Universidade Federal de São Carlos - Campus Sorocaba
Centro de Ciências Humanas e Biológicas
Departamento de Biologia

Guilherme Eduardo Ciamponi

Genetic diversity of Neotropical rove beetles in the Alto da Figueira Private Natural Heritage Reserve

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Trabalho de Conclusão de Curso apresentado à Coordenação do curso de Graduação em Ciências Biológicas da Universidade Federal de São Carlos, Campus Sorocaba, para obtenção do título de bacharel em Ciências Biológicas

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Trabalho de Conclusão de Curso

Universidade Federal de São Carlos – c*ampus* Sorocaba

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Resumo

A Mata Atlântica é um bioma de grande importância devido à riqueza da sua diversidade biológica, o que justifica sua de alta relevância para esforços na conservação das espécies conhecidas e desconhecidas. Sua conservação pode ser feita pela criação e manutenção de áreas protegidas. Uma delas é a RPPN Alto da Figueira, que tem como objetivo estimular o desenvolvimento de pesquisas científicas sobre a diversidade biológica do bioma, gerando conhecimentos úteis para programas de conservação. A caracterização da fauna da família Staphylinidae (ordem: Coleoptera), via análises morfológicas e moleculares, permite inferir o grau de preservação natural da unidade. Uma das principais dificuldades para o estudo dessa fauna é obter ou confirmar a identidade taxonômica dos espécimes devido à baixa representatividade de sequências nucleotídicas de amostras neotropicais nas bases de dados, a ausência de chaves de identificação adequadas para diagnóstico espécie-específico desta fauna específica, e a escassez de conhecimento sobre a ecologia do grupo. Neste estudo foram obtidas 27 sequências nucleotídicas referentes à região barcode do gene da subunidade I da Citocromo Oxidase c (marcador COI), de indivíduos identificados morfologicamente até o nível de gênero com os objetivos de: 1) investigar o potencial informativo deste marcador para integrar esforços de identificação taxonômica; 2) verificar sua utilidade na inferência de relações filogenéticas dentro do grupo, 3) ampliar a representatividade da família Staphylinidae nas bases de dados (BoLD e GenBank) e 4) associar a diversidade deste fauna ao status de preservação ambiental da reserva. De acordo com os táxons identificados, a unidade de conservação se caracteriza como uma floresta bem conservada em estágios avançados de sucessão. As árvores filogenéticas geradas a partir da análise do marcador COI apresentaram baixa resolução (apesar da alta variabilidade), assim como a análise mais conservadora utilizando comparações entre sequências de aminoácidos, indicando a importância de ampliar a amostragem desta rica fauna de coleópteros para obter um cenário mais representativo da diversidade presente na Mata Atlântica, incluindo um acervo de dados morfológicos e moleculares mais completo e informativo.

Palavras-chave: Mata Atlântica; Staphylinidae; Áreas Protegidas; Identificação Morfológica; Identificação Molecular; RPPN Alto da Figueira

Abstract

The Atlantic Forest is a biome of critical importance due to the richness of its biological diversity, underlining its high priority for the conservation of both known and yet-undescribed species. Conservation strategies for this biome include the establishment and maintenance of protected areas. One such initiative is the Alto da Figueira Private Natural Heritage Reserve (RPPN), which seeks to foster scientific research on its biological diversity, generating valuable data to inform conservation programs. The characterization of fauna belonging to the family *Staphylinidae* (order: *Coleoptera*), through combined morphological and molecular analyses, provides insights into the ecological integrity and conservation status of the reserve. A major challenge in studying this group is the difficulty of accurately identifying or confirming the taxonomic status of specimens. This is mainly due to the underrepresentation of nucleotide sequences from Neotropical taxa in databases, the lack of suitable taxonomic keys for species-level identification for these fauna and limited ecological knowledge of the group. In this study, 27 nucleotide sequences corresponding to the barcode region of the cytochrome c oxidase subunit I gene (COI marker) were generated from specimens that had been morphologically identified to the genus level. The main objectives were to: 1) assess the utility of the COI marker in supporting taxonomic identification; 2) evaluate its effectiveness for inferring phylogenetic relationships within the group; 3) increase the representation of *Staphylinidae* sequences in public repositories (BoLD and GenBank) and 4) associate the diversity of this fauna with the state of environmental preservation of the reserve. The identified taxa indicate that the conservation unit corresponds to a well-preserved forest in an advanced successional stage. Phylogenetic trees derived from the analyses of COI nucleotide sequences showed low resolution (despite high genetic variability). Similar results were obtained for a more conservative approach based on amino acid sequence comparisons. These findings emphasize the need to expand sampling efforts of this diverse beetle group to produce a more representative scenario of the biodiversity from the Atlantic Forest based on a more comprehensive and informative dataset that integrates morphological and molecular information.

Keywords: Atlantic Forest; Staphylinidae; Protected Areas; Morphological Identification; Molecular Identification; RPPN Alto da Figueira.

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Table 5 - Representation of rove beetle genera in BOLD, relating the number of species with barcodes with the current number of species described for each genus to calculate a Taxon Coverage percentage. Numbers of Brazilian data are shown.

Summary:

1. Introduction:

The Atlantic Forest

The Brazilian Atlantic Forest biome currently occupies 1.422.660 km² (RESENDE et al., 2024), distributed as multiple small and isolated fragments along the coast of Brazil, from the Northeast region to the South region, being part of 17 Brazilian states (RIBEIRO et al., 2009), with smaller territories in Argentina (GIRAUDO, 2003) and Paraguay (CARTES & YANOSKI, 2003).

The Brazilian Atlantic Forest Sensu Lato can be described as a diverse mosaic of forest formations, including Dense Ombrophilous, Mixed Ombrophilous, Open Ombrophilous, Seasonal Deciduous, Seasonal Semidecidous, Mangroves, Restinga Vegetation, High Altitude Fields and Caatinga Moist Forest Enclaves (BRASIL, 2006). This heterogeneity can be explained by a few factors, one of them being the extension of the forest, with a latitudinal range of around 29° and an elevated longitudinal and altitudinal range, thus creating different rainfall and sun exposure patterns along its distribution (RIBEIRO et al., 2009).

Furthermore, the average temperatures and humidity in the Atlantic Forest's main different forest formations have different patterns. The Dense Ombrophilous forest formation is known to not have a biologically dry season and an average temperature of 22°C to 25°C. The Semidecidous and Deciduous forests have 2 to 5 months of dry season, with temperatures similar to the Dense Ombrophilous formation. On the southern portion of the Atlantic Forest, the Mixed Ombrophilous forest occurs in a subtropical mesothermal climate (COLOMBO & JOLY, 2010) resulting in average temperatures ranging from 15°C to 22°C. The Open Ombrophilous formation is similar to the Dense Ombrophilous, but with key differences that create a different pattern of vegetation, those being the presence of clearings, and well defined dry season, lasting for about 60 days (LIMA et al., 2022).

The coastal region of the Atlantic forest is characterized by the presence of the Mangroves and Restinga forest formations, creating areas of transition between the marine and terrestrial environments. The Mangroves are composed mainly of vegetation adapted to long flood periods and soil with low oxygen levels, like *Rhizophora* and *Avicena*, the soil is also characterized by being muddy and salty, with high levels of organic matter (KATHIRESAN & BINGHAM, 2001). The Restinga formation has a very salty and sandy soil, and is dominated by smaller organisms and herbaceous vegetation (PUPIN & NAHAS, 2014).

High Altitude Fields occur in the mountainous areas of the South and Southeast Brazilian states. These areas feature very shallow soil and are dominated by small shrubs and herbs, their hydric regime is maintained primarily by naturally occurring springs (BENITES et al., 2003; MARTINELLI, 1996).

This environmental heterogeneity is only one of the many reasons contributing to the Atlantic Forest's elevated biodiversity, another important aspect is the forest history. According to the Forest Refuge Theory, during the late Pleistocene

(between 18.000 and 13.000 B.C.E.), climatic events in the region led to the formation of isolated forest patches and the later unification of these (VIADANA & CAVALCANTI, 2012). And even prior to these events, diversification of many different groups in the region have been recorded between the Eocene (46 Mya) and Miocene (20 Mya), related to the many climatic and geological events seen in the region (FOUQUET et al., 2012; BACCI et al., 2022; CABRAL et al., 2021).

It is known that currently the Atlantic Forest is highly threatened by accelerated deforestation patterns in its territory (COLOMBO & JOLY, 2010; RIBEIRO et al., 2009), despite its importance to biodiversity on a local and global scale. The Forest history, along with the more recent developments of land use, create a scenario marked by a high degree of endemism and threat, and thus the Atlantic Forest is currently one of the five richest biodiversity hotspots in the world (MYERS et al., 2000) harboring 1–8% of the world's total species diversity (SILVA & CASTELETI, 2003).

Conservation Units: RPPN Alto da Figueira

According to Brazilian federal law, a Private Natural Heritage Reserve (RPPN) is a private area in which the main objective is the preservation of biological diversity, etched in perpetuity (BRASIL, 2000). This specific category of conservation unit is part of the group of Sustainable Use Conservation Units, therefore the usage of its area tends to be less restrictive compared to the Integral Protection Conservation Units. Within the borders of this type of conservation unit, it is exclusively permitted to carry on scientific research and visitation, with touristic, educational and recreational objectives (BRASIL, 2000). The specific activities of the Unit should be done according to the Management Plan, created in partnership between the owner of the area and the associated public organs (BRASIL, 2000). Generally, the state law tends to be more specific towards the regional context of the conservation units within its borders, as previously mentioned in the State law regarding this category of protected area.

The Alto da Figueira Private Natural Heritage Reserve (Figs. 1 and 2), formerly known as Bacchus Private Natural Heritage Reserve, was created in 2009 by the State Environmental Institute/RJ, by the state ordinance n°81 of December 1st 2009, located in the city of Nova Friburgo - Rio de Janeiro, in the Macaé de Cima road (22°22'31.6"S 42°29'45.0"W) (RIO DE JANEIRO, 2009). It is localized within the borders of the Environmental Protection Area (APA) Macaé de Cima and is close to other RPPNs such as the Terra do Sol e da Lua, Sítio Azul, Fattoria Grigia, Vale do Paraíso, and the Rio Bonito de Lumiar RPPNs. It is near the Três Picos State Park, and the Bacia do Rio Macacu APA. The 1,0173 km² of the Alto da Figueira RPPN are composed of typical Atlantic Forest vegetation, being exclusively Dense Ombrophilous Forest. The climate is considered Tropical Central Brazil, mild mesothermic, super-humid subdry (MMA, 2011). Its altitude ranges from 1,3 km to 1,6 km, being inserted in a mountainous region.

