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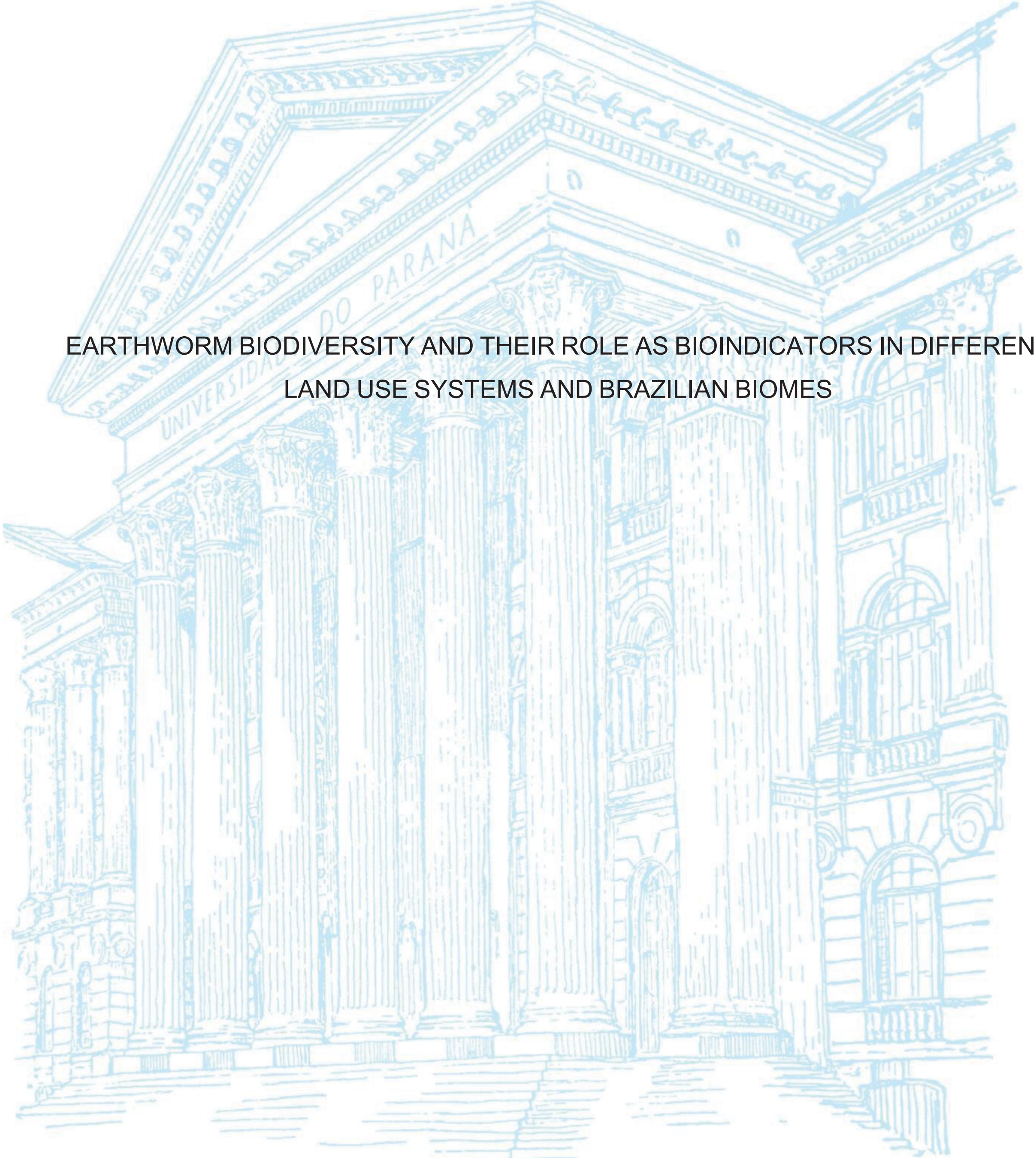

EARTHWORM BIODIVERSITY AND THEIR ROLE AS BIOINDICATORS IN DIFFERENT LAND USE SYSTEMS AND BRAZILIAN BIOMES

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Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciência do Solo, Área de Concentração Solo e Ambiente.

Orientador: Prof. Dr. George Gardner Brown

Coorientadora: Profª. Dra. Marie Luise Carolina Bartz

Coorientador: Prof. Dr. Luís Filipe das Neves Cunha

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **RAFAELA TAVARES DUDAS** intitulada: **EARTHWORM BIODIVERSITY AND THEIR ROLE AS BIOINDICATORS IN DIFFERENT LAND USE SYSTEMS AND BRAZILIAN BIOMES**, sob orientação do Prof. Dr. GEORGE GARDNER BROWN, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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"I'm a shooting star leaping through the sky,
Like a tiger defying the laws of gravity.
I'm a racing car passing by like Lady Godiva,
I'm gonna go, go, go, there's no stopping me.
(Don't stop me now) 'Cause I'm having a good time.
(Don't stop me now) I don't wanna stop at all, yeah!"
(Queen, 1978)

RESUMO

As minhocas têm sido amplamente utilizadas na avaliação da qualidade do solo devido à sua importância ecológica e relação com funções do solo, e o estudo de aspectos como sua abundância e riqueza tem se mostrado uma ferramenta fundamental para esse propósito. No Brasil, são registradas dez famílias de minhocas, sendo a Glossoscolecidae uma das mais biodiversas, especialmente em áreas com menor impacto e melhor saúde ambiental. Este trabalho integra análises ecológicas, taxonômicas e moleculares para explorar o potencial bioindicador das minhocas, além de descrever novas espécies dos gêneros *Glossoscolex* e *Fimoscolex* (Glossoscolecidae) e investigar as relações entre características morfológicas e evolutivas desses táxons. As coletas ocorreram em três sistemas de uso do solo distintos: vegetação nativa (VN), Sistema Plantio Direto (SPD) e área mal manejada (AMM), localizados nos biomas brasileiros Mata Atlântica e Cerrado. Foram coletadas 1769 minhocas, pertencentes a sete famílias e 24 espécies, das quais 13 são nativas e 11 exóticas. Entre as nativas, 11 são novas para a ciência. As maiores abundâncias foram registradas no bioma Mata Atlântica e em áreas de SPD, indicando que manejos mais conservacionistas favorecem a presença de minhocas, incluindo espécies nativas. Em relação ao potencial bioindicador, realizado pela análise de espécies indicadoras (IndVal), espécies exóticas apresentaram valores indicativos superiores a 50% para áreas agrícolas, enquanto espécies e famílias nativas foram associadas a ambientes preservados. Características do solo, como maior umidade, altos teores de argila e melhor fertilidade, mostraram correlação com espécies nativas, reforçando seu valor como bioindicadoras. Das novas espécies, nove foram descritas neste trabalho: sete do gênero *Glossoscolex* e duas do gênero *Fimoscolex*, com base na taxonomia clássica. Os principais caracteres diagnósticos utilizados incluem a quantidade de poros masculinos e câmaras copulatórias. No entanto, novas descobertas sobre essas características destacam a complexidade taxonômica dos gêneros. As análises moleculares foram realizadas com sequências obtidas neste trabalho e dados previamente disponíveis, utilizando o gene do citocromo oxidase subunidade I (COI). Os resultados indicam que os gêneros *Glossoscolex* e *Fimoscolex* são parafiléticos e apresentam convergências evolutivas que podem explicar adaptações específicas ou barreiras evolutivas enfrentadas por algumas espécies. Embora o COI tenha se mostrado eficiente para separar espécies, o uso combinado de outros marcadores genéticos pode ampliar a resolução taxonômica. Por meio de uma abordagem integrativa que combina dados ecológicos, morfológicos e moleculares, este estudo fornece uma visão abrangente da história evolutiva de dois gêneros de minhocas, associando aspectos ecológicos, taxonômicos e genéticos. Além disso, reforça o papel das minhocas como bioindicadoras confiáveis e agentes fundamentais na manutenção da qualidade do solo.

Palavras-chave: Saúde do solo. Oligochaeta. Novas espécies. Taxonomia. Sequenciamento genético. Gene citocromo oxidase subunidade I.

ABSTRACT

Earthworms have been widely used to assess soil quality due to their ecological importance and relationship with soil functions, and the study of aspects such as their abundance and richness has proven to be a fundamental tool for this purpose. In Brazil, ten families of earthworms are recorded, with Glossoscolecidae being one of the most biodiverse, especially in areas with lower impact and better environmental health. This work integrates ecological, taxonomic and molecular analyses to explore the bioindicator potential of earthworms, in addition to describing new species of the genera *Glossoscolex* and *Fimoscolex* (Glossoscolecidae) and investigating the relationships between morphological and evolutionary characteristics of these taxa. The collections occurred in three distinct land use systems: native vegetation (NV), No-Tillage System (NTS) and bad management area (BM), located in the Brazilian Atlantic Forest and Cerrado biomes. A total of 1769 earthworms belonging to seven families and 24 species were collected, of which 13 are native and 11 are exotic. Among the native species, 11 are new to science. The highest abundances were recorded in the Atlantic Forest biome and in areas of NTS, indicating that more conservationist management favors the presence of earthworms, including native species. Regarding the bioindicator potential, performed by the analysis of indicator species (IndVal), exotic species presented indicative values higher than 50% for agricultural areas, while native species and families were associated with preserved environments. Soil characteristics, such as greater moisture, high clay content and better fertility, showed correlation with native species, reinforcing their value as bioindicators. Of the new species, nine were described in this work: seven from the genus *Glossoscolex* and two from the genus *Fimoscolex*, based on classical taxonomy. The main diagnostic characters used include the number of male pores and copulatory chambers. However, new findings on these traits highlight the taxonomic complexity of the genera. Molecular analyses were performed with sequences obtained in this work and previously available data, using the cytochrome oxidase subunit I (COI) gene. The results indicate that the genera *Glossoscolex* and *Fimoscolex* are paraphyletic and present evolutionary convergences that may explain specific adaptations or evolutionary barriers faced by some species. Although COI has proven efficient to separate species, the combined use of other genetic markers can broaden the taxonomic resolution. Through an integrative approach that combines ecological, morphological and molecular data, this study provides a comprehensive view of the evolutionary history of two genera of earthworms, associating ecological, taxonomic and genetic aspects. In addition, it reinforces the role of earthworms as reliable bioindicators and key agents in maintaining soil quality.

Keywords: Soil quality. Oligochaeta. New species. Taxonomy. Genetic sequencing. Cytochrome oxidase subunit I.

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SUMMARY

GENERAL INTRODUCTION

Soil is an essential natural resource along with water and air, in the maintenance of all forms of life on the planet, performing crucial roles in economic, social, and ecological spheres (Bünemann *et al*. 2018). From an economic perspective, soil serves as the foundation for agricultural production and forestry development, sustaining activities that result in food and biofuel production, which are fundamental for economic stability and growth (FAO, 2015). In the social context, soil is intimately connected to food security, human well-being, cultural heritage, and serves as the basis for anthropogenic infrastructure (Lal, 2019). Ecologically, soil plays a significant role in biogeochemical cycles (Carbon, Nitrogen, Oxygen) and water, in climate regulation and biodiversity conservation (Smith *et al.* 2015, Lal *et al.* 2021).

The inappropriate use of soil is a major environmental threat, involving soil loss due to erosion, desertification, compaction, and decrease of organic matter, all of which contribute to soil degradation (Godoi *et al.* 2021). These changes compromise the productivity of agricultural lands, in addition to the negative impact on the entire ecosystem, such as the decrease in biodiversity (Montanarella *et al*. 2016). In light of these problems, the implementation of sustainable soil management and conservation agricultural practices has become an essential tool to preserve and recover degraded areas and maintain ecosystem functionality (Kassam *et al.* 2009; Passinato *et al.* 2021).

Soil animals represent one quarter of the world's biodiversity and play significant roles in soil chemical and physical attributes as well in ecosystem service delivery, resulting in a direct impact on soil quality and productivity (Brown *et al.* 2018). Organisms such as earthworms are fundamental for processes like organic matter decomposition, soil aeration, nutrient cycling, aggregate and structure formation, and increase in soil fertility (Edwards *et al*. 2022). Earthworms are ecosystem engineers that can mitigate some of the negative impacts of soil degradation (Lavelle *et al.* 1997), and can contribute significantly to crop production, increasing global cereal (wheat, maize, rice, and barley) productivity by 6.45% and legume (peas, lentils, alfalfa, soybeans) productivity by 2.3% (Fonte *et al.* 2023).

Furthermore, earthworms are well-established bioindicators of soil quality, primarily because they are sensitive to environmental changes, easy to collect, visible in the field, and cost-effective (Brown; Dominguez, 2010). The presence and diversity of different species provides information on the conservation state of different land use systems. Native species, for example, typically indicate a more preserved environment and/or vegetation type, with sufficient resources for their development and survival. In contrast, exotic species, originating from different locations are found in all kinds of land uses and management conditions, demonstrating environmental changes, disturbance and/or degradation (Demetrio *et al.* 2023).

The global diversity of earthworms is quite remarkable, with Misirlioğlu *et al*. (2023), reporting 23 families, 382 genera, and approximately 5,700 species/subspecies, although the estimated richness worldwide is likely to exceed 30 thousand species (Decaëns *et al.* 2024). In Brazil, 10 of the 23 families, 65 genera, and approximately 355 species are found (Brown *et al*. 2023) (TABLE 1). However, up to 2,170 species have been estimated (Brown; James, 2007), and dozens of new species have already been collected but not yet described (da Silva *et al.* 2017).

TABLE 1 – EARTHWORMS FAMILIES, NUMBER OF GENERA AND SPECIES FOUND IN BRAZIL, NATIVE AND/OR EXOTIC IN BRAZIL, CONTINENT OF ORIGIN AND STATES IN BRAZIL WITH RECORDS OF OCCURRENCE

Family	Genera	Species	Native/Exotic	Origin	States with records/occurrence
Acanthodrilidae	3	6	Exotic	Southern hemisphere	AC, AM, AP, BA, MG, MS, MT, MS, PB, PA, PE, PR, RJ, RO, RS, RR, SC, SP
Almidae	3	3	Native	Africa, Asia and America	AM, PR, SP
Arecoidae	1	1	Native	South America	AM
Benhamiidae	5	19	Native and Exotic	Africa, South America	MT, MS, MG, PR,
Eudrilidae	2	2	Exotic	Africa	BA, RJ, PE, PA, SP, PR, PB, MG, SE, MA
Glossoscolecidae	6	156	Native	South America	AM, AP, SP, PR, SC, RJ, MG, MA, RR, MS, PA, GO, RO, MT, PB
Lumbricidae	7	14	Exotic	Europe, North America	DF, MG, PB, PE, PR, RJ, RS, SC,
Megascolecidae	6	15	Exotic	Asia	AM, DF, BA, MG, PR, RJ, PB, PA PE, RS, SC, SP
Ocnerodrilidae	17	44	Native and Exotic	Africa, South America, Asia	AM, BA, ES, MT, MG, MS, PA, PE, PR, RR, RO, SE, SC, SP, RS, RO, RS
Rhinodrilidae	15	95	Native and Exotic	Americas	AC, AP, AM, BA, DF, ES, GO, MA, MG, MS, MT, PA, PB, PE, PR, RJ, RO, RR, RS, SC, SE, SP

Source: Author (2024).

One of the families with the greatest species richness and distribution in Brazil is Glossoscolecidae, found in all biomes and endemic to Latin America (Fragoso; Brown, 2007). This family has been recorded in areas of native vegetation (Demetrio *et al.* 2018), no-till agricultural crops (Bartz *et al.* 2011; Santos *et al.* 2018), lawns and pastures (Feijoo *et al.* 2017; Ferreira *et al.* 2018), and forest plantations (da Silva *et al.* 2019) in the South and Southeast regions. Glossoscolecidae includes many native and endemic species, restricted to natural habitats, which can be considered indicators of well-preserved environments and habitats. Despite this wide distribution, some species still lack more information regarding their biology, ecology, morphology, genetic diversity and relationships with soil characteristics (da Silva *et al*. 2017; Demetrio *et al.* 2018).

This family contains six genera in total, with *Glossoscolex* (Leuckart, 1835*)* and *Fimoscolex* (Michaelsen, 1900) being the two most significant. *Glossoscolex* has 58 species and *Fimoscolex* nine. However, there are many specimens already collected which represent new, undescribed species (da Silva *et al.* 2017), so the total richness is much higher. These genera have a complex evolutionary history but depending on the species they have morphological similarities. These issues will be addressed in chapter three of this thesis.

The primary reason for the delay in new species descriptions is the scarcity of earthworm taxonomists. In Brazil only three taxonomists are active. Besides taxonomy, comprehensive studies are also lacking that relate species to environmental characteristics and ecology to determine their impact on soil ecosystems. To accelerate and facilitate species identification and new species description, molecular tools have been widely used in earthworm taxonomy (Marchán *et al*. 2020). DNA barcoding, presented by Hebert *et al.* (2003), proposes the use of a DNA sequence for species identification, using a region of the mitochondrial genome called the cytochrome c oxidase subunit I (COI). This region was chosen because it is well preserved and allows differentiation between species and phylogenetic groups within the same species (Hebert *et al*. 2003). The combination of ecological data with molecular tools and classical morphological methods can help identify gaps in earthworm biology and facilitate new earthworm species descriptions (Lone *et al.* 2022).

In this context, the objectives of this thesis were (I) to explore the potential of earthworms as soil health indicators in various land use systems and biomes in Brazil;

(II) to describe new species found in these systems using classical taxonomy combined with molecular tools; and (III) to evaluate how integrative taxonomy using ecological knowledge, morphological and molecular data can aid in species delimitation and the assessment of evolutionary processes and speciation.

1.1 REFERENCES

Bartz, M. L., Brown, G. G., Rosa, M. G., Locatelli, M., James, S. W., & Baretta, D. M. (2011). *Urobenus* sp: das matas para as áreas sob plantio direto. *Rev. Plantio Direto*, 124, 6-7.

Brown, G. G., & James, S. W. (2007). Ecologia, biodiversidade e biogeografia das minhocas no Brasil. Minhocas na América Latina: biodiversidade e ecologia. Londrina: Embrapa Soja, 297-381.

Brown, G. G., & Domínguez, J. (2010). Uso das minhocas como bioindicadoras ambientais: princípios e práticas - o 3° Encontro Latino Americano de Ecologia e Taxonomia de Oligoquetas (ELAETAO3). *Acta Zoológica Mexicana*, 26(spe2), 1-18. Recuperado de: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0065-17372010000500001

Brown, G. G., Silva, E. D., Thomazini, M. J., Niva, C. C., Decaëns, T., Cunha, L. F., Nadolny, H.S., Demetrio, W.C., Santos, A., Ferreira, T., Maia, L.S., Conrado, A.C., Segalla, R.F., Ferreira, A.C., Pasini, A., Bartz, M.L.C., Sautter, K.D., James, S.W., Baretta, D., Antoniolli, Z.I., Briones, M.J.I., Sousa, J.P., Römbke, J. & Lavelle, P. (2018). The role of soil fauna in soil health and delivery of ecosystem services (pp. 197-241). In: Reicosky, D. (Ed.), Managing soil health for sustainable agriculture, v. 1: Fundamentals. Oxford: Burleigh Dodds Science Publishing, 2018. p. 197–241. Recuperado de: https://doi.org/10.19103/AS.2017.0033.11

Brown, G. G., James, S. W., Csuzdi, C., Lapied, E., Decäens, T., Reynolds, J. W., Misirlioglu, M., Mirjana, S., Tanja, T., Jovana, S., Phillips, H.R.P. & Cameron, E. (2024). A checklist of megadrile earthworm (Annelida: Clitellata) species and subspecies of the world. Available from: *Zenodo*. Recuperado de: https://doi.org/10.5281/zenodo.7301848

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., De Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van

Groeningen, J.W. & Brussaard, L. (2018). Soil quality–A critical review. *Soil biology and biochemistry*, 120, 105-125.
Recuperado de: https://doi.org/10.1016/j.soilbio.2018.01.030

da Silva, E., Rosa, M.G., Schuhli, G.S., James, S.W., Decaëns, T., Bartz, M.L.C., Nadolny, H., Feijoo, A. & Brown, G.G. (2017) O potencial do DNA barcoding para a identificação e conservação de espécies de minhocas brasileiras. In: Moreira, F.M.S. & Kasuya, M.C.M. (Eds.), Fertilidade e biologia do solo - Integração e tecnologia para todos, Vol. 2. Lavras, SBCS, pp. 549–570.

da Silva, E., de Lima, O. G., de Andrade, D. P., & Brown, G. G. (2019). Earthworm populations in forestry plantations (*Araucaria angustifolia*, *Pinus elliottii*) and Native Atlantic Forest in Southern Brazil compared using two sampling methods. *Pedobiologia*, 72, 1-7.
Recuperado de: https://doi.org/10.1016/j.pedobi.2018.10.002

Decaëns T, Brown GG, Cameron EK, Csuzdi C, Eisenhauer N, Gerard S, Goulpeau A, Hedde M, James S, Lapied E, Maggia ME, Marchan DF, Mathieu J, Phillips HRP, Marcon E (2024) A can of worms: estimating the global number of earthworm species. bioRxiv, 2024b.09.08.611896. Recuperado de:
https://doi.org/10.1101/2024.09.08.611896

de Faria Godoi, R., Rodrigues, D. B., Borrelli, P., & Oliveira, P. T. S. (2021). High-resolution soil erodibility map of Brazil. *Science of The Total Environment*, 781, 146673. Recuperado de: https://doi.org/10.1016/j.scitotenv.2021.146673

Demetrio, W.C., Santos, A., Ferreira, T., Naldony, H., Cardoso, G.B.X., Torres, J.L.M., Dudas, R., Oliveira, V., Barreto, J.O., James, S.W., Silva, E., Brown, G.G. & Bartz, M.L.C. (2018) Earthworm species in various land use systems in the Campos Gerais region of Lapa, Paraná, Brazil. Zootaxa, 4496, 503. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W., Brown, G., Pupin, B., Novo, R., Dudas, R., Baretta, D., Römbke, J. Bartz, M.L.C. & Borma, L. (2023). Are exotic earthworms threatening soil biodiversity in the Brazilian Atlantic Forest? Applied Soil Ecology, 182, 104693. Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104693

Edwards, C. A., & Arancon, N. Q. (2022). The role of earthworms in organic matter and nutrient cycles. In Biology and ecology of earthworms (pp. 233-274). New York, NY: Springer US.

Food Agriculture Organization - FAO (2015) - Montanarella, L., Badraoui, M., Chude, V., Costa, I. D. S. B., Mamo, T., Yemefack, M., & MCKENZIE, N. (2015). Status of the world's soil resources: main report.

Feijoo, A. M., Brown, G. G., & James, S. W. (2017). New species of *Andiorrhinus* Cognetti, 1908 (Oligochaeta: Rhinodrilidae) from Venezuela and Brazil. *Zootaxa*, 4363(1), 55-78.

Recuperado de: https://doi.org/10.11646/zootaxa.4363.1.2.

Ferreira, T., Santos, A., Demetrio, W. C., Cardoso, G. B., Moraes, R., Assis, O., Niva, C.C., Smokanit, M., Knopik, J., Sautter, K.D., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in public parks in Curitiba, Paraná, Brazil. *Zootaxa*, 4496(1), 535-547. Recuprerado de: https://doi.org/10.11646/zootaxa.4496.1.41.

Fonte, S. J., Hsieh, M., & Mueller, N. D. (2023). Earthworms contribute significantly to global food production. *Nature Communications*, 14(1), 5713. Recuperado de: https://doi.org/10.1016/j.geoderma.2009.12.016

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: *Biological Sciences*, 270(1512), 313-321. Recuperado de: https://doi.org/10.1098/rspb.2002.2218.

