SAMUEL OLUWATOYIN FAMUYIWA

IMPACTS OF THE MARIANA (MG, BRAZIL) MINING TAILINGS PASSAGE ON THE RIPARIAN STAPHYLINID (COLEOPTERA: STAPHYLINIDAE) COMMUNITIES

Dissertation submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Carlos Frankl Sperber
Co-adviser: Neucir Szinwelski

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To God, my parents and my children

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Research is creating new knowledge

(Neil Armstrong)

ABSTRACT

FAMUYIWA, Oluwatoyin Samuel, M.Sc., Universidade Federal de Viçosa, June, 2023. **Impacts of the Mariana (MG, Brazil) mining tailings' passage on the riparian staphylinid (Coleoptera: Staphylinidae) communities.** Adviser: Carlos Frankl Sperber. Co-adviser: Neucir Szinwelski.

Seven years after the 2015 Fundão dam mine tailings disaster in Mariana, Mina Gerais, little is known about the impacts of this disturbance, particularly on the adjacent riparian forest ecosystem. Here, we assessed the impacts of the iron ore tailings' passage through the Doce River on the staphylinid communities in the surrounding riparian forests. We considered eventual environmental drivers as covariables. The species composition between the treatment areas was compared using permutational multivariate analysis of variance (PERMANOVA). To compare community structure between impacted and reference, we adjusted generalized linear mixed models (GLMMs). Our results revealed spatial heterogeneity among sampled regions, with differences in abundance, species richness, and evenness of staphylinids. We detected an effect of the tailings' passage on staphylinid species richness, evidenced by the significant interaction term of the tailings' passage with the local availability of leaves in the litter, even though the tailings passage occurred almost entirely restricted to the river's bed. This effect was subtle, and the statistical significance disappeared when analyzed within each category (affected versus reference areas). No difference in species composition between affected and reference areas was detected. We conclude that even in an ecosystem that is adjacent, but distinct, from the aquatic ecosystem, there are detectable effects of the disturbance provoked by the tailings passage that are present on a long term, seven years after the event. This effect is subtle, but suggests that the disturbance altered local mechanisms of species richness regulation.

Keywords: Community structure. Disturbance. Evenness. Iron ore tailings. Leaf litter. Species abundance.

RESUMO

FAMUYIWA, Oluwatoyin Samuel, M.Sc., Universidade Federal de Viçosa, junho de 2023. **Impactos da passagem dos rejeitos da mineração de Mariana (MG, Brasil) nas comunidades ribeirinhas de estafilinídeos (Coleoptera: Staphylinidae).** Orientador: Carlos Frankl Sperber. Coorientador: Neucir Szinwelski.

Sete anos após o desastre de rejeitos da barragem de Fundão em Mariana, Minas Gerais, em 2015, pouco se sabe sobre os impactos dessa perturbação, particularmente no ecossistema de mata ciliar adjacente. Aqui, avaliamos os impactos da passagem dos rejeitos de minério de ferro pelo Rio Doce nas comunidades de estafilinídeos nas matas ciliares vizinhas. Consideramos eventuais drivers ambientais como covariáveis. A composição de espécies entre as áreas de tratamento foi comparada usando análise de variância multivariada permutacional (PERMANOVA). Para comparar a estrutura da comunidade entre impactada e de referência, ajustamos modelos lineares mistos generalizados (GLMMs). Nossos resultados revelaram heterogeneidade espacial entre as regiões amostradas, com diferenças na abundância, riqueza de espécies e equitabilidade dos estafilinídeos. Detectamos um efeito da passagem de rejeitos na riqueza de espécies de estafilinídeos, evidenciado pelo significativo termo de interação da passagem dos rejeitos com a disponibilidade local de folhas na serapilheira, embora a passagem dos rejeitos tenha ocorrido quase inteiramente restrita ao leito do rio. Este efeito foi sutil e a significância estatística desapareceu quando analisada dentro de cada categoria (áreas afetadas versus áreas de referência). Nenhuma diferença na composição de espécies entre as áreas afetadas e de referência foi detectada. Concluímos que mesmo em um ecossistema adjacente, mas distinto, do ecossistema aquático, existem efeitos detectáveis da perturbação provocada pela passagem de rejeitos que estão presentes a longo prazo, sete anos após o evento. Este efeito é sutil, mas sugere que a perturbação alterou os mecanismos locais de regulação da riqueza de espécies.

Palavras-chave: Abundância de espécies. Estrutura comunitária. Perturbação. Uniformidade. Rejeitos de minério de ferro. Serapilheira

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LIST OF ACRONYMS AND ABBREVIATIONS

Al	Aluminum
ANOVA	Analysis of Variance
Cu	Copper
Cr	Chromium
Fe	Iron
GLMMs	Generalized linear mixed models
MG	Minas Gerais
Mn	Manganese
PERMANOVA	Permutational multivariate analysis of variance

LIST OF SYMBOLS

%- percentage

p- probability

SUMMARY

1. INTRODUCTION

Biodiversity loss has emerged as a significant worldwide concern, with species decline currently occurring at unparalleled rates (Sánchez-Bayo and Wyckhuys, 2019). Ecologists and conservationists have long studied global declines in biodiversity, but insect populations are underrepresented in such assessments (Simmons *et al.,* 2019). Despite being the most diverse and numerous group of animals on Earth, insects remain largely understudied, widely misunderstood by the public, and are confronted with various environmental challenges. Nevertheless, they play vital roles in shaping ecosystem functions in both terrestrial and aquatic environments (Saunders *et al.,* 2020).

In recent years, a drastic decline in insect population has been reported in diverse habitats and ecosystems (Homburg *et al.,* 2019). A reduction in insect diversity jeopardizes ecosystem stability since it results in a diminished number of species capable of pollinating plants, managing pest populations, and acting as a food source for insect-eating vertebrates within the ecological web. Insect groups such as Coleoptera (dung, ground beetles and Saproxylic beetles), Ephemeroptera (mayflies), Hymenoptera (bumble and honey bees, Cuckoo wasps), Hemiptera (planthoppers and leafhoppers), Lepidoptera (butterflies and moths), Odonata (dragonflies and damselflies) and Plecoptera (stoneflies) have been reported to have witnessed a significant decline in their diversity and abundance in the last decade (Sánchez-Bayo and Wyckhuys, 2019). These declines are said to be severe in areas highly impacted by human activity (Forister *et al.,* 2019). Suggested causes of the decline include climate change, alterations in land use, habitat degradation, fragmentation, pollution, and environmental disturbance (Homburg *et al.,* 2019; Cardoso *et al.,* 2020; Kehoe *et al.,* 2021).

The mine tailings overflow following the Fundão dam disaster in Brazil represents a form of environmental disturbance. In November 2015, the Fundão dam collapse led to the release of more than 50 million cubic meters of mining tailings, occurring 600 kilometers inland from the Rio Doce estuary in Eastern Brazil (Oliveira *et al.,* 2017; Nunes *et al.,* 2022). The impact of dam tailings extended to 36 municipalities within the Doce basin and eventually reached the Atlantic Ocean (Santos *et al.,* 2019). The mine tailings had the most significant influence, covering an area of 17 square kilometers within the Doce River basin and spanning across the Gualaxo do Norte and Carmo Rivers, where well-preserved fragments of Atlantic riparian forest were located

(IBAMA, 2016; do Carmo *et al.,* 2017). The composition of the tailings primarily consisted of iron, chromium, cadmium, lead, aluminum, and silicon dioxide (Bonecker *et al.,* 2019). The iron ore tailings flooded and destroyed the riparian vegetation along the Doce River Basin resulting in the loss of major invertebrates fauna and in the largest socio-environmental disaster in Brazil's history (Oliveira *et al.,* 2017; Nunes *et al.,* 2022). Due to the disaster, substantial quantities of tailings amassed within the river basin and riparian ecosystems (Gabriel *et al.,* 2021). The mining waste slurry led to elevated concentrations of iron (Fe), aluminum (Al), manganese (Mn), copper (Cu), and chromium (Cr) in both the soil and the plant tissues within the impacted regions (Nascimento *et al.,* 2021). The sedimentation of mining waste, consisting of finer granular particles (silt), also resulted in changes to the soil's physical attributes (Thomé and Passini, 2018). The impacts of mine tailings are currently understudied and their impacts on terrestrial ecosystems are still poorly elucidated (Oliveira *et al.,* 2017; Buch *et al.,* 2020).

Detecting alterations in natural conditions and identification and/or quantification of the impact of pollutants or contaminants on the environment is achieved by the use of bioindicators (Manickavasagam *et al.,* 2019). Bioindicators are regarded as valuable instruments for observing and tracking environmental changes effectively (Ghannem *et al.,* 2018). Insects and various arthropods possess significant potential as markers for assessing alterations in habitats and their restoration efforts, and their utilization in conservation research is on the rise (Pohl *et al.,* 2007). The abundance, makeup, and diversity of insects are interlinked with other species, climate conditions, and soil properties, making them potential key indicators of environmental transformations (Cajaiba *et al.,* 2017).

Numerous insect groups have been extensively researched and are commonly employed as indicators for detecting environmental shifts, such as those related to land management, habitat fragmentation, and pollution. Frequently utilized insect groups in this context encompass Coleoptera (including Carabidae, Curculionidae, and Staphylinidae), Collembolla (Springtails), Diplura, and Hymenoptera (specifically Formicidae) (Ghannem *et al.,* in 2018). Beetles are used for indicating many kinds of alterations in the environment due to their abundance, easy sampling, bioaccumulation capacity, and diets (Ghannem *et al.,* 2018). Forest floor beetles (Coleoptera) hold significant importance as sensitive indicators of long-term forest change and overall health. This significance stems from their diverse ecological roles, which span from acting as decomposers (such as carrion beetles, Silphidae) to occupying the position of top predators (like rove beetles, Staphylinidae) in the ecosystem (Hoekman *et al.,* 2017; Harris et al., 2019). As a result, the beetle community is likely to respond to alterations in available resources (bottom-up effects) as well as changes in predator-prey interactions (top-down effects) (Harris *et al.,* 2019)

Rove beetles belonging to the Staphylinidae family (Insecta: Coleoptera) have been suggested as potential indicators of environmental disturbance due to their high numbers and diverse species, adaptable feeding habits, intricate life cycles, quick reactions to various types of disturbances (both natural and human-induced), and their presence in a wide range of microhabitats (Cajaiba *et al.,* 2017; Rodríguez *et al.,* 2019). The extensive variety and plentiful population of rove beetles render them an ideal choice for studying, contrasting, and overseeing their complete ecosystems and ecological networks (Salnitska *et al.,* 2022).