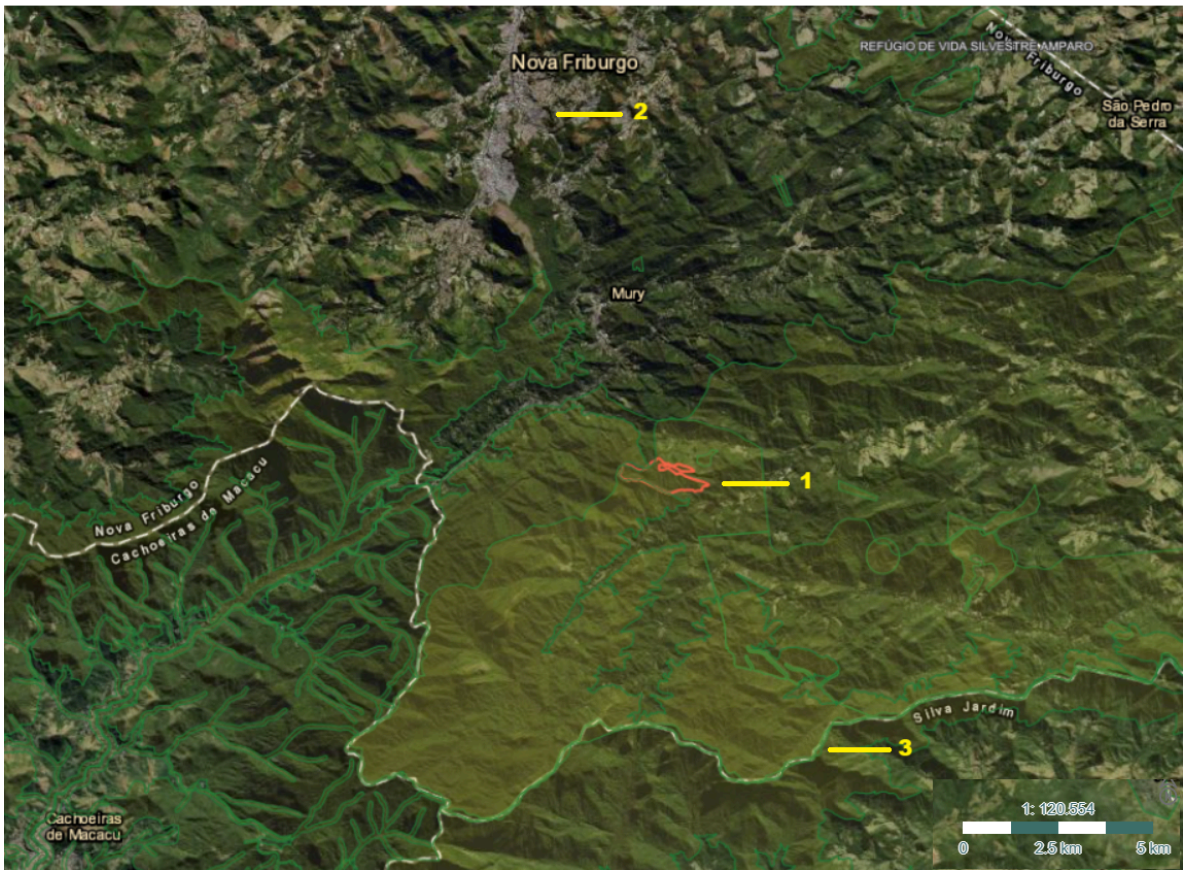

Fig. 1: Map of the Alto da Figueira Private Natural Heritage Reserve area. (CNUC, 2025). 1 - RPPN Alto da Figueira; 2 - City of Nova Friburgo (RJ); 3 - City limits of Silva Jardim (RJ).

Fig. 2: Map of the Alto da Figueira Private Natural Heritage Reserve in the Rio de Janeiro state. (GOOGLE, 2025; Images NASA, 2025; Map data Google, 2025).

The high diversity of the Atlantic Forest can be exemplified by its plants and fungi. This biome has the highest number of species of Fungi, Algae, Bryophytes, Ferns and Lycophytes, Gymnosperms, and Angiosperms, with 19.355 total species, of which 7.646 are endemic (FORZZA et al., 2010). Gymnosperms and Angiosperms are responsible for 70% of this diversity (FORZZA et al., 2010). The Dense Ombrophilous Forest is generally dominated by large trees creating a dense canopy, and consequently species adapted to low luminosity in its lower layers (JÚNIOR et al., 2017).

Originally, the Alto da Figueira RPPN was created with the intent of protecting biodiversity and conducting research (RIO DE JANEIRO, 2018). The area has attracted the interest of many researchers. There have been multiple projects on the characterization of the biodiversity of this RPPN, focusing on different groups such as Lepidoptera, Fungi and *Solanum*, some of these resulting in the discovery of new species and threatened ones (ROSA et al., 2023; ARAÚJO et al., 2015; GIACOMIN & STEHMAN, 2024). This RPPN is also part of the ARAÇÁ Project, organized by the non-profit Antonelli Foundations for Biodiversity Research and Conservation, which has been collaborating with both the Lifeplan and The Red List projects (ARAÚJO et al., 2015).

Rove Beetles: Diversity, Taxonomy and Biology

Coleoptera is the most diverse order of insects, with estimates of between 300.000 and 450.000 species in total (NIELSEN & MOUND, 1999), primarily occupying terrestrial and freshwater habitats There is still a lot to be discovered regarding their diversity, especially considering unknown species which could be endemic and sampling gaps from less studies areas.

Among this extremely diverse order, the families Staphylinidae, Curculionidae, Carabidae, Chrysomelidae, Cerambycidae and Scarabaeidae are considered megadiverse groups, encompassing around 60% of the order's diversity (BOUCHARD et al., 2017). The family Staphylinidae (rove beetles) are considered the most diverse group within the Coleoptera, with 66.928 species across 4.038 genera, accounting for 20% of the order's diversity (BOUCHARD et al., 2017; BÁNKI et al., 2025). Despite this and their importance to the ecosystem, there has been little focus on the Staphylinidae of Atlantic Forest (19 results in Web of Science for searching Staphylindae + Atlantic Forest, Staphylinidae + Atlantic Rainforest and Staphylinidae + Atlantic Rain Forest). Therefore, the characterization of this group has the potential to be of great interest for future research projects, conservationist efforts and the medical field (VIEIRA et al., 2014).

The extreme diversity of rove beetles is also reflected in the morphology, ecology and trophic levels of the group. This taxon is commonly predaceous, mycophagous and saprophagous, but the group includes species of parasitoids, pollen feeders, algal grazers and herbivores (THAYER, 2005; KLIMASZEWSKI et al., 2010). There are also many examples of subsocial behavior in multiple subfamilies

(HANLEY & GOODRICH, 1995), and complex and diverse adaptations of some groups of the subfamilies Aleocharinae and Pselaphinae, with their obligatory association with other social insects (PARKER, 2016). In Brazil, the larvae of a species of Staphylinidae (*Xantholinus* Dejean, 1821 sp.) are the only beetles capable of bioluminescence, outside the Elateriformia group (ROSA, 2010).

The Staphylinidae species are commonly found in humid terrestrial habitats and can be used as bioindicators of the ecological integrity of these areas (BETZ et al., 2018), the richness and diversity of species found in an area can be directly related to its conservation status and forest succession stage (ARENHARDT et al., 2021; ARENHARDT et al., 2024; MÉNDEZ-ROJAS et al., 2021; LÓPEZ-BEDOYA et al, 2021). Also, some of the predator and parasitoid beetles are important for the control of plant pests and other arthropods (HU & FRANK, 1995), especially the genus *Aleochara* Gravenhorst, 1802 (HEMACHANDRA et al., 2007). An important example of Staphylinidae are species from the genus the *Paederus* Fabricius, 1775 known to have a toxin in its hemolymph, which causes effects such as dermatitis and conjunctivitis (VIEIRA et al, 2014). In forensic investigations, species from the Staphylinidae family are common predators of the fauna found in decomposing remains (MADRA et al., 2014).

Rove beetles are generally small, elongated to ovoid-shaped insects ranging from 1 mm to 40 mm, with most species being under 7 mm, and their color is commonly yellowish to very dark. Typically, rove beetles have short elytra, exposing a flexible abdomen that can be telescoped. The abdominal segments are protected by sclerotized plates, a dorsal tergite, one or two pairs of dorso-lateral paratergites, and a ventral sternite, united by long membranous connections. These plates are fused in rings around the abdominal segments in some groups. Some species living in low light environments such as in deep soil layers or caves can have the composite eyes absent. Some more uncommon traits are a pair of ocelli and winglessness, observed in some groups of various habitats (FRANK & THOMAS, 2002; NEWTON, 1990, NAVARRETE-HEREDIA et al., 2002).

In the Staphylinidae family, there are 474 genera and 2829 (RAFAEL et al., 2024) species in Brazil, making it the country with the largest diversity of rove beetles in Latin America. A list of 1961 species from 88 genera are considered endemic, the highest endemism rate for this taxa in Latin America (CARON et al., 2024). This diversity is considerably higher compared to more sampled regions, such as Canada and Alaska with 1.858 species, Fennoscandia with 1.399 species and West Siberia with 726 species (KRIVOSHEEVA et al., 2023).Globally, Aleocharinae is the most diverse subfamily, currently with 16.864 species, followed by Pselaphinae with 10.529 species, Staphylininae with 9.071, and Paederinae with 7.982 species (BÁNKI et al., 2025).

This high level of diversity is also reflected in the species distribution in the taxonomic groups of Staphylinidae. For example, the Aleocharinae subfamily currently has 16.864 species while the Empelinae subfamily is represented by a single species (BÁNKI et al., 2025). Another example of this situation is the Steninae

subfamily, which has 3.378 living species distributed in only two living genera (BÁNKI et al., 2025).

Rove Beetles: Molecular markers and phylogenetic relationships

Rove beetle diversity and systematic distribution are related to challenging attempts to recover a well supported phylogeny for the Staphylinidae family. BETZ et al. (2018), provides an attempt to compile other phylogenetic studies to propose a definitive phylogenetic tree for the family. In this study, various difficulties regarding the phylogeny of the group were highlighted, including the uncertain monophyly of many subfamilies, and the uncertain relationship between them. The result was a supertree with well established subfamilies but many polytomies at multiple taxonomic levels. This was due to few family wide phylogenetic studies, with most being focused on lower taxonomic groups. This lack of phylogenetic studies is even more prevalent in the Atlantic Forest biome (3 results in Web of Science for searching Staphylinidae + Atlantic Forest + Phylogeny, Staphylinidae + Atlantic Rainforest + Phylogeny and Staphylinidae + Atlantic Rain Forest + Phylogeny).

Only four out of 36 phylogenetic studies (BALLARD et al., 1998; IKEDA et al., 2008; GREBENNIKOV & NEWTON, 2009; ZHANG & ZHOU, 2013), focuses on family level relationships. Another important factor contributing to the situation is the lack of a standardized method, while there are commonly used genetic markers such as the nuclear genes wingless(wg), topoisomerase I (TP), arginine kinase (AK), the carbamoyl phosphate synthetase domain of CAD (CAD), and the mitochondrial cytochrome c oxidase subunits I (COI) and II (COII). The use of molecular markers varies a lot among studies which focus on phylogenetic relationships regarding the same taxonomic levels.

The result of this situation is a lack of representativity on the main genetic databases such as BOLD and NCBI. BETZ et al. (2018) compares the amount of nucleotide sequences available in GenBank with the number of described species for every subfamily, calculating an average of 0,062 sequences per species. However, this ratio varies a lot between groups, with smaller subfamilies with a higher number of sequences per species than the larger ones, and in the case of the Protopselaphinae subfamily no sequences were available.

Another important factor is the geographical distribution of the sequences, with some groups having a large number of sequences but mostly of the same region, such as the megadiverse Aleocharinae. The authors highlight specifically that the sampling efforts are mostly focused on the Holarctic region (BETZ et al., 2018). As such, regions with increased diversity and high endemism levels such as Brazil end up not being represented in the databases.

The target gene was chosen due to its reliability for lower taxonomy groups identification and due to it being a rapidly evolving protein coding gene (SIMON et al., 1994; HEBERT, 2003). These properties allowed it to become one of the standard genetic markers used for insects as shown in numerous taxonomic and evolutionary studies (more than 2.000 results in Web of Science for searching COI +

insect). Another reason for choosing this specific marker was due to its common usage in various studies of the Staphylinidae family, as shown in BETZ et al. (2018), focusing on higher taxonomic levels.

The lack of local fauna representation in nucleotide sequence databases could undermine species identification efforts - impacting biodiversity research, lead to incomplete taxa sampling - lowering resolution in phylogenetic inferences, weaken ecological monitoring programs and obscure evolutionary routes. The fast and reliable species-specific identification protocol provided by DNA barcoding is reliant on matching the individual sequence to homologous sequences in the databases (ODAH, 2023), and if a similar sequence from conspecific species is underrepresented in databases this approach loses its informative potential

Objectives

Considering the context of the Staphylinidae family in the Atlantic Forest, this study is motivated by a general objective. To explore the Staphylinidae fauna present in the RPPN Alto da Figueira and to access and expand the current knowledge of this group in this region, along with its potential as a bioindicator. In order to achieve this, 4 specific objectives were established:

1) Assess the utility of the COI marker in supporting taxonomic identification;
2) Evaluate its effectiveness for inferring phylogenetic relationships within the group;
3) Increase the representation of *Staphylinidae* sequences in public databases;
4) Associate the Staphylinidae fauna with the state of environment preservation.