Kassam, A., Friedrich, T., Shaxson, F., & Pretty, J. (2009). The spread of conservation agriculture: justification, sustainability and uptake. *International journal of agricultural sustainability*, 7(4), 292-320. Recuperado de: https://doi.org/10.3763/ijas.2009.0477

Lal, R. (2019). Rights-of-Soil. *Journal of Soil and Water Conservation*. 74. 81A-86A. Recuperado de: https://doi.org/10.2489/jswc.74.4.81A

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P.O.W.H. & Dhillion, S. (1997). Soil functions in a changing world: the role of invertebrate ecosystem engineers.

Lone, A. R., Thakur, S. S., Tiwari, P., James, S. W., & Yadav, S. (2022). Phylogenetic relationships in earthworm Megascolex species (Oligochaeta: Megascolecidae) with addition of two new species. Diversity, 14(11), 1006. Recuperado de: https://doi.org/10.3390/d14111006

Marchán, D. F., Fernández, R., Domínguez, J., Díaz Cosín, D. J., & Novo, M. (2020). Genome-informed integrative taxonomic description of three cryptic species in the earthworm genus Carpetania (Oligochaeta, Hormogastridae). *Systematics and biodiversity*, 18(3), 203-215.

Recuperado de: https://doi.org/10.1080/14772000.2020.1730474

Misirlioğlu, M., Reynolds, J., Stojanović, M., Trakić, T., Sekulić, J., James, S., Csuzdi, C., Decaëns, T., Lapied, E., Phillips, H.R.P., Camreon, E.K., & Brown, G. (2023). Earthworms (Clitellata, Megadrili) of the world: an updated checklist of valid species and families, with notes on their distribution. *Zootaxa*. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.33.

Santos, A., Gorte, T., Demetrio, W.C., Ferreira, T., Nadolny, H., Cardoso, G.B.X., Tonetti, C., Ralisch, R., Nunes, A.P., Coqueiro, A.C.P., Leandro, H.C.L., Wandscheer, C.A.R., Bortoluzzi, J., Brown G.G. & Bartz, M.L.C. (2018) Earthworm species in no-tillage agroecosystems and native Atlantic forests in Western Paraná, Brazil. Zootaxa, 4496, 517–534. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.40

Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A., McDowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I., Sobocká, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya, T., Scholes, R. J., and Scholes, M. C.: Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. *Soil*, 1, 665–685. Recuperado de: https://doi.org/10.5194/soil-1-665-2015, 2015.

2 ARTICLE 1: EARTHWORM ECOLOGY AND BIODIVERSITY IN DIFFERENT LAND USE SYSTEMS AND BRAZILIAN BIOMES

2.1 RESUMO

Dados de ecologia de minhocas, como abundância, biomassa e riqueza, são úteis para indicar como o manejo do solo impacta a biodiversidade, ao mesmo tempo que possibilita inferir o efeito das minhocas nas propriedades do solo. No presente trabalho avaliou-se a comunidade de minhocas em três tipos de manejo do solo em dois biomas brasileiros e determinou-se as relações com os atributos físicos e químicos do solo. As minhocas foram coletadas na Mata Atlântica (estados do Rio Grande do Sul, Santa Catarina, Paraná e São Paulo) e no Cerrado (Minas Gerais, Mato Grosso, Mato Grosso do Sul, Bahia, Piauí e Maranhão), totalizando 20 municípios amostrados. Em cada município, três sistemas de uso do solo foram avaliados: vegetação nativa (VN), Sistema Plantio Direto (SPD) e área mal manejada (AMM). As coletas foram realizadas pelo método TSBF, com retirada de nove monólitos por sistema de uso do solo (SUS), além de coletas qualitativas em pontos específicos. Amostras de solo foram analisadas quanto à granulometria, macro e micronutrientes, e dados de precipitação acumulada levantados. No total, foram coletadas 1769 minhocas, distribuídas em sete famílias e 24 espécies, das quais 13 são nativas e 11 exóticas. Entre as espécies nativas, 11 são novas para a ciência e serão descritas futuramente. A Mata Atlântica apresentou maior abundância de minhocas, possivelmente devido à sua mais alta precipitação. Em relação aos SUS, o SPD apresentou os maiores valores de densidade, biomassa e riqueza, seguido por AMM e VN, indicando que o SPD proporciona condições mais favoráveis para as minhocas. A análise dos atributos do solo revelou que as populações de minhocas se correlacionam mais fortemente com solos argilosos e elevados teores de CTC, umidade, nitrogênio, cobre e manganês. Algumas espécies mostraram valor indicador para espécies (IndVal) acima de 50% em locais específicos, indicando forte associação com certas características locais. Esses resultados reforçam o papel das minhocas como indicadoras de qualidade do solo e mostram a importância de práticas agrícolas conservacionistas para a biodiversidade do solo. No entanto, esforços adicionais que integrem fatores ecológicos (comunidade dos organismos e suas interações), ambientais (clima, propriedades físicas e químicas) e biológicos (presença e ausência de organismos e suas atividades) são necessários para aprofundar o entendimento das dinâmicas das comunidades de minhocas nos diferentes ambientes.

Palavras-chave: Biodiversidade de minhocas. Ecologia do solo. Bioindicadores. Oligochaeta. Fauna do solo.

2.1.1 ABSTRACT

Earthworm ecological data, such as abundance, biomass, and richness, are useful to indicate how soil management affects biodiversity, while also allowing inferences regarding the effect of earthworms on soil properties. In the present study we evaluated earthworm communities in three soil management types in two Brazilian biomes and determined their relationships with soil physical and chemical attributes. Earthworms were collected in the Atlantic Forest (states of Rio Grande do Sul, Santa Catarina, Paraná, and São Paulo) and Cerrado (Minas Gerais, Mato Grosso, Mato Grosso do Sul, Bahia, Piauí, and Maranhão), totaling 20 municipalities. In each municipality, three land use systems were evaluated: native vegetation (NV), No-Tillage system (NTS), and bad management (BM). Sampling was performed using the TSBF method, with the removal of nine monoliths per land use system (LUS), in addition to qualitative sampling at specific points. Soil samples were analyzed for texture, macro and micronutrients, and accumulated precipitation data was collected. In total, 1769 earthworms were found, belonging to seven families and 24 species, of which 13 were native and 11 exotics. Among the native species, 11 were new to science and will be described soon. The Atlantic Forest had the greatest total earthworm abundance, possibly due to its higher rainfall regime. Regarding the LUS, NTS had higher density, biomass and richness, followed by BM and NV, indicating that NTS provides more favorable conditions for earthworm communities. The analysis of soil attributes revealed that earthworm populations correlated more strongly with clayey soils and high levels of CEC, moisture, nitrogen, copper and manganese. Some species showed indicator values (IndVal) over 50% at specific locations, indicating a strong association with certain local characteristics. These results reinforce the role of earthworms as an indicator of soil quality and show the importance of conservation agricultural practices for soil biodiversity. However, additional efforts that integrate ecological (community of organisms and their interactions), environmental (climate, physical and chemical properties) and biological (presence and absence of organisms and their activities) factors are necessary to deepen the understanding of the dynamics of earthworm communities in different environments.

Keywords: Earthworm biodiversity. Soil ecology. Bioindicators. Oligochaeta. Soil fauna.

2.2 INTRODUCTION

Earthworms play fundamental roles in soil ecology and function as ecosystem engineers due to their biological, chemical, and physical effects on soils (Lavelle *et al.* 1997). These include bioturbation, organic matter decomposition and distribution throughout soil layers, aggregate formation, soil aeration through burrow creation, enhancement of microbiota, and nutrient cycling (Fonte *et al.* 2010; Gong *et al.* 2019; Akhila; Entoori, 2022). These soil transformations are essential for improved fertility, structure, and health of the soil and the use of earthworms as bioindicators is directly related to environmental conditions and ecosystem functions (Bonilla-Bedoya *et al.* 2023).

In diverse land use systems (LUS), such as native vegetation (NV) and agricultural systems, earthworm communities can exhibit variation in terms of abundance (number of individuals) and diversity (number of species) (Vršič *et al.* 2021. This principle applies to different ecosystems and biomes as well, because of variations in vegetation types, soil types and climatic conditions, considering that temperature and precipitation/rainfall can affect the soil and, consequently, earthworm populations (Nadolny *et al.* 2017; Singh *et al*. 2019).

Concerning NV, a higher number of earthworms is expected due to the natural characteristics of these areas, although Demetrio *et al.* (2023) found lower density in Atlantic Forest sites and attributed this to human disturbance and the invasion of exotic cosmopolitan earthworm species in agroecosystems. Furthermore, when comparing agricultural management practices, the more conservative No-Tillage System (NTS) has frequently shown higher density and diversity of earthworms than conventional tillage (CT) (Brown *et al.* 2003; Dudas *et al.* 2023). This is because NTS adheres to the principles of minimal soil disturbance, crop rotation and diversification, and permanent soil cover, all of which contribute to a more suitable habitat for earthworm development (Bartz *et al.* 2024). In CT, subsoiling and/or tillage loosens the soil, thereby modifying the upper soil layers, eliminating earthworm burrows and affecting the soil as a habitat (Dudas *et al.* 2023).

Higher earthworm abundance and diversity indicates improved soil management and quality and is crucial for ecosystem balance and resilience (Dewi; Senge 2015). Each species fulfills specific ecological roles, such as enhanced decomposition efficiency, creation of pores (for aeration and infiltration), and nutrient transportation through the soil (Cardoso *et al.* 2013). This functional diversity is based on the ecological categories to which the species belong. For earthworms, there are three major groups: I - epigeic: organic matter feeders that live on the soil surface; II - anecic: species that move through vertical burrows and feed on fresher organic materials and soil; and III - endogeic: species that feed solely on soil and move through horizontal burrows (Fragoso *et al.* 1997). In addition, Capowiez *et al*. (2024) proposed six functional groups based on the activity of earthworms on soil that can be linked to the ecological categories: I - intense tunnelers: surface activity and great extensive burrow system (link with anecics); II – burrowers: pigmented and large earthworms, with surface activity and limited true burrows (link with epi-anecics); III – deep bioturbators: large or average, non-pigmented earthworms, with less activity in soil surface and with deeper galleries in the soil (link with hypo-endogeic); IV – shallow bioturbators: small, non-pigmented earthworms, low surface activity, with shallow galleries (link with epi-endogeics); V – litter dwellers: small and pigmented earthworms, a lot surface activity, with few shallow galleries (link with epigeics). VI – intermediate group: earthworms that don’t presents characteristics such as pigmentation (Capowiez *et al*. 2024).

Therefore, understanding how earthworm communities vary across different land use systems and the implications of this variation on soil physical, chemical, and biological properties is critical for the conservation and sustainable management of ecosystems (Smith *et al.* 2008). Analysis of earthworm ecology in different settings provides valuable insights into agricultural and environmental practices that promote soil health and biodiversity conservation.

Although earthworms have been extensively studied in various soil management systems in the state of Paraná (Brown et al. 2003, Bartz et al. 2013, 2024, Dudas et al. 2020), their populations are much less known in other states (Demetrio et al. 2019). This limits a more general understanding of the relationship between agricultural management and earthworm diversity and their potential relationships with soil and environmental factors. Hence, the aim of this study was to determine the composition of the earthworm community in three LUS in a wide geographical range of sites within the Brazilian Atlantic Forest and Cerrado biomes, and to assess the bioindication potential of the species found, relating them to soil management and soil attributes.

2.3 MATERIAL AND METHODS

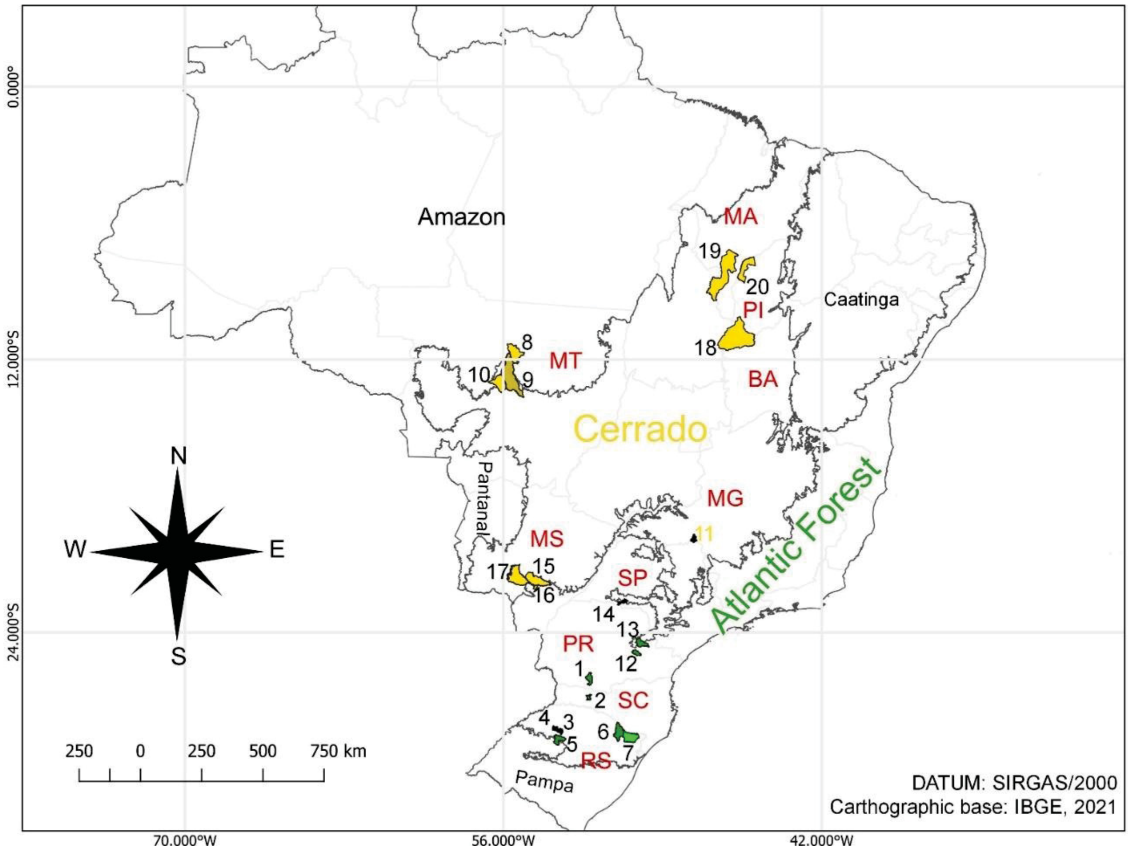
2.3.1 Study Sites

The sampling campaigns were conducted in 20 counties distributed across 10 states of Brazil, with the primary biomes sampled being the Atlantic Forest and Cerrado. Some transitional ecotone environments between Cerrado and Amazonia were sampled, like the city of Sinop (MT), and between the Atlantic Forest and Cerrado, like the cities Rio Brilhante, Douradina and Maracaju (MS) and Conquista (MG), and Pampa and Atlantic Forest, like Panambi, Ajuricaba and Cruz Alta (RS). These counties were classified as belonging to Cerrado and Atlantic Forest, respectively. Thus, overall, our study areas were distributed across 10 sites within the Cerrado biome and 10 sites within the Atlantic Forest (FIGURES 1 and 2).

The Cerrado region, characterized by a seasonally dry tropical climate, accounts for approximately 25% of Brazil’s territory. This climate is distinguished by two predominant seasons: a dry season (from May to October) and a rainy season (from November to June), with average annual precipitation ranging from 750 to 2000 mm (ICMBio, 2024). Furthermore, the Cerrado is recognized as a savanna with high biodiversity and endemism (IBGE, 2004), known as one of the world’s hotspots (Myers *et al.* 2000). The Atlantic Forest, occupying approximately 16% of the Brazilian territory, and in the southern and southeastern region of the country is characterized by a humid tropical climate with precipitation distributed throughout the year and temperatures ranging from 20°C to 30°C (IBF, 2024). It is considered the fifth most diverse area of fauna globally (IBGE 2004) and is another of the world’s biodiversity hotspots (Myers *et al.* 2000).

In each county, three Land Use Systems (LUS) were sampled: Native Vegetation (NV), corresponding to the typical natural vegetation cover of the biome (includes Cerradão, Cerrado strict sensu, steppe forest, semideciduous and mixed ombrophilous forest; No Tillage System (NTS); and an area with an area with some type of inadequate soil management/practice, designated as Bad Management (BM). TABLE 2 presents the characteristics of all the sites. At all sites, a sampling grid with nine monolith points was established (FIGURE 3), totaling 180 samples in each LUS, and an overall total of 540 samples, considering both biomes and the three LUS.

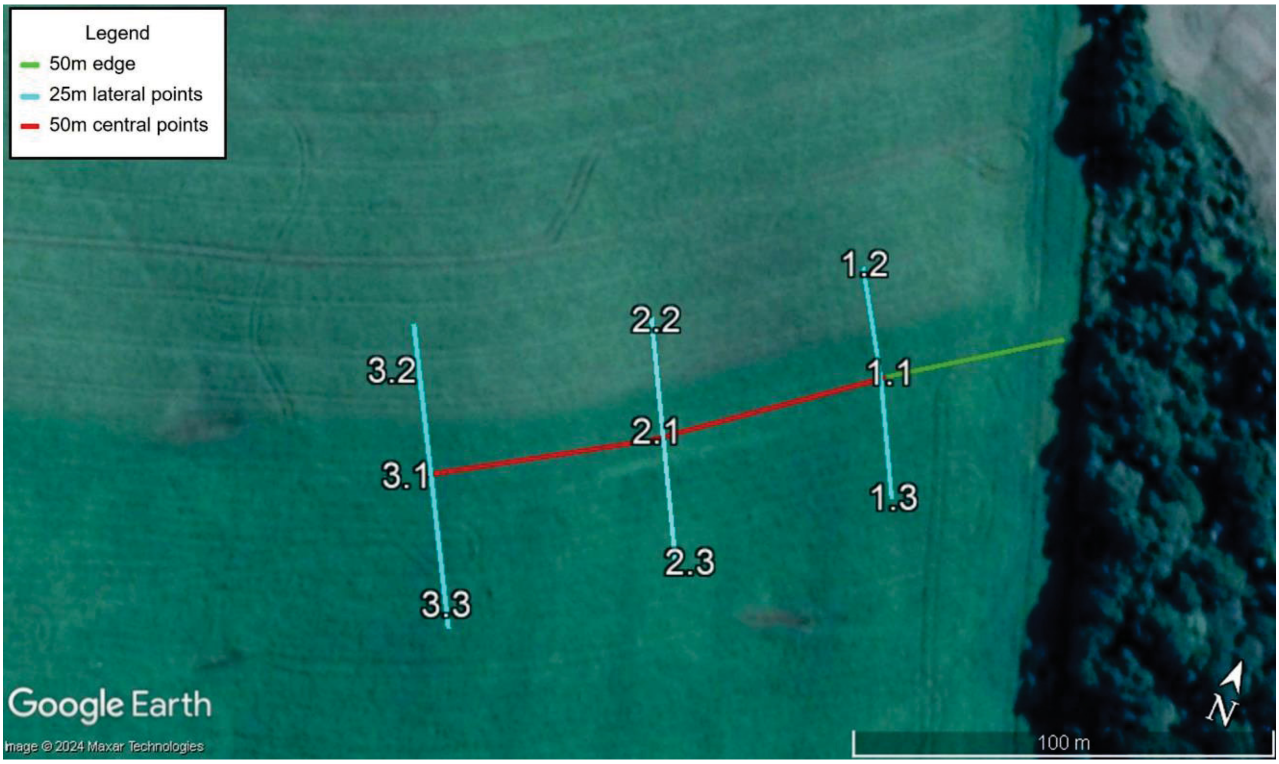
FIGURE 1 – MAP SHOWING THE LOCATIONS OF THE TWENTY COUNTIES IN BRAZIL WHERE SAMPLING WAS CONDUCTED AND THE BOUNDARIES OF EACH BIOME



SOURCE: Author (2024).

LEGEND: Brazilian biomes where sampling occurred: Cerrado (yellow) and Atlantic Forest (green). Brazilian states: MA: Maranhão, PI: Piauí, BA: Bahia, MT: Mato Grosso, MS: Mato Grosso do Sul, MG: Minas Gerais, SP: São Paulo, PR: Paraná, SC: Santa Catarina, RS: Rio Grande do Sul.Cities 1: Mangueirinha (PR), 2: Faxinal dos Guedes (SC), 3: Panambi, 4: Ajuricaba, 5:Cruz Alta, 6: Vacaria, 7: Bom Jesus (RS), 8: Sinop, 9: Sorriso, 10: Lucas do Rio Verde (MT), 11: Conquista (MG), 12: Carambeí, 13: Piraí do Sul (PR), 14: Maracaí (SP), 15: Rio Brilhante, 16: Douradina, 17: Maracaju (MS), 18: Formosa do Rio Preto (BA), 19: Balsas (MA) and 20: Ribeiro Gonçalves (PI). Other Brazilian biomes, where no sampling was conducted in this Project: Amazon, Caatinga, Pantanal and Pampa (black).

FIGURE 2 – SAMPLING GRID SCHEME USED IN ALL AREA



SOURCE: Author (2024).