The occurrence of rove beetles is strongly influenced by the specific microhabitat, with a notable preference for riparian vegetation (Rodríguez *et al.,* 2019). Riparian vegetation is part of the complex forest ecosystem adjacent to a freshwater (river, stream, lake, and pond) or estuary which influences its biotic composition. While riparian ecosystems may cover a relatively small land area, they offer a wide range of ecosystem services, including but not limited to habitat connectivity, the preservation of biodiversity, reinforcement of riverbanks, mitigation of pollution, and recycling of nutrients (Huylenbroeck *et al.,* 2020; Méndez-Rojas *et al.,* 2021). Riparian vegetation, serving as a reservoir for various terrestrial species, often exhibits distinct species composition compared to nearby forests (Méndez-Rojas et al., 2021). Rove beetles play a significant role in the predatory insect fauna within riparian vegetation, primarily preying on leaf litter and decomposing plant material. Moreover, they are versatile in their choice of microhabitats, including fungi, ant and termite nests, burrows, and even the nests of birds and mammals (Ahn *et al.,* 2017).

The diversity patterns of rove beetles observed in different ecosystems is attributed to microenvironmental conditions and habitat structure (Méndez-Rojas *et al.,* 2021). In open riparian areas, predatory rove beetles (Staphylinidae) demonstrate a remarkable ability to withstand alterations in environmental conditions caused by human activities. This adaptability allows them to thrive in disturbed environments (Popescu *et al.,* in 2021). Rove beetles are acknowledged for their susceptibility to fluctuations in temperature, humidity, and precipitation, which can significantly impact the quality of their microenvironments (*Rodríguez et al.*, in 2019).

The Staphylinidae family is renowned for its remarkable diversity and abundance within the neotropical region. However, it is worth noting that research efforts in this area, apart from Mexico, are still in their early stages (Gutierrez-Chacon *et al.,* 2009). Remarkably, there is a dearth of information concerning rove beetles associated with riverine landscapes, with very few studies delving into this group, (Guerrero-Bolaño *et al.,* 2003), which primarily focused on a family-level analysis. This limitation can be attributed to the inherent taxonomic challenges, a lack of comprehensive knowledge, and reference collections specific to the Neotropical region (Gutierrez-Chacon *et al.,* 2009). Given the surprisingly sparse empirical data available on the fauna, particularly with regards to riparian macro-invertebrates, and the significant hurdles faced in riverine ecosystem restoration due to the lack of natural condition knowledge (Ward *et al.,* 2002), conducting research on rove beetles in Neotropical riverine landscapes holds the potential to provide invaluable insights into the functioning of these natural riverine ecosystems (Gutierrez-Chacon *et al.,* 2009).

Seven years after the 2015 Fundão dam mine tailings disaster in Mariana, Mina Gerais, little is known about the impact of the tailings' disturbance on the diversity and abundance of insects in the riparian forests along the Doce River. Previous studies aimed at assessing the ecological impacts of the mine tailings on the fauna in and along the Doce River and its basin focused on the diversity and abundance of fishes and macrobenthic invertebrate communities (Bonecker *et al.,* 2019; Gabriel *et al.,* 2020; Queiroz *et al.,* 2021; Coppo *et al.,* 2023). None of these studies considered the impact of the mine tailings overflow on the staphylinid fauna in the riparian vegetation along the Doce River Basin.

In this study, we assessed the impacts of mining tailings spilled as a result of the Fundão dam collapse on the riparian staphylinid community assemblages along the Doce River Basin. The relationship between the occurrence of Staphylinids and environmental variables was also investigated. We hypothesize that (i) there were long-term effects of the passage of the tailings, that are detectable in the riparian staphylinid fauna, (ii) these disturbance effects were diluted along the Doce River, (iii) staphylinid fauna is locally affected by processes correlated to the distance to the river or other local drivers, related to feeding or hiding resources, correlated to the litter, (iv) staphylinid species composition was altered by the passage of the tailings.

2. MATERIAL AND METHODS

2.1 Study area

The study was carried out in five different areas within the Rio Doce basin, which is situated in the southeastern region of Brazil and extends between the coordinates 17°45′–21°15′ S and 39°55′–43°45′ W. This basin is jointly shared by the states of Minas Gerais and Espírito Santo, as documented by Coelho in 2006. Covering a total length of 853 kilometers and encompassing an expansive drainage area of roughly 83,465 square kilometers, the basin includes a total of 230 municipalities. The selected regions correspond to the municipalities of Mariana, Rio Casca, Ipatinga, Conselheiro Pena and Aimorés (**Fig. 1**). In each of these five regions, two areas of native riparian vegetation, one directly impacted by the passage of tailings from the Fundão dam and one not impacted, which was used as a reference area for the previous state of the basin were selected. The sample areas were selected according to the criteria of the presence and extent of native riparian forest patches. Due to the scarcity of well-preserved riparian forest patches in certain stretches of the Doce River, edaphic and topographical aspects was not considered in the selection of areas.

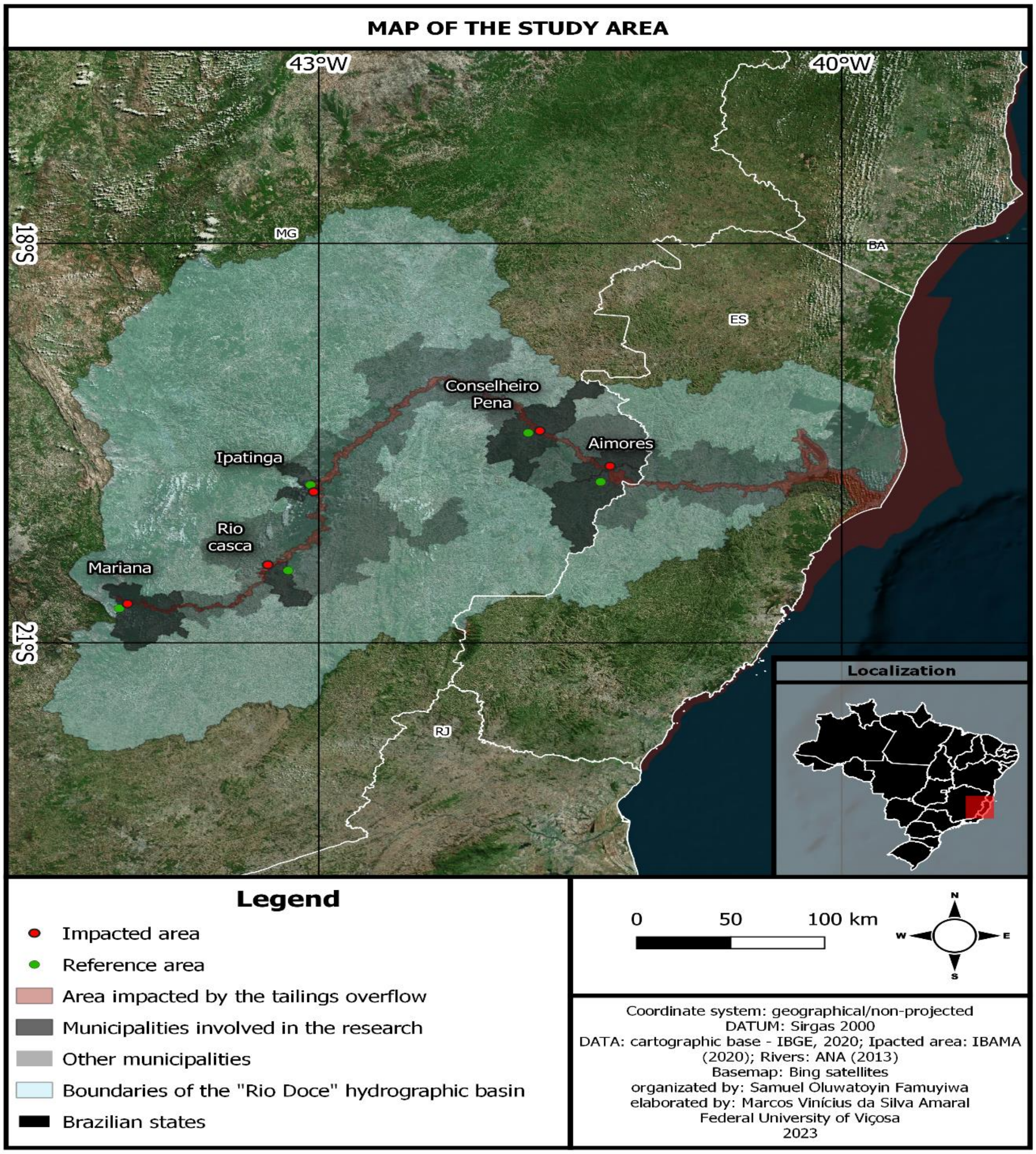


Figure 1**:** Map of the study area

2.2 Sampling design

In each sampling area (impacted/reference), four transects 120 m long each perpendicular to the course of the river were installed. These transects were set up at a distance of 50 m from each other, starting 1 m from the edge of the body of water towards the interior of the forest ciliary (**Fig. 2**). For every 30 meters of the transect, three pitfall traps were set up in a triangular dimension.

