scenario of decreasing ecological niches and altitudinal shifts of species, exacerbating the vulnerability already imposed by local anthropic actions. The mismatch between future climate refuges and current protected areas warns of the urgent need to rethink conservation strategies, taking into account future species distribution dynamics.

Integrating multiple methodological approaches and analytical scales, this work provides an integrated picture of the challenges facing aquatic biodiversity in the affected river basins. The evidence gathered here reinforces the importance of management actions based on the integration of sensitive bioindicators, ecological connectivity, and adaptive planning in the face of environmental change, especially in historically degraded regions such as the Doce River Basin.

APPENDIX

A decade after the tailings dam disaster: Distribution and status of *Smicridea* McLachlan, 1871 (Trichoptera : Hydropsychidae) in the Rio Doce basin, Southeastern Brazil*

The following manuscript presents an updated survey of species of the genera *Smicridea* McLachlan, 1871 (Hydropsychidae: Trichoptera) in the Rio Doce Basin, a region severely affected by the collapse of a mining tailings dam in 2015. The study recorded 12 species of *Smicridea* in the basin, including six new records for the states of Minas Gerais and Espírito Santo, and the description of one new species: *Smicridea* (Rhyacophylax) *exu* sp. nov. The data were obtained through field sampling, literature review and analysis of material from entomological collections, which also resulted in detailed maps of the distribution of the species in the river basin.

The relevance of this article to the thesis lies in expanding knowledge of the diversity and distribution of Trichoptera in a context of intense environmental degradation. As *Smicridea* larvae live in the substrates of rivers and streams and weave nets to capture food in lotic environments, they are susceptible to physical and chemical changes in the river - such as the accumulation of tailings. Thus, documenting theirdiversity provides an essential basis for assessing the impact of the disaster on aquatic fauna, as well as input for the chapters dealing with the dispersal of aquatic communities. This work reinforces the importance of knowledge about the sensitive species of the Trichoptera order. It completes the ecological panorama outlined in the thesis by deepening the taxonomic and spatial understanding of an important group of Neotropical entomofauna.

*This manuscript is formatted according to the instructions of the journal “Biologia (Poland)”

A decade after the tailings dam disaster: distribution and status of *Smicridea* McLachlan, 1871 (Trichoptera: Hydropsychidae) in the Rio Doce basin, southeastern Brazil

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Abstract

The greatest diversity of the genus *Smicridea* (Trichoptera: Hydropsychidae) is concentrated in the Neotropicalregion, where 72 species have been recorded from Brazil:43 from the subgenus *S.* (*Rhyacophylax*), 29 from *S.* (*Smicridea*). Despite this richness, there are gaps in the knowledge of the geographical distribution of the species, especially in areas affected by human activities. The Doce River Basin, hosting the second largest river in the Southeastern region of Brazil, has a history of environmental degradation and intense mineral exploitation, aggravated by the collapse of a tailings dam in 2015, Brazil's largest socio-environmental disaster, which directly affected the local aquatic fauna. With the aim of expanding and integrating the knowledge of the *Smicridea* fauna of the Doce River Basin, this study reports 12 species of the genus in the Doce River Basin, including six new state records: three for the State of Espírito Santo (*S.* (*Rhyacophylax*) *appendiculata* Flint, 1972, *S.* (*Rhyacophylax*) *scalprum* Vilarino, Dias & Calor, 2019, and *S.* (*Rhyacophylax*) *vilela* Flint, 1978), and three for Minas Gerais (*S.* (*Rhyacophylax*) *atrobasis* Flint, 1983, *S.* (*Rhyacophylax*) *palmar* Sganga, 2005, and *S.* (*Rhyacophylax*) *vilela*). In addition, a new species, *Smicridea* (*Rhyacophylax*) *exu* **sp.nov.** is described and illustrated based on males collected in Rio Doce and its tributaries, in the Minas Gerais state. It can be recognised on a combination of genital characters, mainly in the tergum X and the phallic apparatus.

Keywords

Aquatic insects, Smicrideinae, New species, New records, Taxonomy, Atlantic Forest

Introduction

The genus *Smicridea* McLachlan, 1871 is the most species-rich genus of net-spinning caddisfly, or Hydropsychidae, in the New World, with comprises 247 species (Desidério et al. 2020, 2021a; Santana et al. 2021). It is the only genus of Smicrideinae (Hydropsychidae) occurring in this region and is classified into two subgenera: *Smicridea* (*Smicridea*) and *Smicridea* (*Rhyacophylax*) Müller, 1879 (Flint 1974a). These subgenera can be distinguished in the adult stage by differences in wing venation, the male tibial spur formula, and the presence or absence of two pairs of internal reticulate sacs on segments VI and VII (Flint 1974a, 1989).

The Neotropical region harbors the highest species richness of *Smicridea* (Holzenthal and Calor 2017; Santana et al. 2021). In Brazil, *Smicridea* is the most diverse genus of Trichoptera, with 72 recorded species—43 belonging to *S.* (*Rhyacophylax*) and 29 to *S.* (*Smicridea*) (Pes and Desidério 2024). Despite its remarkable diversity, significant knowledge gaps remain regarding the geographical distribution of *Smicridea* (Desidério et al. 2021b; Santana et al. 2021), particularly in regions threatened by anthropogenic activities. Most studies focus on conservation areas (Alves et al. 2018; Martín and Sganga 2021; Queiroz et al. 2023; Rocha et al. 2016) and this bias limits the assess to the full extent of its distribution and the potential impacts of environmental changes on its populations.

With a long history of environmental degradation, the Rio Doce Basin is of particular interest, as it encompasses the second-largest river in Brazil's Southeastern region. The upper and middle portions of the basin, which together account for 87% of its totalarea, are located in the state of Minas Gerais, while the lower portion lies in Espírito Santo. These regions exhibit significant transitions in hydroclimatic and topographic conditions (ANA 2021).

Mining in the region dates back to the 17th century gold rush in Brazil and later expanded with large-scale iron ore extraction, which remains the dominant activity today (Castro 2011). This extensive exploitation has played a major role in shaping the current environmental scenario (de Rodrigues et al. 2013). The situation worsened even after the tragic collapse of a mining tailings dam ten years ago, on the afternoon of November 5, 2015 (Agurto-Detzel et al. 2016). This was considered the largest socio-environmental disaster recorded in the country (Cordeiro et al. 2019), which released 43.8 million cubic meters of mining tailings throughout the river, affecting the soil and substrate at the bottom of the water body and, therefore, the aquatic fauna (Queiroz et al. 2018).

The history of environmental degradation in the Rio Doce basin reinforces the need for studies of its aquatic biodiversity (Ramos et al. 2024). *Smicridea*, an important component of freshwater ecosystems (Wiggins, 2006), remain poorly documented in the degraded areas. Studying their distribution improves our understanding of species responses to environmental changes, contributing to conservation strategies and biomonitoring efforts as well filling the gaps in the scientific knowledge and distribution of these species.

This study aims to expand and integrate knowledge about the composition and distribution of *Smicridea* species in the Doce River Basin, providing new species records and ecological notes. Additionally, it describes a newly discovered *Smicridea* species, identified through samplingefforts conducted to assess the environmental impact of the disaster on aquatic entomofauna.

Materials and methods

This study was conducted in the Doce River Basin in Southeastern Brazil, covering an area of 86,715 km² (Sánchez et al. 2018). The region has an average annualtemperature of 19 °C and receives about 1,375 mm of rainfall per year. The dry season is from April to September, and the rainy season is from October to March, with precipitation peaking during December and January (Alvares et al. 2013).

Sampling was conducted using a collapsible Pennsylvania light trap (Nessimian et al. 2024) equipped with a white fluorescent lamp at 40 sites during the dry season and 16 sites during the rainy season. All specimens were preserved in 80% ethanol. In the laboratory, the abdomens were removed and cleared using a 10% KOH solution at room temperature. The lactic acid (85%) method was employed to evert the genital structures (Blahnik and Holzenthal 2004; Blahnik et al. 2007). The cleared abdomens were then placed on microscope slides with alcoholgel and covered with a coverslip for viewing, photographing, and illustration of the genitalia. Species identification was conducted using a Leica Ivesta 3 stereomicroscope with a integrated vídeo camera and/or an Olympus CX31 compound microscope with an attached Motic A5 vídeo camera. Identification followed taxonomic keys and references from previous studies (Albino et al. 2011; Alves et al. 2018; Desidério et al. 2020, 2021a, b).

Photographs were taken with the same equipments used for identification. Stacks of images of each structure were produced at different focaldistances; these were then combined automatically into a single image with a greater depth of field using Helicon Focus® Pro stackingsoftware (version 6.7.1). Stacked images of the genitalia were used as templates in Adobe Illustrator®.

All photographs and illustrations were edited and assembled into plates using Adobe Photoshop®. Species descriptions were generated using the DELTA (Description Language for Taxonomy) software (Dallwitz et al. 1999), following the descriptive framework established by Desiderio et al. (2020). The distribution maps were created using QGIS® software, incorporating shapefiles from the Instituto Brasileiro de Geografia e Estatística (IBGE 2021), Agência Nacional de Águas e Saneamento Básico (ANA 2017), and Fundação SOS Mata Atlântica (SOSMA 2019), along with terrestrial ecosystems data from the World Wildlife Fund (WWF) (Olson et al. 2001). Additionally, digital elevation model layers from Natural Earth (2023) and OpenTopography were included. Distribution data were compiled from the specimens examined in the present study, documented records in the literature, and data available in the Global Biodiversity Information Facility (GBIF 2024).

Morphological terminology follows Wells and Neboiss (2018) for the head setal warts and Holzenthal and Blahnik (1995) for the male genitalia. The list of examined materialwas compiled using the AUTOMATEX macro in Microsoft Excel (Brown 2013). Type material is deposited in the following collections: Coleção de Invertebrados, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil; Coleção Entomológica do Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil, and Museu de Entomologia, Universidade Federal de Viçosa (UFVB), Viçosa, Brazil. All non-type specimens are deposited at the UFVB.

Taxonomy

New species

Smicridea (*Rhyacophylax*) *exu* Bonfá-Neto, Desidério & Salles **sp. nov.**

(Figs. 1–3)

Zoobank number: https://zoobank.org/urn:lsid:zoobank.org:act:7BEC9912 -2636-4A0B-9A28-B48D61196942

Differential diagnosis

Smicridea (*Rhyacophylax*) *exu* **sp. nov.** is most similar to *S.* (*Rhyacophylax*) *boraceia* Santana, Desidério & Hamada, 2021 mainly based on the shape of phallic apparatus, with enlarged basal section and angulate about 63° with apical section, and by havingtergum X concave at its apex in dorsal view, with lateral margins rounded forming a produced flap, strongly sclerotized, and with the apex of the phallotremal sclerite of the phallic apparatus with subtriangular lateral flap. However, S. (R.) boraceia sp. nov. has six short, stout spines on the ventralregion of the apicomesallobe of tergum X, which are absent in S. (R.) scalprum. In addition, S. (R.) boraceia sp. nov. can be recognized by the two medium-sized, apicolateralspines directed posterad and generally three apicoventralspines directed downward on the phallic apparatus (both absent in S. (R.) scalprum). Additionally, the new species has a pair of subapical, comma - shaped sclerites on the phallotremal sclerite of the phallic apparatus, in dorsal view.

Description

Adult male. Length of forewing 4.90–5.50 mm (n = 10). General color brown (in alcohol) (Fig. 1a, b). *Head* dark brown (Fig. 1c, d), with brown setae on dorsum; dorsally with well-pronounced coronalsuture; with five setal warts; anteromesal wart (*am. wt.*) small, rounded; anterolateral pair (*al. wt.*) undivided and hook-shaped, weakly delimited; posterolateral pair (*pl. wt.*) large, ovoid. Width of one eye, in dorsal view, 1/3 that of interocular distance. *Thorax* brown (Fig. 1a, b), without setae (denuded); legs pale brown, with fore- and mid-legs tarsi yellowish. Forewings (Fig. 2a) with forks I, II, III, V petiolate and IV sessile; *r-m* and *m* crossveins aligned; *m-cu* crossvein located on mid-length of the M cell; in alcohol, brown, with dark bands over *r-m*, *m*, *m-cu* and *cu* crossveins, and three white transverse bands zigzagging over full length; basal half with one band arising on subcosta and reaching anal vein; apical half with one band arising on costa, running through base of R_{2+3} andending on apex of Cu_2 andanother subapically arising on costa, running through half length of R_2 and endingat Cu_{1b}; hindwing (Fig. 2b) with forks II, III, V petiolate, R_4+R_5 vein transversal to R_2+R_3, and s crossvein absent. Sternum V (Fig. 2c) with pair of anterolateral glandular processes 1.2X longer than sternum. Abdominal segments VI and VII without internal glands.

Male genitalia. Segment IX in lateral view (Fig. 3a) with anterolateral margin sinuous, concave mesally, weakly sclerotized; posterodorsal margin produced, rounded, bearing small spicules; ventromesal posterior lobe absent. Tergum X (Fig. 3a, b) elongate; basolateral lobe absent; in lateral view, anterolateral apodeme well-developed, Y-shaped; ventrolateral margin strongly sclerotized, without processes; apex produced and slightly upturned; in dorsal view, lateral margin rounded, produced as flap; dorsomesalsetose area bearingfive medium-sized, thin setae; divided apicomesally by V-shaped incision about 1/2 its length; apex of tergite rounded, with strong, narrow apicomesallobe, each bearingabout six to eight short, stout spines mesally. Inferior appendages (Fig. 3a, b) 2-segmented; basalsegment as long as tergum X, slightly inflated distally, covered with medium-sized to long, thin setae, with pair medium-sized, stout apicalspine-like setae on internalmargin; apicalsegment long, about 1/2 as longas basalsegment, thick, covered with medium-sized, thin setae, curved mesad, apex rounded. Phallic apparatus (Fig. 3e) long and tubular; basalsection, in lateral view, enlarged, 3X diameter of phallotheca at its narrowest portion, forming angle of about 63° with apical section; median section of phallotheca strongly curved ventrad, without processes; apex (Fig. 3f, g, h, i), in dorsal view, slightly enlarged, without processes or plates; in lateral view, with one long, subapicolateral spine directed posterad (*variation* - male paratype UFVBTR 00863 has two spines on left side), one to four medium -sized, dorsal spine directed posterad, and three to four medium-sized, apicoventral spines directed downward; endothecal membranes, without spines; phallotremalsclerite, in dorsalview, distinct, strongly sclerotized, slightly divided distally into two slender, paired lobes with rounded apices, each lobe bearing one subtriangular lateral, flap, accompanied basally with pair of very dark, subapical, comma-shaped sclerites; in lateral view, slender, slightly upturned apically. Ejaculatory duct of endophallus, in lateral view, distinct, sclerotized. When endothecal membranes everted (Fig. 3g, i), distinctly reticulated, with conical sclerite, directed anterad in lateral view.

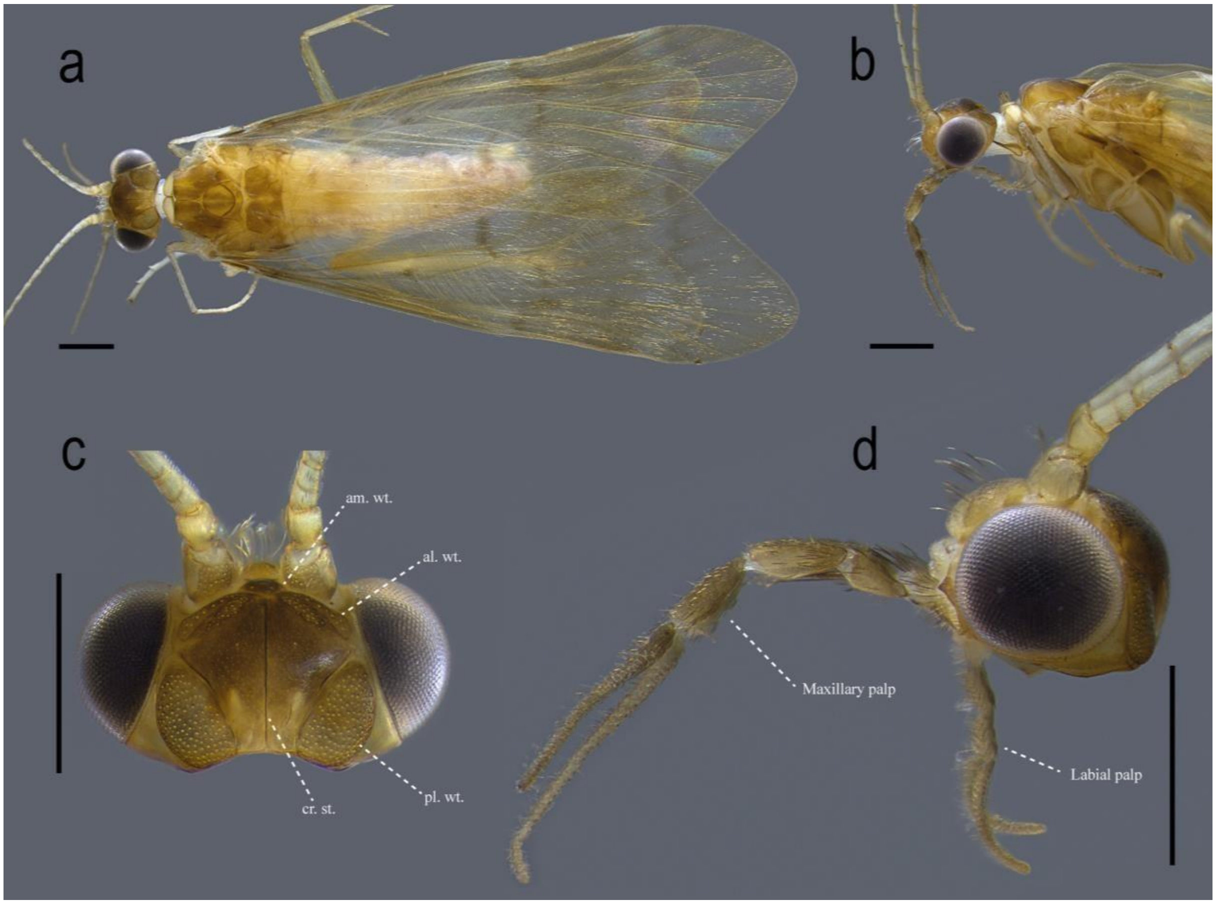

Fig. 1 *Smicridea (Rhyacophylax) exu* **sp. nov.**, male (UFVB TR00865): **a** dorsal habitus; **b** head and thorax, lateral view; **c** head, dorsal view; **d** head, lateral view. Abbreviations: al. wt. = anterolateral wart; am. wt. = anteromesal wart; cr. st. = coronal suture. Scale bars: 0.5 mm.

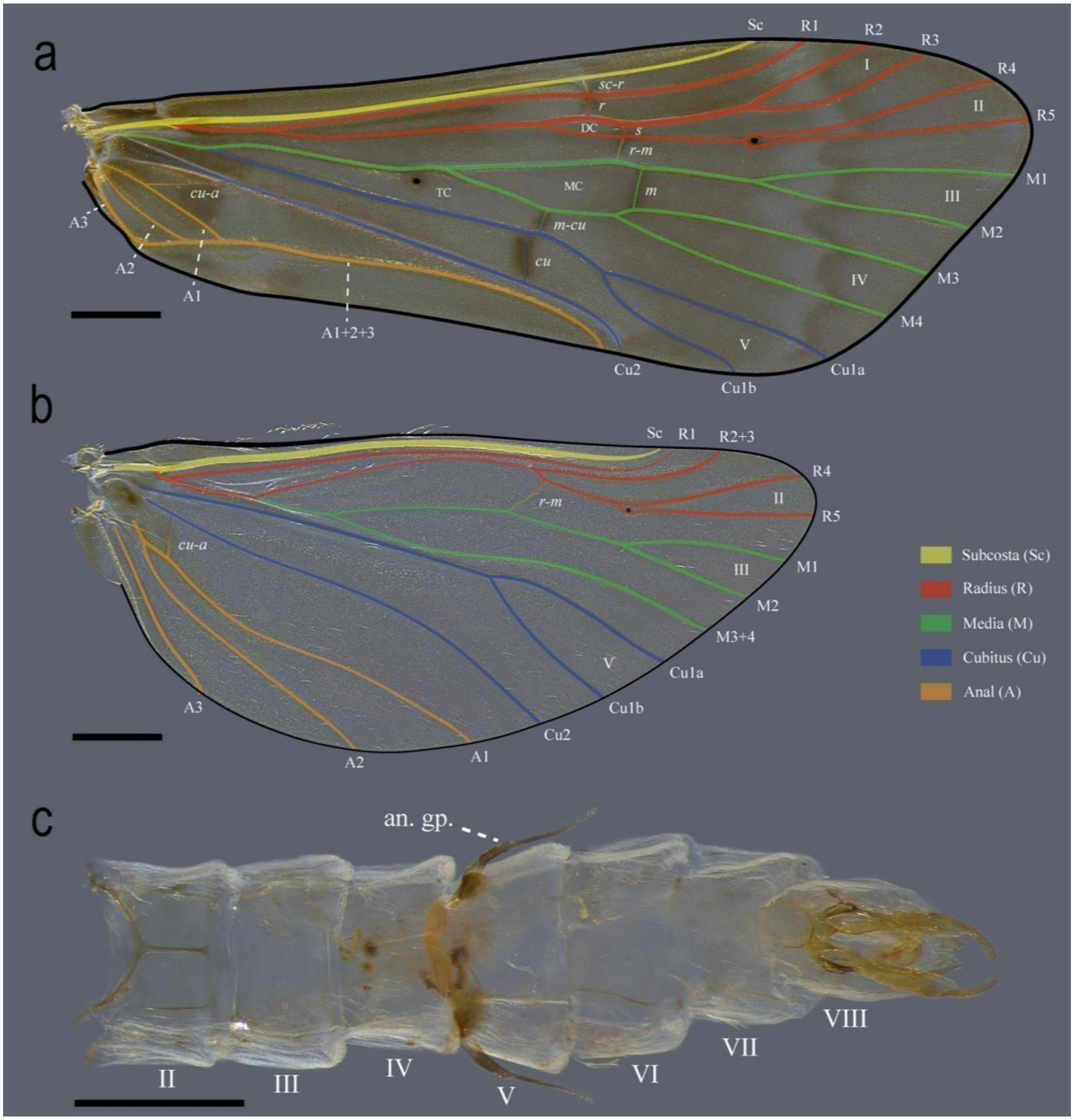

Fig. 2 *Smicridea (Rhyacophylax) exu* **sp. nov.**, male (UFVB TR00865): **a** forewing, dorsal view; **b** hindwing, dorsal view; **c** abdomen, ventral view. Abbreviations: an. gp. = anterolateral glandular process. Scale bars: 0.5 mm.

Female and immature. Unknown.

Holotype. Male.

Material examined:

BRAZIL: Minas Gerais: Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427 m, 1 ♂, 20-21.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataíde, Pennsylvania trap (UFVB TR00862).

Paratypes. Males.

Material examined:

BRAZIL: Minas Gerais: Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427m, 7 ♂, 20-21.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB TR00863), same data, except, 6 ♂, (INPA), same data, except 6 ♂, (MNRJ), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 2 ♂, 14.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB TR00864), Ponte Nova, Rio Piranga, UHE Brito, 20°26'43.10"S 42°59'05.70"W, el. 440m, 1 ♂, 11-12.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB TR00865), 8 ♂, 11-12.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB TR00866), Rio Doce, Rio Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 1 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB TR00867), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 1 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB TR00868), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 1 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB TR00869), Santo Antônio do Rio Abaixo, Rio Santo Antônio, 19°13'52.40"S 43°12'54.80"W, el. 475m, 6 ♂, 19-20.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB TR00870) same data, except, 6 ♂, (INPA), same data, except 6 ♂, (MNRJ).