TABLE 2 – SELECTED CHARACTERISTICS OF THE SAMPLING SITES, INCLUDING STATE, COUNTY, GEOGRAPHICAL COORDINATES, BIOME, CLIMATE, PRECIPITATION AND LAND USE SYSTEM (LUS)

Nº on map	**State**[1]	**County**	**Biome**[2]	**Climate**[3]	**Precipitation (mm)**[4]	**Sampling Date**	**LUS**[5]	**Location (Lat,Long, elevation**	**Site details**[6]
1	PR	Mangueirinha	AF	Cfb	446	08/23	NV	-26.05108, -52.235847, 966m	20 ha Mixed Ombrophilous forest fragment
							NTS	-26.051079, -52.237655, 981m	Annual crops - 27 years of NTS, with straw and a cover crops mixture at sampling. Size 15 ha
							BM	-26.049991, -52.25608, 966m	Annual crops - crop succession (soybean, corn and wheat) with soil preparation, with wheat planted at sampling. Size 33 ha
2	SC	Faxinal do Guedes	AF	Cfb	467	08/23	NV	-26.807988, -52.238571 924m	116 ha Mixed Ombrophilous forest fragment
							NTS	-26.785627,-52.229168, 883m	Annual crops - 28 years of NTS, with straw and a cover crops mixture at sampling. Size 143 ha
							BM	-26.813235, -52.247984, 870m	Annual crops - under no-tillage with crop succession (soybean, wheat or oat), and subsoiling from time to time, with oats planted at sampling. Size 30 ha
3	RS	Panambi	AF	Cfa	474	08/23	NV	-28.375519, -53.445185, 494m	23.8 ha steppe forest fragment
							NTS	-28.348351, -53.41567, 514m	Annual crops - 28 years of NTS, with straw and a oats at sampling. Size 128 ha
							BM	-28.371324, -53.418971, 494m	Annual crops - under no-tillage with crop succession (soybean and wheat or oats), and wheat planted at sampling. Size 37 ha
4	RS	Ajuricaba	AF	Cfa	427	08/23	NV	-28.167072, -53.814564, 419m	16 ha Seasonal Deciduous forest fragment
							NTS	-28.144014, -53.777835, 422m	Annual crops - 30 years of NTS, with straw and oats at sampling . Size 24 ha

Table 2 continuation…

Nº on map	State[1]	County	Biome[2]	Climate[3]	Precipitation (mm)[4]	Sampling Date	LUS[5]	Location (Lat,Long, elevation	Site details[6]
4	RS	Ajuricaba	AF	Cfa	427	8/23	BM	-28.149816, -53.774019, 385m	Annual crops - under no-tillage with crop succession (soybean and oat), and oats planted at sampling. Size 13 ha
5	RS	Cruz Alta	AF	Cfa	419	08/23	NV	-28.763297, -53.59013, 432m	17 ha steppe forest fragment
							NTS	-28.76386, -53.583866, 419m	Annual crops - 30 years of NTS, with straw of rolled cover crop mixture at sampling. Size 36 ha
							BM	-28.765973,-53.603882, 414m	Integrated system - olive grove with annual crops between the rows under conventional tillage, with oats planted at sampling. Size 24 ha
6	RS	Vacaria	AF	Cfb	431	08/23	NV	-28.391828, -51.082774, 879m	12 ha steppe forest fragment
							NTS	-28.370221, -51.078605, 920m	Annual crops - 37 years of NTS, with straw and oats planted at sampling. Size 45 ha
							BM	-28.418021, -51.047461, 918m	Integrated system - under no-tillage with crop succession and livestock grazing, and sporadic soil preparation; oats planted at sampling. Size 28 ha
7	RS	Bom Jesus	AF	Cfb	406	08/23	NV	-28.467102, -50,597372, 1003m	17 ha steppe forest fragment
							NTS	-28.485754, -50.609524, 1043m	Annual crops - 24 years of NTS, with straw and rye and oats planted at sampling. Size 125 ha
							BM	-28.469298, -50.671423, 976m	Integrated system - under no-tillage overgrazed and with oats planted at sampling. Size 12 ha
8	MT	Sinop	Ce	Am	704	02/24	NV	-11.812857, -55.441029, 384m	101 ha fragment of Cerradão-type forest

Table 2 continuation…

Nº on map	**State[1]**	**County**	**Biome [2]**	**Climate[3]**	**Precipitation (mm)[4]**	**Sampling Date**	**LUS [5]**	**Location (Lat,Long, elevation**	**Site details[6]**
8	MT	Sinop	Ce	Am	704	02/24	NTS	-11.814623, -55.441207, 361m	Annual crops - 30 years of NTS, with soil partially covered and straw and maize planted at sampling. Size 224 ha
							BM	-11.837736, -55.454047, 369m	Annual crops - area rented under no-tillage, with small amounts of straw and maize planted for silage at sampling . Size 15ha
9	MT	Sorriso	Ce	Aw	575	02/24	NV	-13.41935, -55.223485, 486m	106 ha Cerradão-type forest fragment
							NTS	-13.407872, -55.234652, 456m	Annual crops - 22 years of NTS with soil partially covered, with straw and maize planted at sampling. Size 82 ha
							BM	-13.409439, -55.230844, 469m	Pasture -with bare soil spots and overgrazed. And brachiaria plantation Size 7 ha
10	MT	Lucas do Rio Verde	Ce	Aw	697	02/24	NV	-13.371176, -56.161247, 403m	181 ha Cerrado sensu-stricto type fragment
							NTS	-13.273261, -56.087176, 429m	Annual crops - 27 years of NTS, with straw and oat plantation. Size 111 ha
							BM	-13.281358, -56.092964, 442m	Bare soil with recently seeded pasture (*Paspalum notatum*). Size 1 ha
11	MG	Conquista	Ce	Cwa	711	02/24	NV	-19.852722, -47.675057, 743m	18 ha Cerradão-type forest fragment
							NTS	-19.854886,-47.675168, 757m	Annual crops - 20 years of NTS with soil partially covered with straw and soybean planted at sampling. Size 23 ha
							BM	-19.856911, -47.672457, 722m	Annual crops - under no-tillage but with frequent tillage and crop succession (soybean and corn); newly planted soybean at sampling. Size 30 ha

TABLE 2 continuation…

Nº on map	State[1]	City	Biome[2]	Clime[3]	Precipitation (mm)[4]	Sampling Date	LUS[5]	Location (lat/lon/elevation	Characterization
12	PR	Carambeí	AF	Cfb	587	03/24	NV	-25.030129, -50.189709, 921m	43 ha Seasonal Semideciduous forest fragment
							NTS	-24.998144, -50.137432, 953m	Annual crops - 46 years of no-tillage without soil cover, corn planted for silage at sampling. Size 100 ha
							BM	-24.989568, -50.146001, 906m	Annual crops - under no-tillage with frequent tillage and crop succession, with soybean planted at sampling. Size 15 ha
13	PR	Piraí do Sul	AF	Cfb	575	03/24	NV	-24.312956, -50.130363, 1032m	51 ha Seasonal Semideciduous forest fragment
							NTS	-24.359189, -50.14082, 992m	Annual crops - 38 years of NTS, with straw of soybean recently harvested at sampling. Size 122 ha
							BM	-24.313634, -50.133242, 998m	Pasture area recently converted into an annual crop - with tillage and soybean planted at sampling. Size 15 ha
14	SP	Maracaí	AF	Cfa	587	03/24	NV	-22.668361, -50.880278, 390m	small 2 ha Seasonal Semideciduous forest fragment
							NTS	-22.666164, -50.878025, 395m	Annual crops - 30 years of NTS, with straw and oats planted at sampling. Size 97 ha
							BM	-22.664104, -50.887685, 386m	Annual crops - under conventional tillage and crop succession, with maize planted at sampling. Size 30 ha
15	MS	Rio Brilhante	Ce	Am	456	04/24	NV	-21.697567, -54.466489, 354m	96 ha Cerradão-type forest fragment
							NTS	-21.717408, -54.482286, 357m	Integrated - 30 years of NTS with soil partially covered with straw and maize planted with *Brachiaria* sp. at sampling. Size 66 ha

TABLE 2 continuation…

Nº on map	**State[1]**	**City**	**Biome[2]**	**Clime[3]**	**Precipitation (mm)[4]**	**Sampling Date**	**LUS[5]**	**Location (lat/lon/elevation**	**Characterization**
15	MS	Rio Brilhante	Ce	Am	456	04/24	BM	-21.711077, -54.459221, 323m	Pasture, with brachiaria, - over 20 years old and spots of bare soil. Size 9 ha
16	MS	Douradina	Ce	Am	442	04/24	NV	-22.034549, -54.592499, 360m	8 ha Cerradão-type forest fragment
							NTS	-22.017011, -54.592261, 324m	Annual crops - 27 years of NTS with soil partially covered with straw and acrop mixture recently planted at sampling. Size 60 ha
							BM	-22.014234, -54.584546, 352m	Pasture with brachiaria, - with signs of degradation (termite mounds and bare soil). Size 17 ha
17	MS	Maracaju	Ce	Am	456	04/24	NV	-21.70826, -55.608399, 609m	12 ha Cerradão-type forest fragment
							NTS	-21.699578, -55.606883, 609m	Annual crops - 37 years of NTS, with straw and maize planted with a mixture of grasses at sampling. Size 108 ha
17	MS	Maracaju	Ce	Am	456	04/24	BM	-21.70238, -55.632698, 610m	Pasture, with brachiaria, - over 10 years old, with no signs of degradation, and native plants growing. Size 11 ha
18	BA	Formosa do Rio Preto	Ce	Aw	370	05/24	NV	-10.416057, -45.543317, 800m	285 ha Cerrado sensu-stricto fragment
							NTS	-10.375206, -45.534562, 807m	Annual crops - 20 years of NTS, with straw and maize planted at sampling. Size 277 ha
							BM	-10.428958, -45.60349, 812m	Annual crops -, under no-tillage and with frequent tillage and crop succession, with almost no soil cover and in fallow at sampling. Size 508 ha
19	MA	Balsas	Ce	Aw	489	05/24	NV	-8.521612, -46.175366, 532m	199 ha Cerrado sensu-stricto fragment

TABLE 2 continuation…

Nº on map	**State[1]**	**City**	**Biome [2]**	**Clime [3]**	**Precipitation (mm)[4]**	**Sampling Date**	**LUS [5]**	**Location (lat/lon/elevation**	**Characterization**
19	MA	Balsas	Ce	Aw	489	05/24	NTS	-8.567386, -46.19787, 555m	Annual crops - 20 years of NTS, with straw and maize planted with *Brachiaria* sp.at sampling. Size 131 ha
							BM	-8.559989, -46.171032, 553m	Annual crops - under no-tillage, with frequent soil tillage and crop succession, with maize planted at sampling. Size 50 ha
20	PI	Ribeiro Gonçalves	Ce	Aw	337	05/24	NV	-8.381882, -45.510471, 570m	724 ha Cerrado sensu-stricto fragment
							NTS	-8.407016, -45.453937, 558m	Annual crops - > 20 years of NTS with soil partially covered with straw and maize planted with *Brachiaria* sp. in low density at sampling. Size 562
							BM	-8.419253, -45.563019, 490m	Annual crop - under no-tillage with crop succession, converted from Cerrado native vegetation to annual crops in 2009/2010, with soil partially covered with straw and nothing planted at sampling. Size 434 ha

Source: Author (2024)

Legend: [1]: corresponding number on the maps in figures 1 and 2; [2]: AF = Atlantic Forest, Ce: Cerrado; [3]Clime according to Köppen (1973); [4]: Accumulated precipitation in the three months prior to sampling; [5]: NV: native vegetation, NTS: No-Tillage System, BM: Bad management; **[6]** For the LUS NTS and BM the description of the crops and plant residues refers to the sampling moment; vegetation types are provided according to the classification of the IBGE (2012).

2.3.2 Earthworm and soil sampling and analysis

Earthworms were collected using two methodologies. The first was an adaptation of the quantitative method developed by the Tropical Soil Biology and Fertility (TSBF) Programme of UNESCO (Anderson; Ingram, 1993), wherein nine monoliths measuring 25 x 25 cm and 20 cm in depth were excavated in all sites. The soil from the monolith was manually sorted, and the specimens found were preserved in 96% ethanol. The second method was qualitative (Bartz *et al.* 2014a) and involved excavating strategic locations at the sites, such as within and beneath logs, in bromeliads, and areas with higher organic matter content, to increase detection of earthworm species and obtain a more accurate species richness estimate. Usually, the number of samples in qualitative is close to the number of monoliths removed in the quantitative sampling, in this case close to nine samples. The strategic locations must be within the sampled area so that at the end of the entire sampling campaign, including both methods, the size corresponds to 1 hectare of sampled area.

Earthworms were identified at the species level through classical taxonomy, using keys from Righi (1971, 1995), Schwert (1991), Chang *et al.* (2016), Feijoo and Brown (2017), Bartz and James (2018), Reynolds and Misirlioğlu (2018), and Feijoo and Brown (2023). Following identification, all specimens were quantified (for abundance - ind m^{-2}) and weighed (for biomass - g m^{-2}) in the laboratory.

For chemical analyses and characterization 300 g of soil was collected from the same location as the monolith, to determine the macro and micronutrient contents (phosphorous, zinc, boron, copper, manganese, sulfur, nitrogen, Cation Exchange Capacity, carbon, iron, potassium, magnesium, calcium) and particle size distribution (sand, silt and clay content). The soil was analyzed following standard methods of IAC (2001), at the IBRA Megalab in Sumaré, São Paulo. For physical analysis, a soil core was extracted, adjacent to the monolith to measure bulk density and moisture content (Teixeira *et al.* 2017).

2.3.3 Data analysis

The total abundance and species richness were utilized to compare the sampling methods. To demonstrate the sampling effort, a rarefaction curve was generated using iNExt package from R software (R Core Team, 2024). To compare the total results between different collection methods, we applied the Student’s test for independent samples, assuming different variations between groups. This test allowed us to identify significant differences between the methods, with a significance level of 5%. The comparison between the methods was carried out in order to demonstrate the efficiency of both methods and to prove that the use of more than one method is more effective for collecting more species of earthworms. The Indicator Value Analysis (IndVal) employed data on abundance from both methods to determine the indicator potential of species and/or families; we utilized the packages labdsv, readxl, and dplyr from R (R Core Team, 2024) to conduct all the analyses.

For comparison between biomes and LUS, considering only the quantitative method (TSBF), the earthworm data employed were species richness, and abundance (individuals) and biomass (g), transformed to per square meter (m^{-2}). To evaluate the differences in earthworm abundance and biomass between the LUS and biomes, we employed the Kruskal-Wallis test, suitable for nonparametric data and for the comparison of multiple groups. When the Kruskal-Wallis test indicated significant difference, post-hoc comparisons were conducted using the Dunn test with Bonferroni adjustment to control for type I errors. Statistical differences between groups are represented by lowercase letters for comparisons between LUS within the same biome, and uppercase letters for comparisons between the same LUS in different biomes.

Data visualization was conducted using violin plots, generated with the ggplot2 package in the R software. The violin charts incorporated all the data, providing a comprehensive representation of data variation within each group. Transformed data previously selected to reduce redundancy between highly correlated variables were used for correlation analyses between the biological, chemical and physical attributes, conducted using the 'ADE4' package of R (R Core Team, 2024).

2.4 RESULTS AND DISCUSSION

2.4.1 Soil properties

The results of soil chemical and physical attributes showed important differences regarding the influence of management and biomes in the sampling sites (TABLE 3, FIGURE 3). TABLE 3 presents the characteristics of each LUS, considering texture, clay, silt and sand content, bulk density and moisture, in addition to macro and micronutrients. Statistical analyses (Kruskal-Wallis) were performed to determine whether there were differences between the attributes within the same city but under different management systems. Only a few sites showed differences for sum of bases (SB) and the micronutrients boron (B), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn), represented by the letters next to the values. FIGURE 3 indicates the association between these attributes quantified in TABLE 3 and the Atlantic Forest and Cerrado biomes and between the three LUS sampled.

The pH exhibited low values in NV areas, ranging from 3.7 to 5.8. This observation was anticipated, as natural soils tend to be more acidic due to the higher content of organic matter and, consequently, its decomposition and leaching (Menezes *et al*. 2017). For NTS, pH values ranged from 4.6 to 6.1, which can be attributed to liming practices applied in this type of management (Neves *et al*. 2012). For BM areas, pH ranged from 4.3 to 5.7, which may be indicative of inadequate management (TABLE 3).

Soil P values were low in NV areas, ranging from 1.8 mg/dm^3 to 23.9 mg/dm^3, which underscores the low natural availability of this nutrient, as previously noted in other studies (Brady and Weil 2008). In contrast, NTS exhibited a greater variation, from 12.2 mg/dm^3 to 69.7 mg/dm^3, which may be attributed to phosphate fertilization and the implementation of conservation management (Ogle *et al.* 2014). For BM, phosphorus ranged from 4.0 mg/dm^3 to 50.7 mg/dm^3, which may indicate either uncontrolled fertilization or increased loss through leaching (TABLE 3).

The CEC was higher in clayey soils of the Atlantic Forest, with values ranging from 80 to 340 mmolc/kg. For NTS, the variation was from 86 to 206 mmol/kg, which demonstrates partial conservation, indicating adequate management, while in BM, due to compaction and/or loss of organic matter, the CEC was lower.

Regarding organic matter (OM), the NV areas had higher levels, ranging from 7.6 to 68.8 g/kg, due to a constant addition of plant residues (Inkotte *et al.* 2022). The NTS exhibited moderate values ranging from 15.8 to 46 g/kg, attributable to the permanent straw cover, which is one of the principles of this management system. For BM, the values ranged from 1 to 51 g/kg, reflecting the absence of vegetation cover or the presence of pasture grass in the sampling sites. Associated with OM, the carbon values followed a similar pattern, with the Atlantic Forest having higher levels. Soils with high C content promote greater biological activity due to the benefits resulting from an adequate content of this nutrient (Lal, 2007). According to Chang *et al.* (2009), soils with higher clay and OM contents can retain more basic cations, which enhances soil CEC, fertility and biodiversity. Nitrogen (N) in the NV area ranged from 0.1 to 0.5 g/kg, with higher values corresponding to greater OM contributions in forest areas. The Cerrado exhibited naturally low levels due to reduced accumulation of organic residues. For agricultural areas, both NTS and BM, the values ranged from 0.1 to 0.4 g/kg.

The sum of bases (SB) in NV ranged from 6.7 to 98.4 mmolc/kg, in NTS from 25.8 to 165.8 mmolc/kg, and in BM from 10.7 to 140 mmolc/kg. In the agricultural systems, liming and fertilization increases available bases and soil fertility (Inkotte *et al.* 2022). Soil micronutrients (S, B, Cu, Mn, Fe and Zn) exhibited variations between the LUS and biomes. In NV, there was a balance in Fe and Mn levels, due to soil acidity resulting from OM accumulation. Regarding biomes, the Atlantic Forest had higher values for micronutrients compared to the Cerrado. This may be associated with higher clay levels and higher CEC, which can store these micronutrients for gradual release, whereas in soils with lower CEC, these nutrients are lost more rapidly, and some nutrients are frequently deficient (Fageria *et al.* 2002; Alloway, 2008).

In agricultural areas, NTS exhibited higher levels of micronutrients compared to BM, due to the quantity of OM and the fertilizers used. Essential elements for plant and organism development, such as Zn and B, were more available in NTS (Brennan, 2005). In BM, low levels of Cu and Mn may reflect the impact of soil erosion and compaction. These results emphasize the importance of more conservationist management practices to mitigate nutrient loss and ensure soil fertility, nutrient cycling, and consequently, soil biodiversity (Ogle *et al*. 2019).

The first two axes of the principal component analyses (PCA) explained 66.4% of the data variation (FIGURE 3). Soils with higher clay content were associated with the Atlantic Forest, while soils in the Cerrado had greater sand contents. The highest nutrient contents were observed in Atlantic Forest sites, while higher bulk density was observed in the Cerrado sites. Regarding LUS, there was a clear relationship between NV and lower pH, higher exchangeable acidity, higher soil C contents and lower bulk density. On the other hand the agricultural sites had higher bulk density and pH and lower exchangeable acidity.

TABLE 3 – SOIL PROPORTIES OF ALL THE SAMPLED SITES PER LAND USE SYSTEM

Biome^1	**State^2**	**City**	**LUS^3**	**Clay**	**Silt**	**Sand**	**Texture**	**Bulk density (g/cm^3)**	**Moisture %**	**C**	**N**	**OM**	**pH**	**P**	**Al ^{3}**	**H° + Al ^{3}**	**SB**	**CEC**	**S**	**B**	**Cu**	**Mn**	**Fe**	**Zn**
				g/kg						g/kg	NIR %	g/dm^3	CaCl$_2$	mg/dm^3										
AF	PR	Mangueirinha	1NV	517	227	256	Clay	0,55	61	68,8	0,5	44	3,7	21,6	34,9	77,4	27,8c	330	30,2	0,6b	5,4b	7,7a	191a	0,41c
AF	PR	Mangueirinha	1NTS	537	269	194	Clay	0,98	33	46,0	0,3	45	5,5	31,8	0,0	38,1	160,5a	198	17,4	1,1a	9,2a	5,5ab	60b	2,6a
AF	PR	Mangueirinha	1BM	480	253	267	Clay	0,92	53	51,9	0,4	49	5,4	20,8	0,1	58,7	140,1b	199	17,7	0,9a	6,3b	3,4b	68b	1,3b
AF	SC	Faxinal dos Guedes	2NV	709	167	124	Clay	0,56	70	54,1	0,5	45	3,9	6,7	14,1	196,2	23,8c	220	27,2	0,93	1,40	10,9a	82a	0,7b
AF	SC	Faxinal dos Guedes	2NTS	588	292	120	Clay	0,75	52	38,9	0,3	39	4,6	32,7	1,0	96,2	83,03b	179	24,8	1,15	1,37	4,4b	67,3a	0,83b
AF	SC	Faxinal dos Guedes	2BM	656	280	65	Clay	0,96	45	33,7	0,3	35	5,5	40,0	0,0	36,4	126,19	162	15,0	1,11	1,46	6,7b	34,5b	1,1a
AF	RS	Panambi	3NV	486	289	224	Clay	0,77	38	32,3	0,3	33	4,5	6,9	3,9	82,4	84,07	166	19,1	1,1a	6,03a	140a	46,4a	2,1a
AF	RS	Panambi	3NTS	423	182	395	Clay	1,24	29	22,2	0,2	27	5,4	21,1	0,1	35,0	97,49	132	13,0	0,7b	3,4b	12,1c	26,4b	1,1b
AF	RS	Panambi	3BM	496	206	298	Clay	1,16	30	19,6	0,2	24	5,1	4,0	0,6	48,3	85,84	134	10,9	0,66b	3,8b	36,4b	19,1c	0,31c
AF	RS	Ajuricaba	4NV	572	288	140	Clay	0,92	40	38,7	0,3	29	5,3	14,1	0,0	41,4	146,76	188	25,4	2a	9,3ab	158a	35,8a	5,4a
AF	RS	Ajuricaba	4NTS	552	256	192	Clay	0,98	35	23,0	0,3	29	5,3	46,0	0,0	45,2	126,16	171	28,6	1,4ab	10,1a	102b	21,8b	1,4b
AF	RS	Ajuricaba	4BM	601	263	136	Clay	1,00	32	19,4	0,2	20	5,1	27,1	0,0	57,1	107,18	164	22,6	1,3b	7,9b	66b	15,4b	0,96b
AF	RS	Cruz Alta	5NV	376	249	375	Clay loam	1,05	28	30,2	0,3	24	4,4	7,1	4,6	77,8	65,8c	143,6	12,8	0,77	6,1a	84a	51,7a	1,60
AF	RS	Cruz Alta	5NTS	437	234	329	Clay	1,33	26	24,9	0,2	28	5,6	12,2	0,0	25,8	125,8a	151	16,1	0,79	3,4a	20,2b	22b	4,23
AF	RS	Cruz Alta	5BM	468	233	299	Clay	2,27	15	15,4	0,2	21	5,7	9,6	0,8	30,4	92,7b	123	14,0	0,72	6,4b	18,6b	24,5b	1,64
AF	RS	Vacaria	6NV	564	299	137	Clay	1,06	39	26,9	0,3	28	4,7	18,4	0,0	75,2	98,2b	173	21,7	1,62	10,9a	125,1a	92,8a	2,1a
AF	RS	Vacaria	6NTS	546	262	193	Clay	1,44	28	33,0	0,3	37	5,1	64,6	0,0	53,2	130,3a	183	28,1	1,32	6,5b	11,5b	43b	1,08b
AF	RS	Vacaria	6BM	612	277	111	Clay	1,93	34	28,0	0,3	32	5,2	43,4	0,0	50,0	124,8a	174	18,9	1,15	6,6b	10,7b	60b	0,5c
AF	RS	Bom Jesus	7NV	381	369	249	Clay loam	1,03	64	46,8	0,3	42	3,7	7,4	36,7	300,8	39,7c	340	15,3	0,59b	1,7c	7,64	200a	0,5ab
AF	RS	Bom Jesus	7NTS	461	254	285	Loam	1,38	31	45,0	0,3	44	5,5	19,2	0,0	40,4	165,8a	206	13,9	1,05a	3,3b	10,64	60c	0,7a
AF	RS	Bom Jesus	7BM	402	362	236	Clay	1,65	31	31,9	0,3	39	4,8	6,0	1,4	85,3	114,2b	199	15,0	0,85ab	4,3a	7,60	102b	0,4b
Ce	MT	Sinop	8NV	440	194	366	Clay	1,22	32	24,9	0,1	21	3,9	3,9	8,6	89,7	10,7b	100	9,8	0,79b	0,3b	4,8a	182a	0,36b
Ce	MT	Sinop	8NTS	491	134	375	Clay	1,45	38	29,1	0,1	24	5,9	44,0	< 0,05	25,1	63a	88	9,8	1,7a	0,6a	1,7b	46b	2,06a
Ce	MT	Sinop	8BM	537	136	326	Clay	1,52	30	23,5	0,1	17	5,5	50,7	< 0,05	27,7	47,97	75	18,6	1,3a	0,3b	1,7b	49b	1,26a
Ce	MT	Sorriso	9NV	538	243	219	Clay	1,14	36	32,0	0,2	26	4,0	4,4	8,4	92,4	7,5c	100	6,2	0,74b	0,42b	2,24a	141,7a	0,24b