The traps were 2 meters apart and filled with 500 ml of fuel alcohol as a killer and conservative solution, as recommended in the literature (Szinwelski; Fialho; *et al.,* 2012). The traps were kept in the field for 48 hours and after that period, they are collected and the insects sorted and stored in plastic bottles with screw caps containing 90% ethanol.

Each set of three traps was considered a sampling unit, so each transect had a total of five sampling units. Thus, each region had four transects x two areas (impacted x reference) x five sets of pitfall traps x three traps per set, totaling 120 traps and composing 40 sample units. Thus, the total sampling effort for collections of Staphylinidae from the litter is, therefore, 600 pitfall traps and 200 sampling units comprising of five areas.

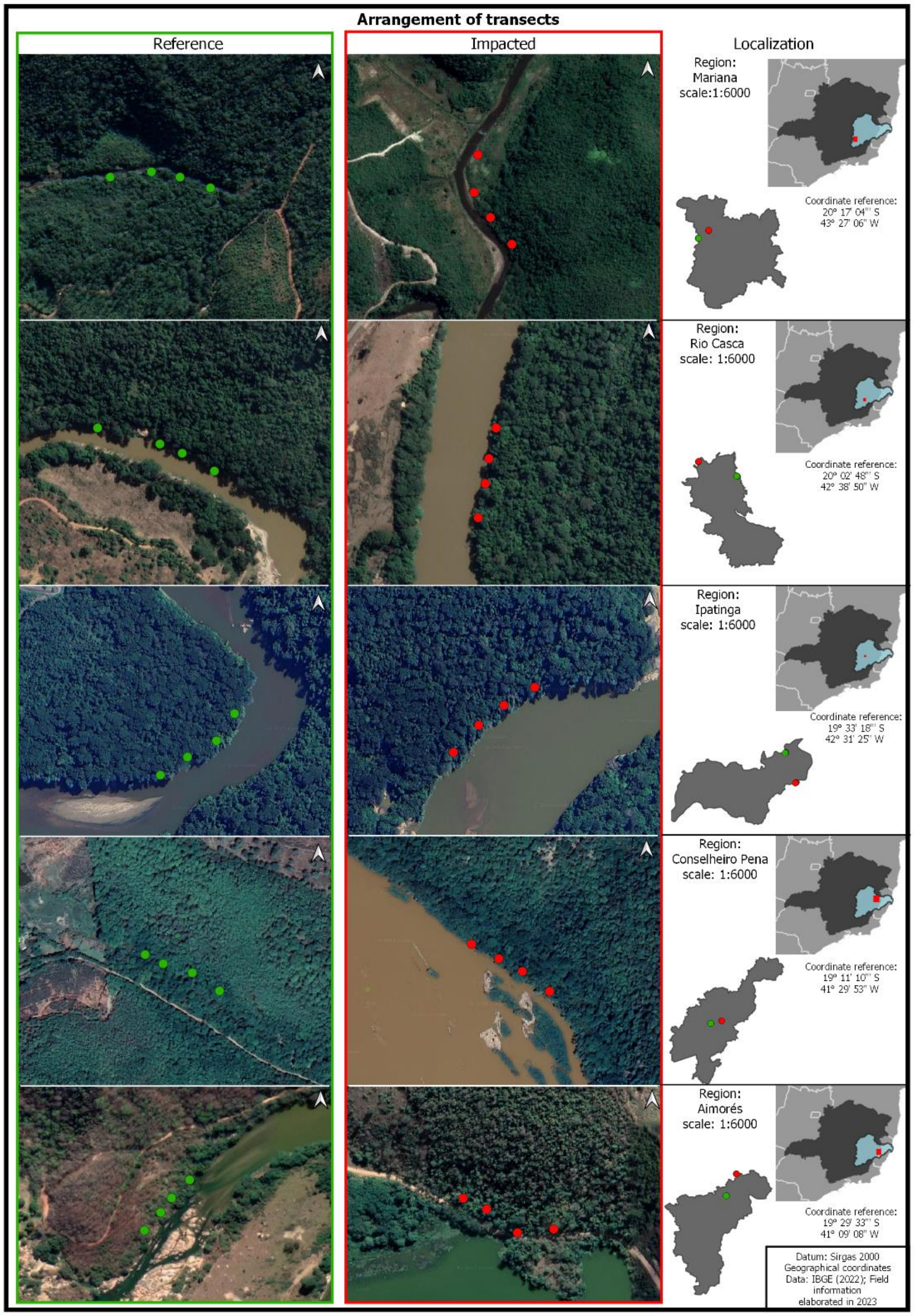


Figure 2: Arrangement of sets of pitfall traps along each transect perpendicular to the river

2.3 Environmental variables

Together with each collection area and sampling unit (set of three buried traps - pitfall), a series of environmental variables that represent important resources and habitat conditions for the staphylinids community were measured (**Table 1**).

Table 1. Details of the environmental variables sampled in this study.

S/N	Sampled Variable	Proxy for Sampling	Scale Summary	Sampling Methodology
1.	Depth of litter (cm)	Conditions and features	In every set of traps	We use a ruler and measure the depth of the soil to the top of the bed
2.	Weight of litter (g)	Conditions and features	In every set of traps	Litter sample using 50×50cm squares and dried in an oven at 50°C for 72h.
3.	Composition of litter (g)	Conditions and features	In every set of traps	Dry weight of total biomass, leaves, branches, trunks, reproductive structures and miscellaneous (i.e. fragmented organic matter)
4.	Distance from the edge of the body of water (m)	Dilution of the effect of the passage of tailings	Transect	Correlated to the arrangement of traps in the transect
5.	Distance from the origin of the impact (km)	Dilution of the effect of the passage of tailings	Hydrographic basin	Estimated by following the course of the affected channel

2.4 Morphospecies identification

For the sake of simplicity, adult staphylinids with similar morphological features were grouped as "morphospecies". Sorting and grouping of the staphylinids were done with the aid of a stereo microscope. At least one individual of each morphospecies was deposited in the

Coleoptera collection section of the Leibniz Institute for the Analysis of Biodiversity Change, for identification and preservation by curator Dr Dagmara Zyla.

2.5 Statistical analysis

To analyze the species composition between impacted and reference areas, a permutational multivariate analysis of variance (PERMANOVA) was performed using the vegan 2.6-4 package (Oksanen *et al.,* 2022). To compare community structure (richness, abundance, and equitability) between impacted and reference environments in the Rio Doce basin, generalized linear mixed models (GLMMs) were adjusted, adopting the significantly simplest model (Crawley 2012). We adjusted GLMMs for the following response variables: staphylinid diversity, estimated by species richness, staphylinid abundance, and staphylinid community evenness (Pileou's J index), with the appropriate distribution for each, adjusting transects, nested within treatment, nested within region.

All complete models had the following explanatory variables: (i) treatment - a factor with two levels: affected, where there was a direct contact of the riparian forest with the tailings, and unaffected, for forest that had no direct contact with the tailings, (ii) distance from each sampling unit to the river, (iii) distance from each sampling unit to the origin of the disaster, (iv) amount of leaves, twigs, wood and humus in the litter, to evaluate local feeding or hiding resource availability, and (v) all second order interaction terms with treatment. Either of region/treatment/transect was used as a random effect, as this grouping best represented the experimental design. All GLMMs were constructed using the glmer function of the lme 4 1.1-31 package (Bates *et al.,* 2015). Residual analysis was performed to determine the best data distribution, and contrast analysis was conducted on qualitative variables to find differences between categories. All statistical analyses were conducted using the R software (R Development Core Team, 2023), and significance was determined at the $p < 0.05$ level.

3. RESULTS

3.1 Riparian staphylinids abundance and species richness

In total, 964 adult rove beetles belonging to 9 subfamilies and 26 genera were sampled (**Table 2**). The abundance was 600 individuals in the riparian forest patches in direct contact with Rio Doce (impacted areas) and 364 individuals in the riparian forest patches that are not in direct contact with the Rio Doce but in contact with other tributaries (reference areas). The species richness of the rove beetles collected from the study 33, since this is the number of different staphylinid groups identified (**Table 2**). Although some of the staphylinids groups (morphospecies) belonged to the same subfamily and genus, slight morphological differences between them suggest that they were different species and thus were treated as such. The species name of some staphylinid groups was indicated as undetermined due to a lack of sufficient data on the nomenclature of these groups.

Subfamilies Staphylininae, Aleocharinae and Paederinae were the most frequent in the samples analyzed. Staphylinids belonging to the subfamily Aleocharinae were the most abundant with 772 individuals across the sampled regions (**Table 3**). This represents approximately 80% of the total abundance of staphylinids in the study area. This was followed by the subfamily Staphylininae with 117 individual staphylinids, representing approximately 12% of the staphylinids sampled in the study area (**Table 3**). The least frequent and abundant subfamilies were Mycetoporinae, Megalopsidiinae, Osoriinae, Oxytelinae and Steninae (Table 3). Staphylinids belonging to subtribe Cryptobiina were being grouped into the genus Homaeotarsus for the first time, while *Ontholestes brasilianus* was recorded in Minas Gerais for the first time since 1987 (**Fig. 3**).