Etymology

The specific epithet *exu* represents a central figure in Afro-Brazilian traditions, written Esù in its language of origin, Yoruba. In religious syncretism with Christian religions, exu corresponds to Santo Antônio, the name of the river where the species was found. The specific epithet is used in apposition.

Distribution

BRAZIL: Atlantic Forest biome (Minas Gerais state) (Fig. 4).

Remarks

In the non-everted phallic apparatus, the phallotremal sclerite remains almost entirely inside the structure (Fig. 3f, h). However, in the fully everted state, it becomes fully exposed. Another key difference between these states in the orientation of the sclerite (sc.), which points posteriorly when not everted (Fig. 3f, h) but shifts anteriorly when fully everted (Fig. 3g, i). The new species was primarily found in two sub-basins, the Rio Piranga and Rio Santo Antônio basins, with only a single specimen collected from the main stem of the Rio Doce (Fig. 4).

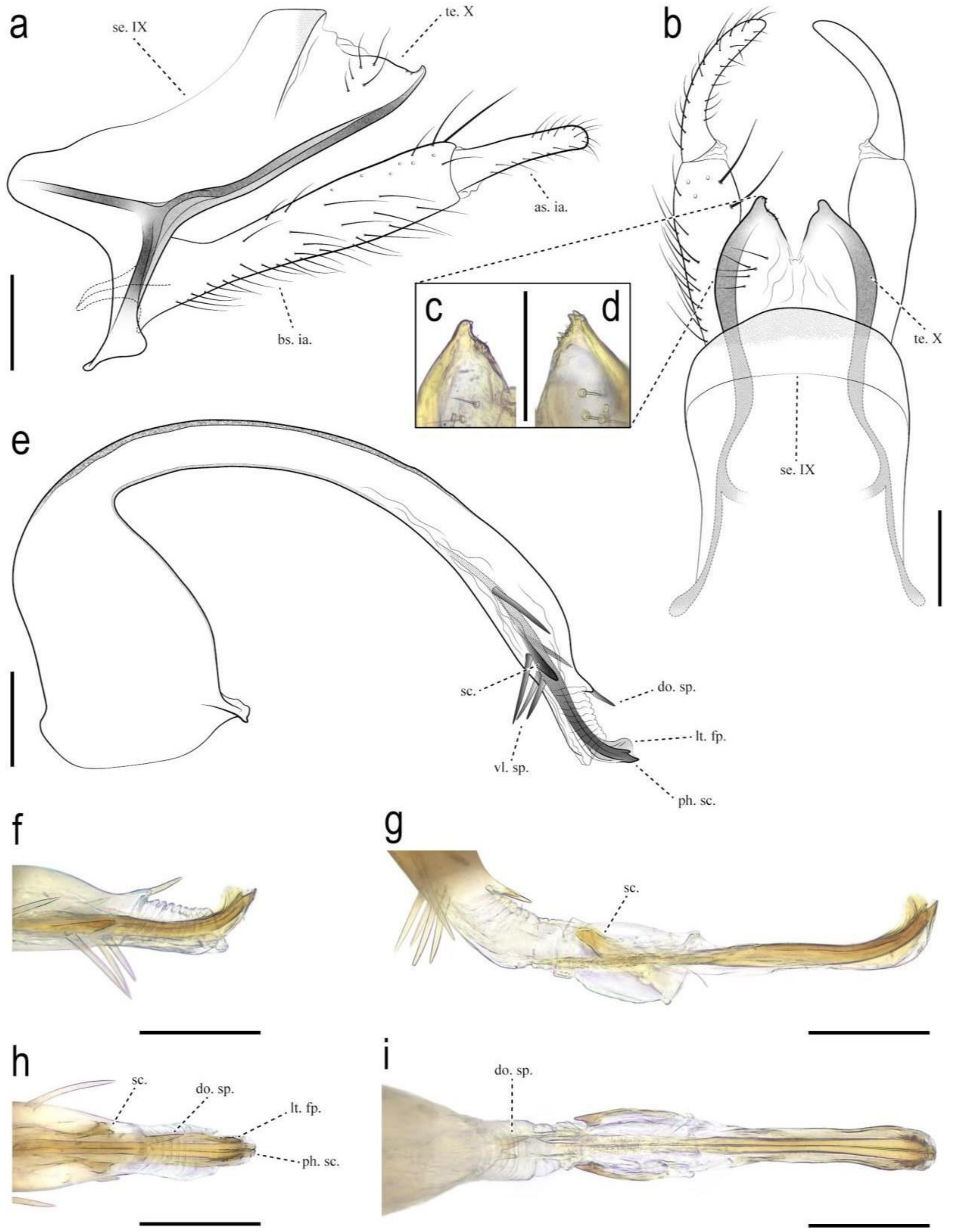

Fig. 3 *Smicridea (Rhyacophylax) exu* **sp. nov.**, male genitalia (**a**–**c**, **e**, **f**, **h** = Holotype UFVB TR00862) (**d**, **g**, **i** = Paratype UFVB TR00863): **a** lateral view; **b** dorsal view; **c**–**d** apex of tergum X, dorsal; **e** phallic apparatus, lateral

view; **f** apex phallic apparatus, lateral view; **g** apex of everted phallic apparatus, lateral view; **h** apex phallic apparatus, dorsal view; **i** apex of everted phallic apparatus, dorsal view. Abbreviations: as. ia. = apical segment of inferior appendage; bs. ia. = basal segment of inferior appendage; do. sp. = dorsal spine; lt. fp. = lateral flap; ph. sc. = phallotremal sclerite; sc. = sclerite; se. = segment; te. = tergum; vl. sp. = ventrolateral spine. Scale bars: 0.01 mm.

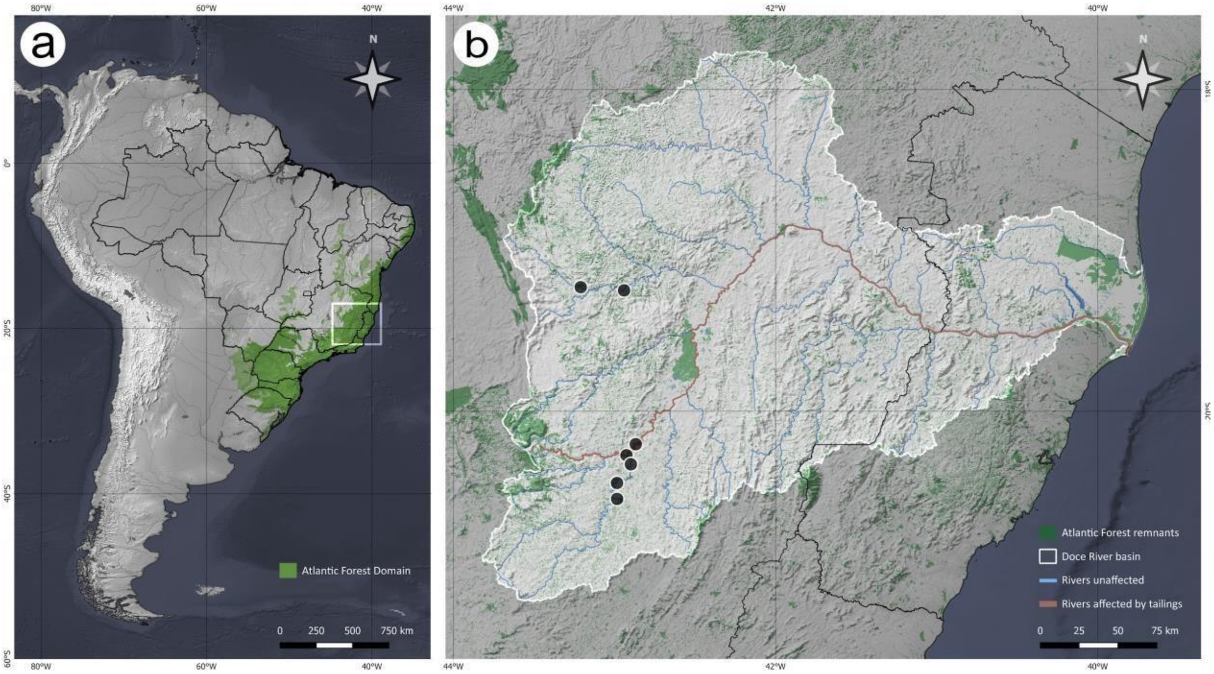

Fig. 4 Geographical distribution maps of the *Smicridea (Rhyacophylax) exu* **sp. nov.**: **a** South America map with the extent of the Atlantic Forest in green and the location of the Doce River basin highlighted; **b** Rio Doce basin with sampling sites of the new species (black dots) and the local river network.

Checklist

Smicridea* (*Rhyacophylax*) *appendiculata Flint, 1972

Type locality

Argentina, Pcia. Santa Fe, Arroyo Saladillo, near Santa Fe.

Literature

Flint, 1972: 238, figs 32–34 (♂, NMNH); Flint, 1978: 377 (distribution); Flint et al., 1999: 72 (catalog); Blahnik et al., 2004: 4 (distribution); Cohen, 2004: 75 (distribution); Paprocki et al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Albino et al., 2011: 21 (♂ phallus, larva, pupa, biology, distribution); Nogueira and Cabette, 2011: 351 (distribution); Paprocki and França, 2014: 32 (checklist); Desidério et al., 2017: 162 (distribution); Holzenthal and Calor, 2017: 162 (catalog); Sganga and Gibon, 2018: 403 (distribution); Moreno et al., 2020: 263 (distribution).

Material examined

BRAZIL: Espírito Santo: Linhares, Rio Doce, 19°26'06.40"S 39°57'04.80"W, el. 12m, 100 ♂, 13-14.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Santa Fe province, BOLIVIA: Beni department, BRAZIL: Amazonas, **Espírito Santo** (**new record**), Maranhão, Mato Grosso, Minas Gerais, Piauí, Roraima states.

Remarks

This species has been known in the Atlantic Forest since its original description (Flint, 1972), with the paratype designated from the Atlantic Forest in the Argentine province of Misiones. There were no previous records of *S. appendiculata* from the Brazilian Atlantic Forest, so the specimen collected in the lower Rio Doce basin, represents its first record (Fig. 5).

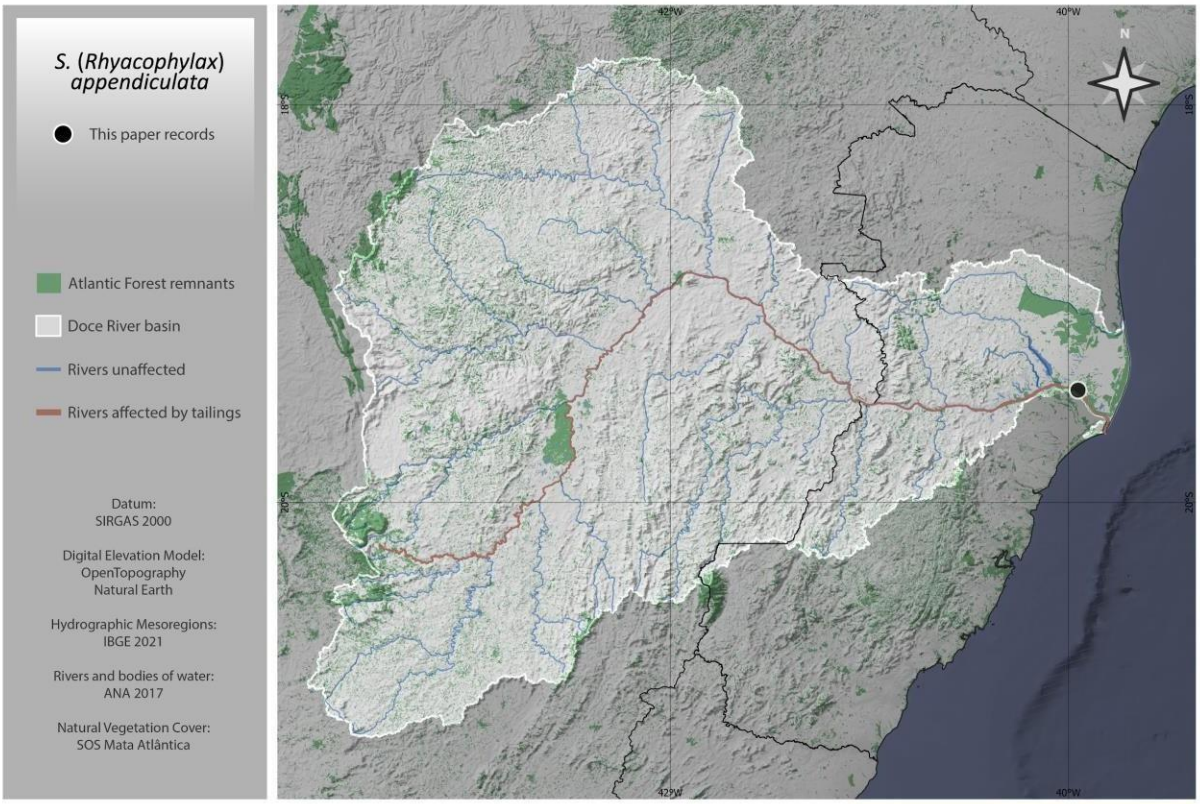

Fig. 5 Geographical distribution maps of *Smicridea (Rhyacophylax) appendiculata* Flint, 1972 in the Rio Doce basin.

Smicridea (Rhyacophylax) atrobasis Flint, 1983

Type locality

Argentina, Pcia. Entre Ríos, Salto Grande, Río Uruguay.

Literature

Flint, 1983:63, figs 227–229 (♂, NMNH); Flint et al., 1999: 72 (catalog); Paprocki et al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Sganga and Angrisano, 2005: 132 (♂; distribution); Rueda-Martín and Sganga, 2011: 2225 (♂; distribution); Paprocki and França, 2014: 32 (checklist); Isa Miranda and Rueda-Martín, 2014: 200 (distribution); Holzenthal and Calor, 2017: 163 (catalog); Sganga et al., 2022: 400 (♂; distribution).

Material examined

BRAZIL: Minas Gerais: Barra Longa, Rio do Carmo, 20°16'50.90"S 43°02'26.80"W, el. 378m, 6 ♂, 02-03.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 4 ♂, 14.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Jequiri, Rio Casca, 20°28'55.70"S 42°40'31.80"W, el. 415m, 1 ♂, 12-13.viii.2022, ADL Viana, P Bonfá- Neto, MCD Correia, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 1 ♂, 09-10.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 3 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 1 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Santo Antônio do Rio Abaixo, Rio Santo Antônio, 19°13'52.40"S 43°12'54.80"W, el. 475m, 2 ♂, 19-20.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Entre Ríos, Misiones, Jujuy, Salta, Tucumán provinces, BOLIVIA: Tarija, BRAZIL: Santa Catarina, **Minas Gerais** (**new record**) states, URUGUAY: Artigas, Paysandú, Salto departments.

Remarks

This species has been known in the Atlantic Forest since its original description (Flint, 1983), with the paratypes designated from the Argentine and Brazilian Atlantic Forests, specifically in the state of Santa Catarina. There are no previous records of the species from the Rio Doce basin. In the present study, specimens were found in the upper Rio Doce region, in the sub-basins of the Santo Antônio, Carmo, Piranga, and Casca rivers, as well as in the main stream of the Rio Doce (Fig. 6).

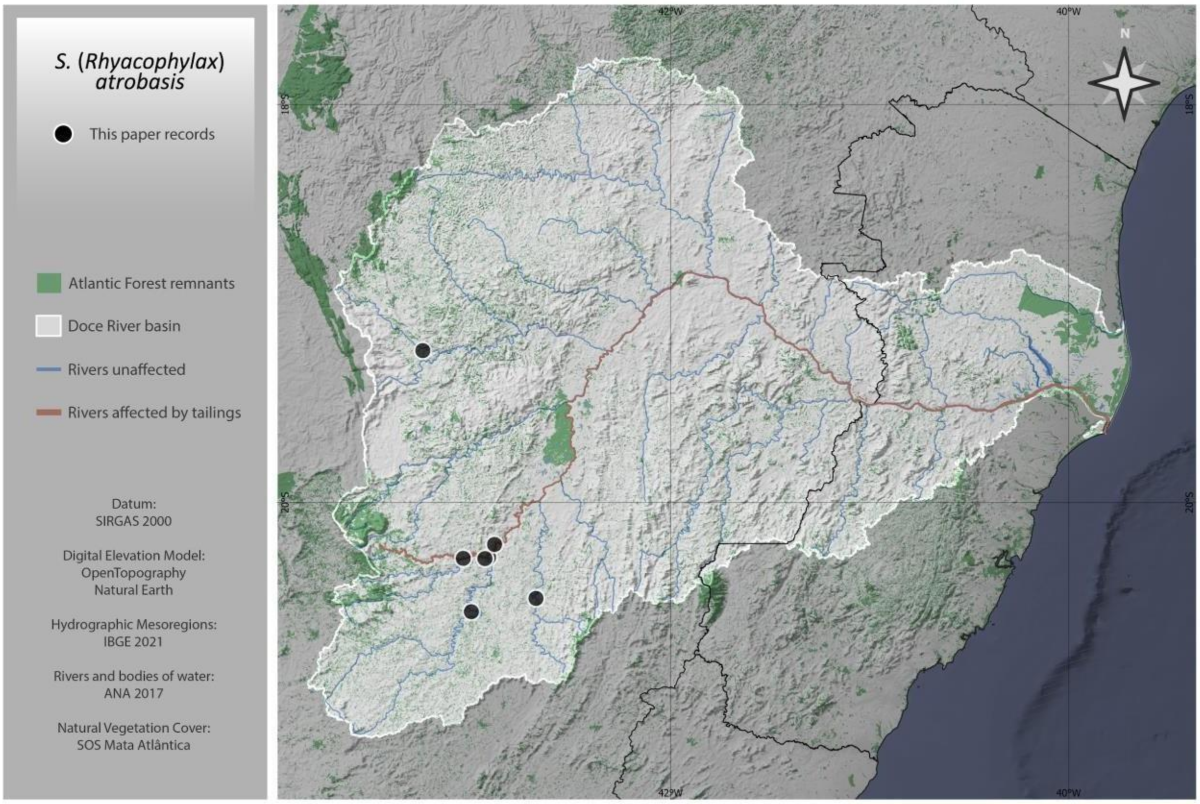

Fig. 6 Geographical distribution maps of *Smicridea (Rhyacophylax) atrobasis* Flint, 1983 in the Rio Doce basin.

Smicridea (Rhyacophylax) coronata Flint, 1980

Type locality

Argentina, Pcia. Cordoba, Villa Anizacate.

Literature

Flint, 1980: 138, figs 5–10 (♂, NMNH); Oliveira and Froehlich, 1996: 757 (biology); Flint et al., 1999: 74 (catalog); Mangeaud, 1996: 154 (distribution); Paprockiet al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Sganga and Angrisano, 2005: 133 (♂; ♀; distribution); Calor, 2011: 321 (checklist); Albino et al., 2011: 33 (♂; variation, distribution); Nogueira and Cabette, 2011: 351 (distribution); Barcelos-Silva et al., 2012: 1278 (distribution); Paprocki and França, 2014: 33 (checklist); Moretto and Bispo, 2015: 126 (distribution); Holzenthal and Calor, 2017: 167 (catalog); Souza et al., 2017: 352 (distribution); Vilarino and Calor, 2017: 511 (distribution); Alves et al., 2018: 393 (distribution); Moreno et al., 2020: 263 (checklist); Andrade Soares et al., 2024: 3 (biology, distribution).

Material examined

BRAZIL: Espírito Santo: Baixo Guandu, Rio Doce, 19°30'23.00"S 41°01'01.80"W, el. 60m, 14 ♂, 09-10.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Baixo Guandu, Rio Guandu, 19°37'30.00"S 41°01'07.80"W, el. 90m, 2 ♂, 09-10.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Colatina, Rio Doce, 19°32'14.00"S 40°38'23.46"W, el. 35m, 58 ♂, 05.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 72 ♂, 11-12.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Lagoa Juparanã Mirin (Lagoa Nova), 19°19'49.00"S 40°10'12.30"W, el. 16m, 6 ♂, 12-13.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Rio Doce, 19°24'23.10"S 40°04'03.70"W, el. 17m, 25 ♂, 12-13.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Rio Doce, 19°26'06.40"S 39°57'04.80"W, el. 12m, 491 ♂, 13-14.ix.2022, ADL Viana, P

Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB); Minas Gerais: Aimorés, Rio Doce, 19°29'11.20"S 41°04'30.20"W, el. 80m, 8 ♂, 06.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 1 ♂, 08-09.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 4 ♂, 06.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'29.10"S 41°11'13.30"W, el. 98m, 4 ♂, 10- 11.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Barra do Cuieté, Rio Caratinga, 19°03'49.90"S 41°31'50.20"W, el. 120m, 1 ♂, 06-07.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Barra Longa, Rio do Carmo, 20°16'50.90"S 43°02'26.80"W, el. 378m, 3 ♂, 02-03.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Governador Valadares, Rio Doce, 18°55'50.27"S 42°01'20.23"W, el. 163m, 9 ♂, 04-05.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), 3

♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 23 ♂, 14.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Jaguaraçu, Rio Piracicaba, 19°37'01.00"S 42°46'46.50"W, el. 245m, 4 ♂, 11.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Jequiri, Rio Casca, 20°28'55.70"S 42°40'31.80"W, el. 415m, 2 ♂, 12-13.viii.2022, ADL Viana, P Bonfá-Neto, MCD Correia, Pennsylvania trap (UFVB), Mariana, Rio Gualaxo do Norte, 20°16'35.10"S 43°25'53.90"W, el. 731m, 16 ♂, 08-09.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 59 ♂, 13.i.2022, ADL Viana, P

Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Matias Lobato, Rio Suaçuí Grande, 18°34'23.50"S 41°56'52.30"W, el. 197m, 70 ♂, 05-06.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Naque, Rio Doce, 19°15'05.56"S 42°18'50.50"W, el. 190m, 12 ♂, 03-04.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), 791 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB), Naque, Rio Santo Antônio, 19°14'04.10"S 42°20'24.80"W, el. 195m, 62 ♂, 03- 04.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Periquito, Rio Corrente, 19°00'21.10"S 42°10'32.00"W, el. 190m, 13 ♂, 04-05.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 2 ♂, 07.ii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Raul Soares, Rio Matipó, 19°54'58.10"S 42°32'26.60"W, el. 263m, 1 ♂, 16-17.viii.2022, ADL Viana, P Bonfá -Neto, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 2 ♂, 09-10.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 25 ♂, 09- 10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47.20"S 42°51'07.20"W, el. 280m, 14 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 2 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 24 ♂, 12.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 15 ♂, 10-11.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 24 ♂, 12.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 1 ♂, 13-14.viii.2022, ADL Viana, P Bonfá -Neto, MCD Correia, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Córdoba, Corrientes, Entre Ríos, Misiones provinces, BRAZIL: Bahia, Ceará, Espírito Santo, Mato Grosso, Minas Gerais, Piauí, São Paulo, Rio Grande do Sul states, PARAGUAY: Guaira departments, URUGUAY: Artigas, Lavalleja, Paysandú, Salto, Tacuarembó departments.

Remarks

This species has been known in the Atlantic Forest since its description (Flint, 1980) with paratypes designated from the Argentine and Brazilian Atlantic Forests. This species was previously recorded in the Rio Doce basin, with some of the type materialcollected in the municipality of Viçosa, MG, located in the upper Rio Doce region, and subsequent records from other locations in the upper Rio Doce region. In the present, specimens were found to be widely distributed throughout the Rio Doce basin (Fig. 7).