2. Materials and Methods:

Samples

The samples were collected by the team of the Laboratório de Borboletas (LABBOR) at the Universidade Estadual de Campinas (UNICAMP) along with members of the Żyła Lab, in the Alto da Figueira RPPN in July 2022 during a 10-day trip, in the winter season. The trip was possible thanks to the courtesy of the ARAÇÁ project, responsible for the RPPN. The sampling method chosen for this was sifting the soil and leaf-litter, with focus on collecting common morphotypes found in the family, Winkler extractors were also used (Figs. 3 and 4). The samples were preserved in 96% ethanol at - 4°C until further use.

Fig. 3: Leaf litter and soil sifting done during the sampling process.

Fig. 4: Winkler extractors used in the sampling process.

Digitization and image editing of individual diagnostic characters for morphological taxonomic identification were provided by using a Zeiss Stereo Microscope model Discovery V.20 and the Axiovision software version 4.8.2. Each individual was photographed in the ventral, dorsal and lateral positions in a petri dish with water.

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DNA Extraction

The DNA from the samples was extracted using a modified version of the DNeasy Blood & Tissue Kit Quick-Start Protocol (QIAGEN), as described by TOKAREVA et al. (2021) for Staphylinidae species. Regarding the tissue, DNA extraction from entire individuals were used for smaller specimens, while only the abdomen was used from samples over 2 mm in length. Tissues were incubated at 56°C in lysis solution with proteinase K Protein, denaturation with Buffer AL was followed by incubation for 10 minutes at 56°C. Subsequently, Ethanol (96-100%) was added to precipitate the DNA. DNA was binded to spin columns, centrifuged for 1

minute at 8000 rpm and washed twice with the kit Washing Buffers AW 1 and AW2. After the washing step, 100 µL of the Buffer AE were aliquoted in the spin columns and incubated at room temperature to elute the DNA.

PCR Amplification and Purification

The thermocycler was programed as follows: an initial step of 95°C for 3' followed by 35 cycles of a 3-step temperature routine of 94°C for 30'', 45°C for 1' and 72°C for 1'30'' with a final elongation step at 72°C for 10'. PCR amplifications were arranged in two batches related to alternative mixtures of primers suitable for different sets of DNA samples. The COI region amplification was tested for batch 1 reactions using the pair of primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG -3') (FOLMER et al., 1994) and C1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC -3') (SIMON et al., 1994). The batch 2 was also tested for rove beetles COI amplification combining the Folmers' universal primers LCO 1490 and HCO 2198 (5'- TAAACTTCAGGGTGACCAAAAAATCA -3') (FOLMER et al., 1994).

A 32 sample set was prepared for sequencing and DNA purification by the ExoSAP-IT™ PCR Product Cleanup. Purified and quantified DNA was sent to Macrogen Europe sequencing by the Sanger Sequencing Method.

Sequence Analyses

Chromatograms of each sequence were analyzed and edited using the platform Geospiza's FinchTV version 1.4.0 (https://digitalworldbiology.com/FinchTV) (GEOSPIZA, 2006) and the Geneious Prime version 2025.1.2 (GRAPHPAD, 2025). Sequences were tested for the presence of stop codon (not expected) by translation. Both nucleotide and amino acid sequences were used in alignments to provide phylogenetic informative sites for evolutionary analyses. The final sequences representing the rove beetles specimens sampled in this study were used as queries in searching for nucleotide identities in the more comprehensive genetic sequence database publicly available, GenBank (www.ncbi.nlm.nih.gov) using BLAST (Basic Local Alignment Search Tool). The sequences were also used for screening potential taxonomic identities in BoLD (Barcode of Life Database - www.boldsystems.org)..

Morphological Identification

The samples were identified to the genus level using different taxonomic keys based on morphological analysis. The "Staphylinidae of Eastern Canada and Adjacent United States" (BRUNKE et al., 2011) and the "Guía Ilustrada para los Géneros de Staphylinidae (Coleoptera) de México" (NAVARRETE-HEREDIA et al., 2002) keys were used for subfamily level identification. The Navarrete-Heredia et al. (2002) key was used for genus level identification, with the exception of one individual that was evaluated using identification keys of "The Beetles of Europe"

(LOMPE et al., 2011) and “The interactive digital key to rove beetles (Coleoptera:Staphylinidae) of Denmark” (KOSZELA et al., 2018). The “Catálogo Taxonômico da Fauna do Brasil (CTFB)” (NEWTON, 2025) was also used to confirm specimens taxonomic status.

Phylogenetic Trees

Alignments of homologous nucleotide and aminoacid sequences from the COI region were used for comparisons and identification of genetic variability. Phylogenetic trees were inferred based on both sequence alignments and by testing the option of ignoring the 3rd codon position to reduce eventual homoplasies from highly variable sites. Phylogenetic analyses were performed using the program MEGA12 Molecular Evolutionary Genetics Analysis version 12 (KUMAR et al., 2024). The phylogenetic analyses considered different sets of samples, including the complete sample pool and members related to the same subfamilies for the taxa Paederinae, Staphylininae and Xantholininae. The consensus trees were recovered showing only node bootstrap values above 50, topology for branches with values lower than 50 were shown as polytomies.

For the outgroup position, a representative of the Scarabaeidae family was selected due to its close and well-established relationship with the Staphylinoidea superfamily. A *Oryctes nasicornis* COI sequence was taken from NCBI, access number OK484312, version OK484312.1.

Database Representativity Evaluation

The current representation status of Staphylinidae subfamilies and genera in sequence databases was evaluated by a similar method used in BETZ et al. (2018). The number of species with DNA barcodes found in the BOLD system was related to the current number of species described in The Catalogue of Life (BÁNKI, et al., 2025), as of June 2025. By relating these two parameters, it was possible to determine the numerical relation between the number of known species and the number of species with sequences available in the BOLD base. The absolute number of entries assigned to Brazilian samples in the BOLD taxonomy browser for each of the Staphylinidae subfamilies and genera was also evaluated.

3. Results:

Taxonomic identification

The taxonomic identification for the Rove Beetles specimens sampled in this study is presented in Table 1, with each individual assigned to a subfamily and the lowest identifiable taxon. Based on the Navarrete-Heredia et al. (2002) key, the lowest taxonomic level assigned for each sample is the genus. However, the sample “02d - 2” (item 17 of Table 1) showed a discrepancy between both reference system used: the Brunke et al. (2011) key identified it as Scydmaeninae, while the Navarrete-Heredia et al. (2002) identified it as Piestinae following the taxonomic revision of this subfamily. It is also worth mentioning that two specimens (items 31 and 32) were collected as larvae, therefore they were unable to be identified using the available keys. Tribe was the lowest taxonomic level identified for the individuals of items 19, 28, 29 and 30.

Table 1 - Proposed taxonomic identification for Rove Beetles of this study. Note: Inaccurate classification as these identifications were based on morphological analyses from available taxonomic keys not designed for Atlantic Forest fauna. * indicates an immature specimen (larvae), subfamily not identified.

Item	Sample Code	Subfamily	Genus/Tribe
1	03b - 4	Aleocharinae	*Aleochara*
2	06a - 1	Aleocharinae	*Myrmecocephalus*
3	03k - 3	Euaesthetinae	*Edaphus*
4	03a - 1	Megalopsidiinae	*Megalopinus*
5	01f - 1	Osoriinae	*Leptochirus*
6	01e - 1	Osoriinae	*Lispinus*
7	06f - 6	Paederinae	*Biocrypta*
8	07a - 1	Paederinae	*Biocrypta*
9	02g - 1	Paederinae	*Haplonazeris*
10	01m - 2	Paederinae	*Lathropinus*
11	02d - 1	Paederinae	*Lathropinus*
12	03b - 1	Paederinae	*Oedichirus*
13	02f - 2	Paederinae	*Palaminus*
14	03k - 2	Paederinae	*Pinophilus*
15	01l - 2	Paederinae	*Sciocharis*
16	03b - 3	Paederinae	*Sciocharis*
17	02d - 2	Scydmaeninae	*Euconnus*

18	**02g - 2**	Staphylininae	*Philonthus*
19	**03c - 1**	Staphylininae	*Philontina morphotype 1*
20	**02k - 1**	Staphylininae	*Quediomacrus*
21	**07a - 3**	Staphylininae	*Quediomacrus*
22	**01l - 1**	Steninae	*Stenus*
23	**06f - 7**	Tachyporinae	*Sepedophilus*
24	**06f - 3**	Tachyporinae	*Vatesus*
25	**03i - 3**	Xantholininae	*Diochus*
26	**06f - 4**	Xantholininae	*Neohypnus*
27	**06f - 5**	Xantholininae	*Neohypnus*
28	**03c - 3**	Xantholininae	*Xantholinini morphotype 1*
29	**02f - 3**	Xantholininae	*Xantholinini morphotype 2*
30	**03b - 2**	Xantholininae	*Xantholinini morphotype 3*
31	**01f - 2**	*	*
32	**01l - 4**	*	*

A total of 10 subfamilies (Fig. 5) and 21 genera (Fig. 6) were identified in this study. The total number of individual/subfamily ranges from 1 (in Steninae, Euaesthetinae, Megalopsidiinae and Scydmaeninae) to 10 (in Paederinae).

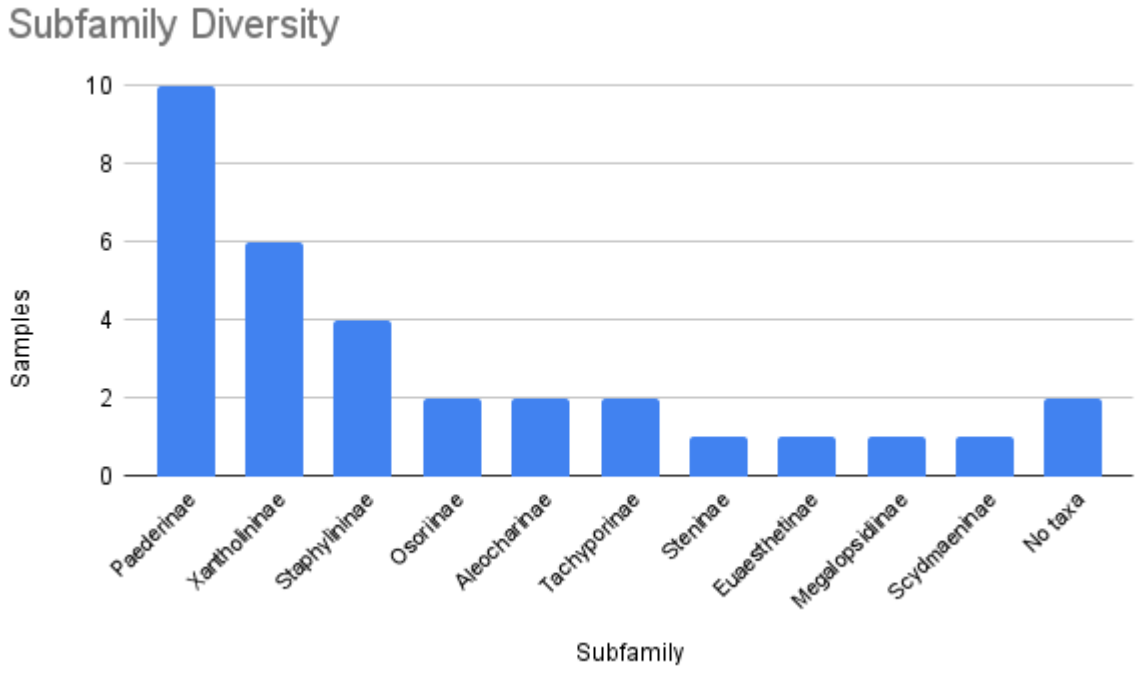

Fig. 5: Number of individuals related to the ten different Staphylinidae subfamilies sampled in this study (based on available taxonomic keys not designed for Atlantic Forest fauna).