TABLE 3 continuation…

Biome^1	**State^2**	**City**	**LUS^3**	**Clay**	**Silt**	**Sand**	**Texture**	**Bulk density** (g/cm3)	**Moisture %**	**C**	**N**	**OM**	**pH**	**P**	**Al ^{3}**	**H° + Al ^{3}**	**SB**	**CEC**	**S**	**B**	**Cu**	**Mn**	**Fe**	**Zn**
				g/kg						g/kg	NIR %	g/dm³	CaCl2	mg/dm³										
Ce	MT	Sorriso	9NTS	575	251	174	Clay	1,37	38	32,6	0,2	28	5,7	26,8	< 0,05	27,6	62,4a	90	20,6	1,29a	1,08a	0,91b	23,3b	5,08a
Ce	MT	Sorriso	9BM	546	251	203	Clay	1,51	36	33,0	0,2	29	4,9	24,2	1,0	47,1	35,7b	83	10,3	0,96b	1,3a	1,08b	52,4b	5,5a
Ce	MG	Conquista	11NV	511	268	221	Clay	0,95	38	47,5	0,4	38	5,8	23,9	< 0,05	40,8	178,8a	219	5,8	1,96a	10,7a	76a	25,6b	2,2b
Ce	MG	Conquista	11NTS	501	262	238	Clay	1,11	36	28,7	0,2	31	5,8	29,2	< 0,05	27,8	76,8b	104	13,6	1,6a	7,6b	7,4b	14,4c	0,87c
Ce	MG	Conquista	11BM	553	238	209	Clay	1,20	34	36,9	0,2	31	5,0	15,8	< 0,05	47,3	41,1b	88	6,3	0,74b	2,9c	1,84b	63,3a	5,9a
AF	PR	Carambeí	12NV	262	153	586	Sandy clay loam	0,58	27	48,5	0,4	35	4,0	7,7	22,9	186,1	18,7c	204	30,0	0,72b	1,3b	17,13a	154a	1,42b
AF	PR	Carambeí	12NTS	517	169	314	Clay	1,05	25	31,2	0,3	32	5,0	69,7	1,5	64,1	67,1a	131	37,6	1,14a	10,2a	3,84b	36b	8,7a
AF	PR	Carambeí	12BM	215	94	691	Sandy clay loam	1,42	24	12,1	0,2	19	5,0	16,7	1,0	32,8	33,02b	66	9,8	0,52c	0,95b	2,2b	39b	0,77b
AF	PR	Piraí do Sul	13NV	262	161	577	Clay	0,61	30	49,4	0,4	39	3,9	5,4	27,0	215,9	13,c	229	26,1	0,50b	1,3ab	4,5a	150a	0,7a
AF	PR	Piraí do Sul	13NTS	561	202	238	Sandy clay loam	0,90	39	40,1	0,4	36	5,4	29,6	1,0	44,3	111,17a	155	19,6	1a	1,1b	1,7b	45b	0,52b
AF	PR	Piraí do Sul	13BM	370	88	542	Sandy clay loam	1,00	26	30,3	0,3	28	5,2	13,0	< 0,05	41,9	69,5b	111	78,9	1a	1,5a	2,8ab	57b	0,64a
AF	SP	Maracaí	14NV	607	206	186	Clay	0,82	29	29,5	0,3	30	6,0	12,2	< 0,05	23,2	119a	142	12,1	2,03a	6,2c	47,5b	7,4b	3,3b
AF	SP	Maracaí	14NTS	663	144	192	Clay	1,15	25	17,2	0,2	23	5,7	49,3	< 0,05	27,9	82b	110	30,1	0,92b	8,9b	51b	8,6b	4,5ab
AF	SP	Maracaí	14BM	603	186	211	Clay	1,17	23	15,4	0,2	22	5,3	32,8	< 0,05	37,6	79b	117	26,7	0,80b	12,3a	92a	13,7a	5,9a
Ce	MS	Rio Brilhante	15NV	625	226	149	Clay	0,89	31	23,9	0,2	26	5,4	8,4	4,5	40,3	93,28	133	8,6	1,40	6,93	56,89	13,22	0,86
Ce	MS	Douradina	16NV	618	215	167	Clay	0,85	26	16,6	0,2	21	4,2	6,4	12,7	93,6	30,7b	124	11,9	0,92b	9,1b	50,2b	17,67	1,03b
Ce	MS	Douradina	16NTS	578	196	226	Clay	1,26	17	19,2	0,3	30	5,4	41,2	-	37,8	139a	177	17,0	1,62a	18,7a	88a	14,67	12,1a
Ce	MS	Douradina	16BM	599	179	222	Clay	1,19	16	9,6	0,2	19	4,3	4,8	12,7	81,1	27,2b	108	8,3	0,66b	7,67b	38,6b	15,56	0,55b
Ce	MS	Maracaju	17NV	226	348	427	Loam	0,67	75	30,4	0,2	34	4,2	10,3	14,7	135,0	20,04b	155	13,0	0,52b	7,2b	7,6b	311a	0,85b
Ce	MS	Maracaju	17NTS	264	174	562	Sandy clay loam	1,13	14	26,7	0,2	28	5,6	27,6	< 0,05	24,9	79,1a	104	15,8	0,96a	2,4a	2,2c	34b	2,5a
Ce	MS	Maracaju	17BM	487	192	321	Clay	1,18	15	13,8	0,2	23	4,3	4,1	10,4	82,7	25,9b	108	15,9	0,41b	10,2a	33,5a	26b	0,68b
Ce	BA	Formosa do rio Preto	18NV	325	237	438	Clay loam	1,04	11	24,0	0,1	19	3,9	1,8	9,7	73,7	6,7c	80	6,0	0,48b	0,1b	1,17b	80,4a	0,12c

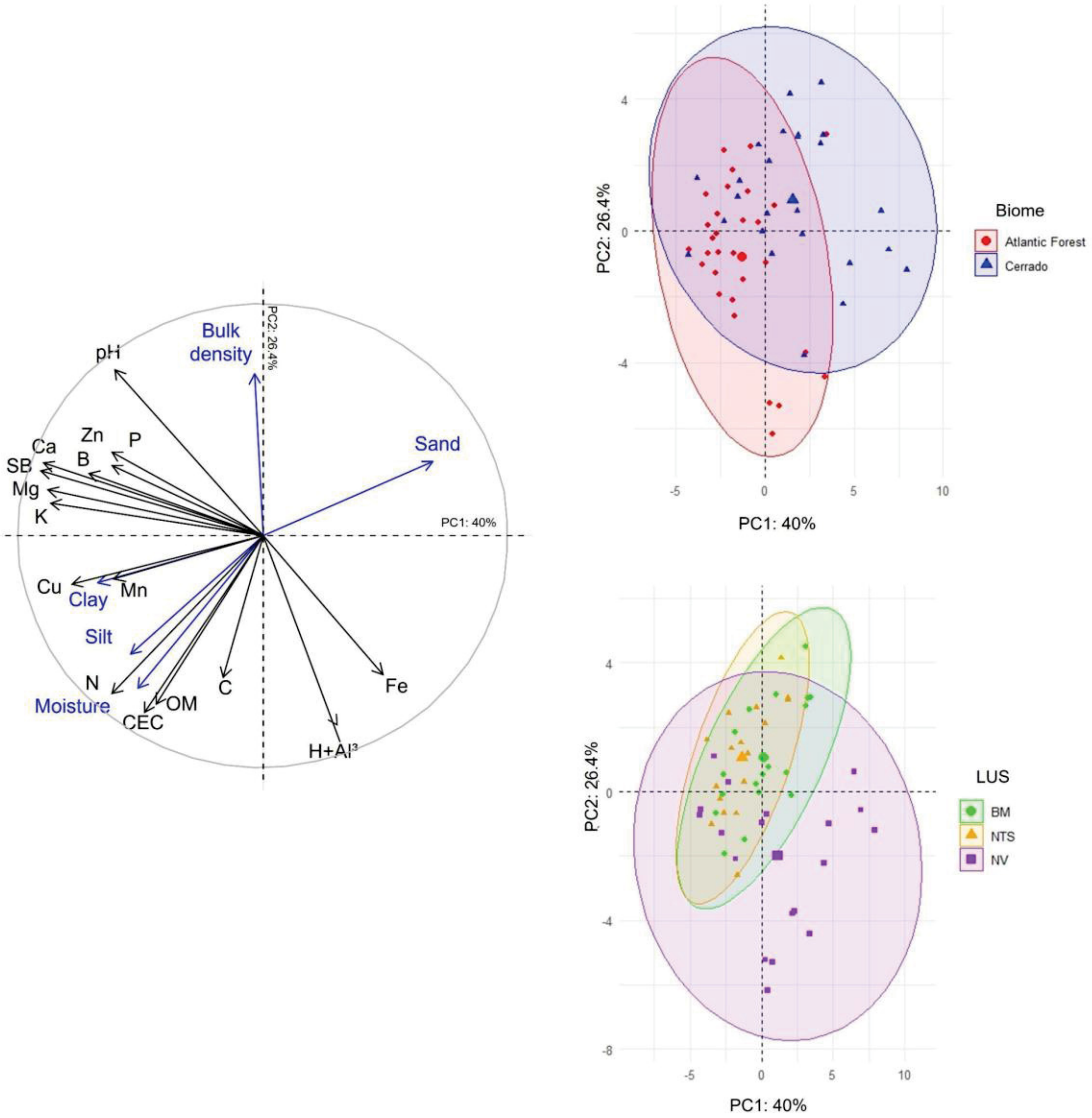
TABLE 3 continuation…

Biome[1]	**State**[2]	**City**	**LUS**[3]	**Clay**	**Silt**	**Sand**	**Texture**	**Bulk density**	**Moisture**	**C**	**N**	**OM**	**pH**	**P**	**Al ^{3}**	**H° + Al ^{3}**	**SB**	**CEC**	**S**	**B**	**Cu**	**Mn**	**Fe**	**Zn**
				g/kg				(g/cm3)	**%**	g/kg	NIR %	g/dm³	CaCl2	mg/dm³										
Ce	BA	Formosa do rio Preto	18NTS	246	141	612	Sandy clay loam	1,33	15	27,0	0,1	17	6,1	15,8	< 0,05	13,9	72,2a	86	8,9	1,26a	0,54a	2,2a	19,5b	1,16a
Ce	BA	Formosa do rio Preto	18BM	259	138	603	Sandy clay loam	1,27	6	23,7	0,1	17	5,0	16,9	< 0,05	39,2	37,7b	77	8,3	0,83b	0,33ab	2,5a	78a	0,55b
Ce	MA	Balsas	19NV	294	102	604	Sandy clay loam	1,12	10	25,1	0,1	23	4,3	2,0	7,6	73,3	12,6b	86	9,3	0,60b	0,1b	0,66b	75,5a	0,15b
Ce	MA	Balsas	19NTS	229	123	648	Sandy clay loam	1,16	9	28,8	0,1	24	5,3	28,9	< 0,05	33,1	58,2a	91	5,1	1,09a	0,78a	1,3a	54,6b	1,7a
Ce	MA	Balsas	19BM	280	133	586	Sandy clay loam	1,18	9	26,0	0,1	23	5,2	13,8	< 0,05	31,4	50,7a	82	6,7	0,80ab	0,08b	0,73b	50,2b	0,47b
Ce	PI	Ribeiro Gonçalves	20NV	231	113	655	Sandy clay loam	0,94	10	28,1	0,1	27	3,8	2,2	13,9	120,9	5,2c	126	6,0	0,47b	0,1b	0,64b	100a	0,16c
Ce	PI	Ribeiro Gonçalves	20NTS	242	109	649	Sandy clay loam	1,19	9	31,6	0,1	24	5,3	55,8	< 0,05	37,1	63,3a	100	8,1	1,03a	0,43a	2,08a	68b	3,9a
Ce	PI	Ribeiro Gonçalves	20BM	186	104	710	Sandy loam	1,39	7	23,9	0,1	17	5,3	30,0	< 0,05	27,4	51,9b	79	6,1	1,06a	0,12b	1,1b	41c	1,3b

Source: Author (2024)

Legend: [1] Brazilian biomes - AF: Atlantic Forest, Ce: Cerrado; [2]Brazilian states - PR: Paraná, SC: Santa Catariana, RS: Rio Grande do Sul, MT: Mato Grosso, SP: São Paulo, MS: Mato Grosso do Sul, BA: Bahia, MA: Maranhão; PI: Piauí. [3]Land Use System: NV: native vegetation, NTS: No-Tillage System, BM: Bad management. The numbers correspond to the location on the map (FIGURE 1). Chemical properties: C: carbon, N: nitrogen, OM: organic matter, P: phosphorous, Al: aluminum, $H+Al^{3}$: total acidity, SB: sum of bases (K^{+}, Ca^{+} and Mg^{+}), CEC: cation exchange capacity, S: sulfur, B: bore, Cu: copper, Mn: manganese, Fe: iron and Zn: zinc. Letters indicate statistical differences among LUS in the same city.

FIGURE 3 – PRINCIPAL COMPONENT ANALYSES WITH SOIL CHEMICAL AND PHYSICAL ATTRIBUTES



Source: Author (2024)

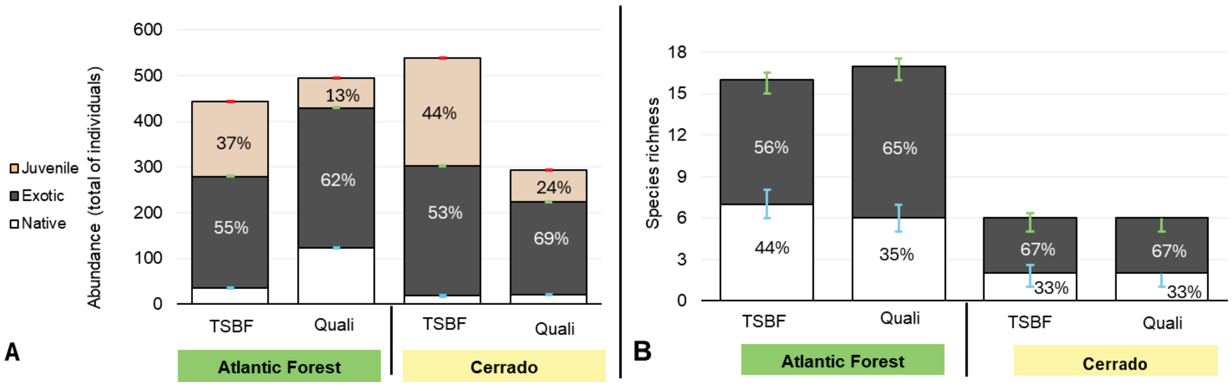
Legend: Chemical properties in black: C: carbon, N: nitrogen, OM: organic matter, P: phosphorous, Al: aluminum, H+Al^3: total acidity, SB: sum of bases, K potassium, Ca: calcium, Mg: magnesium, CEC: cation exchange capacity, S: sulfur, B: bore, Cu: copper, Mn: manganese, Fe: iron and Zn: zinc. Physical properties in blue: bulk density, moisture, clay, sand and silt. Land Use System (LUS): NV native vegetation, NTS: No-Tillage System, BM: bad management.

2.4.2 Earthworm abundance and diversity

A total of 1769 earthworms were collected across all LUS and biomes sampled, using both methods (quantitative + qualitative). The Atlantic Forest had higher abundance (n = 938), with 577 in No-Tillage System (NTS), 199 in Bad Management (BM), and 162 in Native Vegetation (NV). In the Cerrado, 831 individuals were distributed as 370 worms in NTS, 309 individuals in BM, and 152 in NV. Of the total earthworms collected, 981 were obtained using TSBF and 791 with qualitative sampling.

When comparing the methods in both biomes, abundance was approximately equivalent for the Atlantic Forest (TSBF=443; qualitative= 495); however, the number of native, exotic, and juvenile individuals differed. Despite differences in quantity, exotic species were predominant in this biome. The same pattern applies to the Cerrado, but with a higher abundance observed using the TSBF method (TSBF= 538; qualitative= 293) as can be observed in FIGURE 4A. Regarding species richness, when combining both methods, exotic species predominated in both biomes, with the Atlantic Forest exhibiting higher richness with 21 species overall (where Cerrado had six species), and a greater number of native species despite the predominance of exotics (FIGURE 4B). Statistical analysis using Student t-test revealed no significant differences at the 5% level between methods for either of the biomes.

FIGURE 4 – COMPARISON BETWEEN EARTHWORM SAMPLING METHODS: TROPICAL SOIL BIOLOGY FERTILITY (TSBF) AND QUALITATIVE (QUALI), IN DIFFERENT BRAZILIAN BIOMES

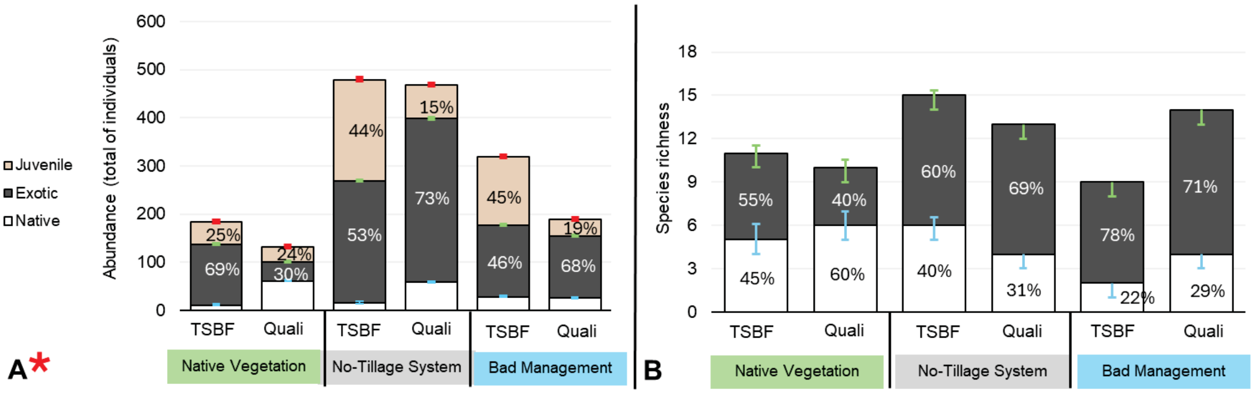


Source: Author (2024)

Legend: Earthworms found in Atlantic Forest and Cerrado biomes using two methods **A.** Total abundance and number of native/exotic/juvenile individuals. **B.** Species richness and number of native/exotic species. Standard error bars are indicated within each column according to the worm category. Red: juvenile; green: exotic and blue: native. The percentage corresponds to juvenile, exotic and native individuals in each of the samples.

In relation to the LUS, NTS had higher abundance for both methodologies (TSBF=479; Quali=468), followed by BM (TSBF=319; Quali=189) and NV (TSBF=183; Quali=131). The TSBF method yielded a higher number of individuals across all sites, and when compared with the qualitative method in the LUS, it was the sole comparison that demonstrated statistically significant differences using Student's t-test, this difference was for all of the LUS (FIGURE 5A). For species richness, a similar pattern was observed, with greater richness in NTS, and apart from BM, a higher number of species was found by TSBF (FIGURE 5B).

FIGURE 5 – COMPARISON BETWEEN EARTHWORM SAMPLING METHODS: TROPICAL SOIL BIOLOGY FERTILITY (TSBF) AND QUALITATIVE (QUALI), IN DIFFERENT LAND USE SYSTEMS

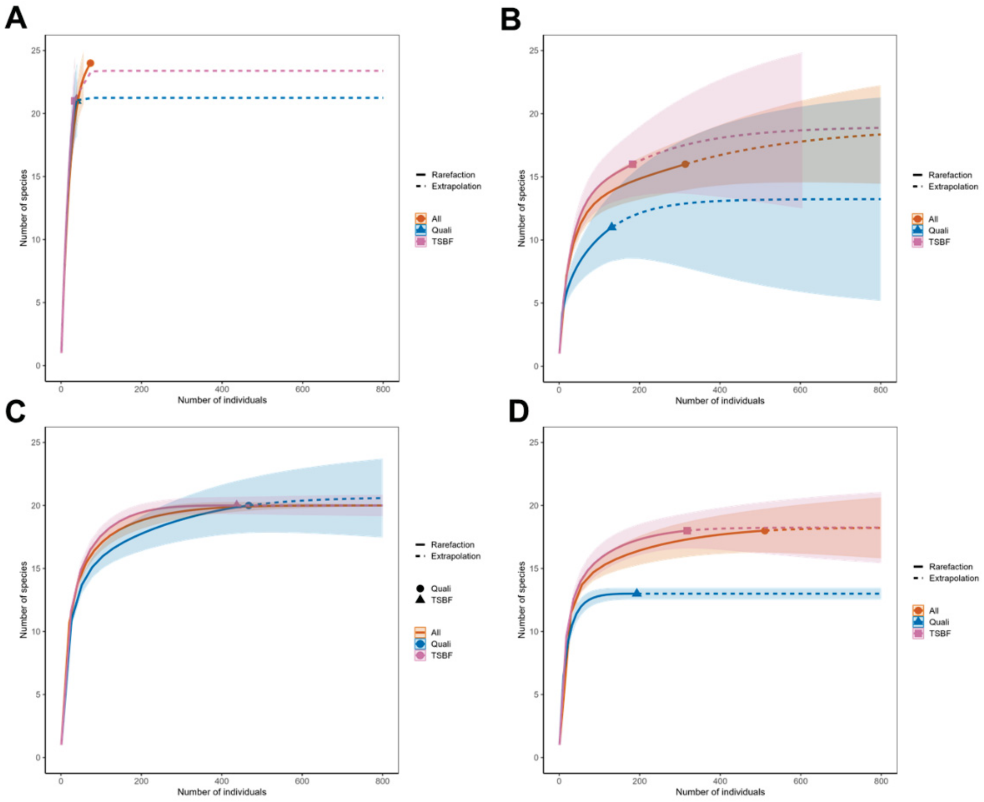


Source: Author (2024)

Legend: Worms found in three Land Use Systems using two methods **A**. Total abundance and number of native/exotic/juvenile individuals. **B.** Species richness, and number of native/exotic species. Standard error bars are indicated within each column according to the worm category. Red: juvenile; green: exotic; blue: native. The asterisk in **A** indicates the statistical difference in the comparison between methods among all LUS. The percentage corresponds to juvenile, exotic and native individuals in each of the samples.

Rarefaction curves for total species richness (FIGURE 6A) and effort for the different LUS (FIGURE 6, B, C, D), indicated that sampling with both methods is more efficient. For the BM area, the qualitative rarefaction curve was lower than that of the TSBF and the combined methodology, despite demonstrating greater richness when compared to the TSBF (FIGURE 5B).

FIGURE 6 – RAREFACTION CURVES COMPARING QUALITATIVE AND TSBF METHODOLOGIES BASED ON EARTHWORM DATA OBTAINED FROM EACH SAMPLING METHOD



Source: Author (2024)

Legend: **A:** Total specie richness in all Land Use System; **B:** Native vegetation; **C:** No-Tillage system; **D:** Bad management

Of the 23 earthworm families catalogued globally, in Brazil 10 are present and seven were found in the presented study - Acanthodrilidae, Benhamiidae, Glossoscolecidae, Lumbricidae, Megascolecidae, Ocnerodrilidae and Rhinodrilidae - (TABLE 1, Appendix 1) (Misirlioğlu *et al.*; 2023; Brown *et al.* 2023). And, overall, 24 were identified.

The species that were found exclusively through qualitative sampling but not in the TSBF monoliths were *Fimoscolex* n. sp.52, a potential new species in the city of Mangueirinha (Paraná) in the NV site. Unfortunately, only one subadult specimen was collected, which prevented definitive taxonomic identification. Other species found solely in qualitative samples were *Glossoscolex dallavecchiai, Glossoscolex strobelli,* and *Andiorrhinus duseni* (Michaelsen, 1918). Both *Glossoscolex* species are new and were described in chapter two of this thesis. Conversely, the species found exclusively in TSBF samples were the native *Fimoscolex* n. sp.51 (Mangueirinha – Paraná) and

Glossoscolex n. sp.77 (Maracaí – São Paulo), both new and described in chapter two, and the exotic *Bimastos parvus* (Eisen, 1874), identified in NTS of Mangueirinha (Paraná) and Panambi (Rio Grande do Sul), and NV of Vacaria (Rio Grande do Sul).