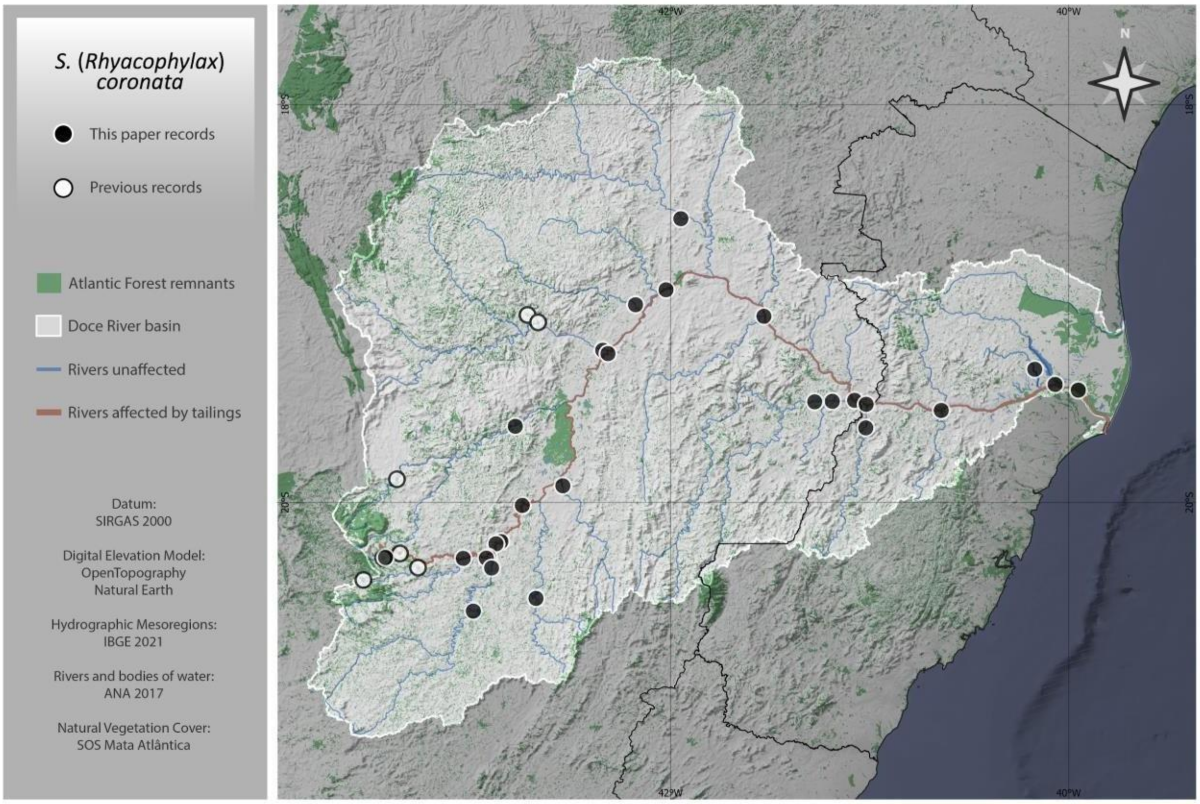

Fig. 7 Geographical distribution maps of *Smicridea (Rhyacophylax) coronata* Flint, 1980.

Smicridea (Rhyacophylax) iguazu Flint, 1983

Type locality

Argentina, Pcia. Misiones, Río Iguazú, Camp Nañdu.

Literature

Flint, 1983: 60, figs 214–220 (♂, NMNH); Flint et al., 1999: 75 (catalog); Marinoni and Almeida, 2000: 286 (distribution, biology); Blahnik et al., 2004: 4 (distribution); Paprocki et al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Dumas et al., 2009: 359 (distribution); Dumas and Nessimian, 2012: 25 (distribution); Barcelos-Silva et al., 2012: 1278 (distribution); Paprocki and França, 2014: 34 (checklist); Moretto and Bispo, 2015: 126 (distribution); Holzenthal and Calor, 2017: 172 (catalog); Souza et al., 2017: 353 (distribution); Moreno et al., 2020: 263 (distribution); Costa et al., 2024: 2 (♂, distribution).

Material examined

BRAZIL: Espírito Santo: Baixo Guandu, Rio Guandu, 19°37'30.00"S 41°01'07.80"W, el. 90m, 27 ♂, 09-10.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Colatina, Rio Doce, 19°32'14.00"S 40°38'23.46"W, el. 35m, 1 ♂, 11-12.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Rio Doce, 19°26'06.40"S 39°57'04.80"W, el. 12m, 1 ♂, 13-14.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB); Minas Gerais: Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 55 ♂, 06.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'29.10"S 41°11'13.30"W, el. 98m, 3 ♂, 10-11.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 8 ♂, 10-11.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Barra do Cuieté, Rio Caratinga, 19°03'49.90"S 41°31'50.20"W, el. 120m, 1 ♂, 06-07.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Barra Longa, Rio do Carmo, 20°16'50.90"S 43°02'26.80"W, el. 378m, 8 ♂, 02-03.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427m, 3 ♂, 20- 21.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 342 ♂, 14.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB), Jequiri, Rio Casca, 20°28'55.70"S 42°40'31.80"W, el. 415m, 2 ♂, 12-13.viii.2022, ADL Viana, P Bonfá-Neto, MCD Correia, Pennsylvania trap (UFVB), Mariana, Rio Gualaxo do Norte, 20°16'35.10"S 43°25'53.90"W, el. 731m, 7 ♂, 13.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Matias Lobato, Rio Suaçuí Grande, 18°34'23.50"S 41°56'52.30"W, el. 197m, 332 ♂, 05-06.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Periquito, Rio Corrente, 19°00'21.10"S 42°10'32.00"W, el. 190m, 1 ♂, 04-05.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 3 ♂, 07.ii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Ponte Nova, Rio Piranga, UHE Brito, 20°26'43.10"S 42°59'05.70"W, el. 440m, 20 ♂, 11-12.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 8 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 963 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47.20"S 42°51'07.20"W, el. 280m, 15 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 2 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 32 ♂, 12.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 460 ♂, 10-11.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Santo Antônio do Rio Abaixo, Rio Santo Antônio, 19°13'52.40"S 43°12'54.80"W, el. 475m, 4 ♂, 09.i.2022, ADL Viana, P Bonfá- Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 101 ♂, 19-20.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), São José do Goiabal, Rio Casca, 20°02'56.40"S 42°38'40.80"W, el. 260m, 2 ♂, 13-14.viii.2022, ADL Viana, P Bonfá -Neto, MCD Correia, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 9 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Misiones provinces, BRAZIL: Bahia, Espírito Santo, Minas Gerais, Paraná, Piauí, Rio de Janeiro, Santa Catarina states.

Remarks

This species has been known in the Atlantic Forest since its description (Flint 1983) with type material designated from the Argentine and Brazilian Atlantic Forests in the states of Santa Catarina and Rio de Janeiro. This species has previous records in the Rio Doce basin, in both the upper and lower regions of the basin. In the present study, specimens were found to be widely distributed throughout the Rio Doce basin (Fig. 8).

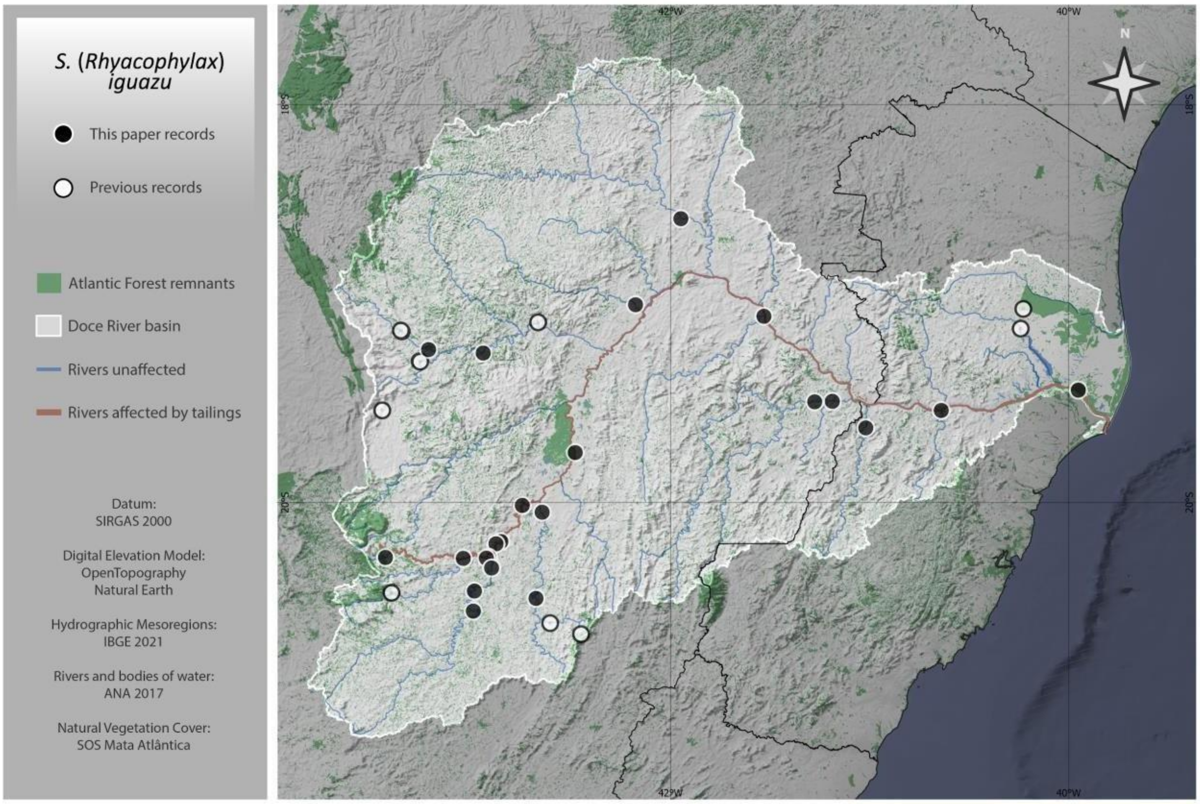

Fig. 8 Geographicaldistribution maps of *Smicridea (Rhyacophylax) iguazu* Flint, 1983.

Smicridea (Rhyacophylax) jundiai Almeida & Flint, 2002

Type locality

Brazil, Espírito Santo, 15 km SE. of Santa Teresa, Fazenda Santa Clara.

Literature

Almeida and Flint, 2002: 769 figs 2A–D (♂, MZUSP); Paprocki et al., 2004: 9 (checklist); Dumas et al., 2009: 360 (distribution); Dumas et al., 2010:8 (distribution); Calor, 2011: 321 (checklist); Dumas and Nessimian, 2012: 14 (distribution); Barcelos-Silva et al., 2012: 1278 (distribution); Paprockiand França, 2014: 34 (checklist); Moretto and Bispo, 2015: 126 (distribution); Holzenthal and Calor, 2017: 173 (catalog); Souza et al., 2017: 353 (distribution); Vilarino and Calor, 2017: 511 (distribution); Alves et al., 2018: 393 (distribution); Santos et al., 2022: 517 (distribution).

Material examined

BRAZIL: Minas Gerais: Barra Longa, Rio do Carmo, 20°16'50.90"S 43°02'26.80"W, el. 378m, 261 ♂, 02-03.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427m, 9 ♂, 20-21.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 1 ♂, 11-12.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 681 ♂, 14.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Jaguaraçu, Rio Piracicaba, 19°37'01.00"S 42°46'46.50"W, el. 245m, 1 ♂, 11.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Jequiri, Rio Casca, 20°28'55.70"S 42°40'31.80"W, el. 415m, 3 ♂, 12-13.viii.2022, ADL Viana, P Bonfá-Neto, MCD Correia, Pennsylvania trap (UFVB), Mariana, Rio Gualaxo do Norte, 20°16'35.10"S 43°25'53.90"W, el. 731m, 3 ♂, 13.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 1 ♂, 07.ii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Ponte Nova, Rio Piranga, UHE Brito, 20°26'43.10"S 42°59'05.70"W, el. 440m, 53 ♂, 11-12.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 3 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 50 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47.20"S 42°51'07.20"W, el. 280m, 6 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 76 ♂, 10-11.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), 101 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 25 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Santo Antônio do Rio Abaixo, Rio Santo Antônio, 19°13'52.40"S 43°12'54.80"W, el. 475m, 1 ♂, 09.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 107 ♂, 19-20.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), São José do Goiabal, Rio Casca, 20°02'56.40"S 42°38'40.80"W, el. 260m, 2 ♂, 13- 14.viii.2022, ADL Viana, P Bonfá-Neto, MCD Correia, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 1 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB).

Distribution

BRAZIL: Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Paraná, Rio de Janeiro, São Paulo states.

Remarks

This species has been known in the Atlantic Forest since its description (Almeida and Flint, 2002) with type material from many locations in the Brazilian Atlantic Forest. This species has previous records in the Rio Doce basin, in both the upper and lower regions of the basin. In the present study, specimens were found only in the upper region of the Rio Doce (Fig. 9).

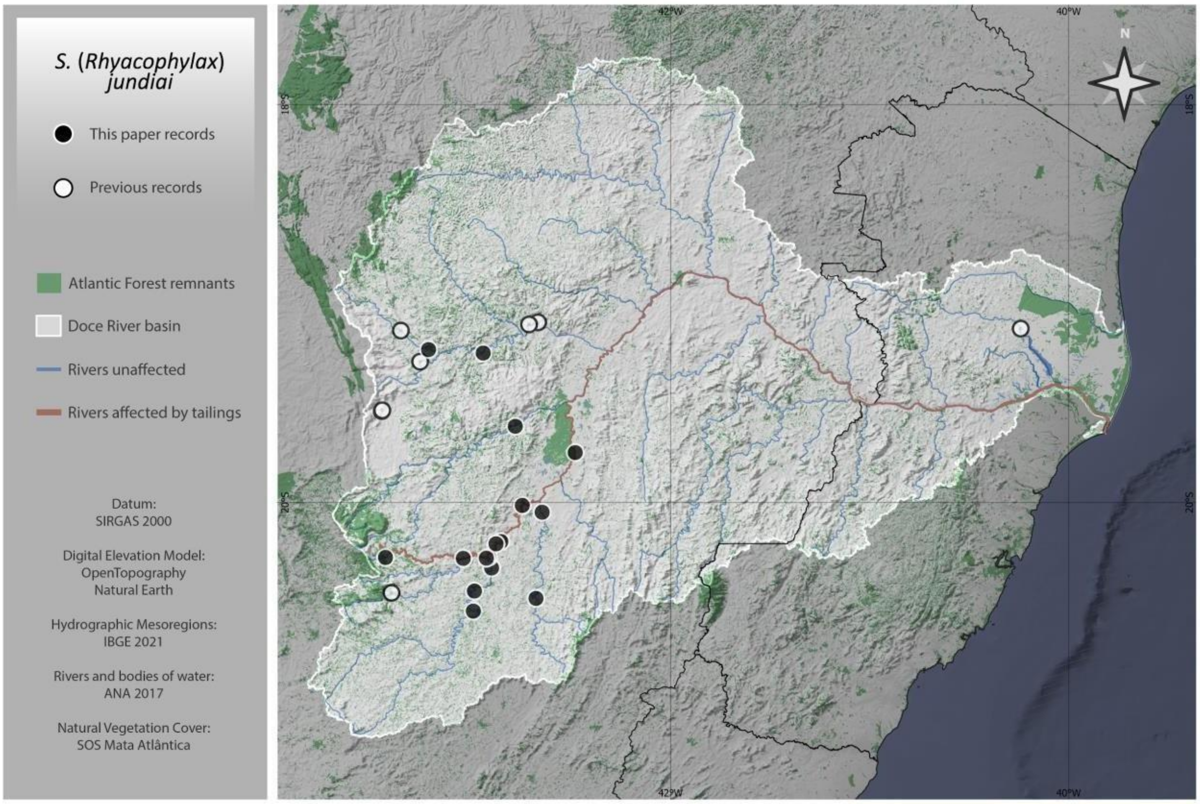

Fig. 9 Geographical distribution maps of *Smicridea (Rhyacophylax) jundiai* Almeida & Flint, 2002.

Smicridea (Rhyacophylax) palmar Sganga, 2005

Type locality

Argentina, Entre Ríos, arroyo El Palmar, Ruta Nacional 14.

Literature

Sganga, 2005: 143 figs 1–7 (♂ ♀, MACN); Angrisano and Sganga, 2007: 13 (♂; ♀; distribution); Albino et al., 2011: 3 (distribution); Nogueira and Cabette, 2011: 351 (distribution); Barcelos-Silva et al., 2012: 1278 (distribution); Souza et al., 2013: 5 (distribution); Paprocki and França, 2014: 35 (checklist); Holzenthal and Calor, 2017: 179 (catalog); Desidério et al., 2017: 162 (distribution); Alves et al., 2018: 393 (distribution); Sganga and Gibon, 2018: 405 (distribution).

Material examined

BRAZIL: Minas Gerais: Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 3 ♂, 06.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Governador Valadares, Rio Doce, 18°55'50.27"S 42°01'20.23"W, el. 163m, 2 ♂, 04-05.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Jaguaraçu, Rio Piracicaba, 19°37'01.00"S 42°46'46.50"W, el. 245m, 1 ♂, 11.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Mariana, Rio Gualaxo do Norte, 20°16'35.10"S 43°25'53.90"W, el. 731m, 3 ♂, 08-09.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Naque, Rio Doce, 19°15'05.56"S 42°18'50.50"W, el. 190m, 13 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 1 ♂, 17-18.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 67 ♂, 09-10.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47.20"S 42°51'07.20"W, el. 280m, 2 ♂, 10- 11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 6 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 89 ♂, 10- 11.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 2 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Tumiritinga, Rio Doce, 18°58'07.56"S 41°39'49.36"W, el. 133m, 2 ♂, 07.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Entre Ríos province, BOLIVIA: Beni, Cochabamba departments, BRAZIL: Espírito Santo, Maranhão, Mato Grosso, **Minas Gerais** (**new record**), Pernambuco, Goiás states.

Remarks

This species was first recorded in the Atlantic Forest in the state of Espírito Santo (Barcelos-Silva et al., 2012). The record is located in the municipality of Sooretama, in the lower region of the Rio Doce. In the present study, specimens were found in the upper and middle regions of the basin, in the main river or near it (Fig. 10).

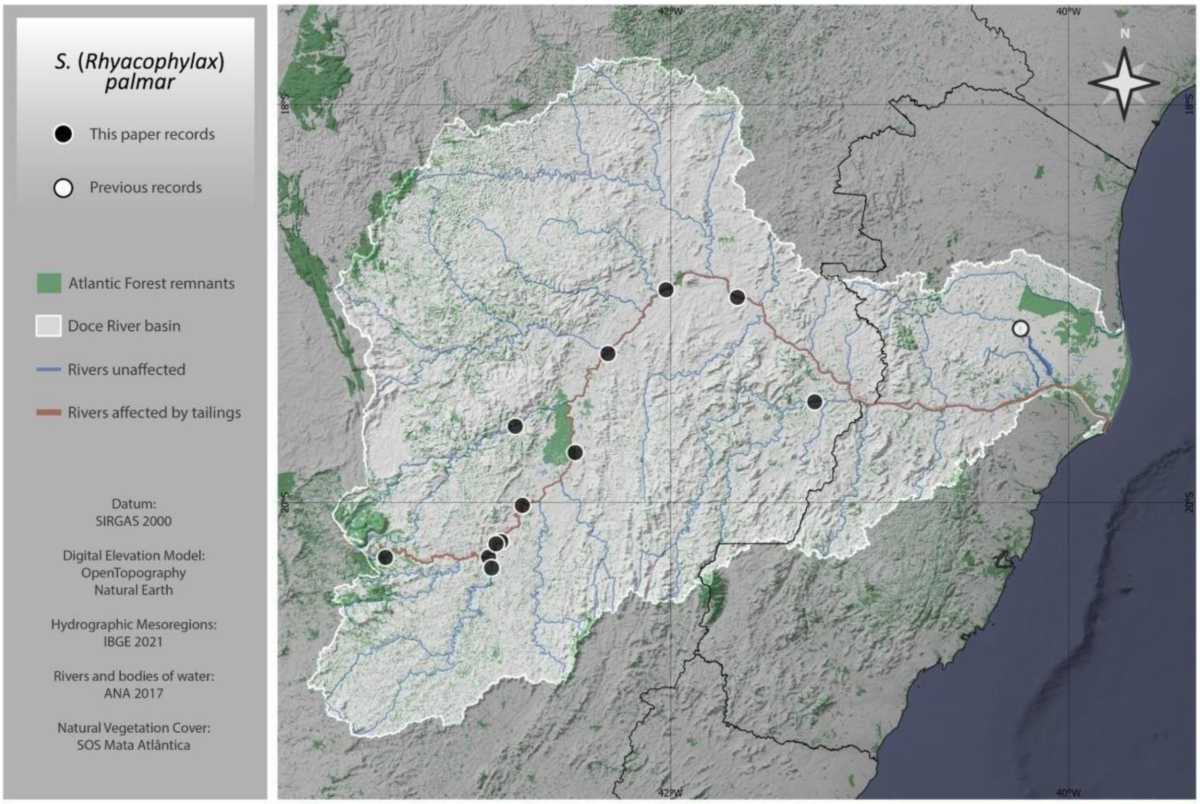

Fig. 10 Geographical distribution maps of *Smicridea (Rhyacophylax) palmar* Sganga, 2005.

Smicridea (Rhyacophylax) piraya Flint, 1983

Type locality

Argentina, Pcia. Misiones, Arroyo Piray Guazú, N San Pedro.

Literature

Flint, 1983: 58 figs 210–213 (♂, NMNH); Flint et al., 1999: 78 (catalog); Marinoni and Almeida, 2000: 286 (distribution; biology); Blahnik et al., 2004: 4 (distribution); Paprocki et al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Calor, 2011: 321 (checklist); Barcelos-Silva et al., 2012: 1278 (distribution); Paprocki and França, 2014: 35 (checklist); Moretto and Bispo, 2015: 126 (distribution); Holzenthaland Calor, 2017: 180 (catalog); Vilarino and Calor, 2017: 511 (distribution).

Material examined

Material examined: BRAZIL: Minas Gerais: Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427m, 1 ♂, 20-21.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Naque, Rio Doce, 19°15'05.56"S 42°18'50.50"W, el. 190m, 1 ♂, 03-04.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Misiones province, BRAZIL: Bahia, Espírito Santo, Minas Gerais, Paraná, Santa Catarina, São Paulo states.

Remarks

This species has been known in the Atlantic Forest since its description (Flint 1983) with type material designated from the Argentine and Brazilian Atlantic Forests in the states of Santa Catarina and São Paulo. This species has previous records in the Rio Doce basin, in the upper region of the basin. In the present study, only two specimens were found, one in the Santo Antônio River sub-basin and the other near this sub-basin, both in the upper region of the Rio Doce basin (Fig. 11).