Regarding the genera identified in the inventory 16 genera were represented by 1 specimen each, the genera *Sciocaris, Lathropinus, Biocrypta, Neohypnus* and *Quediomacrus* were represented by 2 specimens each, and 6 individuals were not identified to genus level, comprising the two unidentified larvae and four samples identified to the tribe level (Fig. 6).

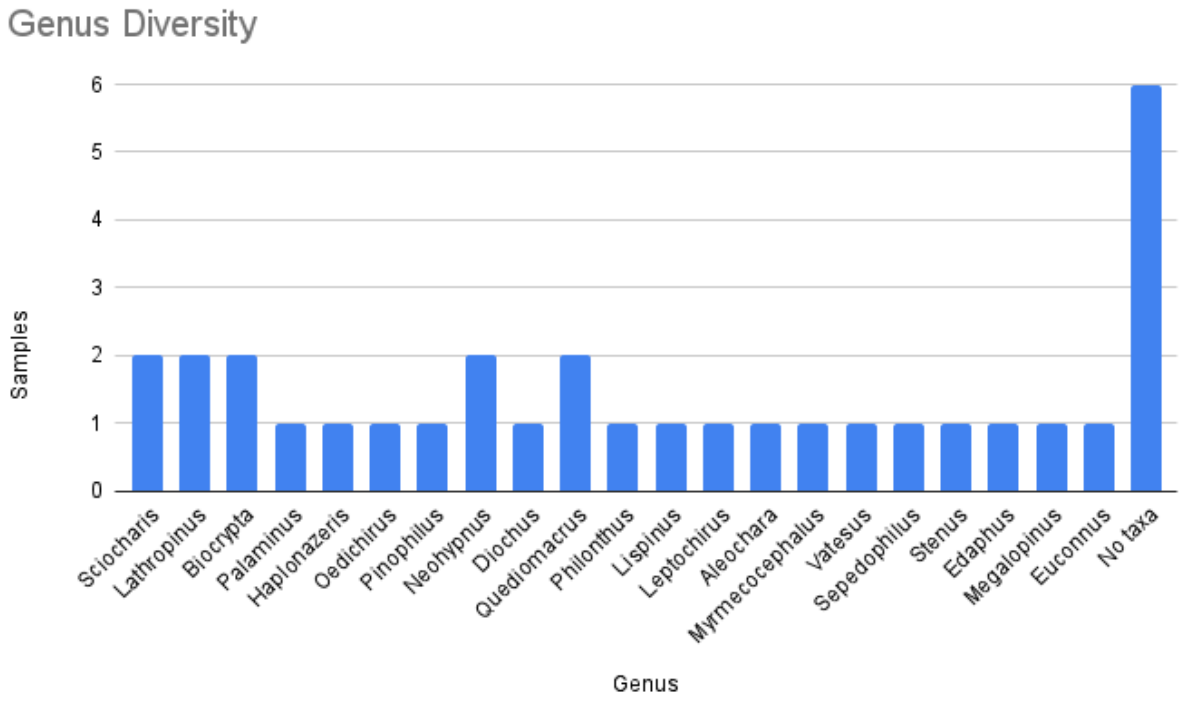

Fig. 6: Number of individuals related to different Staphylinidae genus sampled in this study (based on available taxonomic keys not designed for Neotropical fauna).

Analyses in databases (Bold and GenBank)

Amplification results for batch 1 (see methods) yielded 12 successful products, while batch 2 yielded 20 successful products. Primers from batch 2 allowed successful amplification of the COI region from samples which failed to amplify at batch 1 condition. Five sequences, related to the samples 01e-1, 02d-1, 07a-3, 06f-7, 06f-5, were not used due to low sequencing quality. The remaining 27 sequences used in further analyses showed high HQ levels, ranging from 87,78% to 96,83%.

The results recovered from BOLD analysis of each COI sequence indicated similarities ranging from 86,67% to 96,83% (Table 2).

Table 2 - COI sequences closest match retrieved from BOLD barcode ID engine search for the samples of this study. * no sequence data.

Item	Sample Code	BOLD closest match	Similarity %
1	03b - 4	Staphylinidae (fam.)	91,33
2	06a - 1	Aleocharinae (subf.)	86,67

3	03k - 3	Staphylinidae (fam.)	89,04
4	03a - 1	Coleoptera (ord.)	90,8
5	01f - 1	Staphylinidae (fam.)	89,7
6	01e - 1	*	*
7	06f - 6	Staphylinidae (fam.)	91,3
8	07a - 1	Coleoptera (ord.)	88,32
9	02g - 1	*Aleochara pacifica*	88,15
10	01m - 2	Aleocharinae (subf.)	87,78
11	02d - 1	*	*
12	03b - 1	*Onthophagus acuminatus*	89,52
13	02f - 2	Paederinae (subf.)	88,85
14	03k - 2	Staphylinidae (fam.)	89,37
15	01l - 2	Paederinae (subf.)	91,49
16	03b - 3	Paederinae (subf.)	96,83
17	02d - 2	Scydmaeninae (subf.)	92,67
18	02g - 2	Platydracus violaceus	90,41
19	03c - 1	Tachinidae (fam.)	88,34
20	02k - 1	*Chroaptomus*	88,87
21	07a - 3	*	*
22	01l - 1	*Stenus intrusus*	88,27
23	06f - 7	*	*
24	06f - 3	Ptilodactylidae (fam.)	89,08
25	03i - 3	*Tyrus mucronatus*	89,02
26	06f - 4	*Ochthebius sculptoides*	87,39
27	06f - 5	*	*
28	03c - 3	Carabidae (fam.)	89,96
29	02f - 3	Staphylinidae (fam.)	89,1
30	03b - 2	*Eulissus ACS4441*	88,73
31	01f - 2	Ptilodactylidae (fam.)	91,44
32	01l - 4	Tachyporinae (subf.)	88,16

A similar search on the NCBI nucleotide database using BLAST resulted in nucleotide identities of 84,89% up to 96,76 for the rove beetle's COI sequences of this study (Table 3).

Table 3 - COI sequences closest match retrieved from the NCBI nucleotide database via BLAST for the samples of this study. * no sequence data.

Item	Sample Code	Genbank closest match	% nucleotide identity
1	03b - 4	Staphylinidae sp.	90,64
2	06a - 1	Staphylinidae sp.	85,71
3	03k - 3	*Caccobius diminutivus*	85,66
4	03a - 1	*Tachinus fimetarius*	87,93
5	01f - 1	*Mantura chrysanthemi*	87,88
6	01e - 1	*	*
7	06f - 6	Staphylinidae sp.	87,95
8	07a - 1	Lathrobiini sp.	86,56
9	02g - 1	*Olisthaerus megacephalus*	86,36
10	01m - 2	*Earota dentata*	87,15
11	02d - 1	*	*
12	03b -1	*Onthophagus pullus*	87,4
13	02f - 2	Staphylinidae sp.	88,04
14	03k - 2	*Scaptodrosophila maculata*	86,87
15	01l - 2	Staphylinidae sp.	91,57
16	03b - 3	Staphylinidae sp.	96,76
17	02d - 2	Staphylinidae sp.	91,5
18	02g - 2	*Platydracus chalcocephalus*	88,57
19	03c - 1	*Senometopia quarta*	86,97
20	02k - 1	*Philonthus politus*	84,89
21	07a - 3	*	*
22	01l - 1	*Dianous Banghaasi*	86,48
23	06f - 7	*	*

24	06f - 3	*Tachyporus nitidulus*	88,66
25	03i - 3	*Brachyta interrogationis*	88,55
26	06f - 4	Staphylinidae sp.	86,47
27	06f - 5	*	*
28	03c - 3	*Neomyia indica*	89,27
29	02f - 3	Staphylinidae sp.	88,18
30	03b - 2	*Eulissus sp.*	88,05
31	01f - 2	Ptilodactylidae sp.	90,61
32	01l - 4	*Phacophallus pallidipenis*	86,78

The use of DNA barcodes (COI marker) in taxonomic studies relies on the nucleotide substitution rate of this gene, which is suitable for comparative analyses among species samples within the same genus or species complexes. These sequences exhibit low divergence in intraspecific comparisons but become highly variable in interspecific comparisons among taxa at/above the genus level, as evidenced by the low similarity percentages shown in tables 2 (BOLD) and table 3 (NCBI/GenBank). These percentages are shown for a general overview only, as this approach lacks the precision required to recover accurate taxonomic identities when comparing genus of subfamily levels.

Representativeness

Data from Table 4 and 5 were analyzed following the same structure shown in Betz et al. (2018). This analysis highlights that the taxon coverage in BOLD for COI sequences related to species from the Staphylinidae subfamilies (Table 4) and genera (Table 5) are extremely low. For the subfamilies, the highest coverage was for Tachyporinae (10,2%) and no DNA barcode sequence was registered for the subfamily Megalopsidiinae, despite having 431 described species.

Table 4 - Representation of rove beetle subfamilies in BOLD, relating the number of species with barcodes with the current number of species described for each subfamily to calculate a Taxon Coverage percentage. Numbers of Brazilian data are shown.

Subfamily	Species with barcodes (RATNASINGHAM, 2024)	Described Species (BÁNKI et al., 2025)	Taxon Coverage (%)	Brazilian data
Aleocharinae	965	16.864	5,7	63
Euaesthetinae	15	1.157	1,3	2

Megalopsidiinae	0	431	0	1
Osoriinae	13	2.389	0,5	0
Paederinae	153	7.982	1,9	13
Scydmaeninae	50	5.824	0,9	61
Staphylininae + Xantholininae*	510	9.071	5,6	1
Steninae	191	3.396	5,6	0
Tachyporinae	123	1.205	10,2	2

*tribes formerly recognized as Staphylininae were reclassified as Xantholinini after revision (ZYLA & SOLODOVNIKOV, 2019), not followed by an update of BOLD and The Catalogue of Life databases.

An overview of taxon coverage for rove beetle genera also indicates this lack of DNA sequences in the database: there are no sequences for the six genera *Biocrypta*, *Gnathymenus*, *Lispinus*, *Megalopinus*, *Quediomacrus* and *Sciocharis;* and only four out of 21 genera show a taxon coverage higher than 10%. The number of Brazilian entries for each subfamily is extremely low.

Table 5 - Representation of rove beetle genera in BOLD, relating the number of species with barcodes with the current number of species described for each genus to calculate a Taxon Coverage percentage. Numbers of Brazilian data are shown.

Genus	**Species with barcodes (RATNASINGHAM, 2024)**	**Described Species (BÁNKI et al., 2025)**	**Taxon Coverage (%)**	**Brazilian data**
Aleochara	102	545	18,7	1
Biocrypta	0	23	0	0
Diochus	2	82	2,4	0
Edaphus	1	596	0,2	0
Euconnus	15	2.600	0,6	0
Gnathymenus	0	79	0	0
Haplonazeris	1	3	33	0
Lathropinus	1	22	4,5	0
Leptochirus	1	62	1,6	0
Lispinus	0	162	0	0
Megalopinus	0	431	0	1
Myrmecocephalus	5	119	4,2	0
Neohypnus	6	58	10,3	0
Oedichirus	1	420	0,2	0
Palaminus	3	309	1	0
Philonthus	116	1.323	8,8	0
Pinophilus	2	128	1,6	0

Quediomacrus	0	2	0	0
Sciocharis	0	69	0	0
Sepedophilus	23	362	6,4	0
Stenus	182	3.128	5,8	0
Vatesus	6	27	22,2	0

Phylogenetic analyses

The dataset used to investigate the phylogenetic relationships among rove beetle specimens from the Atlantic Forest likely reflects incomplete taxon sampling, due to the underrepresentation of Staphylinidae species in public databases such as GenBank and BOLD (as described above). As a result, the phylogenetic trees presented here were constructed from sequence data generated from newly collected samples (except accession number OK484312), despite the limitations associated with this non-ideal approach.