Table 2: Staphylinids abundance and species richness

S/N	Subfamily	Tribe	Subtribe	Genus	Morphospecies	Number of Individuals (Abundance)
1	Aleocharinae	Undetermined	Undetermined	Genus 1	Genus 1, sp.1	85
2	Aleocharinae	Undetermined	Undetermined	Genus 2	Undetermined	17
3	Aleocharinae	Lomechusini	Undetermined	Genus 3	Undetermined	213
4	Staphylininae	Staphylinini	Xanthopygina	Xenopygus	Undetermined	40
5	Staphylininae	Staphylinini	Philonthina	Genus 2	Undetermined	8
6	Aleocharinae	Lomechusini	Undetermined	Genus 5	Undetermined	414
7	Staphylininae	Staphylinini	Philonthina	Genus 1	Genus 1, sp.1	2
8	Staphylininae	Staphylinini	Xanthopygina	Genus 4	Undetermined	29
9	Staphylininae	Staphylinini	Philonthina	Genus 1	Genus 1, sp.2	5
10	Xantholininae	Xantholinini	Undetermined	Xantholinus	Undetermined	3
11	Aleocharinae	Lomechusini	Undetermined	Genus 3	Genus 3, sp.2	5
12	Staphylininae	Cyrtoquediini	Undetermined	Cyrtoquedius	Undetermined	10
13	Paederinae	Paederini	Cryptobiina	Homaeotarsus	Homaeotarsus sp.1	1
14	Xantholininae	Xantholinini	Undetermined	Thyreocephalus	Undetermined	7
15	Paederinae	Paederini	Cryptobiina	Homaeotarsus	Homaeotarsus sp.1	23
16	Staphylininae	Staphylinini	Staphylinina	Platydracus	Platydracus, sp.3	2
17	Aleocharinae	Lomechusini	Undetermined	Genus 3	Genus 3, sp. 3	1
18	Staphylininae	Staphylinini	Xanthopygina	Genus 3	Undetermined	14
19	Paederinae	Lathrobiini	Stilicina	Rugilus	*R. punctatus*	7
20	Mycetoporinae	Undetermined	Undetermined	Bryoporus	Undetermined	13
21	Aleocharinae	Undetermined	Undetermined	Genus 1	Genus 1, sp.2	35
22	Paederinae	Lathrobiini	Medonina	Suniotrichus	Suniotrichus sp.2	8
23	Paederinae	Pinophilini	Pinophilina	Pinophilus	Pinophilus sp.1	1
24	Paederinae	Lathrobiini	Scopaeina	Scopaeus	Undetermined	4
25	Paederinae	Lathrobiini	Echiasterina	Ronetus	Undetermined	2
26	Staphylininae	Undetermined	Philonthina	Genus 3	Undetermined	4
27	Megalopsidiinae	Undetermined	Undetermined	Megalopinus	Undetermined	1
28	Steninae	Undetermined	Undetermined	Stenus	Undetermined	1
29	Oxytelinae	Oxytelini	Undetermined	Apocellus	Undetermined	1
30	Staphylininae	Staphylinini	Staphylinina	Undetermined	*Ontholestes brasilianus*	3
31	Aleocharinae	Undetermined	Undetermined	Genus 7	-	2
32	Paederinae	Pinophilini	Pinophilina	Pinophilus	Pinophilus sp.2	1
33	Osoriinae	Undetermined	Undetermined	Lispinus	-	2
					Total	964

Table 3: Staphylinid subfamilies' relative frequency and abundance

Subfamily	Relative frequency	Total abundance
Aleocharinae	24.2	772
Staphylininae	30.3	117
Xantholininae	6.1	10
Paederinae	24.2	47
Mycetoporinae	3.03	13
Megalopsidiinae	3.03	1
Steninae	3.03	1
Oxytelinae	3.03	1
Osoriinae	3.03	2

Note: Relative frequency (RF) = Subgroups frequency / Total frequency or f/n

Where f = number of times the data occurred in an observation, n = total frequency

Expressed as %; % RF = f/n x 100

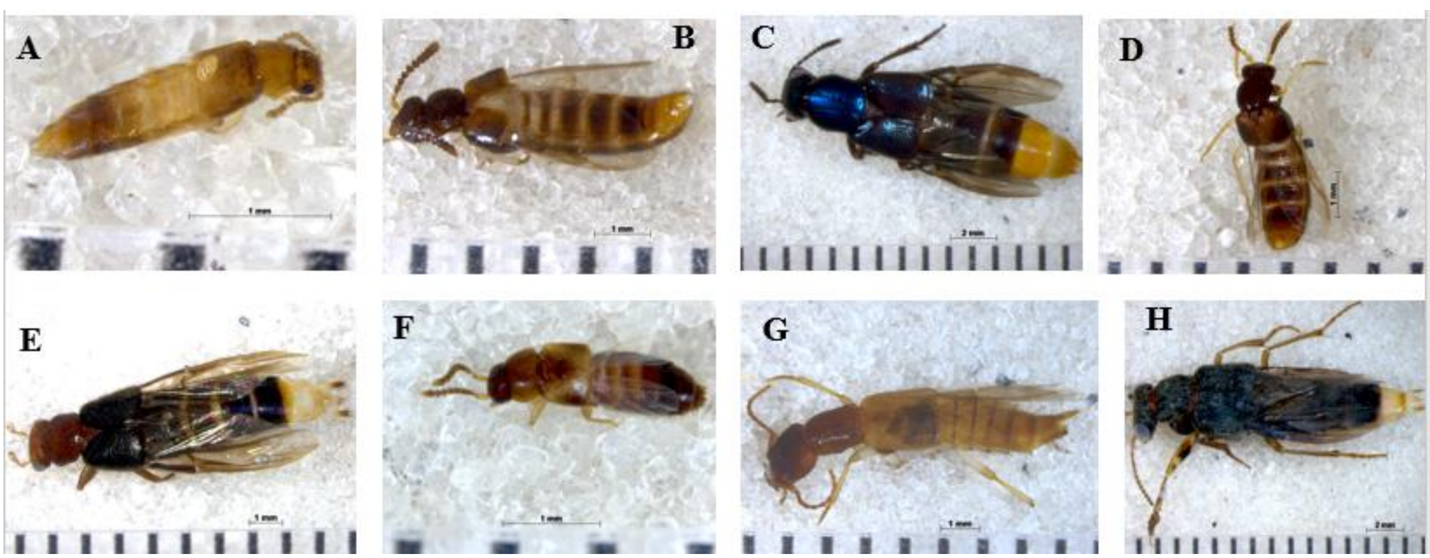


Figure 3: Selected staphylinid morphospecies recovered from the samples.
A- Aleocharinae, *Genus 1*, sp.1; **B**- Aleocharinae (Lomechusini), Genus 3; **C**- Staphylininae (Staphylinini), *Xenopygus*;
D- Aleocharinae (Lomechusini), Genus 5; **E-** Staphylininae (Staphylinini), Genus 4**; F-** Aleocharinae, *Genus 1*,sp.2; **G-** Paederinae (Paederini), *Homaeotarsus*, sp.1. **First time** it is classified to genus level; **H-** Staphylininae (Staphylinini), *Ontholestes brasilianus.* First confirmed record in Minas Gerais since 1897.

3.2 Impact of tailings disturbance and other measured environmental variables on staphylinids community composition

Staphylinid richness was influenced by the interaction between treatment and leaf litter weight (Chi-square = 4.0683, p = 0.0437). Specifically, richness increased with increasing leaf litter weight (**Fig. 4**). However, when analyzing the treatments individually, the same effect was not found (affected: Chi-square = 0.35864, p = 0.5493 and reference: Chi-square = 2.485, p = 0.1149). The abundance of Staphylinids was influenced by the distance to the river, although the effect size was small. There was a positive relationship between abundance and increasing distance to the river (Chi-square = 8.0066, p = 0.0046, **Fig. 5**). On the other hand, the presence or absence of the tailings (affected and reference areas) had no effect on abundance (Chi-square = 0.0081, p = 0.9283). Interestingly, no significant relationship was found between evenness and any of the variables tested. Finally, there was no significant difference in community composition (F = 2.1826, p = 0.1149) between the affected and reference treatments.

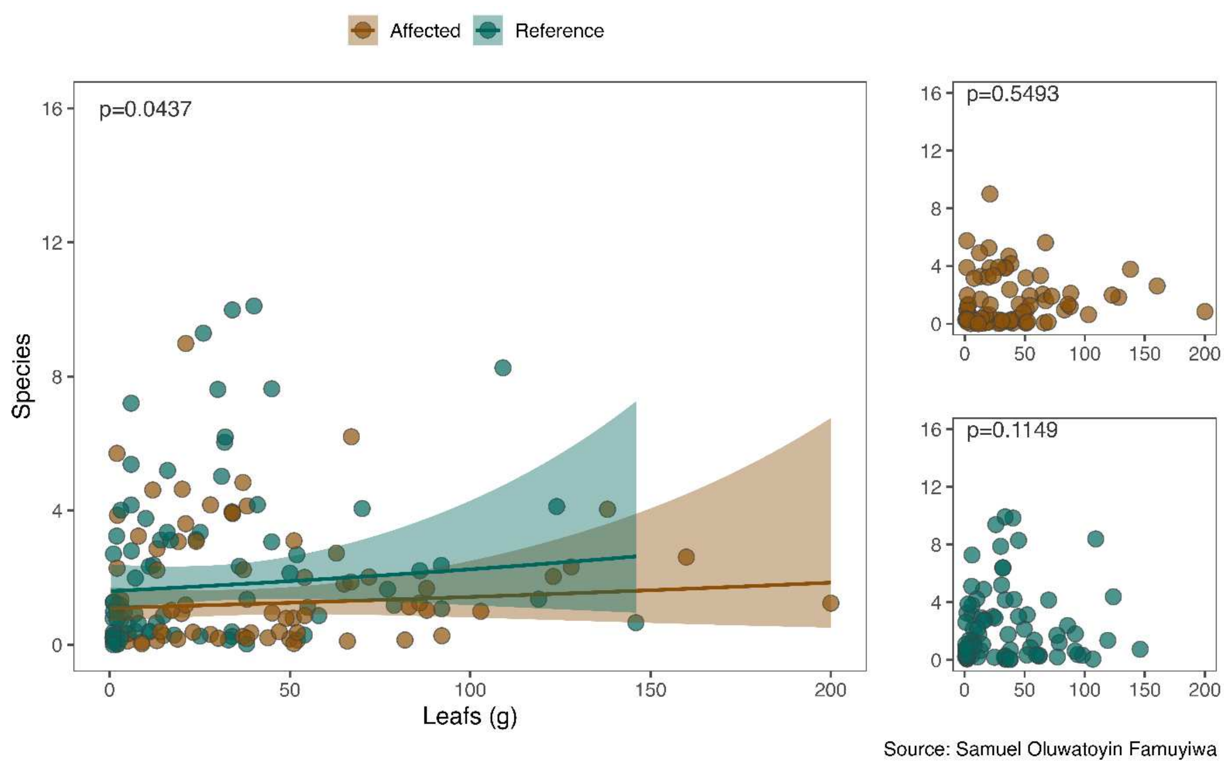


Figure 4: Staphylinid richness in relation to leaf litter weight in areas affected and unaffected by tailings along the Doce River basin, MG, Brazil.