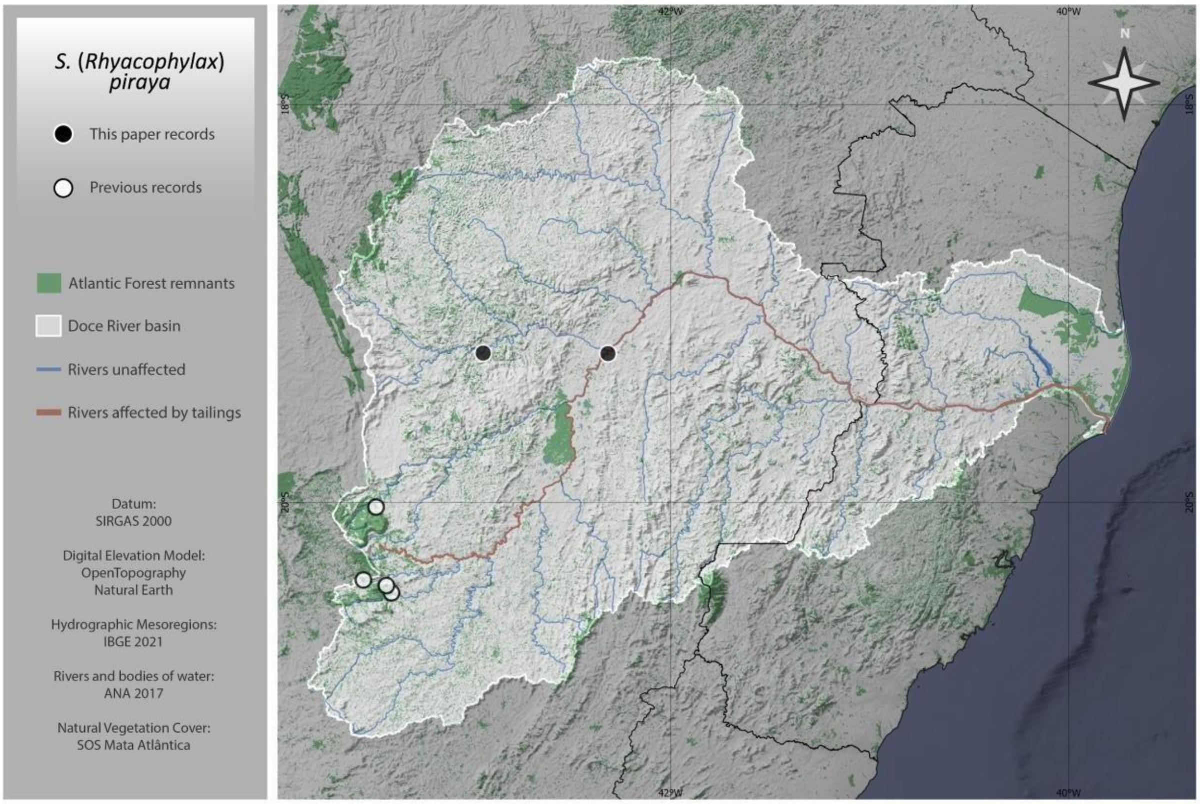

Fig. 11 Geographical distribution maps of *Smicridea (Rhyacophylax) piraya* Flint, 1983.

Smicridea (Rhyacophylax) radula Flint, 1974

Type locality

Costa Rica, San Jose, Río General, Pacuare.

Literature

Flint, 1974a: 36 figs 31, 40, 162–165, 199–202 (♂ ♀, NMNH); Bueno-Soria and Flint, 1978: 208 (distribution); Holzenthal, 1988: 69 (distribution); Aguila, 1992: 542 (distribution); Flint et al., 1999: 78 (catalog); Maes, 1999: 1186 (checklist); Blahnik et al., 2004: 4 (distribution); Paprocki et al., 2004: 9 (checklist); Chamorro -Lacayo et al., 2007: 42 (checklist); Dumas et al., 2009: 360 (distribution); Bueno-Soria and Barba-Álvarez, 2011: 356 (checklist); Calor, 2011: 321 (checklist); Dumas and Nessimian, 2012: 25 (distribution); Barcelos-Silva et al., 2012: 1278 (distribution); Paprocki and França, 2014: 35 (checklist); Armitage et al., 2015: 6 (checklist); Armitage and Cornejo, 2015: 194 (checklist); Moretto and Bispo, 2015: 126 (distribution); Holzenthaland Calor, 2017: 181 (catalog); Ríos-Touma et al. 2017: 8 (distribution); Souza et al. 2017: 353 (distribution); Rázuri-Gonzales and Armitage, 2019: 2 (checklist); Razo-González et al., 2023: 6 (community ecology, distribution); Armitage et al., 2024: 281 (checklist).

Material examined

BRAZIL: Espírito Santo: Baixo Guandu, Rio Doce, 19°30'23.00"S 41°01'01.80"W, el. 60m, 1 ♂, 09-10.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB); Minas Gerais: Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 1 ♂, 06.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 6 ♂, 10-11.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Ferros, Rio Santo Antônio, 19°14'55.40"S 42°56'27.40"W, el. 427m, 91 ♂, 20-21.viii.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Governador Valadares, Rio Doce, 18°55'50.27"S 42°01'20.23"W, el. 163m, 14 ♂, 04-05.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 4 ♂, 11-12.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 129 ♂, 14.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Matias Lobato, Rio Suaçuí Grande, 18°34'23.50"S 41°56'52.30"W, el. 197m, 872 ♂, 05-06.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Naque, Rio Doce, 19°15'05.56"S 42°18'50.50"W, el. 190m, 10 ♂, 03-04.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), 21 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Periquito, Rio Corrente, 19°00'21.10"S 42°10'32.00"W, el. 190m, 2 ♂, 04-05.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 797 ♂, 07.ii.2022, ADL

Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 112 ♂, 17-18.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Raul Soares, Rio Matipó, 19°54'58.10"S 42°32'26.60"W, el. 263m, 1 ♂, 16-17.viii.2022, ADL Viana, P Bonfá -Neto, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 434 ♂, 09-10.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 801 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47 .20"S 42°51'07.20"W, el. 280m, 58 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 642 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 323 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P

Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 2133 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Santo Antônio do Rio Abaixo, Rio Santo Antônio, 19°13'52.40"S 43°12'54.80"W, el. 475m, 62 ♂, 19-20.viii.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), São José do Goiabal, Rio Casca, 20°02'56.40"S 42°38'40.80"W, el. 260m, 32 ♂, 13-14.viii.2022, ADL Viana, P Bonfá -Neto, MCD Correia, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 4 ♂, 12.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 8 ♂, 13-14.viii.2022, ADL Viana, P Bonfá -Neto, MCD Correia, Pennsylvania trap (UFVB), Tumiritinga, Rio Doce, 18°58'07.56"S 41°39'49.36"W, el. 133m, 1 ♂, 07.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB).

Distribution

BRAZIL: Bahia, Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, São Paulo states, COSTA RICA: Carta go, Guanacaste, Puntarenas, San Jose provinces, ECUADOR: Imbabura province, EL SALVADOR: La Libertad, Usulutan departments, GUATEMALA: Chimaltenango, Escuintla, Suchitepequez departments, HONDURAS: Atlantida, Comayagua departments, MEXICO: Chiapas, Oaxaca, Veracruz states, NICARAGUA: Zelaya department, PANAMA: Canal Zone, Chiriqui provinces.

Remarks

This species was first recorded in the Atlantic Forest (Blahnik et al. 2004) in several states of the southern and southeastern regions of Brazil. Previous records have been made in the Rio Doce basin, specifically in the upper region. In the present study, specimens were found in the basin's upper and middle regions (Fig. 12).

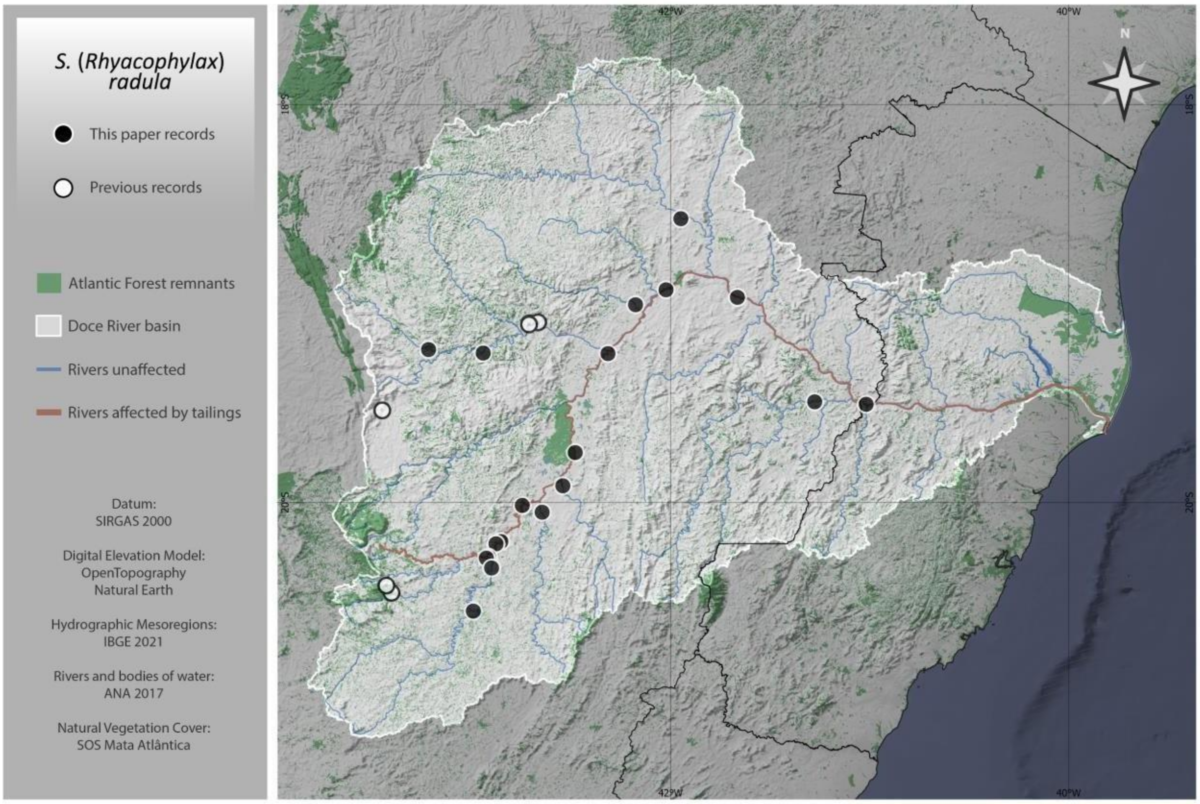

Fig. 12 Geographical distribution maps of *Smicridea (Rhyacophylax) radula* Flint, 1974.

Smicridea (Rhyacophylax) scalprum Vilarino, Dias & Calor, 2019

Type locality

Brazil, Bahia, Iaçu, Balneário O Pote, Rio Paraguaçu.

Literature

Vilarino et al., 2019: 710 figs 1–12 (♂, MZSP).

Material examined

Espírito Santo: Colatina, Rio Doce, 19°32'14.00"S 40°38'23.46"W, el. 35m, 1 ♂, 05.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB).

Distribution

BRAZIL: Bahia, **Espírito Santo** (**new record**) states.

Remarks

This species has been known in the Atlantic Forest since its description (Vilarino et al. 2019) with type material designated from the Atlantic Forest in the state of Bahia. There are no previous records of this species in the Rio Doce basin, making this the first record for the basin and for the state of Espírito Santo. In the present study, a single specimen was found in the basin's main river in the Colatina municipality (Fig. 13).

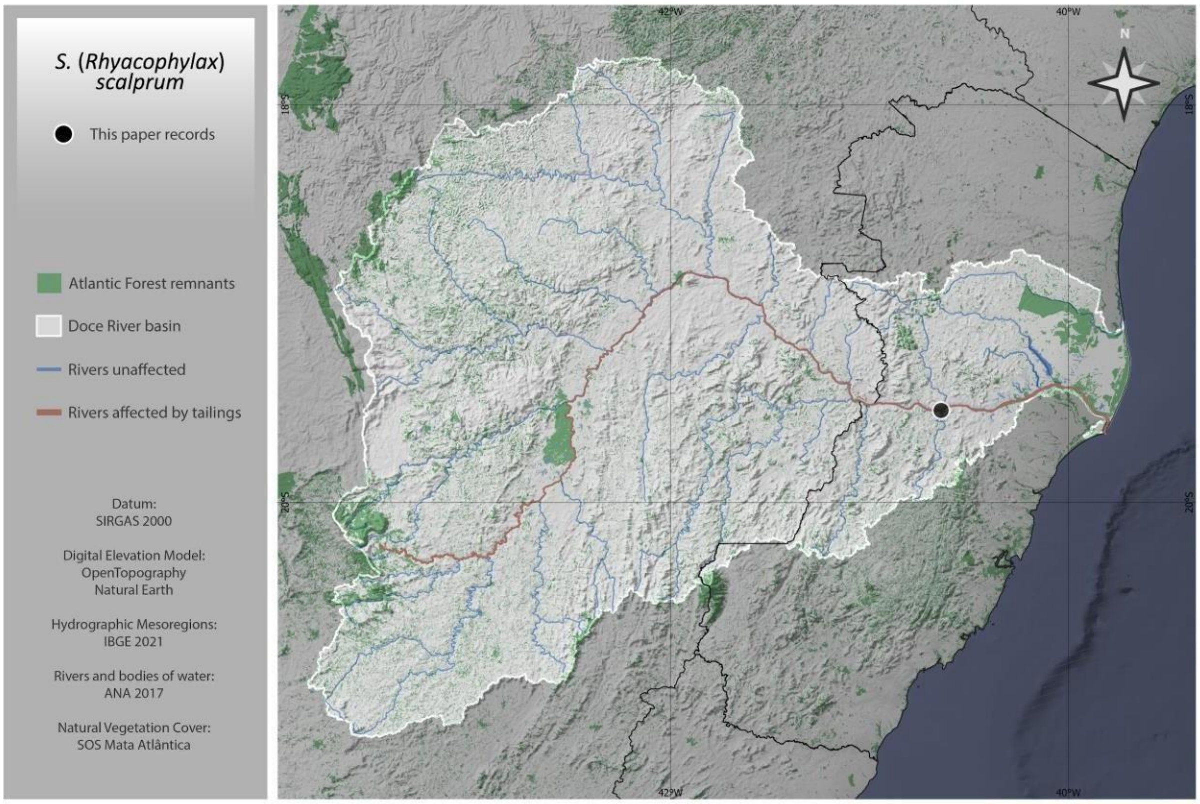

Fig. 13 Geographical distribution maps of *Smicridea (Rhyacophylax) scalprum* Vilarino, Dias & Calor, 2019 in the Rio Doce basin.

Smicridea (Rhyacophylax) vilela Flint, 1978

Type locality

Argentina, Prov. Chaco. Rcho, Barranqueras, Puerto Vilelas.

Literature

Flint, 1978: 382 figs 48–50 (♂, NMNH); Flint, 1982: 29 (distribution); Flint et al., 1999: 80 (catalog); Paprocki et al., 2004: 9 (checklist); Sganga, 2005: 142 (distribution); Paprocki and França, 2014: 36 (checklist); Holzenthal and Calor, 2017: 187 (catalog).

Material examined

BRAZIL: Espírito Santo: Baixo Guandu, Rio Doce, 19°30'23.00"S 41°01'01.80"W, el. 60m, 1 ♂, 09-10.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Baixo Guandu, Rio Guandu, 19°37'30.00"S 41°01'07.80"W, el. 90m, 52 ♂, 09-10.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Colatina, Rio Doce, 19°32'14.00"S 40°38'23.46"W, el. 35m, 3 ♂, 11-12.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Rio Doce, 19°24'23.10"S 40°04'03.70"W, el. 17m, 12 ♂, 12-13.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Linhares, Rio Doce, 19°26'06.40"S 39°57'04.80"W, el. 12m, 175 ♂, 13-14.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB); Minas Gerais: Aimorés, Rio Doce, 19°29'11.20"S 41°04'30.20"W, el. 80m, 10 ♂, 08-09.ix.2022, ADL Viana, P Bonfá- Neto, AD Ataide, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 15 ♂, 06.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'29.10"S 41°11'13.30"W, el. 98m, 8 ♂, 10-11.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Aimorés, Rio Manhuaçu, 19°29'38.07"S 41°16'31.73"W, el. 124m, 9 ♂, 10- 11.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), Barra do Cuieté, Rio Caratinga, 19°03'49.90"S 41°31'50.20"W, el. 120m, 1 ♂, 06-07.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Governador Valadares, Rio Doce, 18°55'50.27"S 42°01'20.23"W, el. 163m, 42 ♂, 04-05.ix.2022, ADL Viana,P Bonfá- Neto, AD Ataide, Pennsylvania trap (UFVB), 2 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB), Guaraciaba, Rio Piranga, 20°32'46.30"S 42°59'25.25"W, el. 526m, 14 ♂, 14.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Matias Lobato, Rio Suaçuí Grande, 18°34'23.50"S 41°56'52.30"W, el. 197m, 57 ♂, 05-06.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Naque, Rio Doce, 19°15'05.56"S 42°18'50.50"W, el. 190m, 3 ♂, 03-04.ix.2022, ADL Viana, P Bonfá-Neto, AD Ataide, Pennsylvania trap (UFVB), 366 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P

Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Naque, Rio Santo Antônio, 19°14'04.10"S 42°20'24.80"W, el. 195m, 15 ♂, 03-04.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Periquito, Rio Corrente, 19°00'21.10"S 42°10'32.00"W, el. 190m, 12 ♂, 04-05.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), Pingo D'agua, Rio Doce, PERD, 19°44'56.80"S 42°28'42.30"W, el. 239m, 246 ♂, 07.ii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio do Carmo, 20°16'47.40"S 42°55'29.50"W, el. 357m, 8 ♂, 09-10.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°16'30.70"S 42°54'49.60"W, el. 349m, 122 ♂, 09-10.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, 20°11'47.20"S 42°51'07.20"W, el. 280m, 12 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), Rio Doce, Rio Doce, UHE Risoleta Neves, 20°12'28.70"S 42°52'34.80"W, el. 317m, 193 ♂, 10-11.viii.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, Pennsylvania trap (UFVB), 163 ♂, 12.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), Rio Doce, Rio Piranga, 20°19'45.20"S 42°53'55.00"W, el. 372m, 111 ♂, 10-11.viii.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, Pennsylvania trap (UFVB), São José do Goiabal, Rio Casca, 20°02'56.40"S 42°38'40.80"W, el. 260m, 15 ♂, 13-14.viii.2022, ADL Viana, P Bonfá-Neto, MCD Correia, Pennsylvania trap (UFVB), São José do Goiabal, Rio Doce, 20°00'52.70"S 42°44'40.10"W, el. 265m, 88 ♂, 12.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB), 3 ♂, 13- 14.viii.2022, ADL Viana, P Bonfá -Neto, MCD Correia, Pennsylvania trap (UFVB), Tumiritinga, Rio Doce, 18°58'07.56"S 41°39'49.36"W, el. 133m, 956 ♂, 07.i.2022, ADL Viana, P Bonfá -Neto, P Rodrigues, MT Rothe- Neves, Pennsylvania trap (UFVB).

Distribution

ARGENTINA: Corrientes, Chaco, Entre Ríos provinces, BRAZIL: Amazonas, **Espírito Santo** (**new record**), **Minas Gerais** (**new record**), Pará states.

Remarks

Currently, this species is distributed in Argentina and northern Brazil, but there are no prior records of it in the Atlantic Forest. Specimens were found to be widely distributed throughout the Rio Doce basin, near the main stream of the Rio Doce (Fig. 14).

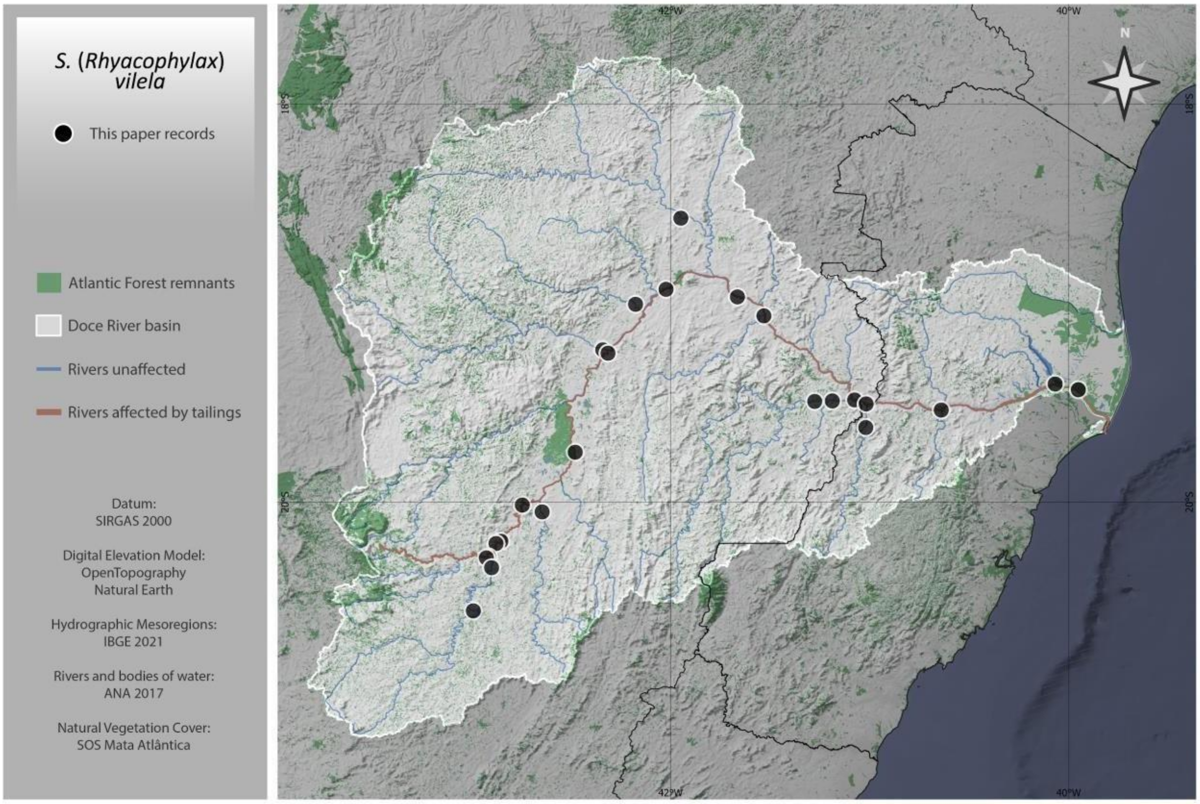

Fig. 14 Geographical distribution maps of *Smicridea (Rhyacophylax) vilela* Flint, 1978 in the Rio Doce basin.

Smicridea (Smicridea) bivittata (Hagen, 1861)

Type locality

Panama.

Literature

Hagen, 1861: 291 (♀ in Hydropsyche, MCZ); Ross, 1952: 33 (lectotype); Flint, 1967: 13 (♂); Flint, 1974a: 16 (♂; ♀; distribution; redescription; larva; pupa); Flint, 1974b: 90 (♂; distribution); Bueno-Soria and Flint, 1978: 207 (distribution); Flint, 1981: 22 (♂; distribution). Maes and Flint, 1988: 5 (distribution); Holzenthal, 1988: 70 (distribution); Botosaneanu, 1989: 205 (distribution); Flint, 1991: 63 (♂; distribution); Flint and Reyes, 1991: 481 (distribution); Aguila, 1992: 541 (distribution); Botosaneanu and Sakal, 1992: 203 (distribution; ecology); Botosaneanu and Alkins-Koo, 1993: 34 (distribution); Blahnik, 1995: 88 (♂; ♀; diagnosis; redescription; distribution);

Flint, 1996: 82 (distribution); Flint et al., 1999: 73 (catalog); Maes, 1999: 1186 (checklist); Muñoz-Quesada, 2000: 277 (checklist); Botosaneanu, 2002: 94 (checklist); Botosaneanu and Viloria, 2002: 108 (distribution); Blahnik et al., 2004: 4 (distribution); Paprocki et al., 2004: 10 (checklist); Bueno-Soria et al., 2005: 75 (distribution); Chamorro-Lacayo et al., 2007: 42 (checklist); Albino et al., 2011: 3 (distribution); Bueno-Soria and Barba-Álvarez, 2011: 356 (checklist); Calor, 2011: 321 (checklist); Oláh and Johanson, 2012: 246 (distribution); Paprockiand França, 2014: 37 (checklist); Armitage et al., 2015: 5 (checklist); Armitage and Cornejo, 2015: 194 (checklist); Takiya et al., 2016: 77 (distribution); Holzenthal and Calor, 2017: 164 (catalog); Sganga and Gibon, 2018: 410 (distribution); Harris and Armitage, 2019: 4 (distribution); Rázuri-Gonzales and Armitage, 2019: 3 (checklist); Desidério et al., 2021a: 183 (distribution); Armitage et al., 2024: 282 (checklist).