One limitation of these analyses was the low bootstrap support related to almost every node for the neighbour joining (NJ) tree retrieved from the COI marker used for inferring phylogenetic relationships among all specimens of this study (Fig. 7). Only two groupings were identified considering bootstrap support above 50: a group with the two *Sciocaris* individuals and a group joining two specimens from the Paederini genera *Pinophilus* and *Lathropinus*. A sample classified as belonging to the family Staphylininae (*Quediomacrus*) was placed out from the main topology.

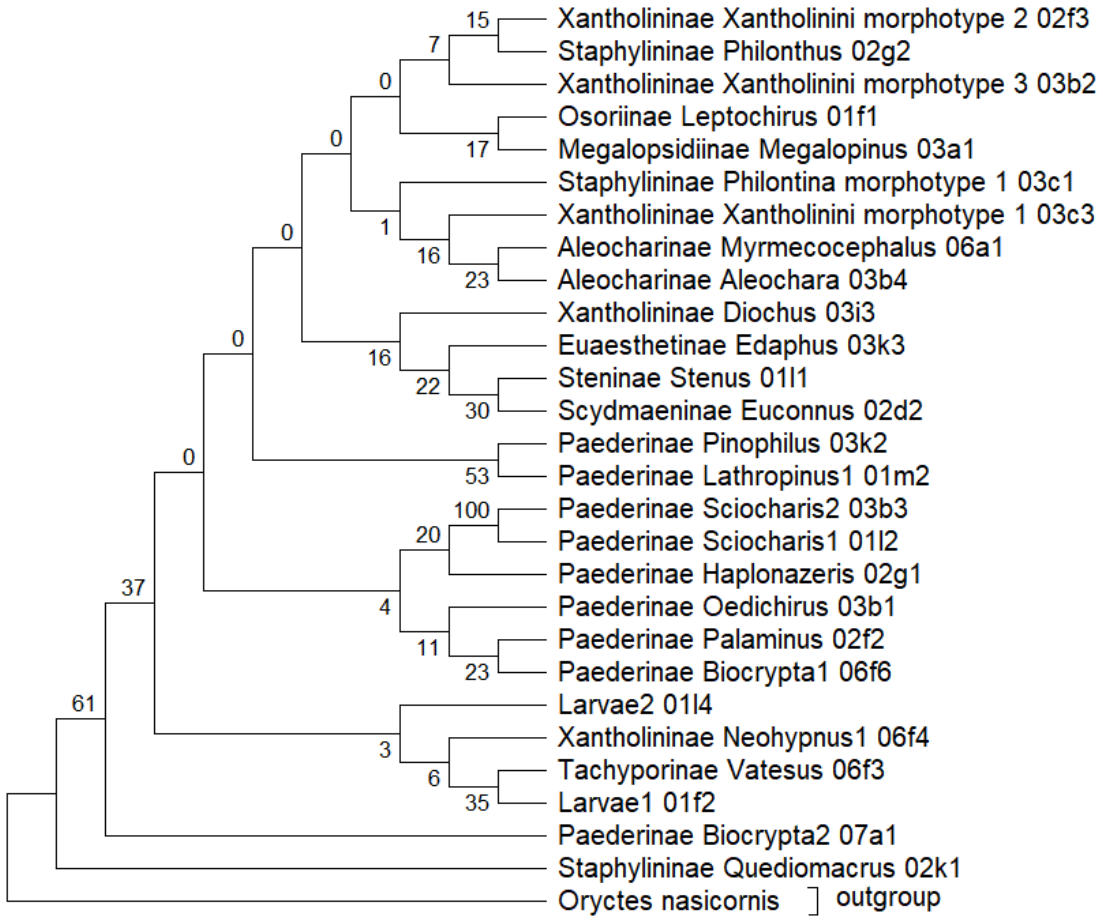

Fig. 7: Neighbour joining tree recovered for all sampled taxa and outgroup (NCBI accession number OK484312) based on the COI sequences. Bootstrap support is indicated in branching nodes.

To enhance phylogenetic resolution and facilitate clearer visualization of relationships, nodes with bootstrap support values below 50 were collapsed, resulting in trees characterized by extensive polytomies. To further investigate the phylogenetic signal of the COI marker, three complementary strategies were adopted: (1) tree reconstruction based on amino acid translations; (2) exclusion of third codon positions to mitigate homoplasy; and (3) subfamily-level phylogenetic analyses restricted to groups with higher specimen representation (see below).

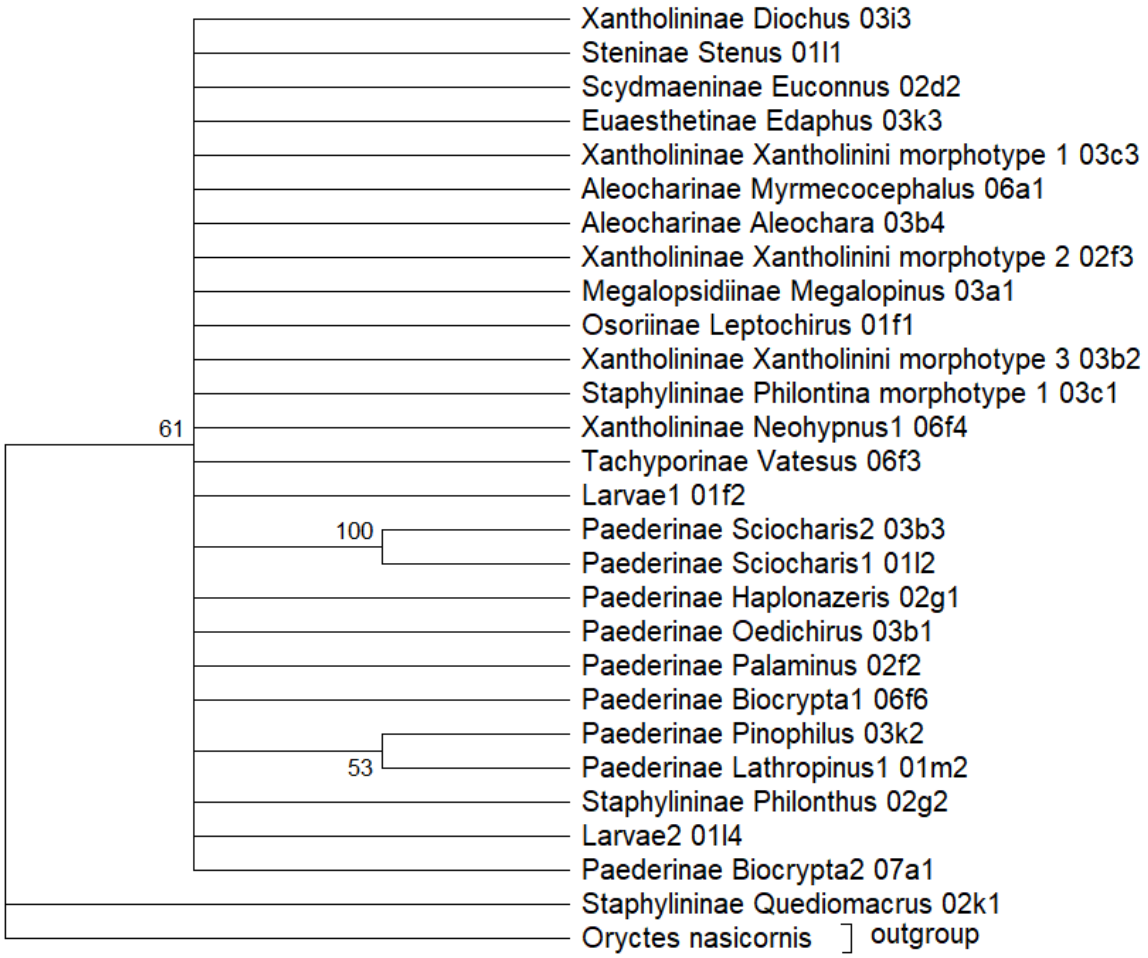

Fig. 8: Neighbour joining tree of all sampled taxa and outgroup based on the COI sequences, nodes with bootstrap values below 50 are collapsed.

The mainly unresolved topology of Figure 8 recovers only the relationships between the *Sciocharis* samples (03b3 and 01l2) and grouping the Paederinae *Pinophilus* with *Lathropinus* (samples 03k2 and 01m2). This same pattern was recovered for other NJ analyses: on a Paederinae subfamily exclusive tree, while considering (Fig. 9) or not (Fig. 10) the third codon position in the analyses.

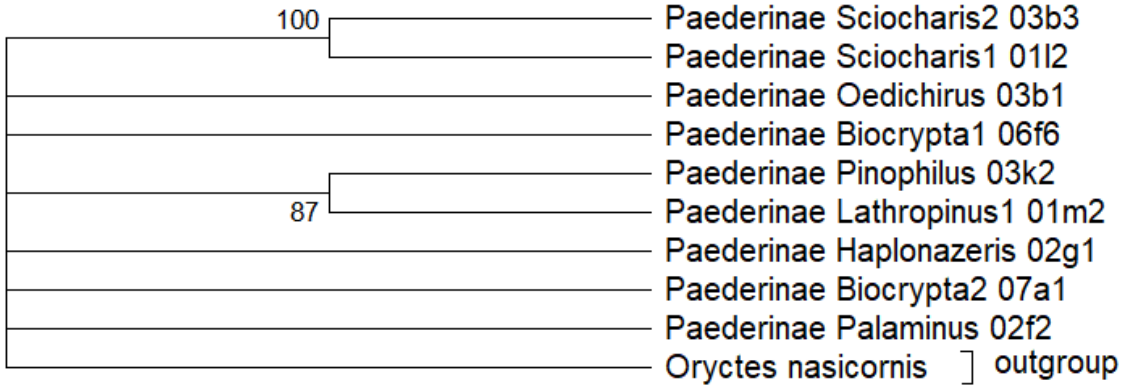

Fig. 9: Neighbour joining tree of the Paederinae subfamily samples and outgroup, based on the COI sequences.

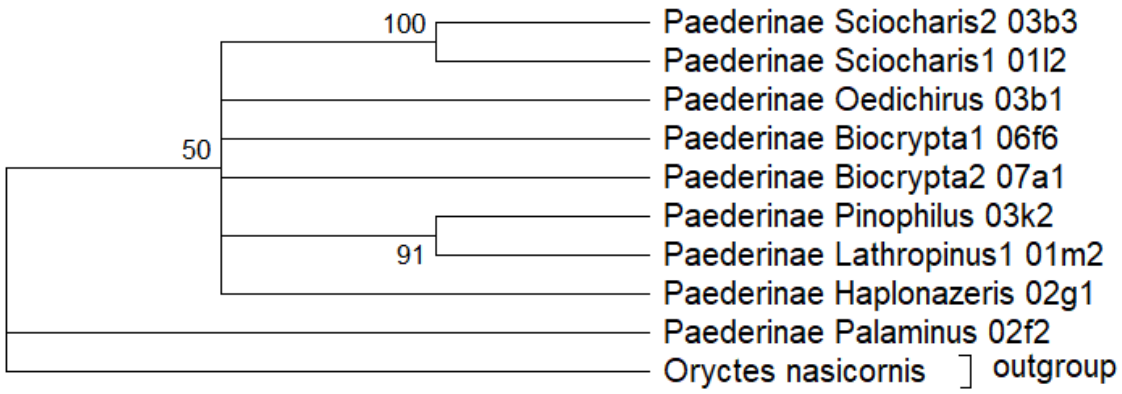

Fig. 10: Neighbour joining tree of the Paederinae subfamily samples and outgroup, using COI sequences without considering the 3rd codon position.