The use of multiple methods to collect earthworms increases the number of individuals and species collected (Römbke *et al.* 2006). Handsorting methods like the TSBF and qualitative approaches are effective in collecting earthworms from all ecological categories (Andriuzzi *et al.* 2017). These methods are complementary, because the qualitative method lacks specific measurements, unlike TSBF monoliths. Coja *et al.* (2008) evaluated five distinct methods for earthworm collection, including handsorting and plus chemical expulsion; consequently, none of the five methods proved 100% effective in collecting all species present. Singh *et al.* (2015) examined handsorting, chemical, and electrical collection methods, and reached a similar conclusion that each method possesses advantages and disadvantages, yet all are efficient when combined. The results of our study demonstrate an increase in earthworm diversity when both methodologies were employed, corroborating that the utilization of multiple methods serves as a complementary approach to estimating earthworm diversity. The species and families of earthworms are found in TABLE 4, indicating the environments in which they were found.

TABLE 4 – EARTHWORMS PRESENT IN DIFFERENT BIOMES AND LAND USE SYSTEMS

Earthworms	Atlantic Forest			Cerrado		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Acanthodrilidae family						
Microscolex dubius	+	+	-	-	-	-
Benhamidae family						
Dichogaster affinis	+	+	+	+	+	+
Dichogaster saliens	+	+	-	+	+	+
Benhamidae juveniles	-	-	-	+	+	+
Lumbricidae family						
Bimastos parvus	-	+	+	-	-	-
Octolasion tyrtaeum	+	+	-	-	-	-
Megascolecidae family						
Amynthas corticis	+	+	+	-	-	-

TABLE 4 continuation...

Earthworms	Atlantic Forest			Cerrado		
	BM	NTS	NV	BM	NTS	NV
Amynthas gracilis	+	+	+	-	-	-
Metaphire californica	-	+	-	-	-	-
Megascolecidae juveniles	+	+	+	+	-	-
Ocnerodrilidae family						
Eukerria saltensis	+	+	+	-	+	+
Ocnerodrilidae sp.1[2]	+	+	-	-	-	-
Ocnerodrilidae juveniles	+	+	+	+	+	+
Rhinodrilidae family						
Pontoscolex corethrurus	-	-	+	+	-	+
Native species						
Glossoscolecidae family						
Fimoscolex fridrichi	+	+	+	-	-	-
Fimoscolex n.sp. 51[3]	-	+	-	-	-	-
Fimoscolex n.sp. 52[3]	-	-	+	-	-	-
Glossoscolex arnsi	-	+	+	-	-	-
Glossoscolex alessioi	-	+	+	-	-	-
Glossoscolex dallavechiai	-	-	+	-	-	-
Glossoscolex debortoli	-	+	+	-	-	-
Glossoscolex strobeli	-	+	-	-	-	-
Glossoscolex *fuchsi*	-	-	-	+	-	-
Glossoscolex n.sp. 77[3]	-	-	+	-	-	-
Glossoscolecidae juveniles	+	+	+	-	-	-
Rhinodrilidae family						
Andiorhinus duseni	-	+	-	-	-	-
Urobenus brasiliensis	+	+	+	-	-	-
Andiorrhinus (Turedrilus) sp. 1[3]	-	-	-	+	-	+
Rhinodrilidae juveniles	+	-	-	-	+	-
Unidentifiable juveniles	+	+	+	+	+	+
Total species richness	10	18	14	5	3	5

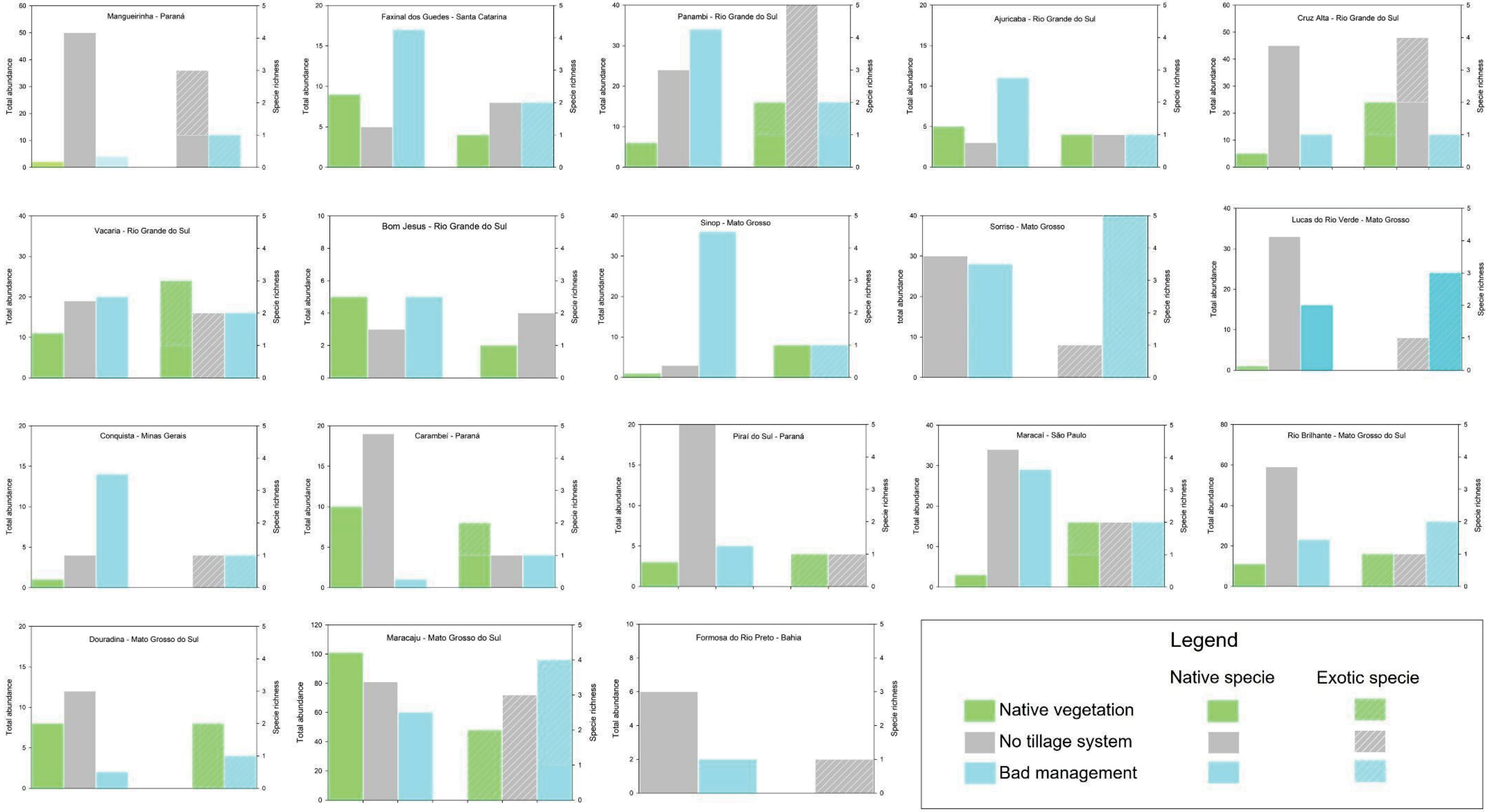
Source: Author (2024)

Legend: BM: bad management; NTS: No-Tillage System; NV: Native vegetation[2]: possible new specie; [3]: sequential numbers refer to the number assigned to each undescribed species in the Fritz

Müller Oligochaeta Collection (COFM); [4] Unidentified juveniles. The “+” = presence and “-” = absence of the species/family.

Comparing results of the TSBF sampling among all sampling sites (FIGURE 7), total abundance was highest in Maracaju (around 100 indiv. in NV), Rio Brilhante (~ 80 indiv. in NTS) and Mangueirinha (~60 indiv in NTS). Total species richness was highest in Panambi (5 spp. in NTS) and Sorriso (5 spp. in BM), Cruz Alta (4 spp. in NTS) and Maracaju (4 spp. in BM). Lowest abundance was observed in Formosa do Rio Preto and Balsas, both in the Cerrado region (5 or less individuals per LUS), and lowest species richness was also observed in the former site (1 species). Several other counties displayed low numbers and diversity of earthworms (Douradina, Piraí do Sul, Carambeí, Faxinal dos Guedes, Ajuricaba and Conquista), with the exception of Douradina and Conquista, all of them in the Atlantic Forest biome. The city of Balsas (in the Cerrado of Maranhão) yielded only six juvenile individuals, while no specimens were found in the city of Ribeiro Gonçalves in the Cerrado of Piauí. Therefore, these cities are not represented in Figure 7. Local species richness (alpha diversity) is generally low in both Cerrado and Atlantic Forest, both in native vegetation and agroecosystems. These values tend to match those already presented by Phillips et al. (2019) for the world and shown as low diversity and abundance in Brazil. However, exceptions clearly exist, and reasons for these should be further explored.

FIGURE 7 – TOTAL ABUNDANCE (NUMBER OF INDIVIDUALS COLLECTED) AND RICHNESS (NUMBER OF SPECIES FOUND) USING THE TSBF METHOD ACCORDING TO LAND USE SYSTEMS AND COUNTIES SAMPLED



SOURCE: Author (2024)

Of all the species found, 13 were native to Brazil and 11 were exotic. Of the native species, 10 belong to the Glossoscolecidae family, and all of them are new species to science, comprising three *Fimoscolex* and seven *Glossoscolex.* The other three species belong to the family Rhinodrilidae: i.e., *Urobenus brasiliensis* and the minhocoçu *A. duseni,* as well as a new species of *Andiorrhinus* (*Turedrilus*) from Sinop.

The Acanthodrilidae family had only one species, *Microscolex dubius* (Fletcher, 1887), endemic to South America, but found worldwide as a cosmopolitan earthworm (Rota; Jong, 2015). In Brazil, there are records of this species in São Paulo (Luederwaldt, 1927) and Rio Grande do Sul (Steffen *et al.* 2018; Ferreira *et al.* 2023a). According to Hughes *et al.* (1994) *M. dubius* is associated with organic matter soils, living in the first few centimeters of soil (<15cm). In this study we only found the species in a NTS area of Panambi (RS).

The Benhamiidae family has several small sized representatives in Brazil, including native and exotic species. However, here we found only two species in the exotic genus *Dichogaster* (*D. affinis* and *D. saliens*) comprising small individuals measuring ≤ 5 cm. These species were distributed across all LUS and biomes in the present study. This genus of African origin is widely distributed in Brazil and is frequently observed in disturbed agricultural and pasture sites (Bruz *et al.* 2023). Bartz *et al.* (2024) and Demetrio *et al*. (2019) also highlighted widespread occurrence of *Dichogaster* species in NTS sites throughout Brazil.

The Glossoscolecidae family is widespread in southeastern and southern Brazil (Brown; James, 2007). Currently, approximately 160 species have been recognized, all of which are native to South America (Mirsiloglu *et al.* 2023). A substantial number of new species have been collected (more than 60), but remain undescribed (da Silva *et al.* 2017). Here we identified ten new species, eight of *Glossoscolex* and two of *Fimoscolex*, which are the most prevalent genera in Brazil. These earthworms tend to be indicators of more preserved habitats, although *Glossoscolex fuchsi* was found exclusively in a BM site in Maracaju (MS). However, this site was a pasture with little to no sign of degradation and had native grasses, highlighting the role of native pastures for the conservation of native earthworm species, already mentioned by Bartz et al. (2014) for Santa Catarina state (TABLE 2).

Two exotic species were found belonging to the Lumbricidae family: *Bimastos parvus* and *Octolasion tyrtaeum* (Savigny, 1826). The former is a small species (2 - 3,5 cm), originating from North America, that is believed to have arrived in other continents through the shipment of soil and plant seedlings (Gates, 1972). It is often associated with humic/moist, mildly acidic soil with high organic matter content (Csuzdi *et al.* 2017). In the present study, *B. parvus* was found in NTS of Mangueirinha (PR) and Panambi (RS) and NV areas in Vacaria (RS). *O. tyrtaeum* can be slightly larger than *B. parvus* (4-14 cm) and is known from many temperate climate regions worldwide (Shekhovtsov *et al.* 2014). This species can affect the soil, plant nutrients, and microbial activity (Callaham; Blair 2001). In this study *O. tyrtaeum* was collected from Vacaria - RS, in NTS and BM sites.

The Megascolecidae family includes species commonly known as "jumping" or "crazy" worms, due to the frenetic movement they exhibit when disturbed. This family includes many exotic earthworms of Asian origin well-adapted to disturbed sites in Brazil and worldwide. In some cases, these worms have become invasive and caused negative impacts on nutrient cycling and plant growth, as has been reported in North America (Chang *et al.* 2021), although in Brazil, the effects have generally been positive (Pivello *et al.* 2024). We identified three species from this family: *Amynthas corticis* (Kinberg, 1866), *Amynthas gracilis* (Kinberg, 1867), and *Metaphire californica* (Kinberg, 1867), in the three LUS, but only in the Atlantic Forest biome.

Two species of the Ocnerodrilidae family were identified: *Eukerria saltensis* (Beddard, 1895) and Ocnerodrilidae sp.1. *E. saltensis* is a semi-aquatic cosmopolitan species that can reduce soil compaction through its burrowing activities (Blakemore *et al.* 2006), though it is usually associated with very wet habitats like stream banks or rice fields. In the present case, it was found in BM of Panambi, Cruz Alta and Vacaria (RS), NTS of Panambi, Cruz Alta, Rio Brilhante and Maracaju (MS), and NV of Panambi, Cruz Alta, Vacaria and Maracaju . The other species was found in south region in Mangueirinha (PR), Faxinal dos Guedes (SC) Ajuricaba and Bom Jesus (RS) may be native and must be further studied in order to reach a proper identification.

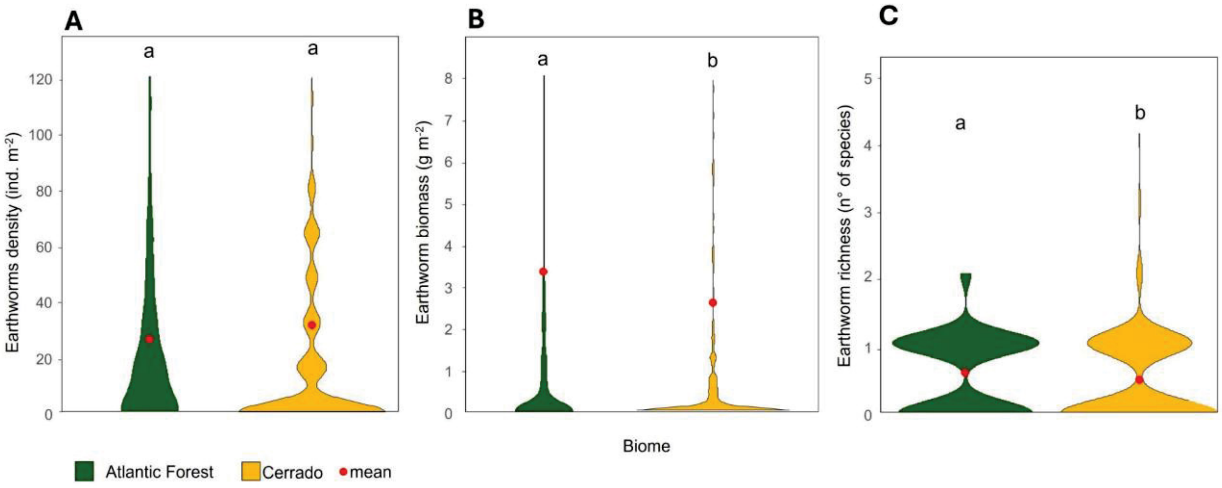
The Rhinodrilidae family included four representative species of which three were native, one of them being a new species from Mato Grosso, where other new *Andiorrhinus* species were also found in a recent study (Feijoo *et al.* 2017). The other species was a widespread exotic cosmopolitan worm, probably the most widely distributed in the world (*P. corethrurus* (Muller, 1856)). Originally from French Guiana (Golpeau 2024) it rapidly spread throughout Brazil and can be found from Amapá and Roraima to Rio Grande do Sul (Brown; James 2007; Steffen *et al.* 2013). This highly adaptable species can be found in crops, native vegetation, pasture, grass lawns, etc., and is highly invasive, due to its wide tolerance to many soil and climate types, and its parthenogenetic reproduction mode (Buch *et al.* 2011). In the present study, *P. corethrurus* specimens were found in a total of five counties in the states of MS, MT and PR. *A. duseni* is a minhocoçu (worms with ≥15 cm in length), found as only one individual, in NTS of Mangueirinha (PR), though this species is relatively widespread in Brazil (Demetrio et al. 2018, 2023b). Silva *et al.* (2019) called attention to the cryptic diversity of *U. brasiliensis* (Benham, 1887) and the need for additional research, both morphological and genetic to improve our understanding of this species. In the present case, it was found in NV of Faxinal dos Guedes (SC), Carambeí (PR), Vacaria and Bom Jesus (RS) and in NTS of Faxinal dos Guedes and Bom Jesus.

2.4.3 Relationship between earthworm abundance, biomass and richness in different LUSs and biomes

Comparing earthworm density (ind m^{-2}) in each biome, overall, the Cerrado presented a mean of 31 ind m^{-2} and Atlantic Forest 26 ind m^{-2} without statistical difference between biomes (FIGURE 8A). Biomass was higher in the Atlantic Forest (3.32 g m^{-2}) than in the Cerrado (2.58 g m^{-2}; FIGURE 8B), although both values were very small overall, indicating that the mean individual earthworm fresh (alcohol preserved) mass and overall size were small in both biomes (0.13 g in Atlantic Forest; 0.08 in Cerrado). This is related to the higher density of small *Dichogaster* in the Cerrado (TABLE 3), and other small Glossoscolecid and Ocnerodrilid species. As for richness, higher richness was obtained in some samples of the Cerrado, but the mean overall number of species found per monolith (individual sample richness) for Atlantic Forest was higher (0.3 species per monolith) (FIGURE 8C) because several Cerrado sites lacked earthworms or had many samples with no earthworms. Regarding the biomes, despite higher values for all three variables (density, biomass, and richness), the proportion of data with zero values was 58% for Cerrado, and 46% for the Atlantic Forest.

Although it is the second largest biome in Brazil (after the Amazon), the Cerrado still has limited sampling efforts for soil fauna, particularly earthworms (Sousa *et al.* 2020; Bartz *et al.* 2024). Currently 84 species are known (22 exotic, 62 native) from the biome, but many more new species are expected (Bartz *et al.* 2024; Bruz *et al.* 2023; Ferreira *et al.* 2023b), as well as for the Amazon (Maggia et al., 2023), and the Atlantic Forest, as shown in Chapter 2 of this thesis. From the Atlantic Forest we know over 140 species, of which around 35 are exotic (Brown; James, 2007), which tend to dominate in terms of abundance and biomass (Demetrio et al., 2023a). In the present study we found more species in the Atlantic Forest (21) than Cerrado (6), and the overall proportion of native vs exotic species was also higher in the Atlantic Forest (~50%) than in the Cerrado (33%).

FIGURE 8 – EARTHWORM DENSITY (ind m^{-2}), BIOMASS (g m^{-2}) AND SPECIES RICHNESS IN ATLANTIC FOREST AND CERRADO



Source: Author (2024)

Legend: Violin chart representing data distribution, the greater the width, the greater the concentration of data. Red dots represent the mean values. Different letters indicate statistical differences between the Biomes.

In the Atlantic Forest high earthworm densities (>400 individuals m^{-2}) have been observed but comprised mainly of exotic species like *P. corethrurus* (Römbke et al. 2009; Nadolny *et al.* 2020; Demetrio *et al.* 2023a). More favorable soil and climate conditions, like a higher and less variable rainfall, contribute positively to the earthworm populations in this biome compared to the Cerrado (Baretta *et al.* 2007; Bartz *et al.* 2014b; Demetrio *et al.* 2023a). Although more individuals were obtained using the TSBF in the Cerrado, when combined with the qualitative sampling, more specimens were recovered in the Atlantic Forest (FIGURE 4).

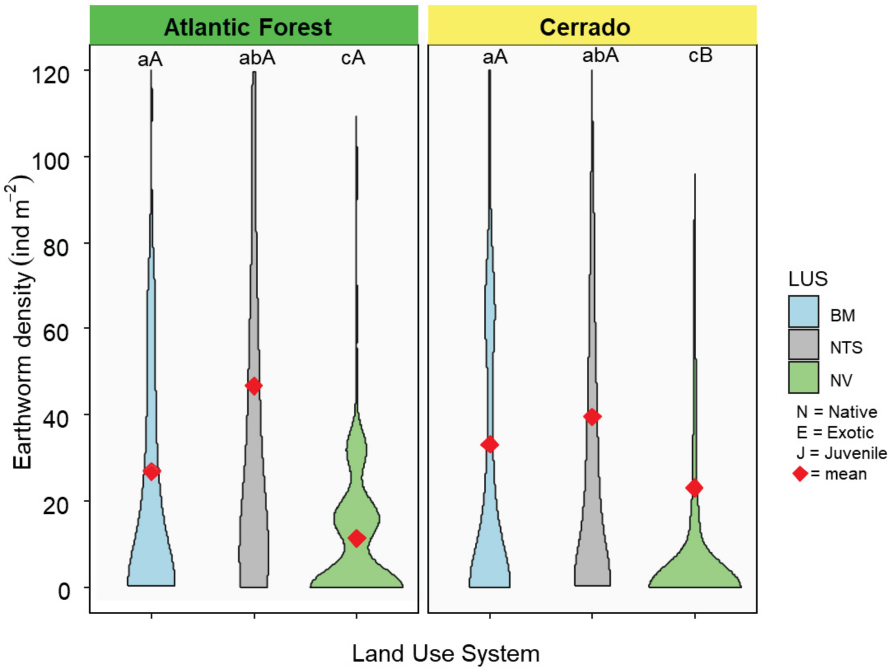
When comparing earthworm data by biome and by LUS, in the Atlantic Forest, density was higher in NTS (44 ind m^{-2}), followed by BM (25 ind m^{-2}) and NV (11 ind m^{-2}), with NTS exhibiting greater variation, and with statistical difference between the crop sites, NTS and BM, with NV. The Cerrado followed the same pattern with NTS = 38 ind m^{-2}, BM = 32 ind m^{-2} and NV = 22 ind m^{-2}. Between the biomes, the only LUS with statistical difference was NV, being higher in the Cerrado than the Atlantic Forest (FIGURE 9). These results, with low density in NV, suggest that agricultural systems provide some level of favorable conditions for earthworm development. However, most of the LUS presented a predominance of juvenile individuals, followed by exotic species, as represented in the bottom of FIGURE 9 by the percentage scores. Despite this dominance, native species were present in most of the sites.

Regarding biomass, the Atlantic Forest exhibited a pattern like that of density, as did the Cerrado (FIGURE 10), with NTS showing higher biomass in Atlantic Forest (6.6 g m^{-2}), followed by BM (1.9 g m^{-2}) and NV (1.5 g m^{-2}). For the Cerrado, higher biomass was found in NV (4.4 g m^{-2}) followed by BM (3 g m^{-2}), and NTS had the lowest mean biomass for Cerrado and for all the sites with 0.52 g m^{-2}.