Material examined

Material examined: BRAZIL: Minas Gerais: Governador Valadares, Rio Doce, 18°55'50.27"S 42°01'20.23"W, el. 163m, 678 ♂, 04-05.ix.2022, ADL Viana, P Bonfá -Neto, AD Ataide, Pennsylvania trap (UFVB), 99 ♂, 08.i.2022, ADL Viana, P Bonfá-Neto, P Rodrigues, MT Rothe-Neves, Pennsylvania trap (UFVB).

Distribution

BOLIVIA: Cochabamba department, BRAZIL: Alagoas, Amazonas, Bahia, Ceará, Distrito Federal, Espírito Santo, Minas Gerais, Pará, Paraíba, Pernambuco, Piauí, Roraima, São Paulo, Tocantins states, COLOMBIA: Antioquia, Chocó, Risaralda, Valle del Cauca departments, COSTA RICA: Cartago, Guanacaste, Heredia, Puntarenas, San Jose provinces, ECUADOR: Cotopaxi, El Oro, Esmeraldas, Guayas, Los Ríos, Manabi, Napo, Pastaza, Pichincha, Tungurahua, Zamora-Chinchipe province, EL SALVADOR: La Libertad department, GUATEMALA: Izabal, Retalhuleu, Suchitepequez departments, HONDURAS: departments, MEXICO: Chiapas, Oaxaca, San Luis Patosi, Tabasco, Veracruz states, NICARAGUA: León, Chontales, Zelaya departments, PANAMA: Bocas del Toro, Canal Zone, Cocle, Chiriqui, Panama, San Blas provinces, PERU: Cuzco, Huanuco, Lambayeque, Madre de Dios, Puno, San Martin department, SURINAME: Brokopondo district, TRINIDAD AND TOBAGO: Couva-Tabaquite-Talparo, Diego Martín, Sangre Grande, Tobago, Tunapuna-Piarco regions, VENEZUELA: Aragua, Barinas, Bolivar, Nueva Esparta states.

Remarks

The first record this species in the Atlantic Forest was in several states of Brazil (Desiderio et al. 2021a). There are no previous records of the species in the Rio Doce basin. In the present study, a single location was found in the middle region of the Rio Doce basin, in the main stream of the Rio Doce (Fig. 15).

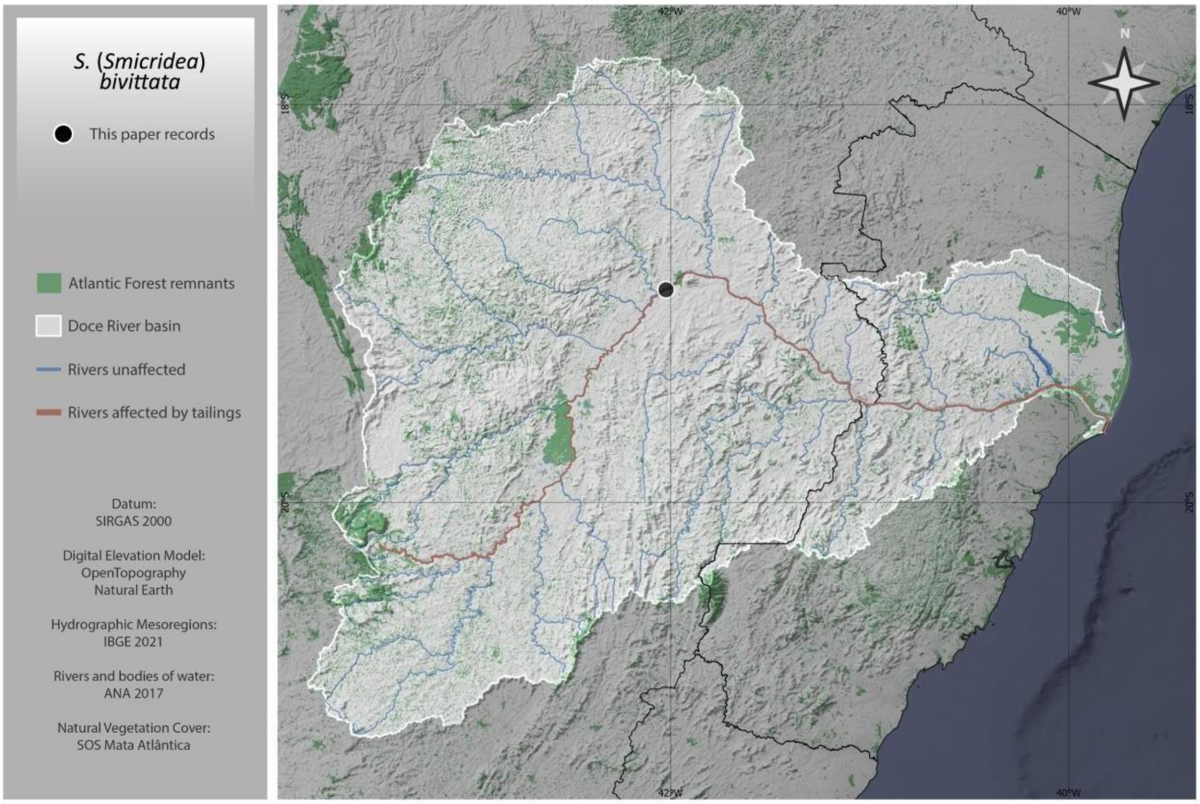

Fig. 15 Geographical distribution maps of *Smicridea (Smicridea) bivittata* (Hagen, 1861) in the Rio Doce basin.

Discussion

After examining 432.135 Trichoptera specimens collected at the forty sampling sites, a total of 36.125 specimens of the genera *Smicridea* were found in twenty-eight different localities. *S. radula* was the most abundant species with 18% of the total species collected. In the literature there were also twenty -eight localities with the occurrence of the genera *Smicridea* within the Rio Doce basin, none of which coincide with the places of occurrence on the sampling of the present study.

The analysis of the distribution of *Smicridea* McLachlan, 1871 (Trichoptera: Hydropsychidae) in the Rio Doce basin, a decade after the tailings dam disaster, highlights the complexity of aquatic biodiversity in the region. The presence of four species previously unrecorded for the Rio Doce basin is rev ealed in this work. Three species, *Smicridea (Rhyacophylax) atrobasis*, *Smicridea (Rhyacophylax) scalprum* and *Smicridea (Rhyacophylax) vilela*, this late one widely distributed in the basin, represent the firsts occurrences of Minas Gerais and Espírito Santo states. The new species *Smicridea (Rhyacophylax) exu* **sp. nov**. was restricted to the upper Rio Doce, in the Rio Santo Antônio and Rio Piranga basins. These new records increase the knowledge concerning the fauna of *Smicridea* of the Rio Doce basin to twenty-three species. The survey lacked eleven species, whose previous records were made by Barcelos-Silva et al. (2012) near the Sooretama Reserve, and by Blahnik et al. (2004) and Vilarino et al. (2019), in the headwaters of the upper Rio Doce. The absence of these species may be associated with the previous surveys being conducted in the headwaters and lower-order rivers of the sub-basins. The sampling design of this study focused on the Rio Doce itself and adjacent rivers, allowed new records in previously unsampled habitats.

The study of the distribution of *Smicridea* species reveals significant gaps, such as the absence of *Smicridea (Rhyacophylax) jundiai* in the middle Rio Doce. This absence may be due to the lack of sampling in tributaries upstream and downstream of the middle Rio Doce, resulting in a data gap for all species in this section in the present study and previous research. (Fig. 8).

Species previously restricted to high altitudes, such as *Smicridea (Rhyacophylax) piraya* and *Smicridea (Rhyacophylax) radula*, have expanded their distribution to the middle and lower Rio Doce, including the sub -basin of Rio Santo Antônio. On the other hand, only two species, *S. scalprum* and *S. atrobasis*, are restricted to the lower Rio Doce in Espírito Santo state.

The description of a new species of *Smicridea*, combined with the new records, highlights the unexplored biological richness of the Rio Doce basin. The identification of these species is crucial for the comprehension of the resilience of aquatic ecosystems after severe disturbances. This study provides valuable information on the biology and ecology of *Smicridea* species and is fundamental to fill gaps in knowledge of their geographical distribution, especially in areas affected by human activities.

Identifying these species is crucial for understanding the resilience of aquatic ecosystems after disturbances and subsidiate information for mitigating impacts in theses disturbed areas. Future studies should expand research on aquatic insects to include other groups beyond *Smicridea*, especially in unsampled areas like the tributaries of the middle Rio Doce. Continued insect collection will enhance our understanding of aquatic fauna dynamics, filling distribution gaps, identifying potencial new species and changes in biodiversity. This study can also serve as a base for future research on the impact of human activities on the region's aquatic ecosystem over time.

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Species composition, distribution, and identification of the Burrowing Mayflies (Polymitarcyidae, Ephemeroptera) from the Doce River Basin

This manuscript presents a detailed study of the composition and distribution of species of the family Polymitarcyidae (Ephemeroptera), known as burrowing mayflies, in the Rio Doce Basin. The research integrated field collection, bibliographic review, and analysis of entomological material from scientific collections. The study identified eight species, including three new regional records and one recently described species. The work also developed an interactive key for identifying the adult males of these species, providing a practical tool for future studies.

The nymphs of Polymitarcyidae live buried in the substrate of water bodies, where they build galleries in fine sediments such as clay and sand. Acting as ecosystem engineers, these nymphs directly influence the physical structure and chemistry of the aquatic bottom. This characteristic makes the group particularly relevant in the mining disaster that has affected the Rio Doce, since the deposition of tailings in the river could compromise the essential habitats for developing these nymphs and, consequently, their survival and distribution.

This article was included in the thesis for its in-depth taxonomic and biogeographical study of a group crucial for assessing environmental quality, especially in the region studied. By providing unpublished data on the local fauna, the study complements the central chapters of the thesis by reinforcing the relevance of Ephemeroptera as a sensitive ecological indicator. This article contributes directly to the overall goal of the thesis, which is to understand and conserve aquatic biodiversity in a site characterized by severe environmental impacts.

Species composition, distribution, and identification of the Burrowing Mayflies (Polymitarcyidae, Ephemeroptera) from the Doce River Basin

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Abstract

Polymitarcyidae (Ephemeroptera) are an essential component of aquatic ecosystems, playing a crucial role in nutrient cycling and as bioindicators of water quality. Despite their ecological significance, taxonomic and distributional knowledge of this family remains limited in many Neotropical regions. This study aims to investigate the species composition, distribution patterns, and taxonomic identification of Polymitarcyidae in the Doce River Basin, southeastern Brazil. Specimens were collected across multiple sampling sites, representing different environmental conditions. Morphological identification was performed using specialized keys, and distribution patterns were analyzed in relation to environmental variables. Our results reveal a diverse assemblage of Polymitarcyidae species, with notable variations in abundance and occurrence across different habitats. This study contributes to the understanding of mayfly diversity in the region, providing baseline data for future ecological assessments and conservation planning.

Introduction

Mayflies in Brazil comprise ten families, 81 genera, and 428 species (Salles et al., 2025). Of the currently recognized families, three belong to a clade usually known as the burrowing mayflies (referência). Comprised within the superfamily Ephemeroidea, the burrowing mayflies of Brazil belong to the families Ephemeridae, Euthyplociidae and Polymitarcyidae (García-Girón et al., 2024), with Polymitarcyidae being the most diverse family (Molineri et al., 2015).

These families' unique features are theirmandibularprocesses, or tusks, which physically allow some burrowers to tunnel into the substrate (Miller et al., 2018). Burrows are primarily builtin mud and silt, with Polymitarcyidae burrowing in solid clay and "sand-clay-rock mixtures" (Edmunds, 1982). Thus, the characteristics of the colonization surface must be ideal for constructing these burrows, which are essential for the development of thedeveloping Polymitarcyidae larvae (Bae & McCafferty, 1995).

They can also be regarded as ecosystem engineers because of their strong influence on substrate structure and geochemistry. It is possible that mayfly larvae colonize the bottom of the river largely independently from the water depth and current speed of flowing (Uchman et al., 2016).

This research was conducted within the geographic boundaries of the Doce River Basin in Brazil, an area that has experienced severe soil degradation due to extensive agricultural and mining endeavors. A tragic mining tailings dam collapse occurred on the afternoon of November 5, 2015, releasing tailings throughoutthe river and destroying the land surface, including soil and substrate at the bottom of water bodies, where the sedimentation of the tailings took place (Fernandes et al., 2016).

In light of the close relationship between colonization and the nature of the substrate and the importance of increasing the knowledge of the most diverse family of burrowing mayflies, Polymitarcyidae, this study aims to expand and integrate knowledge about the composition and distribution of the Polymitarcyidae fauna in the Doce River Basin; to provide new records and notes on their biology; and finally, based on all of this work, to elaborate an interactive key to the male imagoes of Polymitarcyidae's species of Doce River Basin.

Material and Methods

This study was conducted in the Doce River Basin, located in southeastern Brazil, with a drainage area of 86,715 km^2. The headwaters of the Doce River are located in the state of Minas Gerais (MG), in the Mantiqueira and Espinhaço mountain ranges and travel 853 km until they reach the Atlantic Ocean, in the municipality of Regência (Sánchez et al., 2018).

Three methods were used to expand and summarize the knowledge about the species of this family: i) performing a bibliographic review; ii) checking the list and examining the entomological material in the Museu de Entomologia da Universidade Federal de Viçosa (UFVB); and iii) collecting adults with a Pennsylvania light trap at forty sampling points along the Doce River basin, just after the sunset, during dry and rainy seasons. All the material was identified to the taxonomic level of species with the help of papers (Pereira & Da-Silva, 1991; Gonçalves et al., 2011; Molineri et al., 2012; Molineri & Salles, 2017) and a stereoscopic microscope.

To conduct a literature review of Ephemeroidea species with records for the Doce River basin, the EphemBrazil databases and records were consulted first (Salles et al., 2025). Additionally, the "Livro Vermelho da Biota Aquática do Rio Doce Ameaçada de Extinção pós Rompimento da Barragem de Fundão"(Salles, 2021) was checked. Scientific articles published recently, at least since the publication of the "Livro Vermelho," were searched in Google Scholar and the Scientific Electronic Library Online (SciELO). Databases and records such as GBIF (Global Biodiversity Information Facility) and CTFB (Taxonomic Catalog of Brazilian Fauna) were also accessed.

The distributional map was generated using the QGIS® software, incorporating geographical coordinates extracted from the specimens scrutinized in this paper and documented literature records. All collected and examined material is deposited in the Museu de Entomologia, Universidade Federal de Viçosa (UFVB), ensuring the integrity of the data employed in this mapping effort. Digital photos of the specimens were taken with a digital camera Leica Camera (MC170 HD) coupled to a Leica stereomicroscope (M205 A). Photos were edited and improved by Adobe Photoshop CC.

Results

Literature and database search yielded forty-eight species of Polymitarcyidae that had been recorded in Brazil before the present study (Salles et al., 2025). Of these, only five species were recorded in the literature as occurring within the Doce River Basin: *Campsurus latipennis* (Walker, 1853); *Campsurus truncatus* Ulmer, 1920; *Campsurus violaceus* Needham& Murphy, 1924; *Tortopsis canum* Gonçalves, Da-Silva & Nessimian, 2011; and *Tortopus igaranus* Needham & Murphy, 1924 (Salles, 2021).

This study's sampling efforts resulted in the collection of 2,625 adults of Polymitarcyidae, covering three genera and six species. The sampling carried out in this study included three new occurrences in the list of Polymitarcyidae species from the Doce River Basin, *Campsurus cotaxe* Molineri & Salles, 2017, *Torposus harrisi* Traver, 1950, and *Campsurus mirim* Pantoja, Viana & Salles, 2023. The last one, *C. mirim*, was recently described based on specimens also found due to this same survey and described by Pantoja and collaborators (2023) based on the male imago. With the inclusion of these three new species, the list of Polymitarcyidae species from the Doce River Basin is now composed of eight species: *Campsurus cotaxe* Molineri & Salles 2017; *Campsurus latipennis* (Walker,1853); *Campsurus mirim* Pantoja, Viana & Salles, 2023; *Campsurus truncatus* Ulmer, 1920; *Campsurus violaceus* Needham & Murphy, 1924; *Tortopsis canum* Gonçalves, Da-Silva & Nessimian, 2011; *Tortopus igaranus* Needham & Murphy, 1924, and *Torposus harrisi* Traver, 1950.

Twenty-three of the forty sites sampled hosted Polymitarcyidae adults, with more than eighty percent of the individuals collected during the dry season. The greatest abundance occurred in the Suaçuí Grande River, where *Campsurus cotaxe* Molineri & Salles, 2017 was the only species present. The highest species richness occurred in two localities of the Doce River: within the Parque Estadual do Rio Doce, a protected area of Minas Gerais; and in the city of Linhares, Espírito Santo. The records of *T. canum* and *C. truncatus*, previously registered only for the state of Espírito Santo, have been extended to new regions in the Minas Gerais area of the Doce River Basin (Figure 1).

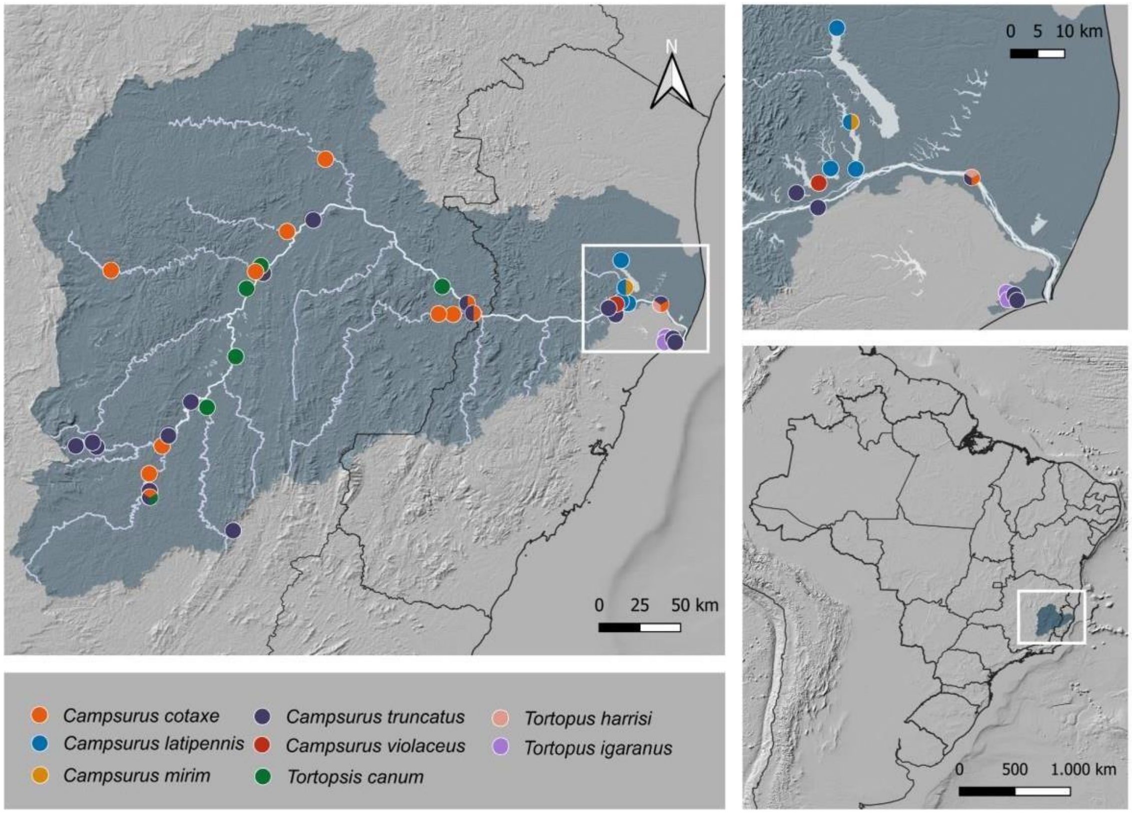

Fig. 1 Map of Doce River Basin with the distributions of the species of Polymitarcyidae family

All Polymitarcyidae species in the Doce River basin are classified as "least concern" according to the Brazilian Fauna Extinction Risk Assessment, except for *C. mirim*, which has not yet been assessed because it is a recently described species. The recorded species have no specialized feeding habits and are mostly filter feeders. So far, no threats have been identified that would soon put the species at risk of extinction.

<u>Registered species</u>

Campsurus cotaxe **Molineri & Salles, 2017**

(Figs. xxx)

Campsurus sp. nov. Angeli et al. 2015: 202.
Campsurus cotaxe Molineri and Salles, 2017: 340; Dominguez et al., 2023:268

Fig. 2. *Campsurus cotaxe*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Minas Gerais State, Rio Doce, Rio Piranga, S 20°19'45.2", W 42°53'55.0", 446 m, 10-11.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P., Correia, M.C.D. col. [1 ♀, 4 ♂]; Brazil, Minas Gerais State, Rio Doce, Rio Piranga, S 20°19'45.2", W 42°53'55.0", 446 m, 13-14.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P., Correia, M.C.D. col. [9 ♀, 5 ♂]; Brazil, Minas Gerais State, Aimorés, Rio Manhuaçu, S 19°29'38.07'', W 41°16'31.73'', 129 m, 06.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P col. [2 ♂]; Brazil, Minas Gerais State, Rio Doce, Rio Doce, S 20°16'30.7", W 42°54'49.6", 349 m, 9-10.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [3 ♀, 2 ♂]; Brazil, Minas Gerais State, Santo Antônio, Rio Santo Antônio, S 19°13'52.40'', W 43°12'54.80'', 474 m, 19-20.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [28 ♀, 2 ♂]; Brazil, Minas Gerais State, Ponte Nova, Rio Piranga, S 20°26'43.1", W 42°59'05.7", 490 m, 11-

12.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [4 ♀, 30 ♂]; Minas Gerais State, Periquito, Rio Corrente, S 19°00'21.1",W 42°10'32.0", 227 m, 04-05.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [36 ♀, 28 ♂]; Minas Gerais State, Aimorés, Rio Doce, S 19°29'11.20", W 41°04'30.20", 124 m, 08-09.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 ♀]; Minas Gerais State, Naque, Rio Santo Antônio, S 19°14'04.1", W 42°20'24.8", 195 m, 03-04.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♀, 1 ♂]; Minas Gerais State, Mathias Lobato, Rio Suaçuí Grande, S 18°34'23.5", W 41°56'52.3", 197 m, 05-06.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [353 ♂ , 773 ♂]; Minas Gerais State, Aimorés, Rio Manhuaçu, S 19°29'29.1", W 41°11'13.3", 135 m, 10-11.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [26 ♀, 3 ♂]; Minas Gerais State, Aimorés, Rio Manhuaçu, S 19°29'38.07'', W 41°16'31.73'', 135 m, 10-11.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [170 ♀, 99 ♂]; Espírito Santo State, Linhares, Rio Doce, S 19°26'06.4", W 39°57'04.8", 23 m, 13-14.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 ♀, 2 ♂].

Distribution: Brazil (Espírito Santo orig., Minas Gerais).

Remarks:

Campsurus cotaxe (fig. 2) was sampled at eleven of the forty sampling sites, accounting for 1,590 individuals, of which less than 1% were collected during the rainy season. Only 12 specimens were collected from the Doce River, the one disturbed by mining tailings.

This was the most abundant species, representing 80% of the total abundance of Polymitarcyidae in the Doce River Basin. In this study, we expanded the knowledge of the distribution of *C. cotaxe*, which had not been previously recorded in this basin.

Suaçuí Grande River was the most frequent location for *C. cotaxe*. At this place, the river bottom consisted mainly of sand and large stones. The portion where they were collected had minimal adjacent vegetation and was nearby a hydroelectric dam, which makes sense considering that nymphs burrow U-shaped tunnels mainly in the bottom of lentic habitats, as dams.