When aminoacid sequences were used for phylogenetic reconstruction comprising all samples, the *Sciocharis* group was maintained, with a high bootstrap support (99). The *Pinophilus* + *Lathropinus* group was lost and a new grouping of Scydmaeninae *Euconnus* (02d2) and Euaesthetinae *Edaphus* (03k3) was formed, with a bootstrap support of 51 (Fig. 11). Aminoacid based tree for the Paederinae subfamily also clustered the *Sciocharis* representatives, with a bootstrap support of 99.

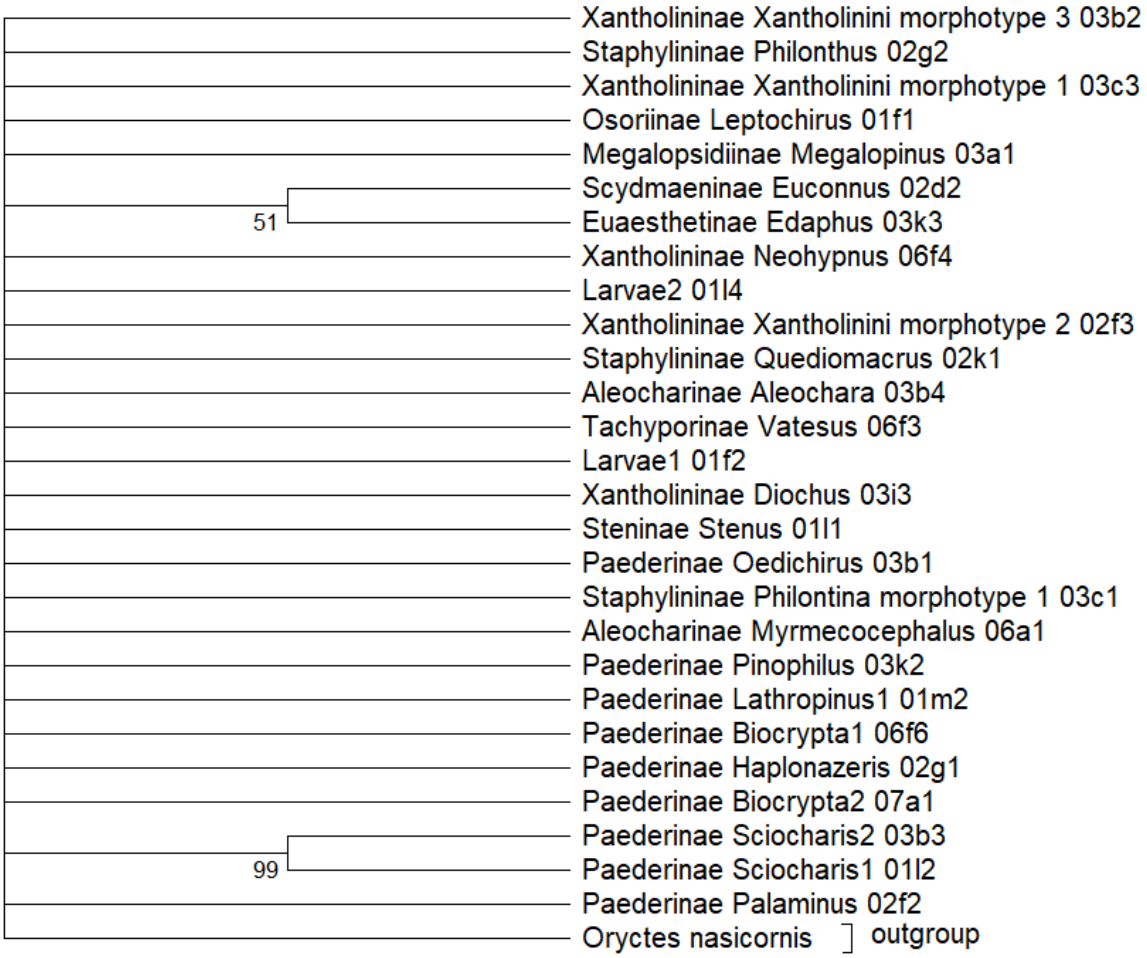

Fig. 11: Neighbour joining tree of all sampled taxa and outgroup, using COI aminoacid sequences.

In the Staphylininae subfamily tree (Fig. 12) it is worth noting that *Quediomacrus* was grouped with *Philontina morphotype 1* (contrasting with its position in Fig. 8). Similarly to the previous trees, there was a dominance of polytomies in the tree topology.

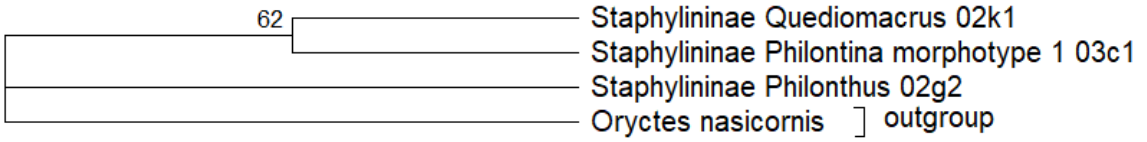

Fig. 12: Neighbour joining tree of the Staphylininae subfamily samples and outgroup, using COI sequences.

In the aminoacid phylogenetic tree for the Staphylininae subfamily, the relations between samples remained the same as in Figure 12. The only difference was that the *Quediomacrus* and *Philontina morphotype 1* node had a lower bootstrap support of 59.

The phylogenetic analysis of Xantholininae subfamily samples resulted in one single polytomy (not shown), however the aminoacid phylogenetic tree for this subfamily (Fig. 13), was a little more informative. There is a polytomy related to the three unidentified morphotypes of Xantholinini, with a bootstrap support of 63 and *Neohypnus* was positioned as the sister group of the Xantholinini cluster.

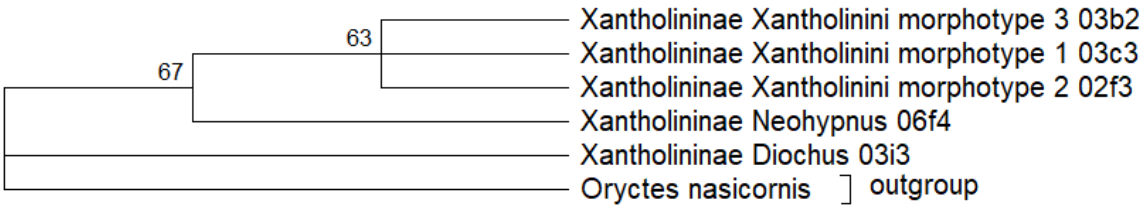

Fig. 13: Neighbour joining tree of the Xantholininae subfamily, using aminoacid sequences.

The phylogeny reconstructed by removing the third codon positions from the analysis (Fig. 14) showed different groupings such as *Euconnus* + *Edaphus*, with a bootstrap value of 82, and *Stenus* + *Diochus*, with a bootstrap support of 58. The *Pinophilus* + *Lathropinus* relationship was not resolved in this approach. However, some of the groupings present in the other trees were similarly shown here, such as the *Sciocharis* cluster. However this phylogenetic tree still presented a large polytomy with few resolved groups.

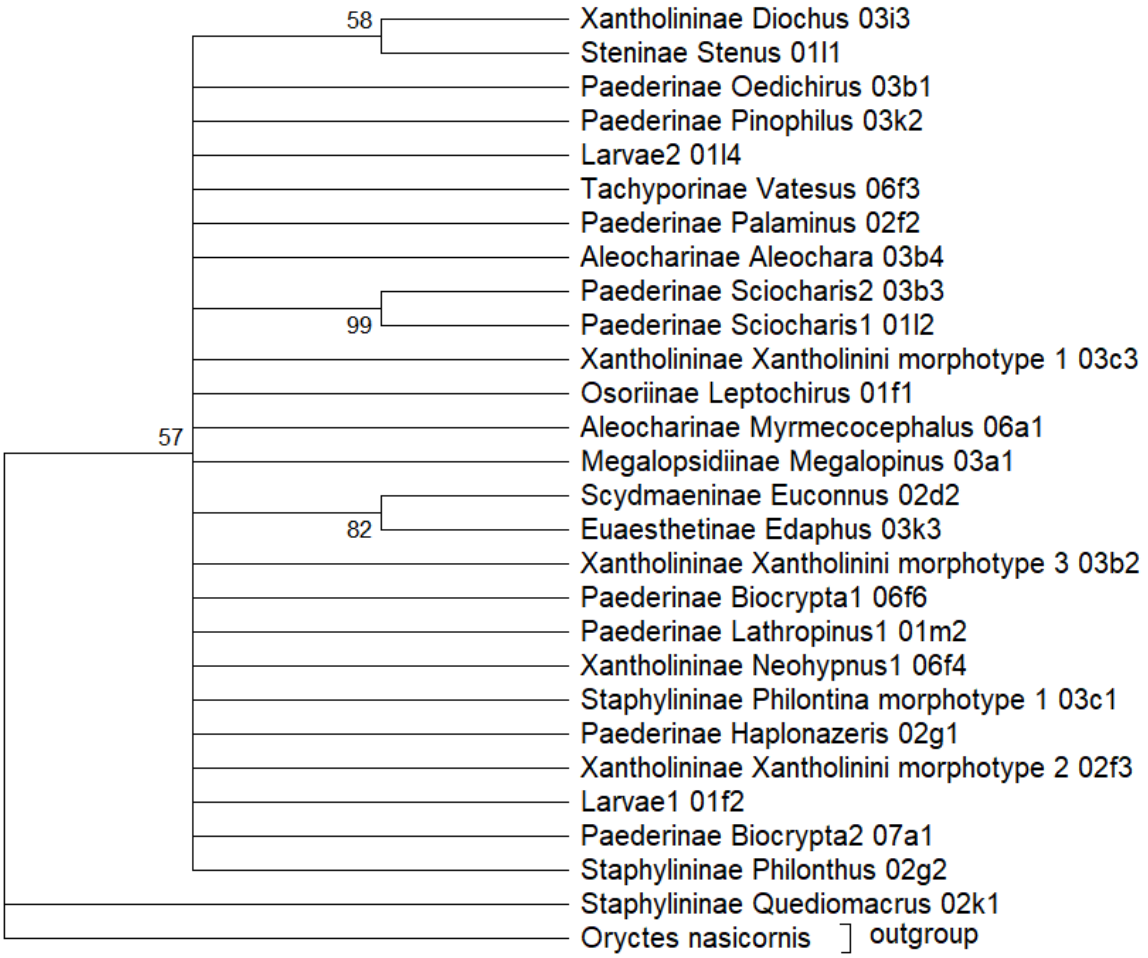

Fig. 14: Neighbour joining tree of all sampled taxa and outgroup, using DNA sequences without the 3rd position of each codon.

4. Discussion

The original motivation of this study was to investigate whether the diversity of Staphylinidae fauna in the natural reserve Alto da Figueira (Nova Friburgo, RJ) could be useful as a bioindicator of forest conservation status. However, this initial motivation faced a series of challenges — despite the advantage of having a pre-existing collection of samples — including difficulties in taxonomic identification due to the use of identification keys developed for non-native fauna; the need to optimize COI region amplification protocols through testing new primer combination; the limited representation of closely related species in available genetic databases; and the inherently high biological diversity of the rove beetle fauna.

Biodiversity surveys of rove beetles from the Brazilian Atlantic Forest are expected to reveal a high number of species, including many still unknown (GROLL, 2025). Unfortunately, despite exploring morphological and molecular approaches for enhancing the diversity knowledge of Staphylinidae fauna, this study didn't provide any taxonomic identification to species level. However, as a result of this research

there are 27 brand new sequences for the COI gene associated with Neotropical rove beetle genera which are significantly divergent (> 7%, except for one) from any other Staphylinidae sequence available in public databases. This was considered a meaningful result, given the complexity of the Staphylinidae family, one of the mega-diverse and abundant families of the ground-living terrestrial arthropods that is taxonomically poorly known even in the regions adjacent to Europe, where the fauna has been investigated for the longest time (KRIVOSHEEVA et al., 2023).