For species richness, higher mean values were found in NTS (0.8 species per monolith) and BM (0.5) compared with NV (0.4 species) in the Atlantic Forest, while in the Cerrado, both NTS and BM had higher richness (around 0.6 species) than NV (0.2 species) (FIGURE 11A). The low species richness encountered is due to the high number of monoliths with zero earthworms. In Cerrado, this occurred more often in NV (79%) than BM (52%) or NTS (44%). In the Atlantic Forest, the same trend was observed, with 57, 50 and 31% zeroes in NV, BM and NTS, respectively. This indicates that despite some high values in the Cerrado, earthworms are encountered more frequently when digging monoliths in the Atlantic Forest.

In terms of alpha diversity (site-level), species richness ranged from 0-4 species in BM, 2-6 species in NTS and 1-4 species in NV in the Atlantic Forest, with no significant difference in the means between LUS in this biome (FIGURE 11B). In the Cerrado, local species richness was lower than in the Atlantic Forest and significant differences were detected for NV and NTS comparing both biomes. Within the Cerrado, significantly lower alpha diversity was detected in NV (mean of 0.5 species) compared with BM (mean of 1.6 species). This overall lower diversity in the Cerrado was reflected in the range of species richness as well, with only 0-4 species in BM, 0-3 species in NTS and 0-2 species in NV, with a much higher proportion of sites having no earthworms (0 species richness).

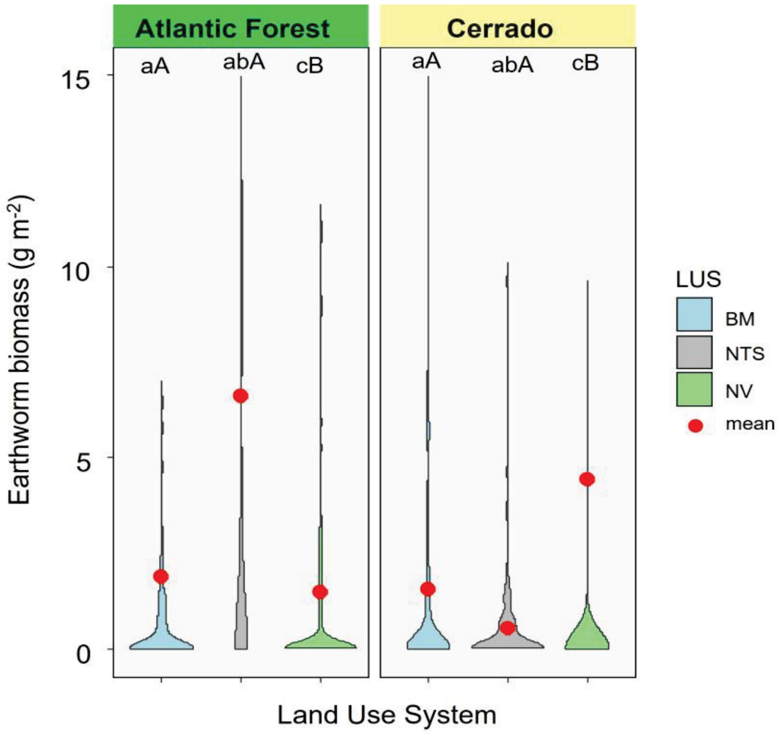
FIGURE 9 – MEAN EARTHWORMS DENSITY (ind m^{-2}) IN ATLANTIC FOREST AND CERRADO, WITH PERCENTAGE OF NATIVE, EXOTIC AND JUVENILE INDIVIDUALS



Source: Author (2024)

Legend: Violin chart representing data distribution, the greater the width, the greater the concentration of data. Red dots are the mean value. Different letters present a statistical difference within the Land Use Systems (lower case) and biomes (upper case). BM: Bad management; NTS: No-Tillage System; NV: Native vegetation.

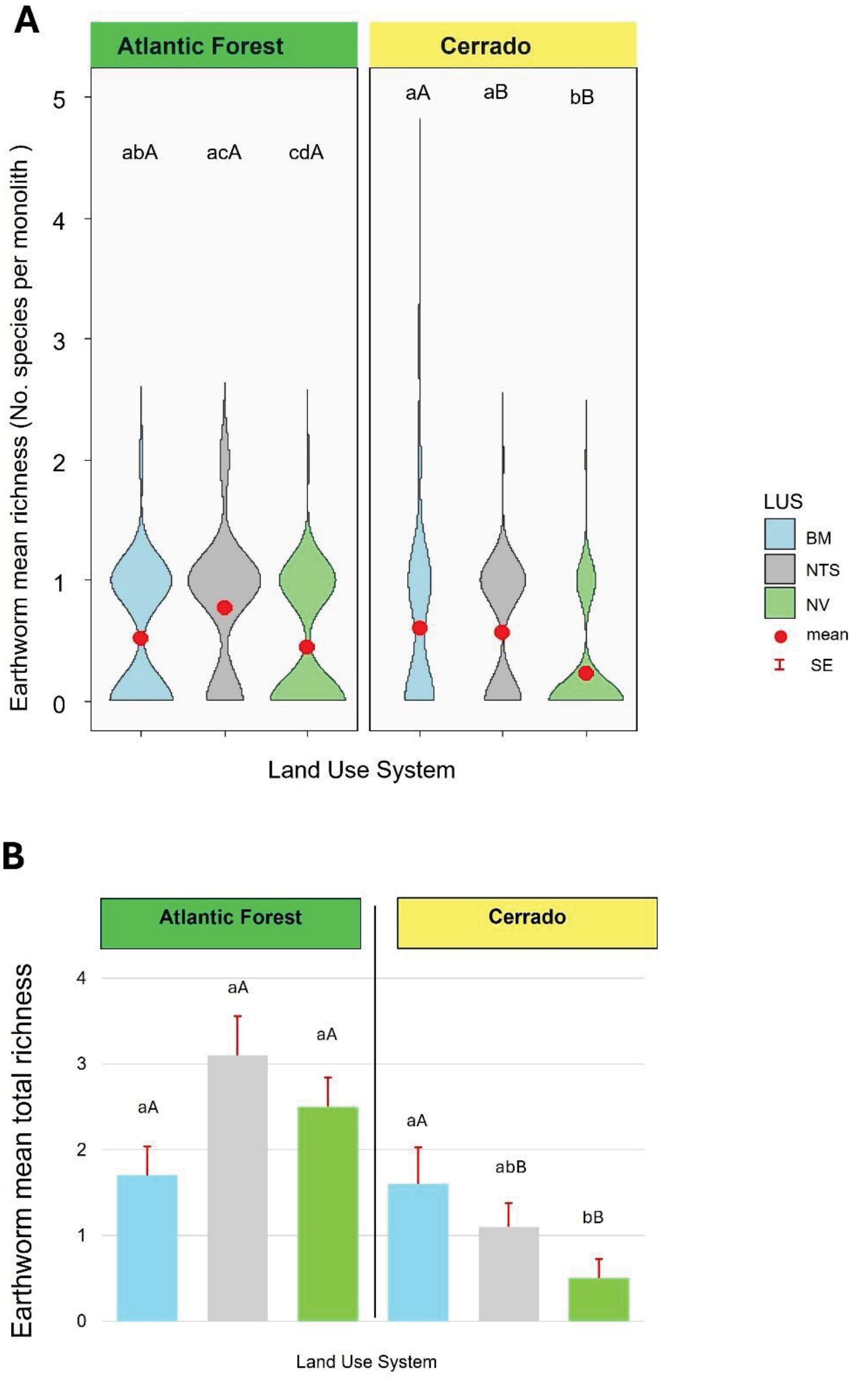
FIGURE 10 – MEAN EARTHWORM BIOMASS (g m^{-2}) IN EACH LUS IN ATLANTIC FOREST AND CERRADO



Source: Author (2024)

Legend: Violin chart representing biomass data distribution. Red dots represent the mean value. Different letters indicate a statistical difference within the Land Use Systems (lower case) and biomes (upper case). BM: Bad management; NTS: No-Tillage System; NV: Native vegetation.

FIGURE 11 – MEAN EARTHWORM RICHNESS (NUMBER OF SPECIES PER MONOLITH) AND ALPHA DIVERSITY (NUMBER OF SPECIES PER SITE WITHIN LUS) ENCOUNTERED IN EACH LUS AND BIOME



Source: Author (2024)

Legend: Earthworm richness. **A.** Mean earthworm richness per monolith. **B.** Mean Alpha diversity (total earthworm species richness in each LUS) in Atlantic Forest and Cerrado, combining sampling methods (TSBF + Quali). Mean of n=10 sites for each LUS and biome. Different letters indicate a statistical difference within the Land Use Systems (lower case) and biomes (upper case). BM: Bad management; NTS: No-Tillage System; NV: Native vegetation.

Lower earthworm densities have been observed in NV than in agricultural ecosystems (Baretta *et al.* 2007, Römbke *et al.* 2009). This is likely due to the soil amelioration processes that occur in the transformation of forests to crops, where lime and fertilizers are added. This process appears to favor the invasion by exotic earthworm species, which predominated in both BM and NTS in the current study. Nevertheless, even in NV exotics were dominant in both biomes, although many juvenile individuals that could not be identified to species level were encountered and may represent different proportions of native to exotic species, that could alter the current results. For this purpose, barcoding of all juveniles would be an interesting option (Richard *et al.* 2010), although financial constraints are an important limiting factor to consider. In their review, Demetrio *et al.* (2023a) also observed a predominance of exotic species in natural areas of the Atlantic Forest. Exotic species are more adaptable to diverse soil and plant habitats, which helps explain their higher abundance in various disturbed land use systems and biomes.

Native vegetation areas usually support higher numbers of species, but with the invasion of exotic species in disturbed LUS these sites can often support larger species richness when compared to native sites (Bartz et al. 2013, 2014). In the present study, this was observed for the Atlantic Forest LUS overall, and for the mean alpha diversity (local or site-level species richness). In the Cerrado region, this was also seen, with NV showing the lowest total richness as well as the lowest alpha diversity.

Areas with a moderate intensity of soil disturbance, such as pastures and cropping systems with rotation, and organic matter inputs are also beneficial to earthworms (Dudas *et al.* 2023). This was evident in some of the BM areas with pastures, for instance (TABLE 2), and BM areas also supported three new earthworm species, two of which were not found in other LUS. Crop rotation positively affects soil quality, and often incorporates residues to the soil and on the soil surface that are also beneficial to earthworms (Torppa; Taylor 2022). Conversely, when intensive tillage occurs for extended periods earthworm populations are negatively affected, resulting in reduced density values (Lees *et al.* 2016; Dekemati *et al.* 2020), by up to 60% (Paolleti, 1999; Briones; Schimdt 2017). In the present case, most of the BM sites that had tillage also had lower earthworm abundances (e.g., Mangueirinha, Faxinal dos Guedes, Ajuricaba, Cruz Alta, Vacaria, Bom Jesus, Lucas do Rio Verde, Conquista, Carambeí, Piraí do Sul, Douradina, Formoso do Rio Pardo, Balsas and Ribeiro Gonçalves).

As for NTS sites, it is well known that this management approach increases earthworm density and can support new native species (Santos *et al.* 2018; Bartz *et al.* 2012, 2024, Demetrio *et al.* 2019). In the present case six new species were found in this system, which maintains surface straw residues, reduces soil temperature and moisture extremes and contributes to a more favorable microclimate for soil organisms (Yang, *et al.* 2018). Additionally, the higher organic matter content, crop diversification, and nutrients contribute to the successful development of these organisms (Rodriguez *et al.* 2020). Despite the higher abundance in this LUS, the mean densities obtained were low, indicating that most of the sites (18 out of 20) would be considered of poor quality (< 50 indiv. m^{-2}), using the classification system proposed by Bartz *et al.* (2024) for earthworms in NTS in Paraná state. Only two sites in Mato Grosso do Sul (Maracaju and Rio Brilhante) had moderate quality (> 50 to less than 100 indiv. m^{-2}), and both were in the Cerrado biome. Although most of the sampling sites were in other states (not in Paraná), this result still highlights the need for further improvement in soil and crop management at these NTS sites to increase earthworm populations and better profit from their beneficial effects on soil functions, plant production (Fonte *et al.* 2023) and other ecosystem services (Blouin *et al.* 2013).

2.4.4 Correlation between soil attributes and earthworms

The first two axes of the principal component analysis (PCA) accounted for 53.8% of the total data variability (FIGURE 12A). The biological factors (density, richness, and biomass) were positively correlated with soils characterized by higher moisture, C, N, CEC, S, Mn, Cu, base (Ca, Mg, K) and clay contents in soils. This indicates a positive relationship between earthworm populations and chemical parameters associated with higher soil fertility.

The Cerrado sites were mainly arranged along the X-axis, associated with soil fertility and texture (sand-clay contents), while the Atlantic Forest sites were arranged along a gradient of bulk density, pH and Fe contents. Due to the absence of earthworms in certain counties within the Cerrado biome (Figure 7), and lack of soil analyses for the Lucas do Rio Verde - all sites - and Rio Brilhante

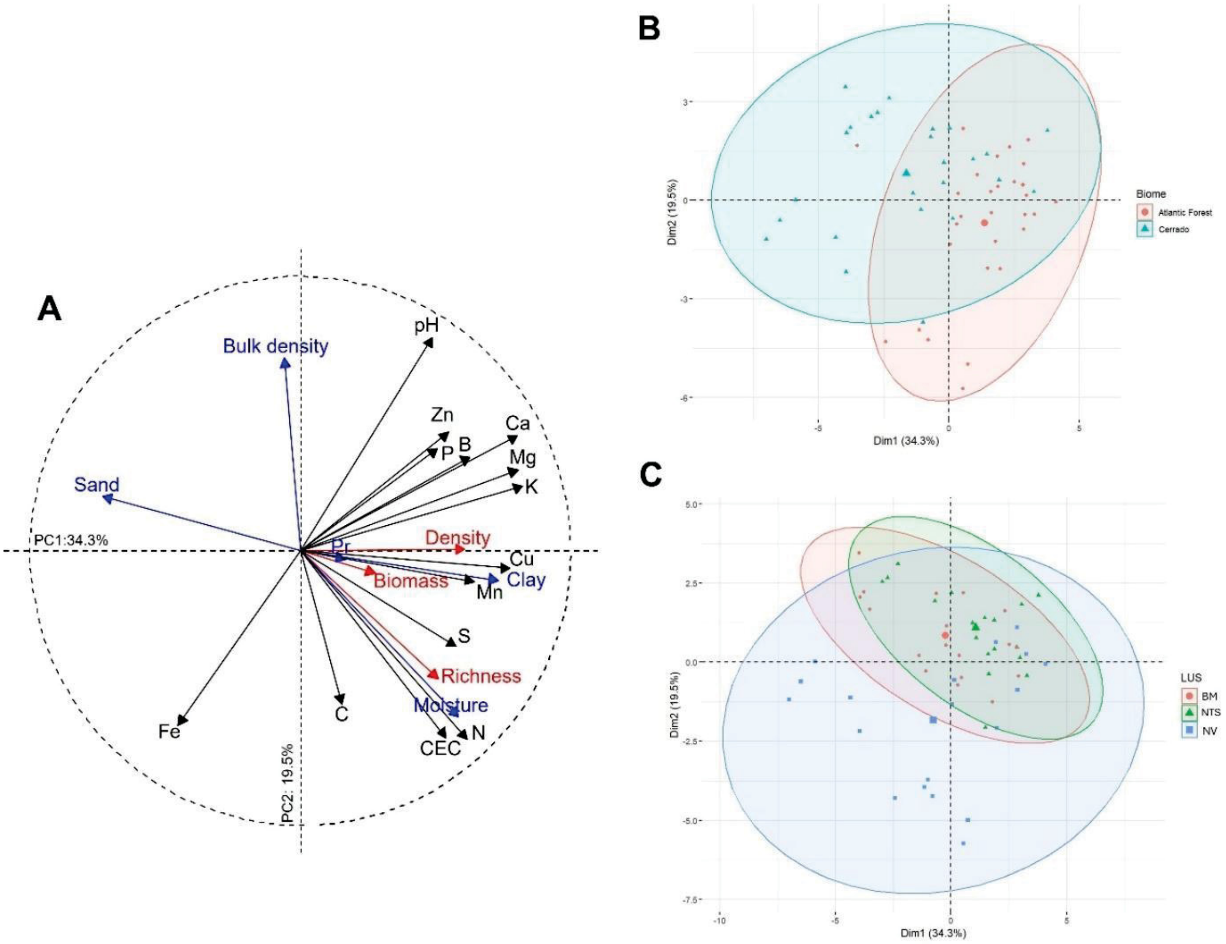
- NTS and BM - the PCA indicates a higher probability of encountering earthworms in the Atlantic Forest (Figure 12B), as mentioned in the previous section (4.4.2). Concerning Land Use Systems (LUS) (FIGURE 12C), BM and NTS management sites were arranged along a gradient of soil fertility and sand, and associated with higher bulk density, whereas NV was positively correlated with moisture and earthworm biomass.

Precipitation and moisture are among the environmental factors that most significantly affect earthworms, and extreme conditions, such as very high or very low temperatures, drought or excessive amounts of water, are detrimental as they can directly impact earthworms, resulting in a decrease in population density and diversity (Rutgers *et al.* 2016; Nadolny *et al*. 2017; Phillips, *et al.* 2019). Hence it is not surprising that soil moisture was a factor closely associated with earthworm species richness, which also was higher in the Atlantic Forest biome, which in most of the regions sampled does not present such an extreme dry period as that observed in the Cerrado. This became evident also when performing the PCA with the earthworm family and origin data (FIGURE 13).

As NV areas are under acid soils in all regions, these had lower values of pH and phosphorus, with these attributes being higher in agricultural areas. The forests had a high C content and lower bulk density, but these parameters were also associated with lower species richness and earthworm abundance. These parameters were lower in the disturbed NTS and BM, which had higher overall soil fertility (both macronutrients and micronutrients), higher pH and bulk density, but higher earthworm abundance and species richness.

Although copper is known to be toxic to earthworms, this only occurs at very high concentrations not normally found in Brazilian soils. For instance, Pelosi *et al.* (2024) showed that earthworm populations can be reduced by half when copper concentration reaches 150 mg/kg, but the copper levels found in the areas sampled in this study did not exceed 20 mg/dm^3. With respect to soil texture, soil grain size has an effect on these organisms, since more clayey soils can contain greater amounts of C, organic matter and water (Costa *et al.* 2013), all of which are important for earthworm populations.

FIGURE 12 – PRINCIPAL COMPONENT ANALYSES WITH SOIL ATTRIBUTES AND SAMPLED SITES – BIOME AND LAND USE SYSTEM



Source: Author (2024)

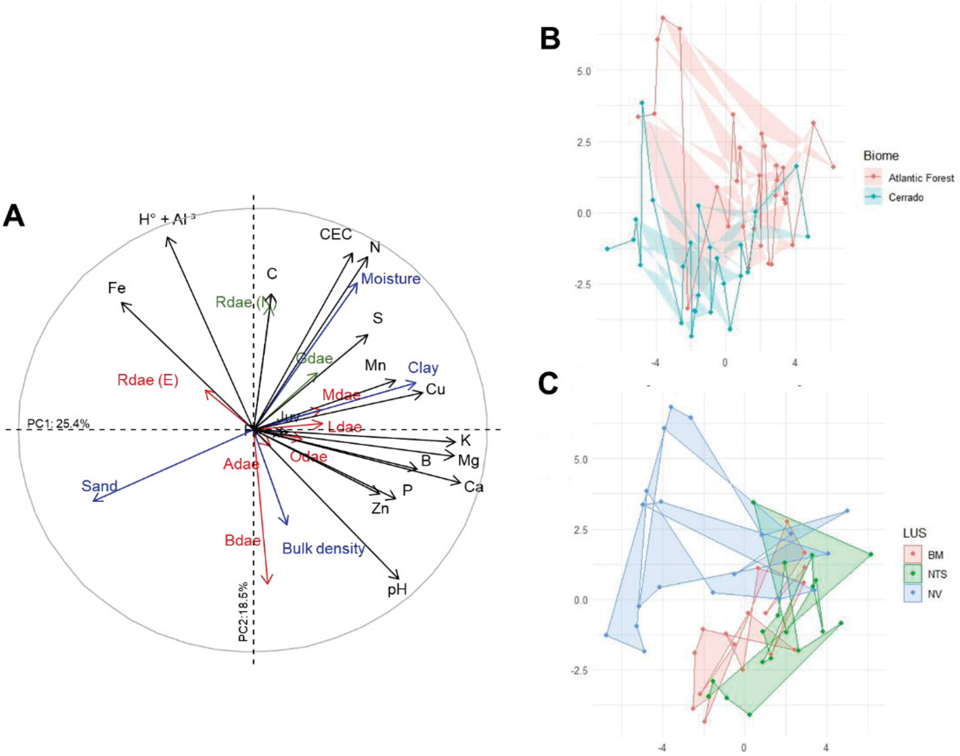
Legend: **A:** Pr: precipitation (mm); P: phosphorous; Zn: zinc; B: bore; Cu: copper; Mn: manganese; S: sulfur, N: nitrogen; CEC: Cation Exchange Capacity; C: carbon; Fe: iron, K: potassium, Mg: magnesium; Ca: calcium. In blue: physical attributes plus precipitation; black: chemical attributes; red: earthworm attributes. **B:** Biomes; **C:** Land Use Systems. BM: bad management; NTS: No Tillage system; NV: native vegetation.

2.4.5 Potential of earthworms as soil indicators

Indicator value analysis (IndVal) was employed to determine how different species can serve as indicators of various environments and/or environmental conditions (TABLE 5). Furthermore, an additional Principal Component Analysis (PCA) was conducted using data from the abundance of native and exotic earthworm species and families (FIGURE 13). On the figure, axis one (PC1 25.4%) explains most of the variance in the data. It usually reflects the largest gradients or patterns in the data. Axis two (PC2 18.5%) explains the second largest portion of the variance but is orthogonal to PC1. Points that are close to each other indicate high similarity in terms of the original variables.

The only family with both native and exotic representatives was the Rhinodrilidae family, with *P. corethrurus* being exotic (Rdae E), and *U. brasiliensis* and *Andiorrhinus* (*Turedrilus*) n. sp.1 being native (Rdae N).

FIGURE 13 – PRINCIPAL COMPONENT ANALYSES WITH SOIL VARIABLES AND EARTHWORMS AND SAMPLED SITES – BIOME AND LAND USE SYSTEM



Source: Author (2024)

Legend: **A:** Pr: precipitation (mm); P: phosphorous; Zn: zinc; B: bore; Cu: coper; Mn: manganese; S: sulfur, N: nitrogen; CEC: Cation Exchange Capacity; C: carbon; Fe: iron, K: potassium, Mg: magnesium; Ca: calcium. In blue: physical attributes plus precipitation; black: chemical attributes; red: exotic earthworms families; green: native earthworms families. **B:** Biomes; **C:** Land Use Systems. BM: bad management; NTS: No Tillage system; NV: native vegetation.

TABLE 5 – INDICATOR SPECIES/families AND VALUES AND THE SITES WHERE THEY SHOW POTENTIAL, ACCORDING TO SAMPLING METHOD. ONLY SPECIES/FAMILIES WITH INDICATOR VALUE >50% AND P<0.001 WERE INCLUDED

Family	**Species**	**Native/Exotic**	**Biome or state**	**Land Use System**	**Method**	**Indicator Value (%)**
Benhamidae	*Dichogaster affinis*	Exotic	Cerrado	NTS+BM	TSBF	76
Benhamidae	*Dichogaster affinis*	Exotic	Atlantic Forest - SP	-	TSBF	100
Benhamidae	*Dichogaster saliens*	Exotic	Cerrado - MS	-	TSBF	87
Benhamidae	-	Exotic	Cerrado - MS	-	TSBF	89
Benhamidae	*Dichogaster affinis*	Exotic	Cerrado	NTS+BM	TSBF+Quali	81
Glossoscolecidae	*Fimoscolex friderich*	Native	Atlantic Forest - RS	-	TSBF	100
Megascolecidade	*Amyntas corticis*	Exotic	Atlantic Forest	NTS	TSBF	60
Megascolecidade	-	Exotic	Atlantic Forest – PR+SC	-	TSBF	100

TABLE 5 continuation...