***Campsurus latipennis* (Walker,1853)**

Palingenia latipennis Walker, 1853: 554; Hagen, 1861: 304.
Campsurus latipennis; Eaton, 1871: 57 ; Eaton, 1873: 388; Eaton, 1883-1888: 39; Banks, 1913: 84; Lestage, 1923: 122; Needham and Murphy, 1924: 15; Traver, 1947: 383; Kimmins, 1960: 313; Hubbard, 1982: 271; Dominguez et al., 2002: 459; Salles et al., 2004: 34; Kluge, 2004: 268; Domínguez et al. 2006: 572; Salles et al., 2010:299; Heckman, 2012: 59-60; Dominguez and Andrés, 2014: 83; Mariano et al., 2014: 74; Dos Santos et al., 2016: 366; Molineri and Salles, 2017: 344; Oliveira et al., 2023: 9

Fig. 3. *Campsurus latipennis*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Espírito Santo State, Lagoa Nova, S 19°19'49.0", W 40°10'12.3", 15 m, 12-13.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [167 ♀, 328 ♂].

Distribution: Brazil (Pará orig., Espírito Santo, Tocantins.)

Remarks:

Campsurus latipennis (fig. 3) was found exclusively in the dry season in Lagoa Nova, State of Espírito Santo. Nevertheless, it constituted almost 20% of the total specimens collected in the sample. A swarm of male imagos, typical in this genus, may have been caught in the trap. The species had already been recorded in the literature in a neighboring site, Lagoa Palminhas.

Campsurus mirim **Pantoja, Viana & Salles, 2023**

Campsurus mirim Pantoja, Viana & Salles, 2023

Fig. 4. *Campsurus mirim*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Espírito Santo State, Lagoa Nova, S 19°19'49.0", W 40°10'12.3", 15 m, 12-13.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [27 ♂].

Distribution: Brazil (Espírito Santo orig.)

Remarks:

Campsurus mirim (fig. 4) is a recently described species belonging to the *Campsurus major* group. So far, its distribution is limited to Lagoa Nova, located in the lower Doce River Valley, where the twenty-seven individuals of the species were originally collected. The substrate profile at the site is predominantly sandy. Although it is classified as an area affected by mining tailings, the sampling was done in a relatively undisturbed area used only for recreational purposes.

***Campsurus truncatus* Ulmer, 1920**

Campsurus truncatus Ulmer, 1920: 2; Lestage, 1923: 123; Puthz, 1973: 94; Hubbard, 1982: 271; Domínguez et al. 2006: 580; Salles et al., 2010: 307; Heckman, 2012: 71-79 Angeli et al., 2015: 202; Dominguez and Andrés, 2014: 83; Lima et al., 2015: 5; Molineri et al., 2015: 61; Campos et al., 2016: 77; Lima et al., 2016: 218; Sartori et al., 2016: 58; Lima and Pamplim, 2017: 1:10; Kuhlmann et al., 2020: ; Sartori and Bauernfeind, 2020: 333; Kuhlmann et al., 2020: 9; Lima and Pamplim, 2023: 53-58

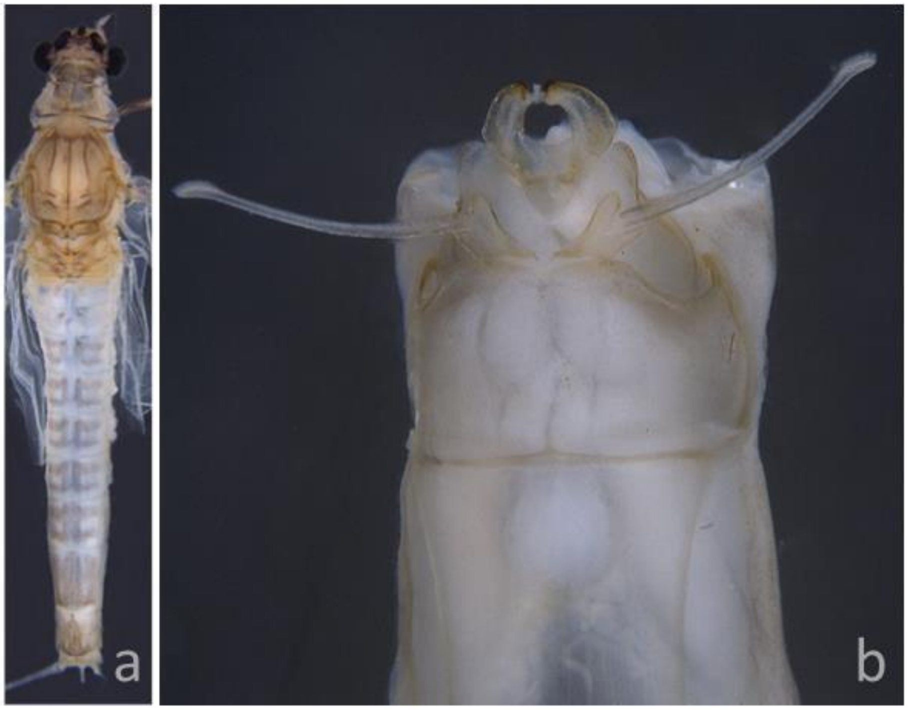

Fig. 5. *Campsurus truncatus*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Espírito Santo State, Linhares, Rio Doce, S 19°26'06.4", W 39°57'04.8", 23 m, 13-14.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 ♂]; Brazil, Minas Gerais State, São José do Goiabal, Rio Doce, S 20°00'52.70'', W 42°44'40.10'', 288m, 13-14.ix.2022, Viana, A.D.L., Bonfá, P., Ataíde, A. col. [3 ♀, 3 ♂]; Brazil, Minas Gerais State, Mariana, Rio Gualaxo do Norte, S 20°16'35.10'', W 43°25'53.90'', 731 m, 08-09.ix.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [1 ♀]; Brazil, Minas Gerais State, Rio Doce, Rio Doce, S 20°12'28.70'', W 42°52'34.80'', 280 m, 12.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [4 ♂]; Brazil, Minas Gerais State, São José do Goiabal, Rio Doce, S 20°00'52.70'', W 42°44'40.10'', 288m, 13-14.ix.2022, Viana, A.D.L., Bonfá, P., Ataíde, A. col. [3 ♀, 35 ♂]; Brazil, Minas Gerais State, Aimorés, Rio Doce, S 19°29'11.20'', W 41°04'30.20'', 124 m, 06-07.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P,. col. [1 ♀]; Brazil, Minas Gerais State, Governador Valadares, Rio Doce, S 18°55'50.27'', W 42°01'20.23'', 162 m, 04-05.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♀, 1 ♂].

Distribution: Bolivia (Santa Cruz); Brazil (Espírito Santo orig. Rio de Janeiro, Goiás, Mato Grosso, Bahia, São Paulo, Pernambuco and Piauí. New record: Minas Gerais).

Remarks:

The species (fig. 5) is widely distributed across Brazil and was found at eight sampling sites along the Doce River Basin. Only two individuals were sampled exclusively during the dry season and all others during the rainy season, showing an inverse seasonality to the other species of the genus found in this basin and other studies on the seasonal distribution of *C. truncatus* (Lima and Pamplin, 2016).

Another inverse pattern is the greater presence in the locations affected by the tailings, with most records for the Doce River. Lima and Pamplin (2016) also found a high productivity value for *C. truncatus* in an area that receives acid drainage from mining tailings. This may indicate how well this species adapts to this environment.

Campsurus violaceus **Needham & Murphy, 1924**

Campsurus violaceus Needham & Murphy, 1924: 18; Traver, 1947: 379; Hubbard, 1982: 271; Hubbard et al., 1992: 227; Melo et al., 1993: 95-107; Dominguez et al, 1994: 93; Takeda and Grzybkowska, 1997: 149-155; Leal and Esteves, 2000: 95-97 De Melo et al., 2004: 374; Domínguez et al., 2006: 581; Zilli et al., 2008: 163-166; Molina et al., 2010: 3387; Molina et al., 2011: 141-149; Zilli and Marchese, 2011: 251-254; Heckman, 2012:55-65; Zilli, 2012: 61–71; Molineri and Salles, 2013: 266; Dominguez and Santos, 2014: 83; Marchese et al, 2014: 24-28; Molineri et al, 2015a: 51-68; Molineri et al, 2015b: 120; Lima and Pamplin, 2016: 1:10; Saigo et al., 2016: 2-5; Molineri and Salles, 2017: 301-354; Tonella et al., 2017; Zili et al., 2017: 3; Tonella et al., 2018: 249; Clemente et al., 2018: 241-253; Jacobus et al., 2019:11; Kuhlmann et al., 2020; Marchese et al., 2020: 104; Saigo et al., 2020: 4-6; Cruz et al, 2022: 435; Hincapié-Montoya, 2022; Shemes et al., 2022: 7; Hurtado-Borrero and Tamaris-Turizo, 2023: 268

Fig. 6. *Campsurus violaceus*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Espírito Santo State, Linhares, Lagoa das Palmas, S 19°26'34.52", W 40°13'13.79", 34 m, 25.ii.2019, Massariol F.C and Gonçalves M.C. col [4♀, 35♂].

Distribution: Argentina; Brazil (Amazonas, Pará, Piauí, Goiás, Mato Grosso, Mato Grosso do Sul, Paraná. New record: Espírito Santo)

Remarks

The record of this species (fig. 6) for the Rio Doce Basin comes from a previous collection at Lagoa das Palmas, Espírito Santo, Brazil. The specimens of *C. violaceus* are deposited in the Museu de Entomologia da Universidade Federal de Viçosa (UFVB). During the review of this material we noticed that males individuals present the relation between length and width of the subretangular pedestal relatively smaller, whereby the pedestal is less enlongate in comparison with the holotype of the species. There were no records of this species in the literature from the Southeastern region of Brazil, including the Doce River Basin. Hence, this is the first record of this species in these areas.

Tortopsis canum **Gonçalves, Da-Silva & Nessimian, 2011**

Tortopsis canum Gonçalves, Da-Silva and Nessimian, 2011: 50-54; Molineri et al., 2012: 466; Dominguez and Dos Santos, 2014: 83; Angeli et al., 2015: 202; Molineri et al., 2019: 13

Fig. 7. *Tortopsis canum*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Brazil, Minas Gerais State, Pingo-D’Água, Parque Estadual do Rio Doce, Doce River, S 19º44’56.8", W 42º28’42", 239m, 07–08.ii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [197 ♀, 42 ♂]; Brazil, Minas Gerais State, Guaraciaba, Rio Piranga, S 20°32'46.30'', W 42°59'25.25'', 526m, 07–08.ii.2022, Viana, A.D.L., Kroth, N., Ataide, A. col. [1 ♀, 1 ♂]; Minas Gerais State, Naque, Rio Santo Antônio, S 19°14'04.1", W 42°20'24.8", 195 m, 07-08.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [19 ♀, 9 ♂]; Minas Gerais State, Rio Doce, Rio Doce, S 20°12'28.70'', W 42°52'34.80'', 280 m, 12.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [4 ♂, 1 ♂]; Brazil, Minas Gerais State, São José do Goiabal, Rio Doce, S 20°00'52.70'', W 42°44'40.10'', 288m, 12-13.i.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [85 ♀, 7 ♂]; Brazil, Minas Gerais State, Pingo-D’Água, Parque Estadual do Rio Doce, Doce River, S 19º44’56.8", W 42º28’42", 239m, 17–88.viii.2022, Viana, A.D.L., Bonfá, P., Ataíde, A. col. [8 ♀, 4 ♂]; Brazil, Minas Gerais State, São José do Goiabal, Rio Doce, S 20°00'52.70'', W

42°44'40.10", 288m, 13-14.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P.,. col. [14 ♂]; Brazil, Minas Gerais State, São José do Goiabal, Rio Casca, S 20°02'56.4", W 42°38'40.8", 325 m, 14.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [8 ♀, 7 ♂]; Minas Gerais State, Aimorés, Rio Doce, S 19°26'03.8", W 41°05'54.1", 90m, 08-0.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A., col. [2 ♂].

Distribution: Brazil (Espírito Santo orig, Rio de Janeiro. New records: Minas Gerais)

Remarks:

Tortopsis canum (fig. 7) was found at seven of the forty sampling sites, five of which were in areas affected by mining tailings. The highestabundance was during the rainy season, according to more sampling sites that had Polymitarcyidae at this season. The type locality of *T. canum* is the Macaé River, Rio de Janeiro state, a deep, large river (6th order) with a sandy bottom and no riparian vegetation. These were also the conditions where the sampled *T. canum* was found, likewise in a 6th-order river, the Doce River.

So far, the species has only been recorded for the states of Rio de Janeiro and Espírito Santo, beyond the boundaries of the Doce River Basin. Thus, this study extends the range to the state of Minas Gerais within the limits of the Basin.

Tortopus igaranus **Needham & Murphy, 1924**

Tortopus igaranus Needham and Murphy, 1924: 24, Ulmer, 1942: 111; Traver, 1950: 600; Hubbard, 1982: 271; Domínguez, 1985: 69; 9; Domínguez et al. 2006: 585; Molineri 2010: 11; Heckman, 2012: 51; Molineri et al., 2012: 466; Molineri and Dos Santos, 2014: 83; Molineri et al., 2021: 166.

Material examined: Brazil, Espírito Santo State, Linhares, Lagoa das Cacimbas, S 19°37’38.89”, W 39°52’31.86”, 2m, 30-31.i.2019, Massariol f. c. and Bramuth, A. C col. [1 ♀]; Brazil, Espírito Santo State, Linhares, Lagoa das Cacimbas, S 19°38’47", W 39°53’29.41”, 5m, 24.ii.2019, Massariol f. c. and Bramuth, A. C col. [1 ♂]

-species photos (INCLUIR?)

Fig. 8. *Campsurus latipennis*, adult. Male imago: habitus, dorsal view.

Distribution:

Peru (Igará-Paraná and Putumayo rivers orig.); Brazil (Amazonas State. New record: Minas Gerais)

Remarks:

The species (fig. 8) was initially described from Peru and represents the type-species of the genus It was later recorded at fourother localities in Amazonas, Brazil. Its distribution was restricted to this region until the present study, which provides an unprecedented record of the species for the southeastern region of Brazil and, consequently, for the Dice River Basin. The single specimen of *T. igaranus specimen*, responsible for the record of the species in the "Livro Vermelho da Biota Aquática do Rio Doce Ameaçada de Extinção pós Rompimento da Barragem de Fundão" is deposited at the Museu de Entomologia da Universidade Federal de Viçosa and was checked in order to confirm its identity. Unfortunately, the genitalia is missing and the presence of this species in the Doce River Basin should be considered with caution.

***Tortopus harrisi* Traver, 1950**

Tortopus harrisi Traver, 1950: 604; Hubbard, 1982: 271; Domínguez, 1985: 69; Domínguez et al. 2006: 586; Molineri, 2010: 8-20; Molineri et al., 2010: 65-68; Heckman, 2012: 86; Molineri et al., 2012: 464-466;

Dominguez and Dos Santos, 2014: 83; Angeli, 2015: 202; Molineri et al., 2015: 48; Sila and Salles, 2017:2; Molineri et al., 2021: 152-169; Wroblewski and Wroblewski, 2022: 4;

Figure 9. *Tortopus harrisi*, adult. Male imago: a, habitus, dorsal view; b, genitalia, ventral view.

Material examined: Espírito Santo State, Linhares, Rio Doce, S 19°26'06.4", W 39°57'04.8", 23 m, 13-14.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♀, 3 ♂].

Distribution: Brazil (Espírito Santo orig, Amazonas, Mato Grosso, Mato Grosso do Sul. New records: Minas Gerais)

Remarks:

Four individuals of *T. harissi* (fig. 9) were sampled, exclusively in the city of Linhares, State of Espírito Santo. The species was found during the rainy season in a very disturbed area along the stretch of the Doce River that runs through the urban area of Linhares.

The substrate at the sampling site was mostly sandy. The nymphs of this species are usually collected on sandy substrate, perhaps because they lack medially directed bristles on the inner margin of the tusks and have a poorly developed fronto-clipeal region, so they do not burrow in clay substrate like other species.

Interactive Key to male imagos of Polymitarcyidae

We propose here an interactive identification key to the male imagos of Polymitarciydae of the Rio Doce Basin. Based on the morphology of male imagos antomy of 8 species spread in 3 genera, the key records 9 discrete characters and 22 character states . almost all of which are illustrated by photographs of fixed exemplars.The majority of morphological characters included need a light microscope to be observed, as 7 from the 9 characters refers to the male genitalia. The proposed identification key was produced with Xper3 software and can be accessed at https://app.xper3.fr:443/xper3GeneratedFiles/publish/identification/-5265334662947911915/

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	Campsurus violaceus	Campsurus truncatus	Campsurus cotaxe	Campsurus latipennis	Campsurus mirim	Tortopsis canum	Tortopus harrisi	Tortopus igaranus
Mid and hind legs	flap-like, tibiae and tarsi absent	flap-like, tibiae and tarsi absent	flap-like, tibiae and tarsi absent	flap-like, tibiae and tarsi absent	flap-like, tibiae and tarsi absent	weak and twisted, all segments present	weak and twisted, all segments present	weak and twisted, all segments present
Abdominal sternum IX	entire	entire	entire	entire	entire	entire	divided medially	divided medially
Penes (main lobe), shape	fingerlike, apically thinner and rounded	fingerlike, apically truncated	cilindrical	subconical	semicircular	bladelike	bladelike	bladelike
Penes, degree of fusion	fused basally	fused basally	fused basally	fused basally	fused basally	not fused	fused basally	fused basally
Penes secondary lobe or "Thumb"	present	present	present	present	present	absent	absent	absent
Penes secondary lobe or "Thumb", length	smaller than half the size of the main lobe	smaller than half the size of the main lobe	larger than half the size of the main lobe	smaller than half the size of the main lobe	smaller than half the size of the main lobe	-	-	-
Distance between pedestals	smaller than pedestal width	smaller than pedestal width	larger than pedestal width	larger than pedestal width	smaller than pedestal width	larger than pedestal width	larger than pedestal width	larger than pedestal width
Relative length of pedestal corners	inner corner longer	equally developed	outer corner longer	outer corner longer	equally developed	outer corner longer	outer corner longer	outer corner longer
Pedestal, outer corner length	shorter than the pedestal base length	-	longer than the pedestal base length	shorter than the pedestal base length	-	longer than the pedestal base length	shorter than the pedestal base length	longer than the pedestal base length

Reducing the Wallacean and the Haeckelian Deficits in Apobaetis Day, 1955 (Ephemeroptera: Baetidae)

The following paper discusses the taxonomy and distribution of two species of the genus *Apobaetis* Day, 1955 (Ephemeroptera: Baetidae). This understudied group is difficult to collect due to its small size and preference for psammophilic habitats. Nymphal and adult specimens of *Apobaetis irai* De Lima, Massariol, Cruz & Hamada, 2022 and *Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002 were found during sampling in the Rio Doce basin following the collapse of a mining tailings dam. The study describes the adults of *Apobaetis irai* for the first time, expands their known ranges, and records unprecedented cases of gynandromorphism in *A. irai*. These results reduce two important gaps in biodiversity knowledge: the Wallacean (geographic distribution) and the Haeckelian (description of different life stages).

The contribution of this article to the thesis consists of providing detailed taxonomic and biogeographic knowledge about a genera that, due to its biology and rarity, is often underestimated in ecological studies. By describing the adults and expanding the distribution of *Apobaetis* in the Rio Doce Basin, the study provides essential data for the chapters dealing with diversity, dispersal and distribution modeling of Ephemeroptera in the region. Furthermore, considering that *Apobaetis* nymphs live in sandy sediments that were directly affected by the tailings deposit, this article also contributes to the assessment of the impact of the disaster on the benthic fauna and to the understanding of the ecological responses of sensitive species to changes in the substrate.

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SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Reducing the Wallacean and the Haeckelian Deficits in *Apobaetis* Day, 1955 (Ephemeroptera: Baetidae)

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Abstract

During an ongoing project aiming to understand the dynamics of Ephemeroptera biodiversity in the Doce River Basin after the disaster, an unusual large number of *Apobaetis* Day, 1955 were analyzed. The occurrence of *Apobaetis* is quite relevant, since the combination of factors such as its small body size and its habitat preferences makes their collection difficult and its presence often neglected. For the first time in science, adults of *Apobaetis irai* De Lima, Massariol, Cruz & Hamada, 2022 and *Apobaetis fiuzai* Salles & Lugo-Ortiz, 2002 are described and diagnosed. *Apobaetis irai* can be easily distinguished, among other characteristics by turbinate portion of compound eyes touching each other along midline; abdominal terga II–III and V–VII with well-marked lines on lateral and posterior margins; posterior margin of subgenital plate with projection broadly rounded to slightly truncate. *Apobaetis fiuzai* are diagnosed by turbinate portion of compound eyes touching each other posteriorly; mesothorax brown, except for broad cream marks on anterior margin of PSp and SLS, SSLi tinged with white, posterior corner of SMS with a red spot; abdominal terga II–VI translucent orangish-brown, terga VII–X brown, terga II–III and VI with conspicuous red medial mark; posterior margin of subgenital plate with subtriangular projection, apex narrowly truncate; forceps base wider at base, inner irregular edges. The new records of *A. irai* and *A. fiuzai* extend the distribution of *Apobaetis* within Minas Gerais and Espírito Santo States, Brazil. Furthermore, it is reported for the first time cases of gynandromorphs for *Apobaetis.*

Keywords Aquatic insects · Taxonomy · Doce River Basin · Distribution · Gynandromorph

Introduction

Baetidae Leach, 1815 is the second richest family of Ephemeroptera with 113 genera (Lima et al. 2024). Among them, *Apobaetis* Day, 1955 presents smallsize and larval shifting-sand habitat preference (Cruz et al. 2020). The genus currently comprises 22 valid species: *Apobaetis biancae* Cruz, Boldrini & Hamada, 2020, *Apobaetis dalcolmoae* De Lima, Cruz & Hamada, 2024, *Apobaetis danielae* De Lima, Cruz & Hamada, 2024, *Apobaetis etowah* (Traver 1935), *Apobaetis fiuzai* Salles& Lugo-Ortiz,2002, *Apobaetis futilis* (McDunnough, 1931), *Apobaetis hamadae* Cruz, Boldrini & Salles, 2011, *Apobaetis insolitus* Nieto, 2006, *Apobaetis irai* De Lima, Massariol, Cruz & Hamada, 2022, *Apobaetis jacobusi* Cruz, Boldrini & Hamada, 2020, *Apobaetis jaquelinae* De Lima, Cruz & Hamada, 2023, *Apobaetis kallawaya* Nieto, 2006, *Apobaetis kethepiali* Cruz & De-Souza, 2014, *Apobaetis lakota* McCafferty, 2000, *Apobaetis luanae* De Lima, Cruz & Hamada, 2023, *Apobaetis niger* Nieto, 2006, *Apobaetis nisiae* De Lima, Cruz & Hamada, 2024, *Apobaetis pasternakae* De Lima, Cruz & Hamada, 2023, *Apobaetis pucupi* Cruz & De-Souza, 2014, *Apobaetis sabinoae* De Lima, Cruz & Hamada, 2024, *Apobaetis signifer* Lugo-Ortiz & McCafferty, 1997, and *Apobaetis trivellae* De Lima, Cruz & Hamada, 2024 (Salles et al.2024; Jacobus and McCafferty 2023; De Lima et al. 2023, 2024). Most of the

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species has been reported from Brazil, seventeen so far, but the genus has also been reported from Argentina, Bolivia, Colombia, Guatemala, and the United States of America (Edmunds et al. 1976; Lugo-Ortiz and McCafferty 1997; Nieto 2006).

There was a remarkable advance in the taxonomy of this genus. Nevertheless, there are only four species of *Apobaetis* in which the imago is described, *Apobaetis futilis* and *Apobaetis etowah* (McDunnough 1931; Traver 1935), both from North America; *Apobaetis hamadae* and *Apobaetis pasternakae* from Brazil (Cruz et al. 2011). This gap in the knowledge of the distinct stages of the known species is called the Haeckelian shortfall. There is also a Wallacean deficit in this genus, which is the scarcity of knowledge on species distribution. These gaps can cause some problems in the study of biodiversity, such as a significant qualitative loss of information, especially for morphology-based systematics and evolution. In addition, the biodiversity distribution of unexplored environments cannot be examined and preserved (Faria et al. 2021).