Current context of the identification methods for Neotropical Staphylinidae

Concerning Neotropical fauna, earliest records of Staphylinidae appear in "*Entomologie, ou, Histoire naturelle des insectes: avec leurs caractères génériques et spécifiques, leur description, leur synonymie, et leur enluminée*" (OLIVIER, 1795), and since then, there have been extensive efforts to catalogue the Neotropical fauna. (BETZ et al., 2018).

Morphological analyses and Identification keys

Morphological identification methods are still a relatively popular way to identify species due to their low cost of application (BEEBE & COOPER, 2000), but there are also crucial limitations. In Brazil, species identification keys for the different regions and biomes are incomplete, despite the fact that the country has the largest species diversity for the group in Latin America (CARON et al., 2024). For the Staphylinidae found in Brazil, there are a few catalogs of type specimens, such as the "Catalog of the types of some families of Coleoptera (Insecta) deposited at Coleção de Entomologia Pe. JS Moure, Curitiba, Brazil" (RIBEIRO-COSTA, et al., 2010), the "Catalogue of Coleoptera type specimens housed in the collection of the Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Brazil" (MOURA & GROLL, 2017) and the "Illustrated catalog of the types of Staphylinidae (Insecta: Coleoptera) deposited in the Museu de Zoologia da Universidade de São Paulo, Brazil" (CARON et al., 2025). While valuable assets, these catalogues, along with different species lists for the country and its regions, only represent a fraction of the described Staphylinidae diversity in Brazil.

A potential problem in the biological classification used in this study: the limitations of the taxonomic keys based on European fauna to accurately identify and classify organisms from Neotropical fauna, as the available keys are not designed for the target group. This can lead to misidentification as new diagnostic traits will not be considered. The sample assembly contains two immature indivídual for which no morphological identity could be assigned due to the absence of diagnostic keys for immature states. Since the larvae have well-resolved COI sequences, their taxonomic status could be recovered by further research.

In this study, the low-ranking taxon was defined to the genus level due to the lack of reliable species identification keys for Neotropical Staphylinidae and because there are no conspecific data in nucleotide sequence databases.

Considering the family's diversity, especially from a hotspot biome, the effort required for confirming species identity would be far beyond the scope of this study. Another limitation relates to the competence needed for accurate morphological identification, requiring an experienced taxonomist, specialized in neotropical Staphylinidae, of which there are very few, as highlighted by BETZ, et al (2018). This scarcity of taxonomic expertise contributes to the current "Taxonomic Impediment", faced by numerous groups (ENGEL et al., 2021). In parallel, the accelerated rate of deforestation in the Atlantic Forest biome (COLOMBO & JOLY, 2010; RIBEIRO et al., 2009) presents an alarming scenario in which current scientific progress on Neotropical Staphylinidae may not keep pace with species extinction rates.

A sample belonging to the subfamily Scydmaeninae, as classified by the Brunke et al. (2011) key, was the only individual not identified by the Navarrete-Heredia et al. (2002) key. This group was not included in the former key, because it was considered its own family when the publication was made, and only became a subfamily of Staphylinidae in 2009 (GREBENNIKOV & NEWTON, 2009). The two keys used in this study are from Europe; considering this, in order to add credibility to the genus identification, the "Catálogo Taxonômico da Fauna do Brasil (CTFB)" (NEWTON, 2025) was searched to determine if there are registers of the genus occurring in the sampled area.

Another important tool for species identification in undersampled areas is the application of reverse taxonomy approaches (MARKMANN & TAUTZ, 2005; GUZZI et al., 2023). By obtaining signature sequences within specific genes, it becomes possible to delimit species limits. In the context of Neotropical Staphylinidae, this has the potential to be an invaluable tool for the species identification and the development of taxonomical keys.

Molecular procedures

Regarding the PCR protocol, the C1-N-2191 primer, while not as commonly used as the HCO 2198 primer, was already described as useful for diverse insect species, including Hemiptera, Diptera and Coleoptera (SIMON et al., 1994; MINGEOT et al., 2024; BEZA et al., 2017; RODRIGUES et al., 2025). The C1-N-2191/LCO 1490 mixture successfully amplified the barcode region of 12 rove beetles specimens, while the COI region were amplified for other 20 individuals combining the primers LCO 1490 and HCO 2198 where the C1-N-2191/LCO 1490 protocol fails. This improved result relates to Folmer's primers high reliability and the degree sequence conservation of their annealing sites for a wide taxonomic range (FOLMER et al., 1994). The HCO 2198/LCO 1490 mix is widely used for barcoding Staphylinidae specimens of diverse taxa and geographic regions (LOPES, 2012; TOKAREVA et al., 2021).

Databases representativeness

Many powerful tools could be used to discover, delimit and describe new species, including an integrated taxonomic approach that combines the analysis of morphological characters, use of identification keys, inference of phylogenetic analyses with multiple molecular markers, and correlations of divergent lineages with spatial species distributional patterns, for enhancing new species descriptions (FEGIES et al., 2021; FERNANDES et al., 2024).

Establishing such a framework requires not only extensive sampling but also comprehensive reference databases with representative sequences to accurately link new lineages to taxonomic information. Even in regions outside recognized biodiversity hotspots, there are concerns over whether current COI barcode libraries are sufficiently representative to support reliable molecular identification of Staphylinidae (KRIVOSHEEVA et al., 2023), what about the Neotropical fauna? The present study is an example of this lack of representativeness: novel sequences didn't find a species-specific match in the Bold system nor in the NCBI nucleotide collection (Tables 2 and 3) and a very low taxon coverage for the subfamilies and genera under analysis (Tables 4 and 5)

The current scenario for Neotropical Staphylinidae creates a condition in which the morphological identification of specimens at species level becomes extremely difficult, and consequently the addition of entries to the appropriate databases also becomes more challenging. With this, molecular identification methods become less useful for studies of the region. Despite the growing effort in collecting and studying the species found in the Neotropical region, the lack of identification keys and low database representativity are still great hurdles that need to be overcome in order to achieve a possible golden standard in both taxonomic knowledge and database representation, and avoid the loss of knowledge for this group.

Rove beetle diversity and insights about the forest preservation status

The application of the Staphylinidae family as a bioindicator has been proven to be a useful method to evaluate the preservation status of their habitat. Where rove beetle fauna are well studied, as in Europe and Asia, their use as bioindicators were tested in multiple environments (PONTÉGNIE et al., 2005; SAKCHOOWONG et al., 2008). Despite less studied, Staphylinidae species were also evaluated as informative bioindicator in Dense and Mixed Ombrophilous áreas of the Atlantic Forest, (ARENHARDT et al., 2024; GANHO & MARINONI., 2005; ARENHARDT et al., 2021; CIRIACO et al., 2019).

Staphylinidae subfamilies are related to diverse behaviors, including predation, phytophagy, fungivory and detritivory (NAVARRETE-HEREDIA et al.,

2002). The group is also considered an important part of the soil fauna, especially the litter-soil interface (CIRIACO et al., 2019; BRITO-SILVA et al., 2016; MARINONI & GANHO, 2003). Other important factors that contribute to the potential of this group as a bioindicator are its sensibility to environmental changes and the ease of taxonomic identification at the family and subfamily level.

A limitation using Neotropical rove beetles as bioindicators are their elevated diversity, the few specialized taxonomists and lack of knowledge on their ecology (BETZ et al., 2018; ARENHARDT et al., 2024). However, despite these limitations, it is currently accepted that family level identification be used for this purpose (ARENHARDT et al., 2021; ARENHARDT et al., 2024). A preliminary evaluation on the conservation status of the Alto da Figueira RPPN, based on the taxa composition found in the reserve (Figs. 5 and 6), was proposed.

Many studies relate conservation status with Staphylinidae species richness and diversity, these being more prevalent in native and well conserved environments (ARENHARDT et al., 2024; MÉNDEZ-ROJAS et al., 2021; LÓPEZ-BEDOYA et al, 2021).The Alto da Figueira RPPN rove beetle fauna is represented by diverse taxa, with predominance of the Paederinae, Staphylininae and Xantholininae subfamilies. The family's overall preference for more complex and well conserved environments along with the diversity of taxa in the area can be indicative of a forest in advanced succession stages.

The information above refers to taxa that were collected in Alto da Figueira RPPN and morphologically identified to subfamily or genus. Habitat characterization was based on NAVARRETE-HEREDIA et al. (2002).

The subfamily **Paederinae** is known to inhabit a wide variety of habitats, but are primarily found in humid areas, fallen trees, decomposing vegetation and the canopy of trees. Some groups are termitophilous or myrmecophilous. The subfamily **Staphylininae** is one of the most diverse Staphylinidae taxa. Their species inhabits a similar environment to Paederinae, but they are also capable of inhabiting temperate, arid and mountainous regions (FIRAT & SERT, 2016). The tribes included in **Xantholininae** have a cosmopolitan distribution and a wide variety of habitats. The subfamily **Osoriinae** is mainly saprophagous, inhabiting humid areas, especially in decomposing organic matter and in the soil. The genus *Stenus* Latreille, 1797, from the subfamily **Steninae** is the most diverse genus in Staphylinidae and has preference for humid habitats, so its presence in the studied area was expected. **Aleocharinae** are an extremely diverse group when it comes to its habitat and ecological niche. They can be found in almost every habitat that other Staphylinidae are found, either as generalist predators in the leaf litter or more specialized habitats. The **Euaesthetinae** are typically found in forest leaf litter, tree canopies and moss formations. These beetles are primarily predators of small arthropods. Both larvae and adults of the **Tachyporinae** family exhibit a wide variety of feeding behaviors, including saprophagy, mycophagy and predation. These beetles are known to primarily inhabit humid areas rich with organic matter, such as decomposing tree trunks or leaf litter. The subfamily **Scydmaeninae** is known to inhabit a variety of humid microhabitats such as leaf litter and other forest debris. The group is known to

inhabit Neotropical humid forest formations (ARNETT & THOMAS, 2000; BLACKWELDER, 1945). The **Megalopsidiinae** subfamily and its only genus, *Megalopinus* Eichelbaum, 1915, are typically found in decomposing tree trunks and fungi formations all across the world, but are primarily found in tropical regions.

Atlantic Forest fauna of Staphylinidae used as bioindicators were classified at higher taxonomic levels (family and subfamily, as here). With the exception of ARENHARDT et al. (2024), in which the Staphylinidae specimens were delimited to the species level.

Phylogenetic relations of the Staphylinidae family

The phylogeny of the Staphylinidae family is a highly debated topic. The most accepted phylogenetic tree (fig. 15) shows that, although many subfamilies and genera form well-supported monophyletic clades, the relationships among them remain largely unresolved (Betz et al., 2018).