Family	**Species**	**Native/Exotic**	**Biome or state**	**Land Use System**	**Method**	**Indicator Value (%)**
Megascolecidade	-	Exotic	Atlantic Forest - PR	-	TSBF	97
Megascolecidade	*Amyntas corticis*	Exotic	Atlantic Forest	NTS	TSBF+Quali	73
Ocnerodrilidae	*Eukerria saltensis*	Exotic	Atlantic Forest - PR	-	TSBF	100
Ocnerodrilidae	*Eukerria saltensis*	Exotic	Atlantic Forest - RS	-	TSBF+Quali	91
Ocnerodrilidae	-	ND	Atlantic Forest – RS	-	TSBF	100
Ocnerodrilidae	-	ND	Cerrado - MS	-	TSBF	100
Rhinodrilidae	-	Native	Atlantic Forest	NV	TSBF+Quali	63

Source: Author (2024)

Legend: BM: Bad management; NTS: No-Tillage System; NV: Native vegetation. The analysis was done using families and species as indicators, in the Species column the “-” means that the indicator value corresponds to the family. And in the Land Use System column the “-” indicates that the indicator value corresponds to the state, in the previous column.

The IndVal analysis results revealed associations between native and exotic families and species and LUS and biomes. The Benhamiidae family has emerged as the most frequent indicator, predominantly in the Cerrado biome. *Dichogaster affinis* exhibited 76% and 81% indicative values for NTS and BM sites in the Cerrado biome, utilizing the TSBF methodology and the TSBF+Quali combination, respectively. Moreover, an indicative value of 100% was observed for the state of São Paulo. For the state of Mato Grosso do Sul, *D. saliens* and the family Benhamidae were important indicators, with values of 87% and 89%, respectively. As previously mentioned, this family, specifically the genus *Dichogaster*, is a good indicator of disturbed agricultural ecosystems (Brown *et al.* 2006, Sautter *et al*. 2007, Bruz *et al*. 2023).

The native species *F. fridrichi* has an indicator value of 100% for the state of Rio Grande do Sul (RS), but further sampling efforts should aim to confirm its possible usefulness as a wider spread indicator for particular LUS in the state. Overall, this earthworm was found in NV and NTS of Panambi and Ajuricaba and NV of Ajuricaba and Cruz Alta sampling sites of RS. Species in the genus *Fimoscolex* occur in pastures (Bartz *et al.* 2014), lawns (Dudas *et al.* 2023b), native vegetation (Nunes et al. 2006), and no-tillage areas (Bartz et al. 2024) of various Brazilian states (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais, Rio de Janeiro and Mato Grosso do Sul). Hence, species in this genus are adapted to diverse locations and LUS, although many *Fimoscolex* species have yet to be described. Concurrently, ecological information must still be obtained on the functional roles of these earthworms in soils.

Megascolecidae had indicator values for the family in the states of Paraná (PR) and Santa Catarina (SC), with values of 97% and 100%, respectively. Furthermore, the species *A. corticis*, was an indicator of NTS areas in the Atlantic Forest biome, with indicator values of 60% and 73%, considering the TSBF methodology and the combination of methods, respectively. This family is a widespread invasive in agricultural ecosystems, not only throughout Brazil (Brown *et al.* 2006), but also throughout the Americas (Chang *et al.* 2021). It was also used as an indicator of disturbance in NV areas and forested sites of the Atlantic Forest biome by Fernandes *et al.* (2010) and Demetrio et al. (2023a,b).

Similar to the two above-mentioned families, the Ocnerodrilidae family was indicative for the states of RS and Mato Grosso do Sul (MS) with 100% for both. Furthermore, *Eukerria saltensis* was an indicator species in the states of PR (100%) and RS (91%), respectively. Individuals of this family have been observed in both native and agricultural areas and are recognized for their resilience to soil disturbances, such as those occurring in croplands (Bartz *et al.* 2013a, 2014b; James *et al.* 2023), as we observed in the present study.

The family Rhinodrilidae (native individuals) functioned as an indicator of NV areas in the Atlantic Forest biome (63%). This family is widely distributed and, in Brazil, has been documented from the Amazon biome (Zicsi *et al.* 2001) to the Pampas (Ferreira *et al.* 2023). As with *Fimoscolex*, despite its extensive distribution, there is still little knowledge on the biology and ecology of the many native species in this family.

The results of the PCA (FIGURE 13), show the relationship between the exotic Benhamidae especially, but also to a lesser degree with the Ocnerodrilidae and soils with higher bulk density and pH. This confirms the association of these exotic earthworms with disturbed environments like BM and NTS in the present study.

On the other hand, the native species in the Rhinodrilidae and Glossoscolecidae were more strongly associated with higher humidity, CEC, N, C attributes more closely associated with the Atlantic Forest biome and NV sites. This confirms the important role of native and exotic species in reflecting the impact of land use practices, and this study confirms that native families are indicators of better soil quality and natural or less degraded habitats, while exotic families serve as good indicators of areas that have undergone by physical and chemical alterations.

2.5 CONCLUSION

This study, covering a large range of two Brazilian biomes, compared earthworm populations in three land use systems, discovering 24 species from seven families, of which 13 were native to Brazil and 11 were exotic. It also calls attention to the important number of new native species to be found, particularly in the Atlantic Forest, as 11 new species were discovered, nine of which were described in the second chapter of this thesis, all of them from the Atlantic Forest biome. This biome also showed the highest species richness, due to its inherent environmental characteristics, particularly a better climate, such as well distributed precipitation and temperatures, for earthworm activities.

Across both biomes higher density and biomass were found in the No-Tillage system and bad management followed by native vegetation. This finding confirms previous studies that showed how the adoption of more conservative agricultural practices is beneficial to earthworm populations, particularly exotic earthworm species. Overall species richness also tended to be higher in NTS than in bad management and native vegetation. Furthermore, even in these disturbed systems native, and new earthworm species were found. Efforts to describe the potential functional roles of these animals in these habitats and soils are sorely needed.

Soil texture, grain size, bulk density, moisture and chemical attributes contribute to explaining how earthworm data correlate with the sampling sites and land use system. The indicator value (IndVal) identified families and/or species most associated with each site, being the exotics more associated with agricultural land use values where soils were limed but denser, while native species/families were more associated with native vegetation sites where soils had higher carbon and nitrogen contents and cation exchange capacity.

However, the earthworm abundance found in most sites was relatively low, which calls for more attention to the use of management practices that can increase their populations such as organic manure additions, cover cropping with legumes and roots crops that can add more organic matter to the soil, thereby providing more food for earthworm populations (Lavelle *et al.* 2001). This is particularly important in warmer regions like the Cerrado, where climate conditions limit soil cover and moisture conditions at certain times of the year.

2.6 REFERENCES

Alloway, B. J. (Ed.). (2008). Micronutrient deficiencies in global crop production. *Springer Science & Business Media*. Brady, N. C., & Weil, R. R. (2008). The Nature and Properties of Soils. Pearson.

Akhila, A., & Entoori, K. (2022). Role of earthworms in soil fertility and its impact on agriculture: A review. *International Journal Fauna and Biological Studies*, 9(3), 55-63. Recuperado de: https://doi.org/10.22271/23940522.2022.v9.i3a.907

Anderson, J. M., & Ingram, J. S. (1994). Tropical soil biology and fertility: a handbook of methods. Soil Science, 157(4), 265.

Bartz, M.L.C., Pasini, A, Brown, G.G. (2013) Earthworms as soil quality indicators in Brazilian No-Tillage systems, *Applied Soil Ecology* 69, 39–48. Recuperado de: https://doi.org/ 10.1016/j.apsoil.2013.01.011.

Bartz, M. L. C., Brown, G. G., da Rosa, M. G., Klauberg Filho, O., James, S. W., Decaëns, T., & Baretta, D. (2014a). Earthworm richness in land-use systems in Santa Catarina, Brazil. *Applied Soil Ecology*, 83, 59-70. Recuperado de: https://doi.org/10.1016/j.apsoil.2014.03.003

Bartz, M. L., Pasini, A., & Brown, G. G. (2014b). Earthworm richness, abundance and biomass in different land use systems in northern Paraná, Brazil (Oligochaeta). Advances in Earthworm Taxonomy VI (Annelida: Oligochaeta). Heidelberg: Kasparek Verlag, 59-73.

Bartz, M. L., James, S. W., Steffen, G. P., Antoniolli, Z., Steffen, R. B., & Brown, G. G. (2018). New species-group taxa of *Glossoscolex* (Clitellata: Glossoscolecidae) from Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 548-561. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.42

Bartz, M. L. C., Dudas, R. T., Demetrio, W. C., & Brown, G. G. (2024). Earthworms as soil health indicators in no-tillage agroecosystems. *European Journal of Soil Biology*, 121, 103605.

Recuperado de: https://doi.org/10.1016/j.ejsobi.2024.103605

Bartz, M.L.C., Demetrio, W.C., Dudas, R.T., Roani, R., & Brown, G.G. (2024). Macrofaua do solo e minhocas em sistemas de uso do solo no Cerrado. In: Reis, A.T.C.C. (ed.), Cerrado: sustentabilidade, agronegócio e inovação. Barreiras: Instituto JCO. pp. 165-193.

Blakemore, R. J., Ito, M. T., & Kaneko, N. (2006). Alien earthworms in the Asia/Pacific region with a checklist of species and the first records of *Eukerria saltensis* (Oligochaeta: Ocnerodrilidae) and *Eiseniella tetraedra* (Lumbricidae) from Japan, and Pontoscolex corethrurus (Glossoscolecidae) from Okinawa. Assessment and control of biological invasion risks, 173-181.

Blouin, M., M. E. Hodson, E. A. Delgado, G. Baker, L. Brussaard, K. R. Butt, J. Dai, L. Dendooven, G. Peres, J. E. Tondoh, D. Cluzeau & J.-J. Brun (2013): A review of earthworm impact on soil function and ecosystem services: Earthworm impact on ecosystem services. European Journal of Soil Science 64, 161–182. https://doi.org/10.1111/ejss.12025.

Bonilla-Bedoya, S., Valencia, K., Herrera, M. Á., López-Ulloa, M., Donoso, D. A., & Pezzopane, J. E. M. (2023). Mapping 50 years of contribution to the development of soil quality biological indicators. *Ecological Indicators*, 148, 110091. Recuperado de: https://doi.org/10.1016/j.ecolind.2023.110091

Brennan, R. F. (2005). Zinc application and its availability in tropical soils. *Soil Science Society of America Journal, 69*, 243–251

Briones, M. J. I., & Schmidt, O. (2017). Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Global Change Biology*, 23(10), 4396-4419. Recuperado de: https://doi.org/10.1111/gcb.13744

Brown, G. G., Benito, N. P., Pasini, A., Sautter, K. D., de F Guimarães, M., & Torres, E. (2003). No-tillage greatly increases earthworm populations in Paraná state, Brazil: The 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, 47(5-6), 764-771.
Recuperado de: https://doi.org/10.1078/0031-4056-00256

Brown, G. G., & James, S. W. (2007). Ecologia, biodiversidade e biogeografia das minhocas no Brasil. Minhocas na América Latina: biodiversidade e ecologia. Londrina: Embrapa Soja, 297-381.

Bruz, L. D. S. M., Santos, A., Demetrio, W. C., Feliciano, L. P., Fernandes, C. H., Bartz, M. L. C.,Bernardi, A.C. de C., Pezzopane, J.R.M. & Brown, G. G. (2023). Earthworms in various agricultural and forest ecosystems in São Carlos-SP, Brazil. *Zootaxa*, 5255(1), 324-335.
Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.26

Buch, A.C., Brown, G.G., Niva, C.C., Sautter, K.D., Lourençato, L.F. (2011). Life cycle of *Pontoscolex corethrurus* (Müller, 1857) in tropical artificial soil. Pedobiologia, 54S, S19-S25.

Callaham, M. A., Blair, J. M., & Hendrix, P. F. (2001). Different behavioral patterns of the earthworms *Octolasion tyrtaeum* and *Diplocardia* spp. in tallgrass prairie soils: potential influences on plant growth. *Biology and Fertility of Soils*, 34, 49-56. Recuperado de: https://doi.org/10.1007/s003740100370

Capowiez, Y., Marchán, D., Decaëns, T., Hedde, M., & Bottinelli, N. (2024). Let earthworms be functional-Definition of new functional groups based on their bioturbation behavior. Soil Biology and Biochemistry, 188, 109209.

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., Santos, C. A. D., Alves, P. R. L., de Paula, Al.M., Nakatani, A.S., Pereiria, J de M. & Nogueira, M. A. (2013). Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, 70, 274-289.

Recuperado de: https://doi.org/10.1590/S0103-90162013000400009

Chang, E. H., Chen, T. H., & Hsu, C. Y. (2009). Soil properties and their relationships with land use in tropical regions. *Soil Biology & Biochemistry, 41*, 221–230.

Chang, C. H., Bartz, M. L., Brown, G., Callaham, M. A., Cameron, E. K., Dávalos, A., Dobson, A., Görres, J.H, Herrick, B.M., Ikeda, H., James, S.W., Johnston, M.R., McCay, T.S., McHugh, D., Minamiya, Y., Nouri-Aii, M., Novo, M., Ortiz-Pachar, J., Pinder, R.A., Ransom, T., Richardson, J.B., Snyder, B.A. & Szlavecz, K. (2021). The second wave of earthworm invasions in North America: biology, environmental impacts, management and control of invasive jumping worms. *Biological Invasions*, 23, 3291-3322.

Recuperado de: https://doi.org/10.1007/s10530-021-02598-1

Costa, A. Da; Albuquerque, J.A.; Costa, A. Da; Pértile, P.; Silva, F.R. da. Water retention and availability in soils of the State of Santa Catarina-Brazil: effect of textural classes, soil classes and lithology. *Revista Brasileira de Ciência do Solo*, v.37, p.1535-1548, 2013. Recuperado de: https://doi.org/10.1590/ s0100-06832013000600010.

Csuzdi, C., Chang, C. H., Pavlícek, T., Szederjesi, T., Esopi, D., & Szlávecz, K. (2017). Molecular phylogeny and systematics of native North American lumbricid earthworms (Clitellata: Megadrili). *PloS one*, 12(8), e0181504. Recuperado de: https://doi.org/10.1371/journal.pone.0181504

Dekemati, I., Simon, B., Bogunovic, I., Kisic, I., Kassai, K., Kende, Z., & Birkás, M. (2020). Long term effects of ploughing and conservation tillage methods on earthworm abundance and crumb ratio. *Agronomy*, 10(10), 1552. Recuperado de: https://doi.org/10.3390/agronomy10101552

Demetrio, W.C., Santos, A., Ferreira, T., Naldony, H., Cardoso, G.B.X., Torres, J.L.M., Dudas, R., Oliveira, V., Barreto, J.O., James, S.W., Silva, E., Brown, G.G. & Bartz, M.L.C. (2018) Earthworm species in various land use systems in the Campos Gerais region of Lapa, Paraná, Brazil. Zootaxa, 4496, 503. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W.C., Ribeiro, R.H., Nadolny, H., Bartz, M.L.C., Brown, G.G. (2019). Earthworms in Brazilian no-tillage agriculture: current status and future challenges, European Journal of Soil Science, 1–18, https://doi.org/10.1111/ejss.12918.

Demetrio, W., Brown, G., Pupin, B., Novo, R., Dudas, R., Baretta, D., Römbke, J. Bartz, M.L.C. & Borma, L. (2023a). Are exotic earthworms threatening soil biodiversity in the Brazilian Atlantic Forest? *Applied Soil Ecology*, 182, 104693. Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104693

Demetrio, W.C.; Da Fonseca, P.M.; Dudas, R.; Zagatto, M.G.R.; Feijoo, A.; Brown, G.G. (2023b) Earthworm species in native and planted forests in Brazil. Zootaxa, 5255, p. 304-323.

Dewi, W. S., & Senge, M. (2015). Earthworm diversity and ecosystem services under threat. *Reviews in Agricultural Science*, 3, 25-35. Recuperado de: https://doi.org/10.7831/ras.3.0_25

Dudas, R. T., Demetrio, W. C., Nadolny, H. S., Brown, G. G., & Bartz, M. L. C. (2023a). Earthworms in the state of Paraná, Brazil: State of the art*. Revista Brasileira de Ciência do Solo*, 47, e0220159. Recuperado de: https://doi.org/10.36783/18069657rbcs20220159

Dudas, R. T., Tavares, A. A., Ercole, C., BL, D. L., Carlos, E. D. S., Torres, J. L., Smoknit, m., Guaranha, R.M., Brown, G.G., & Bartz, M. L. (2023b). Urban green areas as earthworm species maintainers in Curitiba, Paraná, Brazil. *Zootaxa*, 5255(1), 336-346.

Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.27.

Fageria, N. K., Baligar, V. C., & Clark, R. B. (2002). Micronutrients in crop production. *Advances in agronomy*, *77*, 185-268. Recuperado de: https://doi.org/10.1016/S0065-2113(02)77015-6

Feijoo, A. M., Brown, G. G., & James, S. W. (2017). New species of *Andiorrhinus* Cognetti, 1908 (Oligochaeta: Rhinodrilidae) from Venezuela and Brazil. *Zootaxa,* 4363(1), 55-78.

Recuperado de:https://doi.org/10.11646/zootaxa.4363.1.2.

Feijoo, A. & Brown, G.G. (2023) Three new *Glossoscolex* (Annelida: Crassiclitellata: Glossoscolecidae) in the truncatus group from the Brazilian Atlantic Forest. *Zootaxa*, 5225 (1), 220–234. Recuperado de: https://doi.org/ 10.11646/zootaxa.5255.1.21

Fernandes, J.O., Uehara-Prado, M., Brown, G.G. (2010). Minhocas exóticas como indicadoras de perturbação antrópica em áreas de Floresta Atlântica. Acta Zoológica Mexicana, 26 (nueva série), 211–217.

Ferreira, T., James, S. W., Bartz, M. L. C., ACR, D. L., Dudas, R., & Brown, G. G. (2023a). Distribution and diversity of earthworms in different land use systems in Rio Grande do Sul, Brazil. *Zootaxa*, 5255(1), 399-416. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.32.

Ferreira, T., Niva, C. C., Dudas, R., Roani, R., Durães, N., Marchão, R. L., James, S.W., Bartz, M.L.C., & Brown, G. G. (2023b). Earthworm species in different land use systems in the state of Goiás and the Federal District of Brazil. *Zootaxa*, 5255(1), 283-303.

Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.24

Fonte, S. J., Barrios, E., & Six, J. (2010). Earthworms, soil fertility and aggregate-associated soil organic matter dynamics in the Quesungual agroforestry system. *Geoderma*, 155(3-4), 320-328.

Recuperado de: https://doi.org/10.1016/j.geoderma.2009.12.016

Fonte, S. J., Hsieh, M., & Mueller, N. D. (2023). Earthworms contribute significantly to global food production. *Nature Communications*, 14(1), 5713. Recuperado de: https://doi.org/10.1016/j.geoderma.2009.12.016

Fragoso, C., Brown, G. G., Patrón, J. C., Blanchart, E., Lavelle, P., Pashanasi, B., Senapati, B. & Kumar, T. (1997). Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Applied soil ecology*, 6(1), 17-35. Recuperado de: https://doi.org/10.1016/S0929-1393(96)00154-0

Gates, G. E. (1972). Burmese Earthworms - An Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia: *Transactions* of the *American Philosophical Society*, 62, 1-326.

Golpeau, A. (2024). Analyse de la diversité et des déterminismes des communautés de vers de terre dans les écosystèmes de Guyane. PhD Thesis in Community Ecology, University of Montpellier, France. 243 pp.

Gong, X., Wang, S., Wang, Z., Jiang, Y., Hu, Z., Zheng, Y., Chen, X., Li, H., Liu, M. & Scheu, S. (2019). Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. *Geoderma*, 347, 59-69. Recuperado de: https://doi.org/10.1016/j.geoderma.2019.03.043

Hughes, M. S., Bull, C. M., & Doube, B. M. (1994). The effects of sheep manure on the survival and growth of the earthworm *Microscolex dubius* (Annelida: Acanthodrilidae). *Applied Soil Ecology*, 1(4), 291-298. Recuperado de: https://doi.org/10.1016/0929-1393(94)90006-X

Inkotte, J., Bomfim, B., da Silva, S. C., Valadão, M. B. X., da Rosa, M. G., Viana, R. B., & Pereira, R. S. (2022). Linking soil biodiversity and ecosystem function in a Neotropical savanna. *Applied Soil Ecology*, *169*, 104209. Recuperado de: https://doi.org/10.1016/j.apsoil.2021.104209

Instituto Agrônomico (IAC) (2001) Método de análise química para avaliação de solos tropicais. Campinas. 2001.Availabe from: https://lab.iac.sp.gov.br/

Instituto Brasileiro de Geografia e Estatística (IBGE). (2004): "Mapa de biomas e de vegetação". Available from: https://ww2.ibge.gov.br/home/presidencia/noticias/21052004biomashtml.shtm.

James, S. W., Bartz, M. L., & Brown, G. G. (2023). New Ocnerodrilidae genera, species and records from Brazil (Annelida: Crassiclitellata). *Zootaxa.* Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.22

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P.O.W.H. & Dhillion, S. (1997). Soil function in a changing world: the role of invertebrate ecosystem engineers.

Lal, R. (2007). Carbon management in agricultural soils. *Mitigation and adaptation strategies for global change*, *12*, 303-322. Recuperado de: https://doi.org/10.1007/s11027-006-9036-7

Lees, K. J., McKenzie, A, Newell Price, J., Critchley, C. N., Rhymer, C. M., Chambers, B. J., & Whittingham, M. J. (2016). The effects of soil compaction mitigation on below-ground fauna: How earthworms respond to mechanical loosening and power harrow cultivation. *Agriculture, Ecosystems & Environment*, 232, 273–282. Recuperado de: doi:10.1016/j.agee.2016.07.026

Luederwaldt, H. (1927). A coleção de minhocas (Oligochaeta) no Museu Paulista. *Revista do Museu Paulista*, 15, 545-556.

Maggia, M.-E., Decaëns, T., Lapied, E., Dupont, L., Roy, V., Schimann, H., Orivel, J., et al., (2021). At each site its diversity: DNA barcoding reveals remarkable earthworm diversity in neotropical rainforests of French Guiana. Applied Soil Ecology, 164, 103932. https://doi.org/10.1016/j.apsoil.2021.103932

Misirlioğlu, M., Reynolds, J., Stojanović, M., Trakić, T., Sekulić, J., James, S., Csuzdi, C., Decaëns, T., Lapied, E., Phillips, H.R.P., Camreon, E.K., & Brown, G. (2023). Earthworms (Clitellata, Megadrili) of the world: an updated checklist of valid species and families, with notes on their distribution. *Zootaxa*. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.33.

Menezes, C. E. G., Guareschi, R. F., Pereira, M. G., Anjos, L. H. C., Correia, M. E. F., Balieiro, F. C., & Piccolo, M. D. C. (2017). Organic matter in areas under secondary forests and pasture. *Cerne*, *23*(3), 283-290. Recuperado de: https://doi.org/10.1590/01047760201723032333

Nadolny, H. S. (2017). Estado da arte das minhocas como bioindicadoras da qualidade dos solos brasileiros [Tese], Universidade Federal do Paraná, Soils Department, Curitiba.