More recently, new species have been described from Brazil, arising from new surveys and revisions of previously identified material (Cruz et al. 2020; Lima et al. 2023, 2024). Among these new species, *Apobaetis irai* is remarkable as it was described based on a few individuals (as usual for the genus) collected in two localities of the affected area by the mine-tailings in Doce River Basin, Brazil. The collapse of the Fundão dam in Mariana, MG, Brazil, devastated the Doce River watershed with the release of more than 43.8 million cubic meters of mining tailings on 5th November 2015 (Marta-Almeida et al. 2016). This tragedy was considered one of the largest environmental disasters ever recorded in Brazil, causing serious socio-environmental problems throughout the basin (Guerra et al. 2017). Given the biology of the species, its restricted known distribution, and the threats imposed by the consequences of the disaster, Lima et al. (2022) proposed that the species was eligible to be considered threatened (at least vulnerable).

During an ongoing project aiming to understand the dynamics of Ephemeroptera biodiversity in the Doce River Basin after the disaster, some nymphs and several adults of *Apobaetis irai* and *Apobaetis fiuzai* were found. Given the importance of expanding the knowledge of a potentially endangered species (as is the case for *A. irai*), and the importance of describing unknown life stages, the objectives of this study are (i) to describe and diagnose the adults of the species *Apobaetis irai* and *Apobaetis fiuzai* (including cases of gynandromorphism in *A. irai*); (ii) to provide new records and notes on its biology; and (iii) to review the conservation status of *A. irai* based on the new information.

Material and Methods

The Doce River Basin, located between the parallels S 17°30′00″ and S 21°30′00″ and the meridians W 39°30′00″ and W 44°00′00″, in the Southeastern Region of Brazil, is one of the largest hydrographic basins in the region. The entire basin is located in the Atlantic Forest biome, a forest formation that has been reduced to less than 8% of its original area (Rosa et al. 2021).

The biggest environmental disaster in Brazil took place in November 2015, when a mine-tailing dam collapsed and devastated the Doce River Basin (Marta-Almeida et al. 2016). Contamination of soil and water by heavy metals and chemical compounds occurred along the path taken by the tailings, causing changes in the characteristics of the aquatic ecosystem (Cordeiro et al. 2019).

In order to evaluate the effects of the disaster on the aquatic fauna, a survey was carried out 7 years later, covering forty sampling points along the Doce River Basin, of which *Apobaetis irai* were found in twelve and *Apobaetis fiuzai* in ten (Fig. 1). The specimens in nymphal stages were collected with a D net; the imagos and subimagos were captured with a Pennsylvania light trap just after sunset (Frost 1957).

Digital photos of the specimens were taken with a digital camera Leica Camera (MC170 HD) coupled to a Leica stereomicroscope (M205 A). Photos were edited and improved by Adobe Photoshop CC, Adobe Illustrator, and Helicon Focus software. The distributional map was created in the QGIS® software, using geographic coordinates of the material analyzed in this paper and published literature records. Material examined is deposited in the Museu de Entomologia, Universidade Federal de Viçosa (UFVB).

Adults were associated with nymphs by sharing the same abdominal color pattern and for being collected in the same locality. The terminology used to describe the morphology of the thorax of adults follow Kluge (1994; 2004). Abbreviations: ALSC, anterolateral scutal costa; ANp, anteronotal protuberance; MS, medioscutum; PAB:PA, posterior arc of prealar bridge; PSp, Posteior Scutual protuberance; SL, scutellum; SLS, sublateroscutum; SMS, submedioscutum; SSLi, scutoscutellar impression. In the lists of material examined, the numbers inside the brackets indicate the number of specimens in each lot followed by sex (♀ female/ ♂ male) and by the stage examined (N for nymphs, S for subimagos, and I for imagos).

Results

Apobaetis irai De Lima, Massariol, Cruz & Hamada, 2022

(Figures 2, 3, 4, and 5).

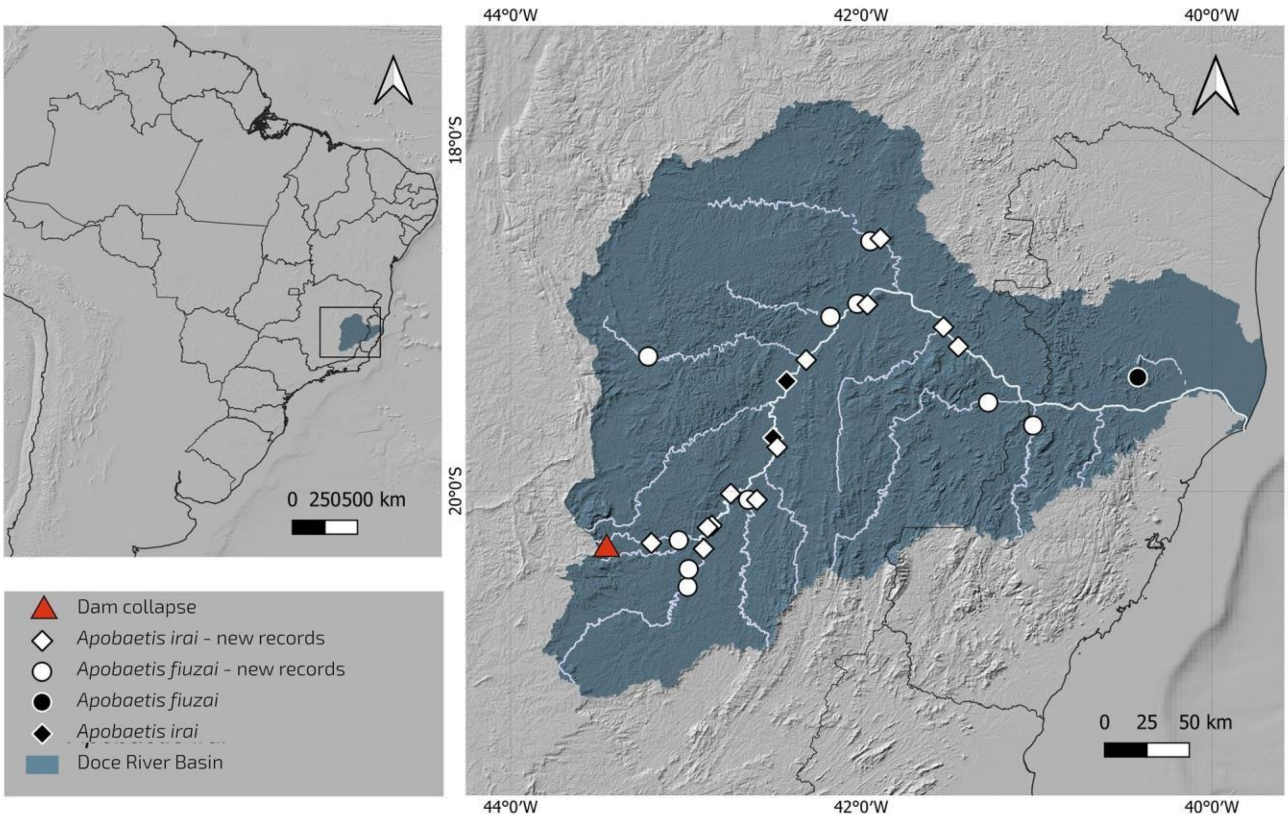

Fig. 1 Map of Doce River Basin with the distributions of the two species of *Apobaetis*

Apobaetis irai De Lima, Massariol, Cruz & Hamada, 2022: 268; De Lima, Cruz and Hamada (2024: 111).

Diagnosis of the male imago: (1) turbinate portion of compound eyes touching each other along midline; (2) mesothorax brown, except for narrow cream marks on anterior margin of PSp and SSLi; (3) abdominal terga II–III and V–VII with well-marked lines on lateral and posterior margins, sigilla strongly marked on terga II–VII; (4) forewing with 6–7 complete stigmatic veins; (5) posterior margin of subgenital plate with projection broadly rounded to slightly truncate; (6) forceps base approximately rectangular with regular edges; (7) inner margin of forceps segment I+II slightly bent on distal ¾.

Description of male imago (in alcohol) (Fig. 2):

Length: body: 2.7–4.5 mm; forewing: 3.3–3.6 mm; antenna: 0.8 mm; tibia: I 1.2–2.1 mm; tibia II: 1.0–1.2 mm; tibia III: 1.0–1.0 mm.

Head (Figs. 2a, b). Colouration whitishcream slightly washed with brown. Turbinate portion of compound eyes cream to light orange; touching each other along midline; turbinate eye: length 0.5 mm; stalk height 0.2–0.3 mm. Antenna: scape, pedicel, and flagellum light brown; scape slightly darker dorsally; antenna up to two times longer than maximum length of head.

Thorax (Fig. 2a, b). Pronotum brown. Mesonotum with PAB:PA with a dark brown triangular mark in anterior portion; MS and ANp brown, except for a longitudinal medial yellowish-brown stripe from posterior portion of ANp up to anterior third of MS; SMS yellowish brown; ALSC, SLS, and PSp dark brown; narrow cream marks on anterior margin of PSp and SSLi. Metanotum brown, except for dark brown macula along the edge between limits of the anterior and posterior portions and a whitish-cream medial stripe. Pleura yellow; pro- and mesosternum brown and metasternum translucent whitish cream to light brown/yellow. Anteronotal protuberance nearly rounded; metascutellar protuberance flattened, posteriorly projected; posterior margin of metathoracic postnotum broadly emarginated. Legs whitish cream. Leg I: tibia 1.7–1.9 length of femur; tarsi 0.1 length of femur; and with 4 segments decreasing in length apically. Leg II: tibia 1.4–1.5 length of femur; tarsi 0.1 length of femur and Leg III: tibia 1.2–1.5 length of femur; tarsi 1.1 length of femur.

Wings (Fig. 2e). Forewing hyaline, distal portion between C and RA opaque white, veins yellowish brown; stigmatic area with 6–7 complete veins, rarely 1 incomplete; marginal intercalary veins paired and divergent, single between veins ICu1 and ICu2, absent between ICu2 and CuP; length of each intercalary vein 1.7–2.2 distance between adjacent longitudinal veins; length of forewing about 0.3 width.

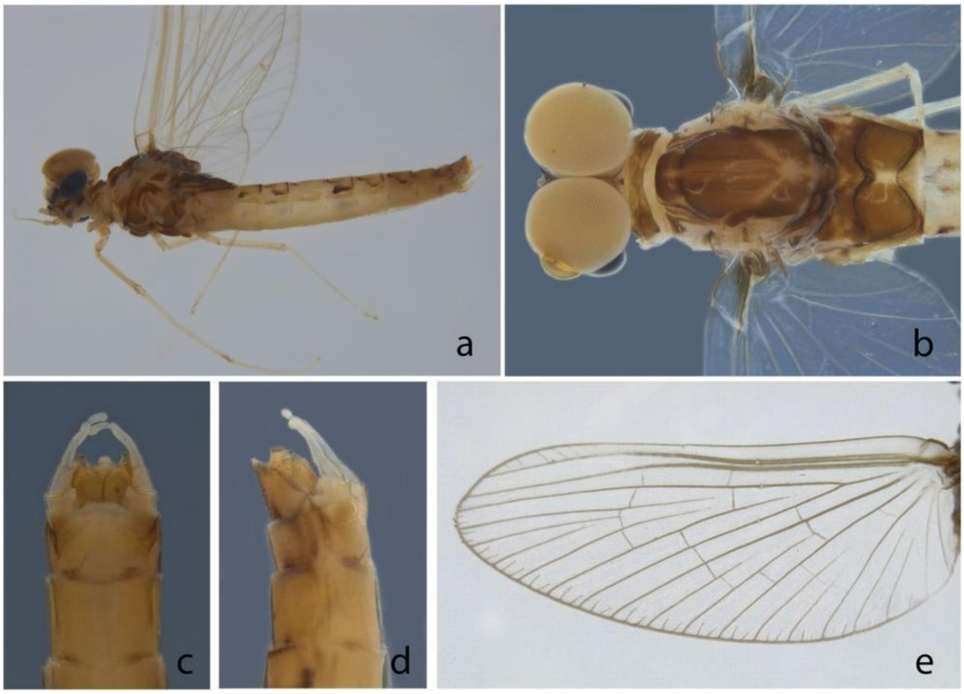

Fig. 2 Male imago of *Apobaetis irai* (UFVB:EP01653). (**a**) habitus lateral view; (**b**) head and torax details, dorsal view; (**c**, **d**) male genitalia, (**c**) ventral view, (**d**) lateral view; (**e**) male forewing

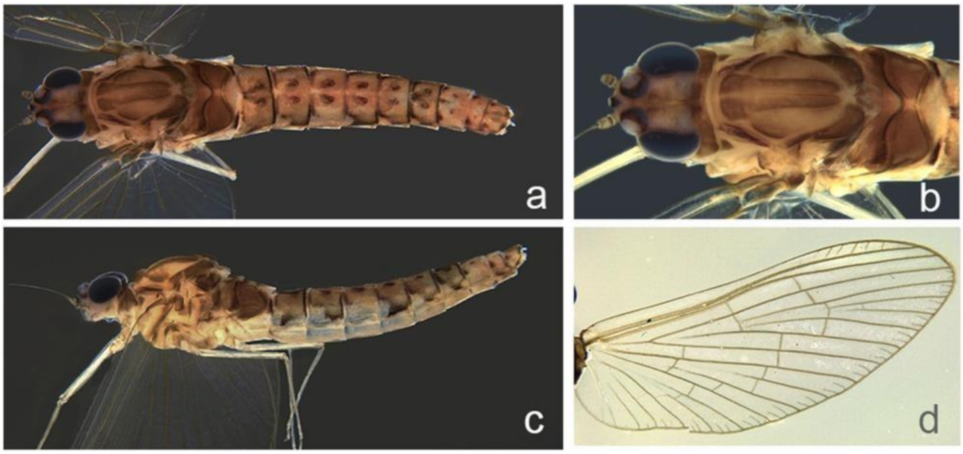

Fig. 3 Female imago of *Apobaetis irai* (UFVB:EP01649). (**a**) habitus dorsal view; (**b**) head and thorax details, dorsal view; (**c**) habitus lateral view; (**d**) female forewing

Abdomen (Fig. 2a). Terga I–X whitish cream with brown to dark brown marks; terga III–IX with pair of brown medial sigilla (smaller on terga VIII and IX) and anterolateral dark brown macula; terga II, III, and V–VII with lateral dark brown macula, larger on terga II, III, and V; abdominal terga II–III and V–VII with well-marked lines on lateral and pos- terior margins, except medially on terga III (eventually on V and VI). Sterna yellow; lateral margins of segment VIII and IX light brown.

Genitalia (Fig. 2c, d). Forceps base approximately rectangular with regular edges. Forceps with segments I + II and III white. Forceps segment I + II with base cylindrical, inner margin slightly bent on distal ¾.; 0.2–0.4 length of segment I + II; segment III oval, wider posteriorly, 2.3–3.2 as long as wide. Posterior margin of subgenital plate with projection broadly rounded to slightly truncate.

Female imago (in alcohol) (Fig.3): Very similar to male imago, except for the following features.

Length: body: 3.7–4.4 mm; forewing: 4.1–4.5 mm; antenna: 0.7–0.8 mm; tibia: I 0.7–0.8 mm; tibia II: 0.7 mm; tibia III: 0.7 mm.

Thorax (Fig. 3a, b). Pro-, meso-, and metasternum usually whitish cream. Leg I: tibia 0.9–1.0 length of femur; tarsi 0.7 length of femur. Leg II: tibia 0.9–1.0 length of femur; tarsi 0.7–0.8 length of femur and Leg III: tibia 0.9 length of femur; tarsi 0.7 length of femur.

Wings (Fig. 3d). Stigmatic area with 5–7 complete veins, incomplete veins ranging from 0 to 6; length of each

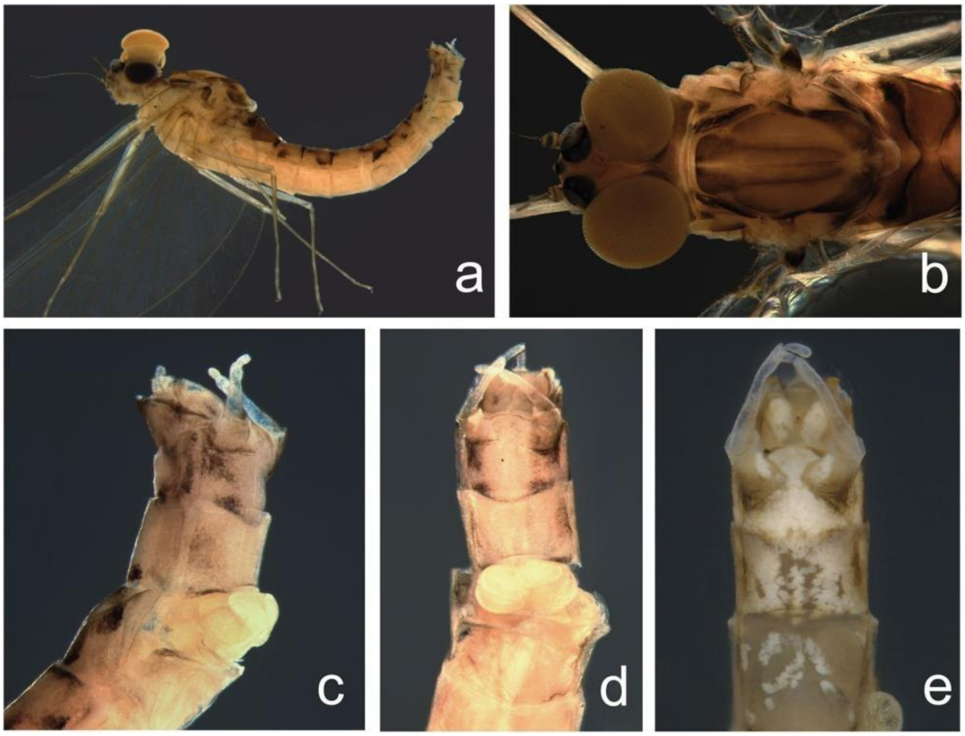

Fig. 4 *Apobaetis irai* gynandromorphs. (**a**–**d**) Gynandromorph 1 (UFVB:EP01650), (**a**) habitus lateral view,(**b**) head and torax details, dorsal view, (**c**) male genitalia and eggs, lateral view, (**d**) male genitalia and eggs, ventral view; (**e**) gynandromorph 2 (UFVB:EP01651), male genitalia, ventral view

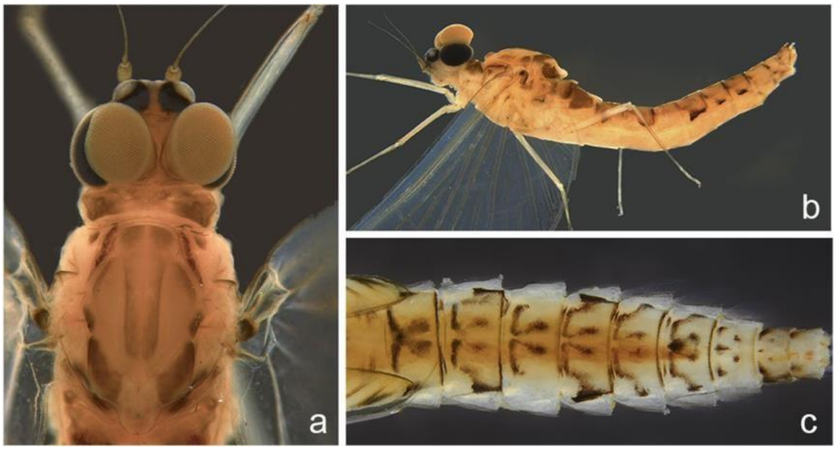

Fig. 5 *Apobaetis irai* gynandromorph (UFVB:EP01650). (**a**) head and thorax details, dorsal view; (**b**) habitus lateral view; (**c**) nymph dorsal abdomen

intercalary vein 1.1 distance between adjacent longitudinal veins; length of forewing about 0.4 width.

Abdomen (Fig. 3a, c). Posterior margin of terga I–III and V–VII dark brown.

Gynandromorph 1 (Fig. 4a–d): Very similar to male imago, except for the following features.

Length: body: 4.2 mm; forewing: 4.4 mm; antenna: 0.8 mm; tibia: I 0.8 mm; tibia II: 0.8 mm; tibia III: 0.7 mm.

Head (Fig. 4b). Colouration yellowish brown. Turbinate portion of compound eye yellow (Fig. 4b). Dorsal portion of left turbinate eye elliptical, length 0.5 mm, stalk height 0.5 mm; dorsal portion of right turbinate eye nearly rounded (Fig. 4b), length 0.8 mm, stalk height 0.7 mm.

Thorax (Fig. 4b). Leg I: tibia 1.0 length of femur; tarsi 0.7 length of femur; and with 4 segments decreasing in length apically. Leg II and III: tibia 0.9 length of femur; tarsi 0.7 length of femur.

Wings. Stigmatic area with 8 veins touching Sc vein; marginal intercalary veins single between veins ICu1 and CuP; length of each intercalary vein 1.9 distance between adjacent longitudinal veins; length of forewing about 0.4 width.

Abdomen (Fig. 4a). All abdominal segments filled with eggs; membranous area between sterna VII and VIII with extruding eggs.

Male genitalia (Fig. 4c, d). Forceps base and base of forceps segment I + II light brown, distal 2/3 of forceps segment

I + II and distal segment yellow. Distal forceps segment, 2.6 as long as wide; 0.3 length of segment I + II. Posterior margin of subgenitalplate with broadly rounded projection.

Gynandromorph 2 (Fig.4e): Similar to gynandromorph 1, except for the following features.

Abdomen (Fig. 4e). Last three segments filled with few eggs.

Gynandromorph 3 (Fig. 5a, b): Similar to gynandromorph 1, except for the following features.

Length: body: 4.3 mm; forewing: 4.3 mm; antenna: 0.7 mm; tibia: I 0.7 mm; tibia II: 0.7 mm; tibia III: 0.6 mm.

Head (Fig. 5a, b): dorsal portion of turbinate eyes smaller, both elliptical; male genitalia completely absent.