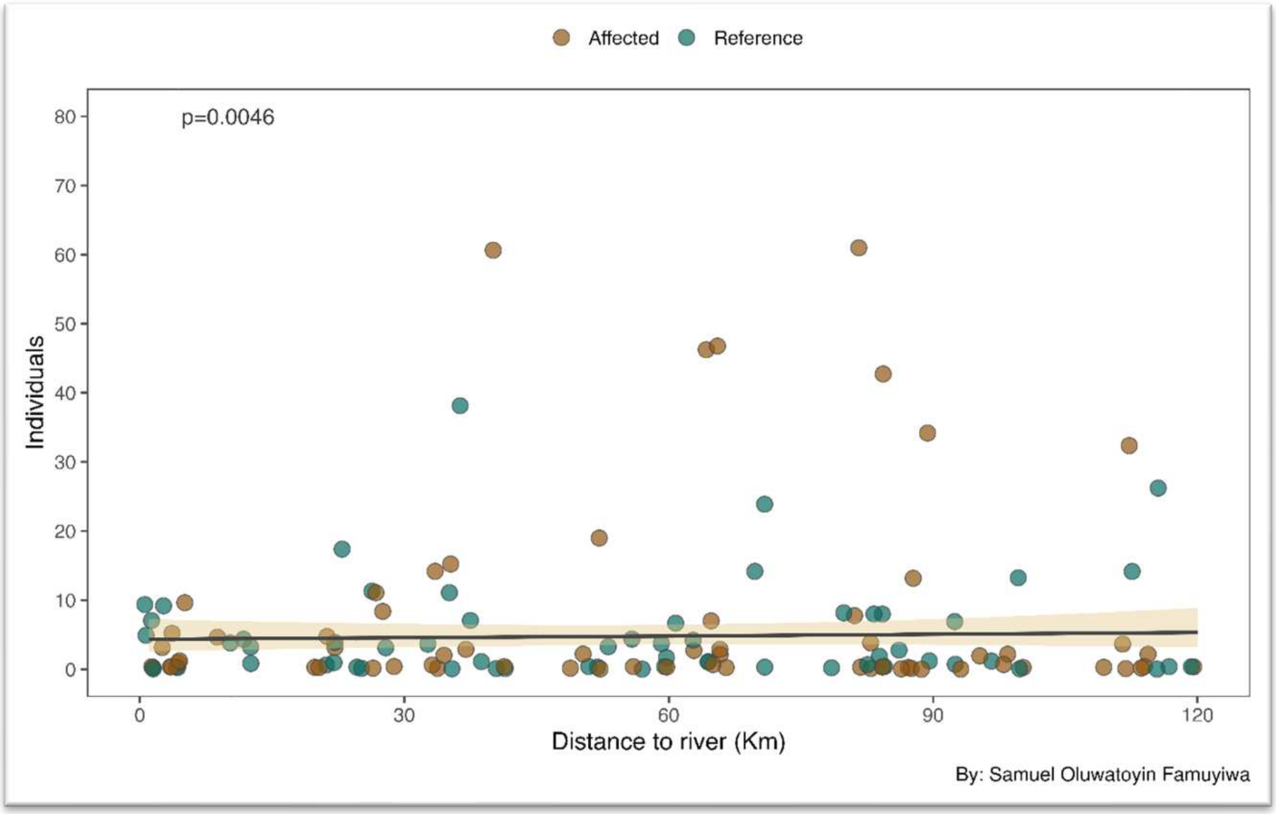
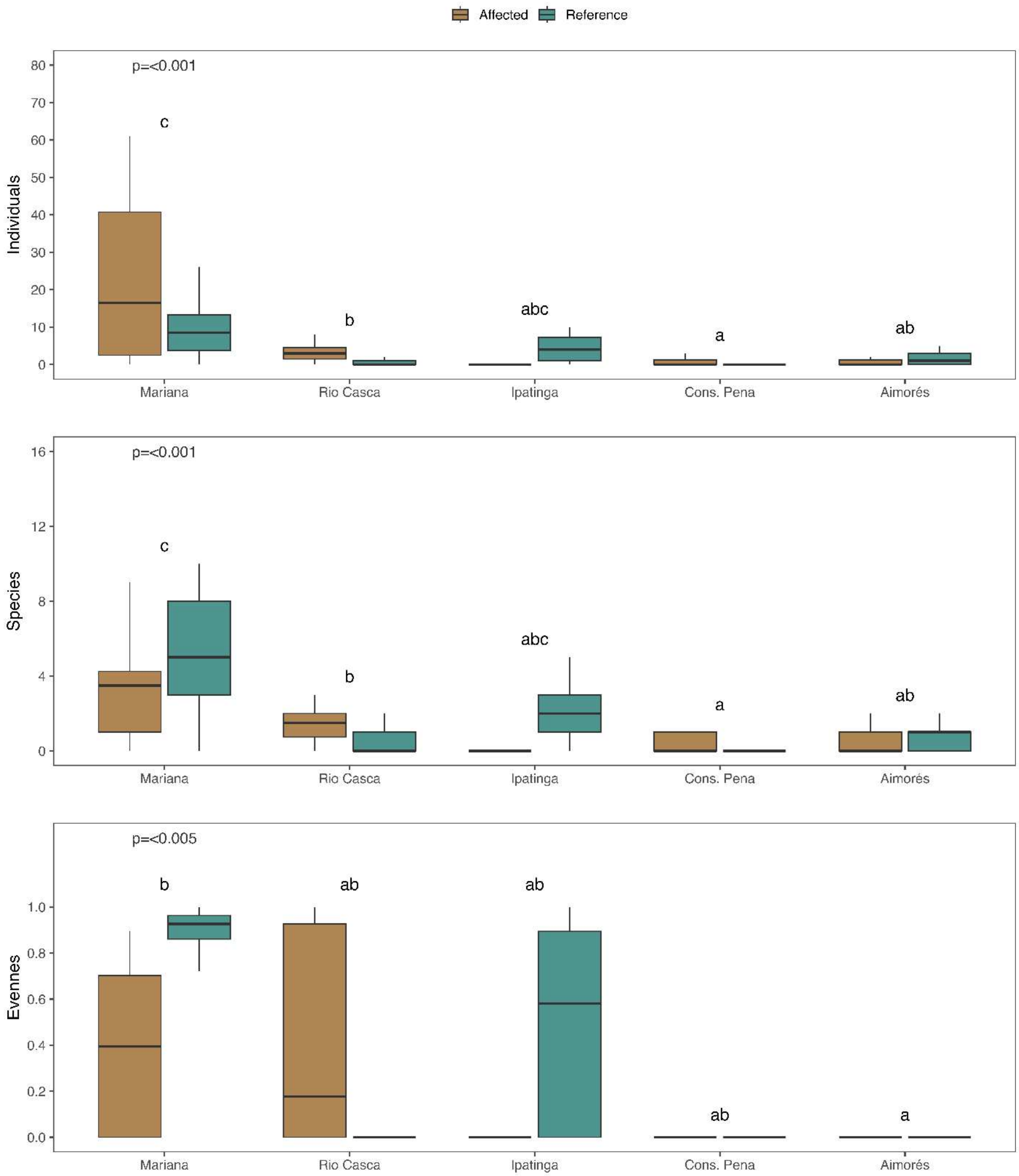


Figure 5: Abundance of Staphylinids in relation to the distance to the river in areas affected and unaffected by tailings along the Doce River basin, MG, Brazil.

3.3 Patterns of distribution of riparian staphylinids across the selected region

The selected regions differed significantly in abundance, species richness, and evenness of staphylinids. The abundance of Staphylinids was found to be significantly higher in Mariana compared to the other sampled regions (**Fig. 6**). The highest richness of Staphylinids was observed in Mariana, followed by Ipatinga and Rio Casca (**Fig. 6**).

This result revealed also revealed an interesting trend in the abundance and species richness of staphylinids across the sampling regions with respect to the distance to the source of the tailings (Fundão dam). Abundance and species richness declined as the distance of these regions increased with respect to the source of the tailings. The abundance of Staphylinids was found to be significantly higher in Mariana (impacted area) which was the closest to the source of the tailings compared to the other sampled regions. The highest richness of Staphylinids was observed in Mariana (reference area), followed by Ipatinga (reference) and Rio Casca (impacted). However, the individuals belonging to the different species of staphylinids in these regions were evenly distributed with no significant difference noticed in the evenness of three of the five regions as indicated by the Pileou's J (evenness). The evenness index (J) was higher among the species found in Mariana, Rio Casca and Ipatinga, suggesting a more balanced distribution of individuals among species in these regions (**Fig. 6**).