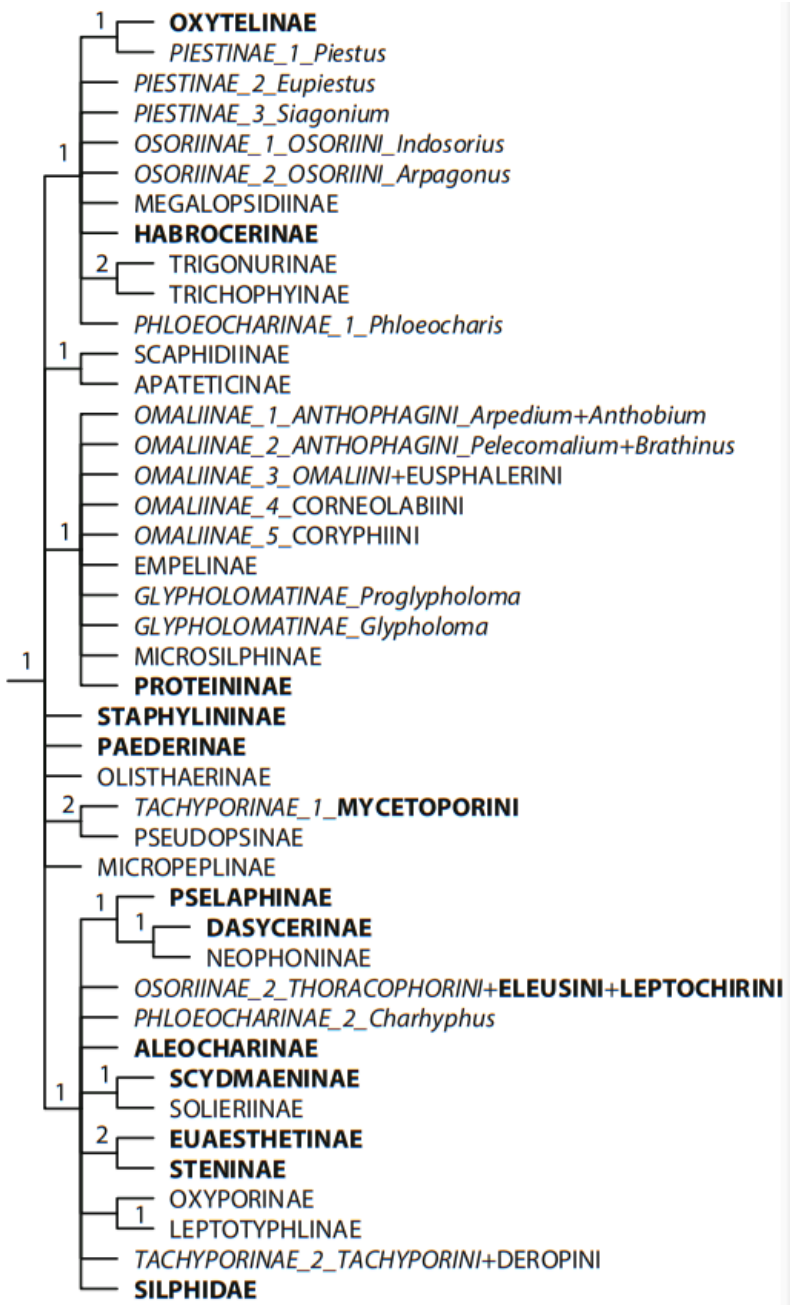

Fig. 15: Phylogeny of Staphylinidae and Silphidae, names in bold indicate monophyletic groups. The tree summarizes the phylogeny of the Silphidae and Staphylinidae clade in McKenna et al. (2015). Adapted from BETZ, et al. (2018).

BETZ et al. (2018) proposed a supertree for the Staphylinidae family. This supertree was a compilation of the phylogenetic relationships from many studies on different taxonomic levels, and used the McKenna et al. (2015) as a basis. As a consequence, this supertree is based on a variety of genetic markers, both nuclear and mitochondrial, and has a much larger and more focused sample pool. Despite the effort of combining data for obtaining a more comprehensive view, the final result is a tree with numerous polytomies and unresolved relations.

The trees from figures 7 to 14 display a similar fate: most branches are related to polytomies. The decision to generate trees using different methods, by

using DNA sequences, with and without the 3rd codon position, and with aminoacid translation sequences was done considering the high variability found in the COI genes of the samples.

5. Conclusion

The Alto da Figueira RPPN has shown a wide variety of Staphylinidae groups, both in subfamily and genus level. This diversity of beetles implies a diverse resource exploitation, so the forest must be able to sustain this diversity. This is indicative of a well conserved Dense Ombrophilous Forest and in advanced succession stages.

There is a lack of representation of COI sequences in the current databases (BOLD and NCBI/GenBank) that slows biodiversity studies, justifying more research for increasing representativeness of Neotropical data.

There is a need for the development of taxonomic identification keys based on Neotropical fauna, justifying training more taxonomists and support for field collections.

There are 27 novel sequences for the COI gene associated with Neotropical rove beetle genera, providing new reference material from a poorly sampled region, increasing the range of geographic distribution of many genera not previously reported in that area (*Myrmecocephalus*, *Biocrypta*, *Haplonazeris*, *Lathropinus*, *Quediomacrus*, *Diochus* and *Neohypnus*).

The current phylogeny of the Staphylinidae family is still rather unclear, possibly due to incomplete taxon sampling, the need of a more slowly evolving gene for resolving subfamily relationships and/or the need of a multilocus approach.

The Staphylinidae family is a megadiverse group and there are many challenges that need to be addressed in order to truly understand it. This study represents a small and initial step towards the characterization of Neotropical rove beetle fauna.

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Appendix

Appendix 1: Digitized specimens collected in the study. Ventral, dorsal and lateral views.

a)

b)

c)

Fig 1: Digitization of specimen 03b - 4 Aleocharinae Aleochara in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 2: Digitization of specimen 06a - 1 Aleocharinae Myrmecocephalus in (a) ventral, (b) dorsal and (c) lateral view.

a)

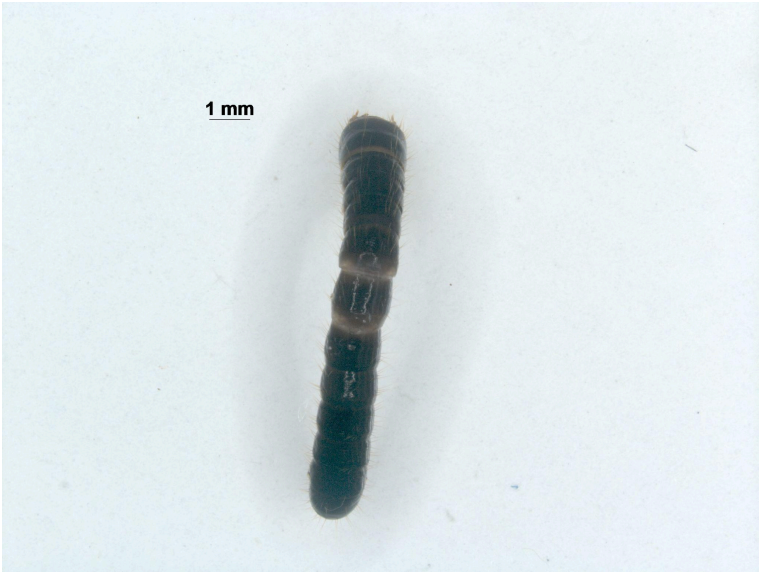
b)

c)

Fig 3: Digitization of specimen 03k - 3 Euaesthetinae Edaphus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)



c)

Fig 4: Digitization of specimen 01f - 2 Larvae in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 5: Digitization of specimen 01l - 4 Larvae in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 6: Digitization of specimen 03a - 1 Megalopsidiinae Megalopinus in (a) ventral, (b) dorsal and (c) lateral view.

a)

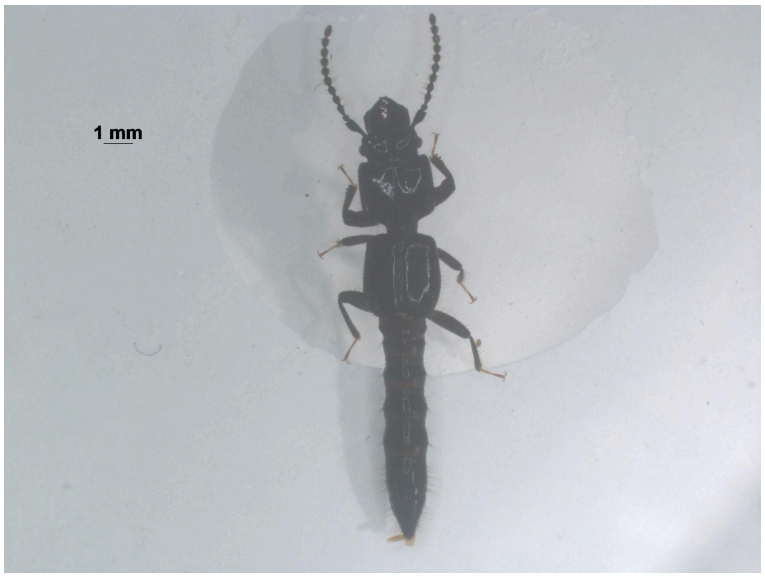
b)

c)

Fig 7: Digitization of specimen 01e - 1 Osoriinae Lispinus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)



c)

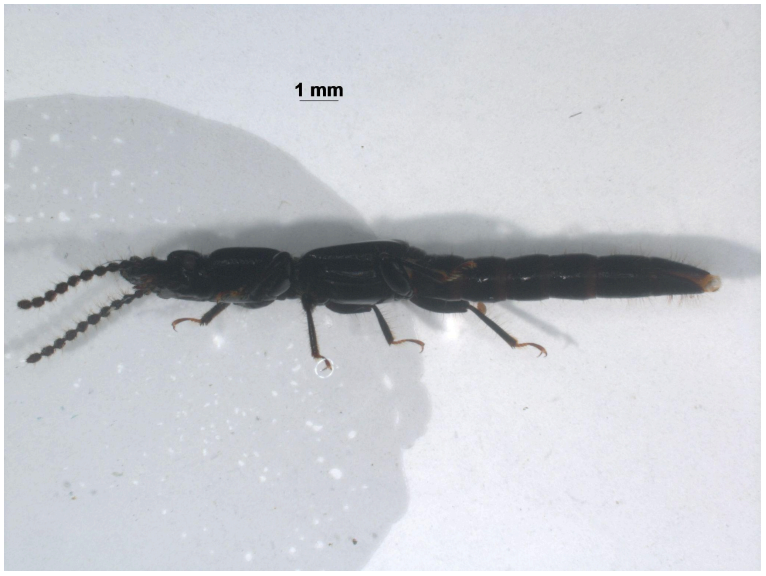


Fig 8: Digitization of specimen 01f - 1 Osoriinae Leptochirus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 9: Digitization of specimen 06f - 6 Paederinae Biocrypta in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 10: Digitization of specimen 07a - 1 Paederinae Biocrypta in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 11: Digitization of specimen 02g - 1 Paederinae Haplonazeris in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 12: Digitization of specimen 01m - 2 Paederinae Lathropinus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 13: Digitization of specimen 02d - 1 Paederinae Lathropinus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 14: Digitization of specimen 03b - 1 Paederinae Oedichirus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 15: Digitization of specimen 02f - 2 Paederinae Palaminus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 16: Digitization of specimen 03k - 2 Paederinae Pinophilus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 17: Digitization of specimen 01I - 2 Paederinae Sciocharis in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 18: Digitization of specimen 03b - 3 Paederinae Sciocharis in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 19: Digitization of specimen 02d - 2 Scydmaeninae Euconnus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 20: Digitization of specimen 02g - 2 Staphilininae Philonthus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 21: Digitization of specimen 03c - 1 Staphylininae Philontina Morphotype 1 in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 22: Digitization of specimen 02k - 1 Staphylininae Quediomacrus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 23: Digitization of specimen 07a - 3 Staphylininae Quediomacrus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 24: Digitization of specimen 01I - 1 Steninae Stenus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 25: Digitization of specimen 06f - 7 Tachyporinae Sepedophilus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 26: Digitization of specimen 06f - 3 Tachyporinae Vatesus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 27: Digitization of specimen 03i - 3 Xantholininae Diochus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 28: Digitization of specimen 06f - 4 Xantholininae Neohypnus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 29: Digitization of specimen 06f - 5 Xantholininae Neohypnus in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 30: Digitization of specimen 03c - 3 Xantholininae Xantholinini Morphotype 1 in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 31: Digitization of specimen 02f - 3 Xantholininae Xantholinini Morphotype 2 in (a) ventral, (b) dorsal and (c) lateral view.

a)

b)

c)

Fig 32: Digitization of specimen 03b - 2 Xantholininae Xantholinini Morphotype 3 in (a) ventral, (b) dorsal and (c) lateral view.