Material examined: UFVB:EP01649: Brazil, Minas Gerais State, São José do Goiabal, Rio Casca, S 20°02′56.4″, W 42°38′40.8″, 325 m, 14.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [9 ♀ I]; UFVB:EP01650: Brazil, Minas Gerais State, São José do Goiabal, Rio Casca, S 20°02′56.4″, W 42°38′40.8″, 325 m, 14.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [2 gynandromorphs I]; UFVB:EP01648: Brazil, Minas Gerais State, Naque, Rio Doce, S 19°15′05.56″, W 42°18′50.50″, 270 m, 04.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col.[2 ♂ I]; UFVB:EP01651: Brazil, Minas Gerais State, Pingo D'agua, Rio Doce, S 19°44′56.80″, W 42°28′42.30″, 279 m, 17–18.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 gynandromorph I]; UFVB:EP01652: Brazil, Minas Gerais State, Rio Doce, Rio Piranga, S 20°19′45.2″, W 42°53′55 .0 ″, 446 m, 10–11.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [1 ♂ I, 1 ♂ S, 1 ♀ I, 1 ♀ S]; UFVB:EP01653: Brazil, Minas Gerais State, Governador Valadares, Rio Doce, S 18°55′50.27″, W 42°01′20.23″, 162 m, 04–05.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [5 ♂ I]; UFVB:EP01654: Brazil, Minas Gerais State, Mathias Lobato, Rio Suaçuí Grande, S 18°34′23.5″, W 41°56′52.3″, 197 m, 05–06.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♂ I]; UFVB:EP01662: Brazil, Minas Gerais State, São José do Goiabal, Rio Casca, S 20°02′56.4″, W 42°38′40.8″, 325 m, 14.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [4 N]; UFVB:EP01663: Brazil, Minas Gerais State, Rio Doce, Rio Doce, S 20°12′28.70″, W 42°52′34.80″, 468 m, 11.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [3 N]; UFVB:EP01661: Brazil, Minas Gerais State, São José do Goiabal, Rio Doce, S 20°00′52.70″, W 42°44′40.10″, 288 m, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 N]; UFVB:EP01660: Brazil, Minas Gerais State, Naque, Rio Doce, S 19°15′05.56″, W 42°18′50.50″, 270 m, 04.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 N]; UFVB:EP01659: Brazil, Minas Gerais State, Rio Doce, Rio Doce, S 20°11′47.2″, W 42°51′07.2″, 431 m, 11.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [6 N]; UFVB:EP01655: Brazil, Minas Gerais State, Conselheiro Pena, Rio Doce, S 19°10′30.7″, W 41°26′45.6″, 270 m, 08.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A .col. [1 N]; UFVB:EP01657: Brazil, Minas Gerais State, Mariana, Rio Gualaxo do Norte, S 20°17′46.20″, W 43°11′48.70″, 533 m, 09.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [3 N]; UFVB:EP01656: Brazil, Minas Gerais State, Barra do Cuieté, Rio Caratinga, S 19°03′49. 9 ″, W 41°31′50.2″, 273 m, 07.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 N]; UFVB EP01658: Brazil, Minas Gerais State, Rio Doce, Rio Piranga, S 20°19′45.2″, W 42°53′55 .0 ″, 515 m, 11.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [48 N].

Distribution: Brazil: Minas Gerais state: Mariana, Rio Gualaxo do Norte (presentstudy); Rio Doce, Rio Piranga and Rio Doce (present study); São José do Goiabal, Rio Casca and Rio Doce (present study); Marliéria, Rio Doce (Lima et al. 2022); Pingo D'água, Rio Doce (present study); Ipaba, Rio Doce (Lima et al. 2022); Naque, Rio Doce (present study); Governador Valadares, Rio Doce (present study); Mathias Lobato, Rio Suaçuí Grande (present study); Barra do Cuieté, Rio Caratinga (present study); Conselheiro Pena, Rio Doce (present study) (Fig. 1).

Biology: Nymphs and adults of *A. irai* were collectedat twelve of the forty sampling sites, exclusively during the dry season. Seventy-six percent of the total abundance of *A. irai* were found in tributaries of the Doce River not affected by mining tailings. All nymphs sampled were collected in sandy habitats and were abundant even in those with anthropogenic disturbance other than the mining tails, such as sand extraction, hydroelectric dams and rivers used for swimming.

Remarks: *Apobaetis irai* was originally described from two localities in the Doce River, Minas Gerais State, based only on nymphs (Lima et al. 2022). In the present paper we not only describe adults for the first time, but of extreme relevance in the context of the conservation status of the species, we greatly expand its distribution (see white diamonds on Fig. 1). According to Lima et al. (2022), *A. irai* should be considered as potentially threatened, or at least vulnerable, due to a sum of small potential extent of occupancy (EOO), small area of occurrence (AOO), and continuing decline in habitat quality. While we concur with Lima et al. (2022) that river silting poses a credible threat capable of rapidly impacting individuals within the species, our recent data significantly broadens the known range and seasonal distribution of the species. These results, including places that have been heavily affected by anthropogenic activities, even those affected by mining tailings, effectively diminishes the possibility of the species being considered threatened.

There are only two species, which the *Apobaetis* imagos were described in South America, *A. hamadae* and *A. pasternakae* (originally described as *A. fiuzai*). *Apobaetis irai* is extremely differentfrom these two speciesbased on its external coloration with abdominal terga II–III and V–VII with well-marked lines on lateral and posterior margins and sigilla strongly marked on terga II–VII (Fig.2a). The posterior margin of subgenital plate is comparatively more rounded than *A. hamadae* (Fig.2c; Cruz et al. 2011: Fig. 9b); *A. pasternakae* is quite different since its subgenital plate presents a subtriangular projection.

The analysis of twenty adults specimens of *A. irai* revealed the presence of three gynandromorphs with differences among them (Figs. 4 and 5). The Gynandromorphs 1 and 2 are similar with the turbinate portion of compound eye developed and male genitalia (Fig. 3); in opposite, the gynandromorph 3 does not have a male genitalia and the turbinate portion of compound eyes is smaller, less developed (Fig. 4). This is the first record of gynandromorphs for the genus *Apobaetis*.

Apobaetis fiuzai Salles & Lugo-Ortiz 2002

(Figures 6 and 7).

Apobaetis fiuzai Salles & Lugo-Ortiz 2002: 2, figs. 1–16; Salles et al. (2003: 5; 2004: 727; 2010: 302); Nieto (2006: 190, Fig. 4, 14); Lima et al. (2012: 306); Cruz (2020: 322, Figs. 1a-b, 4, 5); Lima et al. (2023: 139, Figs. 1–2; 2024: 112).

Diagnosis: Male imago: (1) turbinate portion of compound eyes touching each other posteriorly; (2) mesothorax brown, except for broad cream marks on anterior margin of PSp and SLS, SSLi tinged with white, posterior corner of SMS with a red spot; (3) abdominal terga II–VI translucent orangish-brown, terga VII–X brown, terga II–III and VI with conspicuous red medial mark; (4) forewing with 2–4 complete stigmatic veins; (5) posterior margin of subgenital plate with subtriangular projection, apex narrowly truncate; (6) forceps base wider at base, inner irregular margin; (7) inner margin of forceps segment I + II slightly bent on distal ¾.

Description of male imago (in alcohol) (Fig. 6):

Length: body: 3.2–3.4 mm; forewing: 3.1–3.2 mm; antenna: 0.4–0.7 mm; tibia: I 1.6–1.8 mm; tibia II: 0.8–1.0 mm; tibia III: 0.6–1.0 mm.

Head (Fig. 6a, b). Colouration light brown; lateral branch of epicranial suture brown. Turbinate portion of compound eyes cream to orange, touching each other posteriorly; turbinate eye: length 0.4–0.5 mm width; stalk height 0.3–0.5 mm width of dorsal portion. Antenna: light brown; scape and pedicel darker anteriorly; antenna up to two times longer than maximum length of head.

Thorax (Fig. 6a, b). Pronotum brown. Mesonotum with MS and ANp brown, some specimens with a longitudinal medial yellowish-cream stripe in ANp; SMS brown, with a posterior red dot (not visible in all specimens); ALSC and SLS; PSp and SL dark brown; well-marked cream to light brown line on anterior margin of PSp and SSLi. Metanotum brown, except by a red medial dot posterior to medial hump; whitish cream posteriorly. Pleura yellow; pro- and mesosternum brown and metasternum translucent whitish cream. Anteronotal protuberance nearly rounded; metascutellar protuberance flattened, posteriorly projected; posterior margin of metathoracic postnotum broadly emarginated. Legs whitish cream. Leg I: tibia 2.7–2.9 length of femur; tarsi 0.1

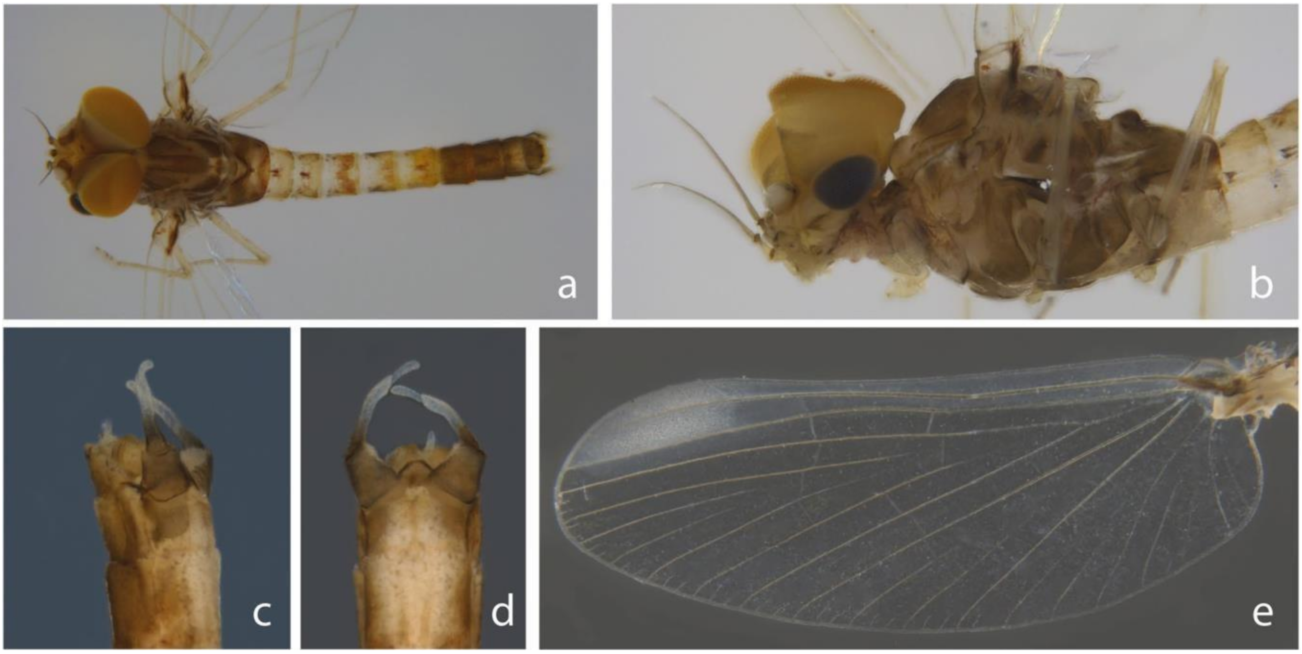

Fig. 6 Male imago of *Apobaetis fiuzai* (UFVB:EP01664). (**a**) habitus dorsal view; (**b**) head and torax details, lateral view; (**c**, **d**) male genitalia, (c) lateral view, (d) ventral view; (e) male wing

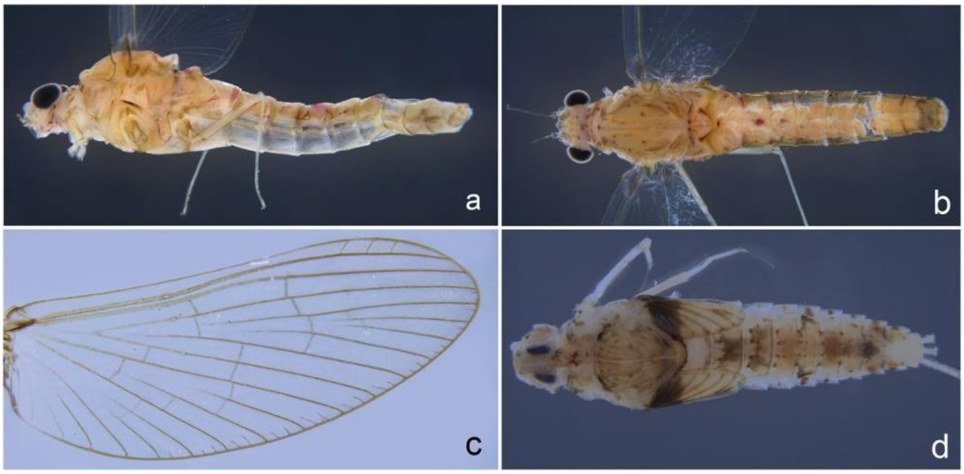

Fig. 7 Female of *Apobaetis fiuzai.* Imago (UFVB:EP01672): (**a**) imago habitus lateral view; (**b**) imago habitus dorsal view; (**c**) female wing. Nymph (UFVB:EP01665): (**d**) dorsal view

length of femur; and with 4 segments decreasing in length apically. Leg II: tibia 1.4–1.5 length of femur; tarsi 0.1–0.2 length of femur and Leg III: tibia 1.2 length of femur; tarsi 0.11–0.16 length of femur.

Wings (Fig. 6e). Forewing hyaline, distal area between C and RA opaque white, veins yellowish; stigmatic area with 2–4 complete veins, rarely 1–2 incompletes; marginal intercalary veins paired and slightly divergent to straight, single between veins IMP1 and IMP2, absent between IMP2 and A; length of each intercalary vein 0.7–1.0 distance between adjacent longitudinal veins; length of forewing about 0.3 width.

Abdomen (Fig. 6a). Terga I reddish brown; terga II–VI whitish cream, some most colored specimens umber in posterior portion of terga; terga II–III and VI with red medial spot; one red spot sublaterally on segments II–VI; terga VII–X brown; tracheation dark brown. Sterna whitish cream.

Genitalia (Fig. 6c, d). Forceps base brown and wider at base, inner margin irregular. Forceps segment I brown on basal half and white on apical half, inner margin slightly bent on distal ¾; segments II and III white. 0.2–0.3 length of segment I + II; segment III ovalated and enlarged posteriorly, 2.7–2.9 as long as wide. Posterior margin of subgenital plate brown with subtriangular projection, apex narrowly truncate.

Female imago (Fig. 7a–c): Very similar to male imago, except for the following features.

Length: Maximallength: body:3.3–3.5 mm; forewing: 3.5–3.6 mm; antenna: 0.6 mm; tibia: I 0.8–0.9 mm; tibia II: 0.6–0.7 mm; tibia III: 0.7–0.8 mm.

Head (Fig. 7a, b). Entirely whitish cream; antennatwo times longer than maximum length of head.

Thorax (Fig. 7b). Pronotum and mesonotum whitish cream, except by a posterior red dot in SMS; metanotum whitish cream, except by a red medial dot posterior to medial hump; medial hump edges red. Pleura yellow; pro- and meso- and metasternum translucent whitish cream. Leg I: tibia 1.4–1.5 length of femur; tarsi 0.1–0.2 length of femur; and with 4 segments decreasing in length apically. Leg II: tibia 0.9–1.4 length of femur; tarsi 0.1 length of femur and leg III: tibia 1.2–1.5 length of femur; tarsi 0.1–0.2 length of femur.

Wings (Fig. 7c). Length of each intercalary vein 1.1–1.3 distance between adjacent longitudinal veins; length of forewing about 0.3 width.

Abdomen (Fig. 7a, b). Terga I–X whitish cream; terga II–III with red spot in midline; terga VI sometimes blotched with red pigment; terga VII–X whitish cream; tracheation dark, not well marked.

Material examined: UFVB:EP01664: Brazil, Minas Gerais State, Ponte Nova, Rio Piranga, S 20°26′43.1″, W 42°59′05.7″, 490 m, 11–12.viii.2022, Viana, A.D.L., Bonfá, P., Rodrigues, P. col. [6 ♂ I, 1 ♀ I]; UFVB:EP01668: Brazil, Minas Gerais State, Periquito, Rio Corrente, S 19°00′21.1 ″,W 42°10′32.0 ″, 227 m, 04–05.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [5 ♂ I]; UFVB:EP01669: Brazil, Minas Gerais State, Barra Longa, Rio do Carmo, S 20°16′50.9″, W 43°02′26.8 ″, 408 m, 02–03.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col.[3 ♂ I]; UFVB:EP01670: Brazil, Minas Gerais State, Guaraciaba, Rio Piranga, S 20°32′46.30″, W 42°59′25.25″, 558 m, 11–12. viii. 2, Viana, A.D.L., Bonfá, P., Rodrigues, P. [1 ♂ I]; UFVB:EP01671: Brazil, Minas Gerais State, Santo Antônio, Rio Santo Antônio, S 19°13′52.40 ″, W 43°12′54.80 ″, 578 m, 19–20.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [6 ♂ I, 11 ♂ S]; UFVB:EP01672: Brazil, Minas Gerais State, São José do Goiabal, Rio Casca, S 20°02′56.4″, W 42°38′40.8 ″, 266 m, 13–14.viii.2022, Viana, A.D.L., Bonfá, P., Correia, M.C.D. col. [1 ♂ I, 1 ♂ S, 16 ♀ I, 2 ♀ S]; UFVB:EP01673: Brazil, Minas Gerais State, Governador Valadares, Rio Doce, S 18°55′50.27″, W 42°01′20.23″, 161 m, 13–14.viii.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [8 ♂ I]; UFVB:EP01674: Brazil, Minas Gerais State, Mathias Lobato, Rio Suaçuí Grande, S 18°34′23.5″, W 41°56′52.3″, 203 m, 05–06.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [7 ♂ I]; UFVB:EP01675: Brazil, Minas Gerais State, Aimorés, Rio Manhuaçu, S 19°29′38.07″, W 41°16′31.73″, 129 m, 10–11.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♂ I]; UFVB:EP01676: Brazil, Espirito Santo State, Baixo Guandu, Rio Guandu, S 19°37′30.0″, W 41°01′07.8″, 91 m, 09–10.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1 ♂ I]; UFVB:EP01665: Brazil, Minas Gerais State, Barra Longa, Rio do Carmo, S 20°16′50.9″, W 43°02′26.8 ″, 384 m, 02–03.ix.2022, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 N]; UFVB:EP01666: Brazil, Minas Gerais State, Santo Antônio, Rio Santo Antônio, S 19°13′52.40″, W 43°12′54.80″, 474 m, 19–20.viii. 2, Viana, A.D.L., Bonfá, P., Ataide, A. col. [1N]; UFVB:EP01667: Brazil, Minas Gerais State, Periquito, Rio Corrente, S 19°00′21.1″, W 42°10′32.0″, 190 m, 04–05.ix. 2, Viana, A.D.L., Bonfá, P., Ataide, A. col. [2 N].

Distribution: Brazil: Rondônia: Alto Alegre dos Parecis, Rolim de Moura (Lima et al. 2024); Piauí: Monsenhor Gil, Rio Grande do Piauí, Valença do Piauí (Lima et al. 2024); Pernambuco state: São Benedito do Sul, Cachoeira Poço do Caboclo (Lima et al. 2012); Bahia: Rio de Contas (putative), Correntina (Lima et al. 2024); Goiás: Colinas do Sul, Paraúna (Lima etal. 2024); Mato Grosso state: Chapada dos Guimarães, Cachoeira do Pulo (Salles and Lugo-Ortiz 2002); Mato Grosso do Sul: Bonito (Lima et al. 2024); São Paulo State: Cananéia, Córrego da Gruta (Salles et al. 2003); Rio de Janeiro state: Comendador Levy Gasparian, Rio Paraibúna (Salles et al. 2003); Minas Gerais state: Arinos, Rio Paraibúna (Salles and Lugo-Ortiz 2002); Descoberto, Cachoeira da Fumaça (Salles and Lugo-Ortiz 2002); Córrego Danta, Rio da Prata (Salles and Lugo-Ortiz 2002); Juiz de Fora (Lima et al. 2024); Santo Antônio, Rio Santo Antônio (present study); Barra Longa, Rio do Carmo (present study); Guaraciaba, Rio Piranga (present study); Ponte Nova, Rio Piranga (present study); Rio Doce, Rio Piranga (present study); Naque, Rio Doce (present study); Periquito, Corrente River (present study); Governador Valadares, Rio Doce (present study); Mathias Lobato, Rio Suaçuí Grande (present study); Aimorés, Rio Manhuaçu (present study); Espírito Santo state: Alto Caparaó, stream between "Cas a Queimada" and Cachoeira da Farofa (Salles etal. 2010); Baixo Guandu, Rio Guandu (present study); Santa Teresa, Córrego Grande (Salles et al. 2010); Linhares (Lima et al. 2024);. Argentina: Tucumán, Río Aranillas (Nieto 2006) (Fig. 1).

Biology: Nymphs and adults of *A. fiuzai* were found in ten of the forty sampling sites, only during the dry season. Eighty-five percent of the abundance took place in rivers that, despite facing other anthropogenic pressures, such as cattle ranching and hydroelectric dams, remained unaffected by the passage of miningtailings. Notably, only two sampling sites on the Doce River, affected by the tailings, exhib- ited occurrences of *A. fiuzai*, one amidst an urban area and the other in an area of sugar cane plantations. All nymphs sampled were found in heavily anthropized cattle ranching areas, with stretches of river lacking vegetation cover, and on substrates comprising sand, small stones or mud.

Remarks: *Apobaetis fiuzai* was originally described based on larvae collected from Mato Grosso and Minas Gerais states (Salles and Lugo-Ortiz 2002). The majority of the subsequent records only expanded their geographic distribution without adding morphological information, which led to a lack of knowledge about the intraspecific variation of the species. (Salles et al. 2003, 2004, 2010; Nieto 2006; Lima et al. 2012; Boldrini et al. 2012). Cruz (2020) made an effort to establish a new morphological diagnosis with the redescription of *A. fiuzai* based on the type material to improve the species delimitation. Few years later, in order to evaluate the wide distribution of *A. fiuzai*, Lima et al. (2023) revised the type material of *A. fiuzai*, part of the literature, the specimens used in previous studies and additional material. They concluded that the records from Amazonas (Cruz et al. 2011), Roraima (Falcão et al. 2011), Rondônia (Boldrini and Cruz 2014), and Maranhão (Boldrini et al. 2012) do not represent the species *A. fiuzai*. Additionally, Lima et al. (2023) described *Apobaetis pasternakae* to abrange the previous records of *A. fiuzai* from Amazonas, Roraima, and Maranhão; and *Apobaetis jaquelinae* from records from Rondônia. Lima et al. (2023) also consider the records from Argentina (Nieto 2006), São Paulo (Salles et al. 2003), Rio de Janeiro (Salles et al. 2004), Espírito Santo (Salles et al. 2010), and Pernambuco states (Lima et al. 2012) as putative, since they did not review this records.

Once the record from Amazonas state (Cruz et al. 2011) is currently accepted as *A. pasternakae* (Limaetal.2023), up until now there was no description of adults for *A. fiuzai*. The specimens analyzed here, nymphs and imagos (Figs. 6a and 7b), were collected near the type locality of *A. fiuzai* and present differences concerning the external morphology of the imagos of *A. pasternakae*, which corroborates withthe species distinction made by Lima et al. (2023). The differences are as follows: *A. pasternakae* has two medial red spots anterior to medial hump (Cruz et al. 2011: Fig. 5), absent in *A. fiuzai* (Figs. 6a, 7b); *A. pasternakae* has stigmatic area with eight veins not touching Sc vein (Cruz et al. 2011: Fig. 8), while in *A. fiuzai* the stigmatic area has 2–4 complete veins, rarely 1–2 incompletes (Figs. 6e and 7c); *A. pasternakae* hastwo pairs of red spots on anterior margin, one submedially and other sublaterally on segments II–V, segments VI–IX only with one pair of red spots submedially (Cruz et al. 2011: Fig. 5), in contrast terga II–III and VI in *A. fiuzai* have one red spot in midline and one red spot sublaterally on segments II–VI (Figs.6a and 7b); *A. pasternakae* has terga tinged with red on middle region (Cruz et al. 2011: Fig. 5), which does not occur in *A. fiuzai*; *A. pasternakae* has the sterna II–VIII with paired red marks sublaterally on anterior margin, one spot medially on middle region and other laterally on middle region(Cruz et al.2011: Fig. 6), while *A. fiuzai* is completely whitish cream; *A. pasternakae* has the forceps base approximately rectangular (Cruz et al. 2011: Fig. 10a-b), in opposite, in *A. fiuzai* the forceps base is wider at base than in the distal portion (Fig. 6d).

The distribution of *A. fiuzai* was expanded to ten municipalities in Minas Gerais state (Santo Antônio, Barra Longa, Guaraciaba, Ponte Nova, Rio Doce, Naque, Periquito, Governador Valadares, Mathias Lobato, Aimorés) and to one municipality in Espírito Santo state (Baixo Guandu) (Fig. 1).

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Author contribution ADLV and JA executed laboratory work, photos, and writing. FFS did the conceptualization, methodology, project administration, supervision, and writing. All authors reviewed and approved the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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