Source: Samuel Oluwatoyin Famuyiwa

Figure 6: Abundance, species richness and evenness of Staphylinids in affected and reference areas of the studied regions along the Doce River basin, MG, Brazil. Same letters do not differ from each other by the Turkey test (p> 0.05) according to two-way analysis of variance (ANOVA)

4. DISCUSSION

Staphylinidae (Insecta, Coleoptera), the rove beetles, a speciose group with over 64,000 species described is the second largest family in the animal kingdom after the family Curculionidae (true weevils). They are highly diverse and abundant, occurring in almost all terrestrial habitats on every continent except Antarctica. Associated with the great diversity and abundance of staphylinids are some pros and cons. While their high diversity and abundance make them a tool for research and monitoring of environmental disturbances, they also make their taxonomic classification complex. According to Salnitska *et al.* (2022), the high taxonomic diversity of rove beetles presents a challenge to accurate species identification. This is because the more rove beetles are discovered around the world, the more there is an urgent need for a taxonomic revision in order to ensure accurate taxonomic grouping of these beetles. Additionally, there remain significant gaps in our understanding of rove beetles, spanning from the insufficient exploration of entire continents' faunas to a multitude of unresolved taxonomic and phylogenetic issues. The species name of some staphylinid groups sampled in this study was indicated as undetermined due to a lack of sufficient data and scarce taxonomic keys that could aid in the naming of these groups. In order to overcome the challenge of scarce taxonomic keys, staphylinids collected were grouped into various taxonomic units as morphospecies on the basis of similarities in their morphology.

Despite not knowing the species name of most of these rove beetles, data obtained from this study was used to draw a conclusion on the effect of the tailings disturbance on the riparian staphylinids community. The most important factor considered was the identification of the assemblage of different riparian staphylinid populations forming a community and the ability to group them into taxonomic units. This is in agreement with the remark by Michaels (2007) in his review of the use of staphylinid and tenebrionid beetles as indicators of sustainable landscape management in Australia where he stated that a species may still be useful as an indicator species even if the scientific name is not known. According to him, the most important thing is the recognition of a taxonomic unit so that populations can be reliably defined.

Subfamilies Staphylininae, Aleocharinae and Paederinae were the most frequent and abundant in the samples analysed. This finding is similar to that of Gutierrez-Chacon, *et al.* (2009) who in their study of the relation between the occurrence of Staphylinidae and environmental variables in riverine systems of Southwest and Central Colombia stated that Oxytelinae, Aleocharinae, Steninae and Staphylininae were the dominant subfamilies. However, contrary to our findings, Paederinae was indicated as one of the least common subfamilies in the study while Steninae which was one of the least abundant subfamilies in our study was designated as abundant. In the most recent checklist provided by Asenjo *et al.* (2013) detailing the abundance and geographical distribution of Staphylinidae of Brazil, Subfamilies Staphylininae, Aleocharinae and Paederinae were listed as having the highest number of genera and species together with Subfamily Pselaphinae. Aleocharinae are one of the largest subfamilies of rove beetles, containing over 12,000 species. Rove beetles belonging to this subfamily are distributed throughout the world in almost all terrestrial habitats. They are common predators in soil communities and leaf litter, frequently inquilines in ant and termite nests or associated with mushrooms and fungi. Paederinae and Staphylininae are generalist predators (except a few specialist predatory species of Staphylininae and inquiline species of Paederinae) that prey upon Nematoda, Oligochaeta, Acarina, Araneae, Collembola, Coleoptera adults, Diptera larvae, and adults and Lepidoptera larvae.

The least frequent and abundant subfamilies were Mycetoporinae, Megalopsidiinae, Osoriinae, Steninae and Xantholininae. The low record of Steninae in this study could be attributed to their preference for macrophytes over riparian vegetation. According to Gutierrez-Chacon *et al.* (2009), although abundant in the study, no member of Stenus, the only known genus of Steninae in the neotropics was collected in the riparian vegetation. Instead, they were mainly collected in macrophytes and in streams suggesting their preference for macrophytes. Steninae are facultative water beetles who are able to swim or skim on water surfaces as well as withstand submergence and thus are common hunters in macrophytes. This amazing feat is accomplished by the release of a surfactant, called stenusin, from their hind ends. Stenusin breaks the surface tension, forcing water molecules suddenly apart. This pushes the lazy beetle along at speeds reaching 70 cm per second.

The distance to the edge of the river and leaf litter weight were the environmental variables that best explained the variation in the riparian staphylinid composition. The abundance of Staphylinids was influenced by the distance to the river regardless of the treatment, although the effect size was small. The positive relationship between abundance and increasing distance to the river can be attributed to the regular river level variations (inundation frequency). There are seasonal or frequent variations in the level of water of the river which sometimes lead to overflowing of the water on the river bank and areas in the riparian forest close to the edge of the river. This overflow disturbance sometimes displaces the riparian terrestrial arthropods. In order to adapt to this frequent disturbance, many riparian forest arthropods including rove beetles have resorted to certain behavioural strategies which include attaching themselves to the closest substrate or escaping the approaching water by flying or moving to areas distant from the edge of the river. Although there is no known literature on how the distance to the edge of the river affects the abundance of staphylinids, there are a few works which described the indirect effect that the variations in the level of the river could have on the riparian staphylinid communities and the strategies deployed by these insects to overcome this challenge (Lott, 2001; Paetzold *et al.,* 2008). According to Paetzold *et al.* (2008), alterations to river flow and morphology widely impact riverine habitats. Lott (2001) stated that rove beetles survive submersion in water by either clinging to submersed vegetation and becoming torpid or actively seeking to escape the advancing waters. However, the ability of ground and rove beetles to survive submersion in water depends on the species, the temperature of the water, and the presence or absence of refuges such as litter or air pockets in the substratum (Lott, 2001). Although the distance to the edge of the river had an impact of a small magnitude on the abundance of the staphylinids at the study area, the impact was however regardless of the treatment (impacted and reference areas). This implies that the presence or absence of the tailings in the Doce River had no significant effect on the abundance of the riparian staphylinids as indicated by our results.

Staphylinid species richness was influenced by the interaction between treatment and leaf litter weight. This result is similar to that of the study conducted by Stašiov *et al.* (2021) to determine the influence of environmental parameters on staphylinid communities in Central European floodplain forests. According to the authors, the thickness of the litter layer had a positive effect on the total dynamic activity as well as the species richness of the staphylinids in the study area. Sites with the highest record of staphylinid species had the thickest leaf litter collected. Rove beetles abundance and richness related to the quality of leaf litter and soil. Stocker *et al.* (2022) described rove beetles as a group of insects that have diversified through their various ways of living in the litter layer. The rove beetles of belonging to the most frequent and abundant subfamilies Aleocharinae, Staphylininae and Paedarinae recorded in this study are known to show a preference for litter as a microhabitat. In their study, Gutierrez-Chacon et al. (2009) discovered that Staphylininae, as a whole, exhibited a strong preference for the litter microhabitat. Although the importance of litter as a microhabitat often goes unnoticed, it harbors a rich diversity of terrestrial and aquatic invertebrates. Nagy et al. (2015) noted that approximately half of all known rove beetle species reside within forest litter. Klimaszewski et al. (2018) emphasized that moist litter plays a crucial role as a habitat for most rove beetles due to their susceptibility to desiccation owing to their small body size. The removal of the litter layer and exposure of mineral soil can lead to decreased soil moisture, making it less suitable for staphylinids.

Irmler and Lipkow (2018) highlighted that the type of litter is the most significant factor influencing the distribution of rove beetles. Forest litter, encompassing fallen leaves, twigs, seeds, and other woody debris, serves as the vital link between the forest and soil systems, with leaf litter constituting more than 70% of its composition. Given the rapid decomposition rate of leaf litter, it expeditiously returns nutrient elements from plants to the soil. Leaf litter contributes at least 90% of nitrogen and phosphorus, as well as 60% of medium and trace mineral elements, serving as a nutrient source for plant root absorption and a reservoir of soil fertility in forested areas. This underscores the pivotal role of leaf litter in nutrient cycling and energy flow within forest ecosystems.

Furthermore, the nitrogen (N) and phosphorus (P) content in the soil indirectly influences the food availability for rove beetles. Soil humus rich in nitrogen and phosphorus becomes an attractive resource for fungi and saprophagous organisms, leading to its faster decomposition through the activities of decomposers and saprophagous species. Consequently, this rich organic material contributes to the rapid recycling of nutrients and supports the diverse food web within forest ecosystems.

Our study also revealed interesting patterns in the abundance, richness, and evenness of Staphylinids across the sampled regions. The abundance of Staphylinids was significantly higher in Mariana compared to the other sampled regions. Additionally, the highest richness of Staphylinids was observed in Mariana, followed by Ipatinga. Interestingly, the evenness index (J) was higher among the species found in Mariana, Ipatinga, and Rio Casca, suggesting a more balanced distribution of individuals among species in these regions. The equitability or evenness of staphylinids communities is related to the contents of N and P in the soil and litter. Regions with high litter composition and soils rich in N and P tend to have a balanced distribution of rove beetles than those with lower contents of N and P. This explains why Mariana, Rio Casca and Ipatinga had similar evenness. These results highlight the importance of considering regional differences in the diversity and distribution of species, as well as the potential role of local environmental factors in shaping these patterns.

Interestingly, the highest abundance of staphylinids was recorded in areas in the riparian forest that had contact with the Doce River. Out of the 964 adult rove beetles collected in this study, 600 individuals were sampled from riparian forest patches in direct contact with Rio Doce (impacted areas) and 364 individuals in the riparian forest patches that are not in direct contact with the Rio Doce but in contact with other tributaries (reference areas). The high abundance of rove beetles recorded in areas designated as impacted areas could be attributed to the ability of these beetles to cope with changes in environmental conditions as well as their feeding preference.

Rove beetles are known to adapt well to environmental disturbances in their natural habitats. Méndez-Rojas *et al.* (2021) observed an increase in the diversity of rove beetles in transformed landscapes of tropical and temperate regions. The presence of iron tailings in an area over a long period of time could alter the soil characteristics of such area. According to Cui *et al.* (2021), iron tailings have been found to result in several adverse effects on soil. These effects include a reduction in particle size, elevated alkalinity, decreased organic matter content, diminished water retention, and reduced permeability. As a consequence of these changes, the soil becomes unsuitable for supporting the growth of both plants and microorganisms. Microorganisms play a crucial role in breaking down organic matter within the soil. Additionally, there are certain specialist rove beetles that act as detritivores, primarily feeding on decaying organic material found on forest floors. The dearth of microorganisms in the soil leads to a restricted availability of food resources for these specialized rove beetles that rely on dead and decaying organic matter as their primary source of nutrition.

Furthermore, the inhibited growth of plants can also have an impact on the availability of food resources, such as pollen, for certain specialist rove beetles that depend on plants for sustenance. Nagy *et al.* (2015) stated that habitat alterations can reduce and slow down the nutrient cycling and decomposition processes in the plantations or forests contributing to the reduction of the diversity of specialist rove beetles. The alteration of habitats by human-induced disturbances and pollution is known to favour generalist rove beetles at the expense of specialist rove beetles. According to Gray *et al.* (2016), changes in the riparian vegetation matrix could alter habitat complexity or food resources, benefiting some species that can take advantage of these changes. This explains why the most abundant subfamilies of rove beetles recorded in this study, particularly in areas designated as impacted areas were generalists while some of the subfamilies with the least frequency and abundance in this study were specialist feeders. Méndez-Rojas *et al.* (2021) noted that the fragmentation of riparian vegetation could impact riparian specialist rove beetles while favouring generalist species that can tolerate a wide range of environmental conditions. Finally, our analysis revealed no significant difference in species composition between the affected and reference treatments.

5. CONCLUSION

Although the abundance and richness of Staphylinids were impacted by the interaction between the treatments and the leaf litter, and the distance to the edge of the river, we detected no significant difference in species composition between affected and reference areas. We interpret as particularly important that, even after seven years of the passage of the tailings through the Doce River, and although this passage occurred almost entirely restricted to the river's bed (exception for the first part of the Doce River, before the Candonga dam), we detected an effect of the tailings' passage on the staphylinid species richness, evidenced by the significant interaction term of the tailings' passage with the local availability of leaves in the litter. We further detected that this effect is subtle, and disappears when analyzed within each category (affected versus reference areas). Thus, we conclude that even in an ecosystem that is adjacent, but distinct, from the aquatic ecosystem, there are detectable effects of the disturbance provoked by the tailings passage that are present on a long term, seven years after the event. This effect is subtle, but suggests that the disturbance altered local mechanisms of species richness regulation.

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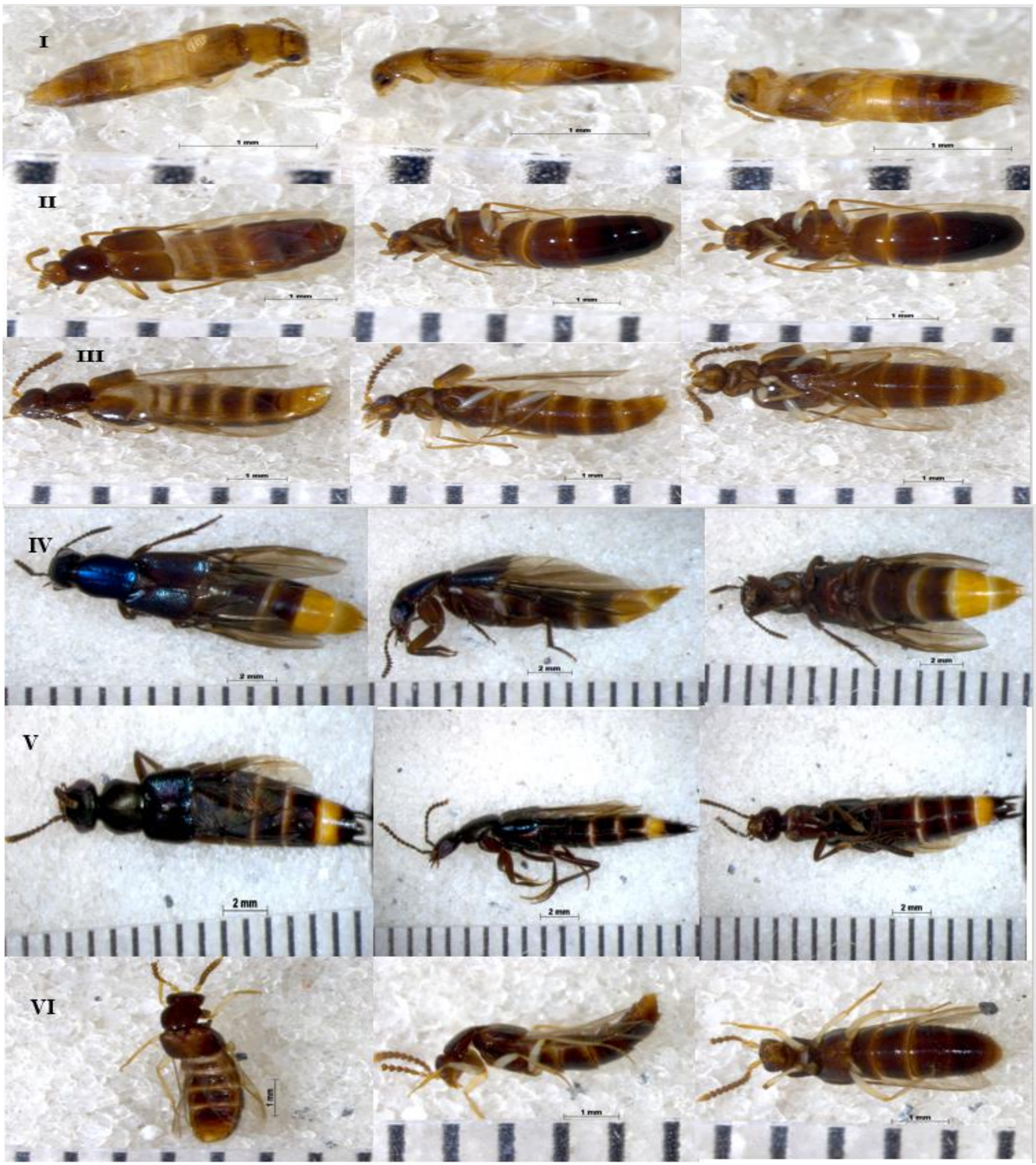
APPENDIX I

MORPHOSPECIES RECOVERED FROM THE SAMPLES (Dorsal, lateral and ventral views)

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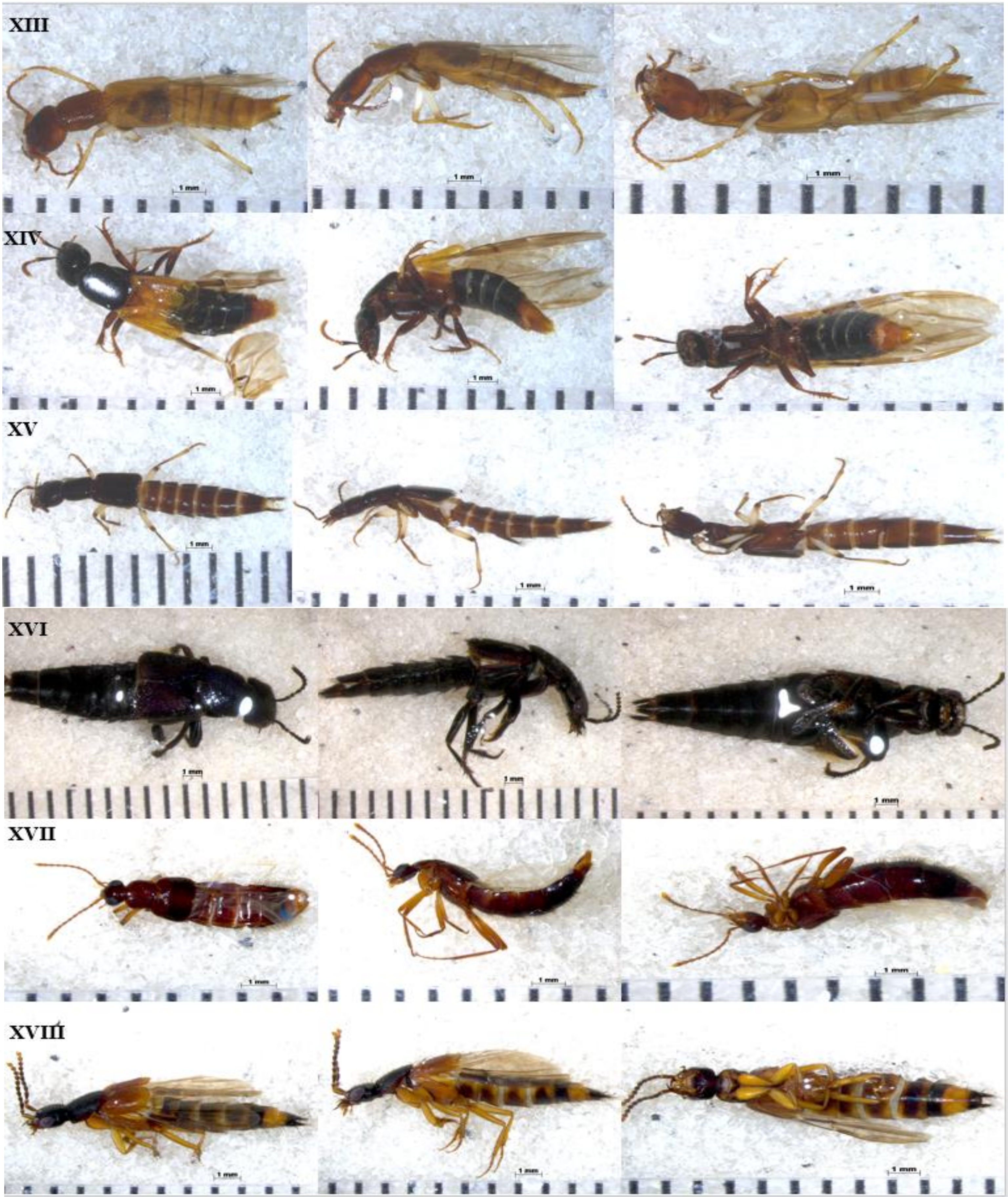
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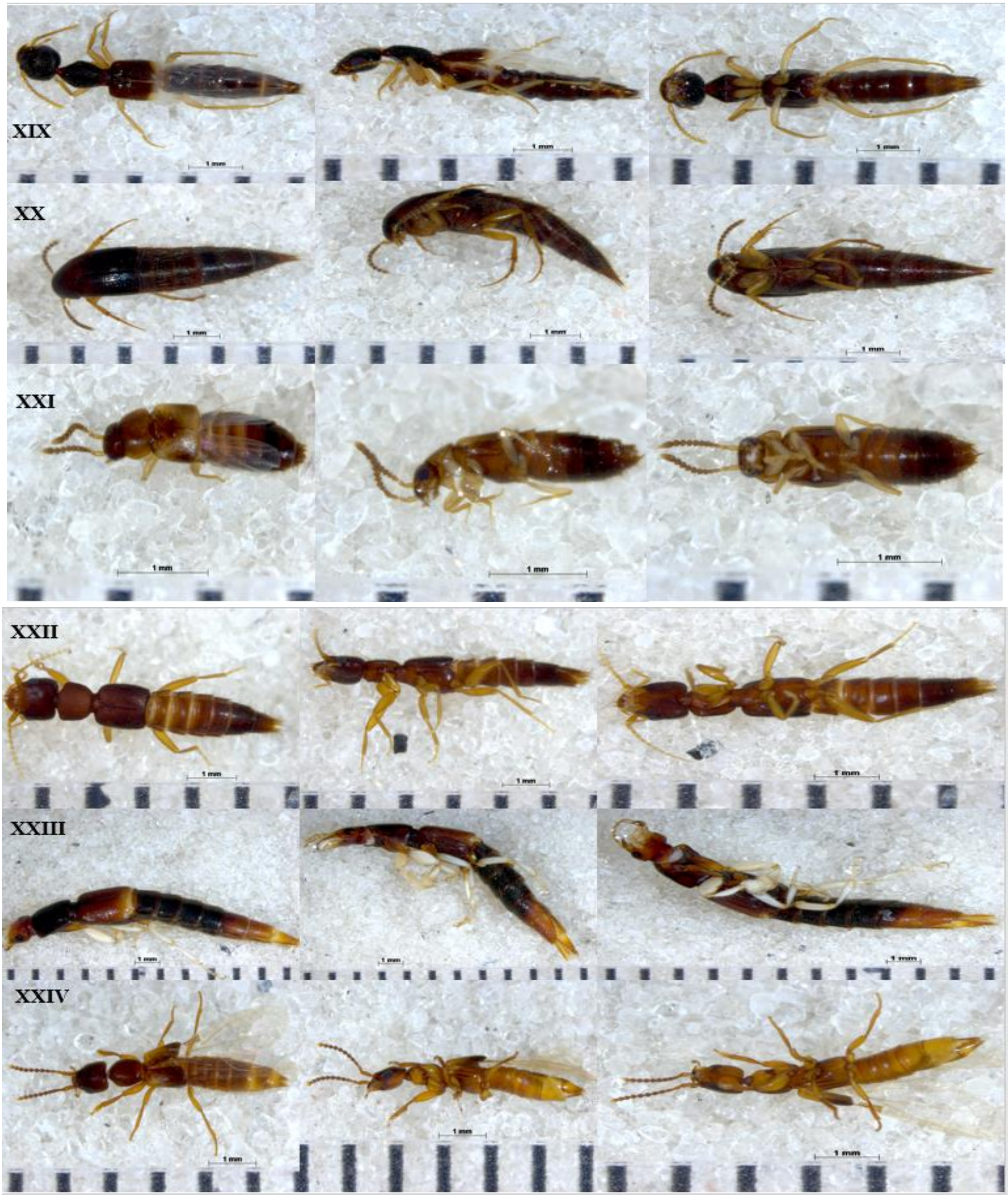
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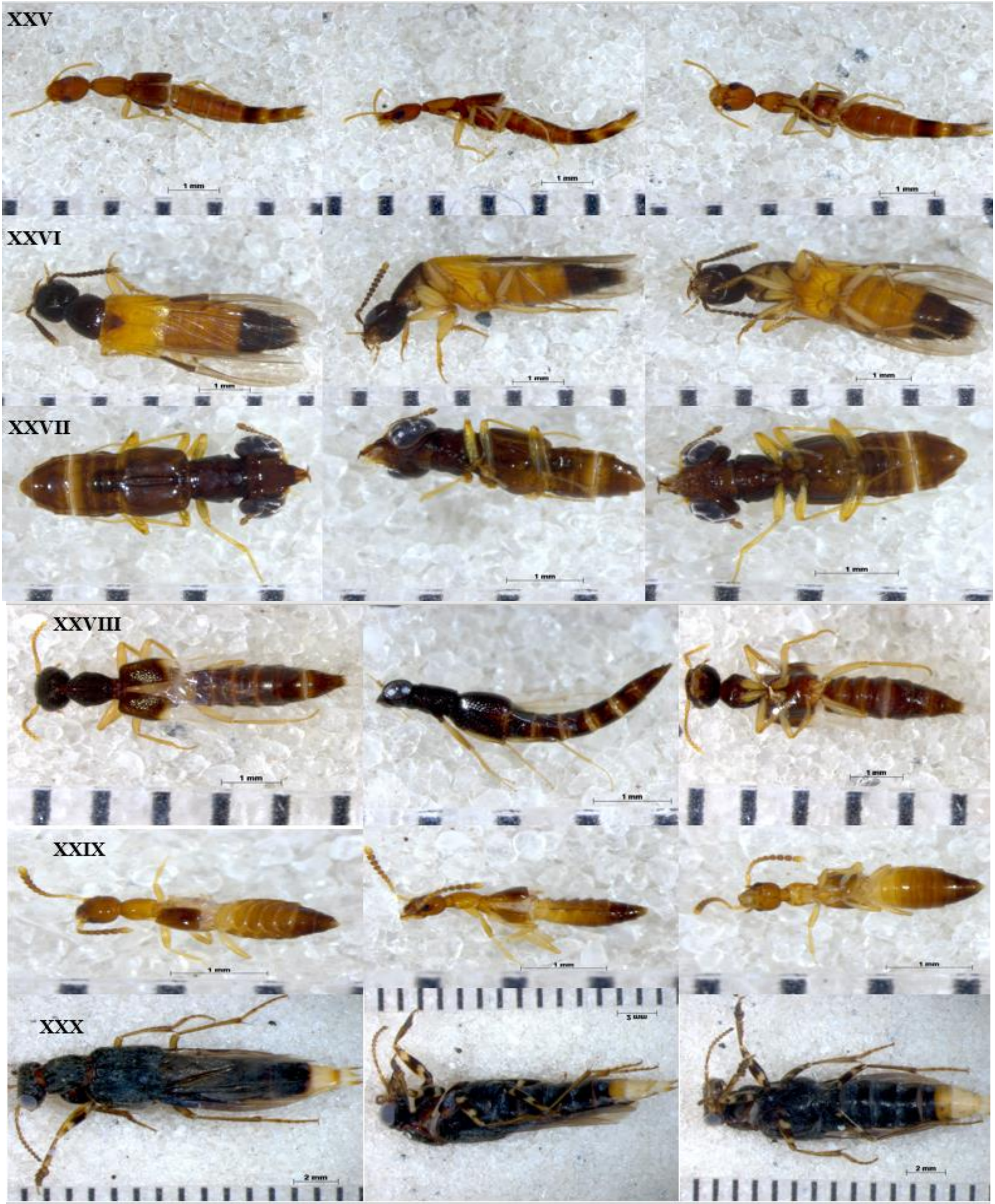
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XXVIII
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XXX
2 mm

I: *Genus 1, sp.1* (Aleocharinae)

II: Genus 2 (Aleocharinae)

III: Genus 3 (Aleocharinae; Lomechusini)

IV: Xenopygus (Staphylininae; Staphylinini)

V: Genus 2 (Staphylininae; Staphylinini)

VI: Genus 5 (Aleocharinae; Lomechusini)

VII: *Genus 1, sp.1* (Staphylininae; Staphylinini)

VIII: Genus 4 (Staphylininae; Staphylinini)

IX: *Genus 1, sp.2* (Staphylininae; Staphylinini)

X: Xantholinus (Xantholininae; Xantholinini)

XI: *Genus 3, sp.2* (Aleocharinae; Lomechusini)

XII: Cyrtoquedius (Staphylininae; Cyrtoquediini)

XIII: *Homaeotarsus sp.1* (Paederinae; Paederini)

XIV: Thyreocephalus (Xantholininae; Xantholinini)

XV: *Homaeotarsus sp.1* (Paederinae; Paederini)

XVI: *Platydracus, sp.3* (Staphylininae; Staphylinini)

XVII: *Genus 3, sp. 3* (Aleocharinae; Lomechusini)

XVIII: Genus 3 (Staphylininae; Staphylinini)

XIX: *R. punctatus* (Paederinae; Lathrobiini)

XX: Bryoporus (Mycetoporinae)

XXI: *Genus 1, sp.2* (Aleocharinae)

XXII: *Suniotrichus sp.2* (Paederinae; Lathrobiini)

XXIII: Pinophilus sp.1 (Paederinae; Pinophilini)

XXIV: Scopaeus (Paederinae; Lathrobiini)

XXV: Ronetus (Paederinae; Lathrobiini)

XXVI: Genus 3 (Staphylininae)

XXVII: Megalopinus (Megalopsidiinae)

XXVIII: Stenus (Steninae)

XXIX: Apocellus (Oxytelinae; Oxytelini)

XXX: *Ontholestes brasilianus* (Staphylininae; Staphylinini)

XXXI: Genus 7 (Aleocharinae)

XXXII: *Pinophilus sp.2* (Paederinae; Pinophilini)

XXXIII: Lispinus (Osoriinae)