Ogle, S. M., Alsaker, C., Baldock, J., Bernoux, M., Breidt, F. J., McConkey, B., Vazquez-Amabile, G. G. (2019). Climate and soil characteristics determine where no-till management can store carbon in soils and mitigate greenhouse gas emissions. *Scientific reports*, *9*(1), 11665. Recuperado de: https://doi.org/10.1038/s41598-019-47861-7

Paoletti, M. G. (1999). The role of earthworms for assessment of sustainability and as bioindicators. *Agriculture, Ecosystems & Environment*, 74(1-3), 137-155. Recuperado de: https://doi.org/10.1016/S0167-8809(99)00034-1

Pelosi, C., Gavinelli, F., Petit-Dit-Grézériat, L., Serbource, C., Schoffer, J. T., Ginocchio, R., & van Gestel, C. A. M. (2024). Copper toxicity to earthworms: A comprehensive review and meta-analysis. *Chemosphere*, 362, 142765. Recuperado de: https://doi.org/10.1016/j.chemosphere.2024.142765

Phillips, H.R.P.; Guerra, C.A.; Bartz, M.L.C.; Briones, M.J.I.; Brown, G.; Crowther, T.W.; Ferlian, O.; Gongalsky, K.B.; Hoogen, J. Van Den; Krebs, J.; Orgiazzi, A.; Routh, D.; Schwarz, B.; Bach, E.M.; Bennett, J.; Brose, U.; Decaëns, T.; König-Ries, B.; Loreau, M.; Mathieu, J.; Mulder, C.; Van Der Putten, W.H.; Ramirez, K.S.; Rillig, M.C.; Russell, D.; Rutgers, M.; Thakur, M.P.; De Vries, F.T.; Wall, D.H.; Wardle, D.A.; Arai, M.; Ayuke, F.O.; Baker, G.H.; Beauséjour, R.; Bedano, J.C.; Birkhofer, K.; Blanchart, E.; Blossey, B.; Bolger, T.; Bradley, R.L.; Callaham, M.A.; Capowiez, Y.; Caulfield, M.E.; Choi, A.; Crotty, F.V.; Dávalos, A.; Cosin, D.J.D.; Dominguez, A.; Duhour, A.E.; Eekeren, N. Van; Christoph Emmerling, C.; Falco, L.B.; Fernández, R.; Fonte, S.J.; Fragoso, C.; Gutiérrez López, M.G.; Hackenberger, D.K.; Hernández, L.M.; Hishi, T.; Holdsworth, A.R.; Holmstrup, M.; Hopfensperger, K.N.; Huerta Lwanga, E.; Huhta, V.; Hurisso, T.T.; Iannone Iii, B.V.; Iordache, M.; Joschko, M.; Kaneko, N.; Kanianska, R.; Aidan M. Keith, A.M.; Kelly, C.A.; Kernecker, M.L.; Klaminder, J.; Koné, A.W.; Kooch, Y.; Kukkonen, S.T.; Lalthanzara, H.; Lammel, D.R.; Lebedev, I.M.; Li, Y.; Jesus Lidon, J.B.; Lincoln, N.K.; Loss, S.R.; Marichal, R.; Matula, R.; Moos, J.H.; Moreno, G.; Morón-Ríos, A.Muys, B.; Neirynck, J.; Norgrove, L.; Novo, M.; Nuutinen, V.; Victoria Nuzzo, V.; Rahman P, M.; Pansu, J.; Shishir Paudel, S.; Pérès, G.; Pérez-Camacho, L.; Piñeiro, R.; Ponge, J.-F.; Rashid, M.I.; Rebollo, S.; Javier Rodeiro-Iglesias, J.; Rodríguez, M.Á.; Roth, A.M.; Rousseau, G.X.; Rozen, A.; Sayad, E.; Van Schaik, L.; Scharenbroch, B.C.; Schirrmann, M.; Schmidt, O.; Schröder, B.; Seeber, J.; Shashkov, M.P.; Singh, J.; Smith, S.M.; Steinwandter, M.; Talavera, J.A.; Trigo, D.; Tsukamoto, J.; Valença, A.W. De; Vanek, S.J.; Virto, I.; Wackett, A.A.; Warren, M.W.; Wehr, N.H.; Whalen, J.K.; Wironen, M.B.; Wolters, V.; Zenkova, I.V.; Zhang, W.; Cameron, E.K.; Eisenhauer, N. Global distribution of earthworm diversity. *Science*, v.366, p.480-485, 2019. Recuperado de: https://doi.org/ 10.1126/science.aax4851

Pivello, V. R., Rocha, R. M., Vitule, J. R.S, Braga, R. R., Brown, G. G., Castro, C. F., Cruz Neto, C. C., Franco, A. S., Heringer, G., Magalhães, A. L. B., Miranda, R. J.10; Mormul, R. P., Oliveira, I., Saulino, H. H. L., Silva Matos, D.M. Capítulo 4: Impactos de espécies exóticas invasoras sobre as Contribuições da Natureza para as Pessoas (CNP), o Desenvolvimento Sustentável e a boa qualidade de vida. In: Dechoum, M.S., Junqueira, A. O. R., Orsi, M.L. (Org.). Relatório Temático sobre Espécies Exóticas Invasoras, Biodiversidade e Serviços Ecossistêmicos. 1a Ed. São Carlos: Editora Cubo, 2024. P. 133-184.
https://doi.org/10.4322/978-65-00-87228-6.cap4

Reynolds, J. W., & Misirlioğlu, Ý. M. (2018). Preliminary key to Tunisian megadriles (Annelida, Clitellata, Oligochaeta), based on external characters, insofar as possible. *Megadrilogica*, 23(11).

Richard, B., Decaëns, T., Rougerie, R., James, S. W., Porco, D., & Hebert, P. D. N. (2010). Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. *Molecular ecology resources*, 10(4), 606-614. Recuperado de: https://doi.org/10.1111/j.1755-0998.2009.02822.x

Righi, G. (1972). Additions to the genus *Glossoscolex* (Oligochaeta, Glossoscolecidae). *Studies on Neotropical Fauna and Environment*, 7(1), 37-47. Recuperado de: https://doi.org/10.1080/01650527209360433

Righi, G. (1995) Colombian earthworms. In: Van der Hammen, T. & Santos, A.G. (Eds.), Studies on Tropical Andean Ecosystems, vol. 4. Cramer, Berlin, pp. 485–607.

Rodríguez, M. P., Domínguez, A., Moreira Ferroni, M., Wall, L. G., & Bedano, J. C. (2020). The diversification and intensification of crop rotations under no-till promote earthworm abundance and biomass. *Agronomy*, 10(7), 919. Recuperado de: https://doi.org/10.3390/agronomy10070919

Rota, E., & de Jong, Y. (2015). Fauna Europaea: Annelida-Terrestrial Oligochaeta (Enchytraeidae and Megadrili), Aphanoneura and Polychaeta. Biodiversity Data Journal, (3). Recuperado de: https://doi.org/10.3897/BDJ.3.e5737

Rutgers, M.; Orgiazzi, A.; Gardi, C.; Römbke, J.; Jänsch, S.; Keith, A.M.; Neilson, R.; Boag, B.; Schmidt, O.; Murchie, A.K.; Blackshaw, R.P.; Pérès, G.; Cluzeau, D.; Guernion, M.; Briones, M.J.I.; Rodeiro, J.; Piñeiro, R.; Cosín, D.J.D.; Sousa, J.P.; Suhadolc, M.; Kos, I.; Krogh, P.-H.; Faber, J.H.; Mulder, C.; Bogte, J.J.; Wijnen, H.J. Van; Schouten, A.J.; Zwart, D. De. Mapping Earthworm Communities in Europe. *Applied Soil Ecology*, V.97, P.9 Recuperado de: https://doi.org/10.1016/j.apsoil.2015.08.015

Schwert, D.P. (1991). Chapter 11: Oligochaeta: Lumbricidae. In: Dindal, D. L. (Ed.). (1991). *Soil biology guide*. John Wiley & Sons.

Silva, R. D., Rodrigues, I., Guimarães, A., & Drumond, M. (2015). Comportamento de construção de câmara de estivação por *Rhinodrilus alatus* e sua relação com a pluviosidade. Encontro Latino-Americano De Ecologia E Taxonomia De Oligoquetas, 5.

Singh, J., Singh, S., & Vig, A. P. (2016). Extraction of earthworm from soil by different sampling methods: a review. *Environment, Development and Sustainability*, 18, 1521-1539. Recuperado de: https://doi.org/10.1007/s10668-015-9703-5

Singh, J., Schädler, M., Demetrio, W., Brown, G. G., & Eisenhauer, N. (2019). Climate change effects on earthworms-a review. *Soil organisms*, 91(3), 114. Recuperado de: https://doi.org/10.25674/so91iss3pp114

Smith, R. G., McSwiney, C. P., Grandy, A. S., Suwanwaree, P., Snider, R. M., & Robertson, G. P. (2008). Diversity and abundance of earthworms across an agricultural land-use intensity gradient. *Soil and Tillage Research*, 100(1-2), 83-88. Recuperado de: https://doi.org/10.1016/j.still.2008.04.009

Steffen, G. P. K., Steffen, R. B., Bartz, M. L. C., James, S. W., Jacques, R. J. S., Brown, G. G., & Antoniolli, Z. I. (2018). Earthworm diversity in Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 562-575.

Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.43

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). Manual de métodos de análise de solo.

Torppa, K. A., & Taylor, A. R. (2022). Alternative combinations of tillage practices and crop rotations can foster earthworm density and bioturbation. *Applied Soil Ecology*, 175, 104460.

Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104460

Vršič, S., Breznik, M., Pulko, B., & Rodrigo-Comino, J. (2021). Earthworm abundance changes depending on soil management practices in slovenian vineyards. *Agronomy*, 11(6), 1241.

Recuperado de: https://doi.org/10.3390/agronomy11061241

Yang, Y., Ding, J., Zhang, Y., Wu, J., Zhang, J., Pan, X., ... & He, F. (2018). Effects of tillage and mulching measures on soil moisture and temperature, photosynthetic characteristics and yield of winter wheat. *Agricultural Water Management*, 201, 299-308.

Recuperado de: https://doi.org/10.1016/j.agwat.2017.11.003

Zicsi, A., Römbke, J. & Garcia, M. (2001) Regenwürmer (Oligochaeta) aus der Umgebung von Manaus (Amazonien). Regenwürmer aus Südamerika 32*. Revue Suisse de Zoologie*, 108 (1), 153–164.

Recuperado de: https://doi.org/10.5962/bhl.part.79624

3 ARTICLE 2: NEW SPECIES OF *GLOSSOSCOLEX* AND *FIMOSCOLEX* (OLIGOCHAETA: GLOSSOSCOLECIDAE) FROM THE BRAZILIAN ATLANTIC FOREST AND CERRADO BIOMES[1]

Rafaela Tavares Dudas, Marie Luise Carolina Bartz, Luís Filipe Das Neves Cunha, George Gardner Brown

3.1 RESUMO

O Brasil apresenta notável biodiversidade, com uma presença estimada de mais de duas mil espécies de minhocas, das quais >80% permanecem não descritas. Aqui descrevemos nove novas espécies de minhocas pertencentes à família Glossoscolecidae, descobertas em áreas de vegetação nativa, sistemas de plantio direto e terras mal manejadas em três regiões do Brasil. Os espécimes de minhocas foram coletados utilizando metodologias quantitativas e qualitativas. Entre as nove espécies encontradas, sete pertencem ao gênero *Glossoscolex*: *Glossoscolex arnsi* n. sp.*, Glossoscolex alessioi* n. sp.*, Glossoscolex dallavecchiai* n. sp.*, Glossoscolex debortolii* n. sp.*, Glossoscolex strobeli* n. sp.*, Glossoscolex* n. sp.77, *Glossoscolex fuchsi* n. sp. Esta última foi a única encontrada no bioma Cerrado; todas as demais eram da Floresta Atlântica. As outras duas pertencem ao gênero *Fimoscolex*: *Fimoscolex fridrichi* n. sp., e *Fimoscolex* n. sp.51 Com exceção de *G. strobeli* n. sp., que pertence ao grupo da *Glossoscolex bergi* (caracterizada por poros masculinos intraclitelares localizados nos segmentos XXI/XXII), as espécies restantes de *Glossoscolex* pertencem ao grupo de *Glossoscolex truncatus*, exibindo poros masculinos intraclitelares no segmento XVII. A espécie *F. fridrichi* n. sp. apresenta uma notável ausência de câmara copulatória, apesar de possuir um poro masculino no segmento XVII. Uma tabela comparativa é fornecida, delineando as semelhanças e diferenças entre as espécies recentemente descritas e espécies previamente documentadas nos mesmos gêneros. É imperativo agilizar a descrição das espécies de minhocas para facilitar a determinação dos padrões de distribuição desses gêneros e elucidar seus papéis ecológicos dentro dos ecossistemas edáficos.

Palavras-chave: Clitellata, Crassiclitellata, Oligochaeta, Mata Atlântica, Cerrado

3.2 ABSTRACT

Brazil exhibits remarkable biodiversity, with an estimated presence of over two thousand earthworm species, of which >80% remain undescribed. Here we describe nine new species of earthworms belonging to the Glossoscolecidae family, discovered in areas of native vegetation, no-tillage systems, and poorly managed lands across Brazil. The earthworm specimens were collected using quantitative and qualitative methodologies. Among the nine species found, seven belong to the genus *Glossoscolex*: *Glossoscolex arnsi* n. sp.*, Glossoscolex alessioi* n. sp.*, Glossoscolex dallavecchiai* n. sp.*, Glossoscolex debortolii* n. sp.*, Glossoscolex strobeli* n. sp.*, Glossoscolex* n. sp.77, and *Glossoscolex fuchsi* n.

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[1] Submitted to Zootaxa

sp. Only this last species came from the Cerrado biome; all others were from the Brazilian Atlantic Forest. The other two belong to the genus *Fimoscolex*: *Fimoscolex fridrichi* n. sp., and *Fimoscolex* n. sp.51. Except for *G. strobeli* n. sp., which belongs to the *Glossoscolex bergi* group (characterized by intraclitelar male pores located in segments XXI/XXII), the remaining *Glossoscolex* species belong to the *Glossoscolex truncatus* group, with intraclittelar male pores in segment XVII. The species *F. fridrichi* n. sp. is remarkable due to the absence of a copulatory bulb, despite possessing a male pore in segment XVII. A comparative table is provided, delineating the similarities and differences between the newly described species and previously documented species within the two genera. It is imperative to expedite earthworm species descriptions to facilitate determining the distribution patterns and ecological roles of these genera in soil ecosystems.

Key-words: Clitellata, Crassiclitellata, Oligochaeta, Atlantic Forest, Cerrado

3.3 INTRODUCTION

Earthworms are important soil quality bioindicators and different species can indicate various environmental conditions. In Brazil, approximately 336 species are known, belonging to nine families (Brown *et al*. 2013). However, nearly 2100 species have been estimated, so >80% remain undescribed (Brown and James, 2007). This is mainly due to the few active taxonomists and earthworm specialists in Brazil, where there are only five persons qualified in classical taxonomy, two of whom are still graduate students.

The most diverse earthworm family in Brazil is Glossoscolecidae, with 156 known species and two main genera found in different soil management systems: *Glossoscolex* (Leuckart, 1835) and *Fimoscolex* (Michaelsen, 1900) (Misirlioglu *et al*. 2023). There are records of *Glossoscolex* in pasture (Bartz *et al.* 2018; Feijoo and Brown 2023), marshes (Bartz et al. 2012), native Atlantic Forest and highland grassland (Cardoso *et al.* 2014; Demetrio *et al.* 2018; Dudas *et al.* 2025), Pampa (Steffen *et al.* 2018), and no-tillage systems (Santos *et al.* 2018). For *Fimoscolex*, there are records in marshes (Brown & James 2007), lawns and highland grassland (Dudas *et al.* 2023, 2025), no-tillage systems (Bartz *et al.* 2023), and Araucaria Forests (Demetrio *et al.* 2023). The distribution of these genera therefore covers diverse vegetation covers, and their interactions with the soil can vary depending on the species and the environment in which they are found (Kanianska *et al*. 2016). Understanding the diversity, taxonomic characteristics, and ecological role of these species is essential for the preservation of tropical soils and for the development of sustainable agricultural practices (Jouquet *et al*. 2018). These genera are often found in the same location (Dudas *et al.* 2025) and seem to occupy similar niches in the soil. In fact, the only morphological feature used to distinguish them is the number of copulatory bulbs (one in *Fimoscolex* and two in *Glossoscolex;* Feijoo and Brown 2018), and their phylogenetic relationships are still not clear (Silva *et al*. 2017; Dudas *et al*. 2025), so further work on these genera is warranted.

Despite the recognized importance of some species of the Glossoscolecidae family, there are significant gaps in the taxonomic knowledge of these earthworms in Brazil (James and Brown 2006). The country has a vast soil animal diversity that has yet to be explored and studied, but this is hindered by the shortage of specialists, the need for taxonomic reviews and species identification keys, and the time required to describe and publish data on new species (Fritz *et al*. 2020). All these factors limit the current understanding of endemic species and their geographic distribution.

In the present study we describe seven new species of the Glossoscolecidae family, six in the genus *Glossoscolex* and one in *Fimoscolex,* collected in different cities located in the Brazilian states of Rio Grande do Sul, Paraná, São Paulo, and Mato Grosso do Sul. Furthermore, we provide morphological data on two other species, aiming to facilitate future description once further specimens are collected.

3.4 MATERIAL AND METHODS

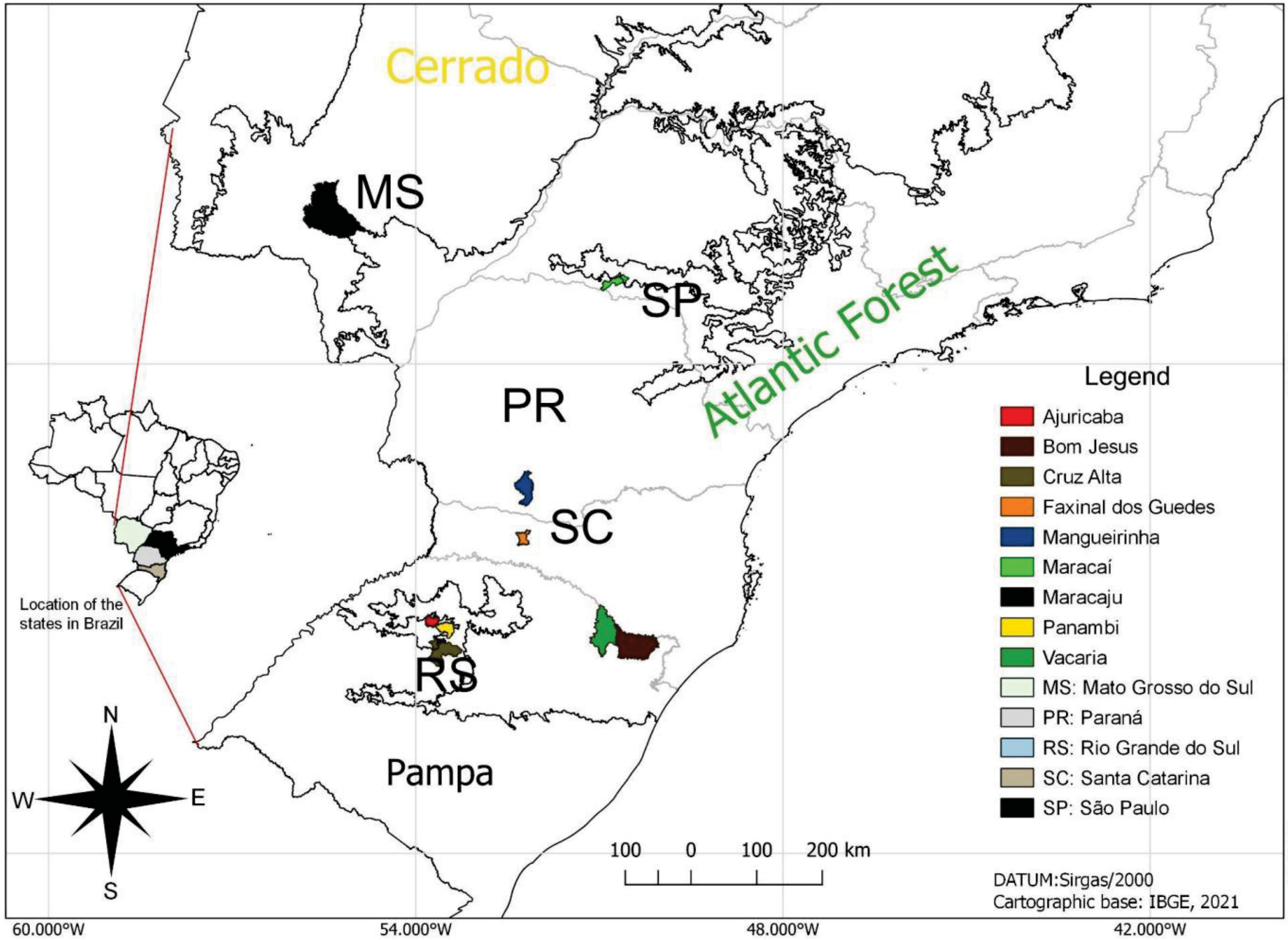
Earthworms were collected via an adaptation of the quantitative method of the Tropical Soil Biology Fertility (TSBF) Program of UNESCO (Anderson; Ingram 1993), by removing nine soil monoliths 25 cm x 25 cm and 20 cm deep at each site. Additionally, earthworms were collected using a qualitative method, by excavating at least an additional nine soil pits of similar dimensions, and by digging in key locations of the sites, such as under logs and areas with high content of organic matter. Earthworms collected in both sampling methods were fixed and preserved in 96% ethanol solution. The specimens were collected in the Brazilian states of Mato Grosso do Sul (Maracaju), São Paulo (Maracaí), Paraná (Mangueirinha), Santa Catarina (Faxinal dos Guedes) and Rio Grande do Sul (Ajuricaba, Bom Jesus, Cruz Alta, Panambi and Vacaria) (FIGURE 14). The sampling sites were in two Brazilian biomes: Atlantic Forest and Cerrado, although the only site in the latter biome was Maracaju. Selected environmental and soil characteristics of the sites where each of the nine new species were found are specified in TABLE 6.

For the identification of the species, taxonomic keys and morphological characteristics of *Fimoscolex* and *Glossoscolex* were utilized following Righi (1972), Bartz *et al*. (2012; 2013b; 2018) and Feijoo and Brown (2018). Initially, the external characters were observed, followed by dorsal dissection for examination of internal structures. All specimens are deposited in the Fritz Müller Oligochaeta Collection (COFM), at Embrapa (Brazilian Agricultural Research Corporation) Forestry in Colombo (Paraná), Brazil, and paratypes in the Museum of Zoology of São Paulo (MZUSP), in São Paulo, Brazil.

For DNA analyses, a piece of tissue of a least two individuals per species, was removed, and the extraction followed the Qiagen protocol - DNeasy® Blood & Tissue Handbook (2011). After extraction, the samples were used for amplification of COI gene on a polymerase chain reaction (PCR) using the universal primers and PCR conditions described in Folmer et al. (1994). ExoSap-IT TM Express PCR was used for PCR Product Cleanup protocol. The DNA sequencing was performed in the Macrogen and Eurofins labs.

The DNA data analysis was performed in the Molecular Evolutionary Genetics Analysis version 11 - MEGA11 (Temura *et al.* 2021). The evolutionary divergence between sequences was calculated by analyses of Maximum Composite Likelihood (Temura *et al*. 2004). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. This analysis involved 11 nucleotide sequences, and 500 replicates. We were unable to amplify the DNA from *Glossoscolex* n. sp.77 and *Glossoscolex fuchsi* n. sp. Sequences from *Pontoscolex corethrurus* (code OQ222854.1), *Glossoscolex riograndensis* (code JX177853) and *Fimoscolex* n. sp.25 (code OQ067384.1) were recovered from the online repository GenBank - NCBI and used as outgroups and to support the phylogenetic analysis.

FIGURE 14 – MAP OF THE MUNICIPALITIES IN BRAZIL WHERE EARTHWORMS WERE COLLECTED



Source: Author (2024)

TABLE 6 – CHARACTERIZATION OF THE SAMPLING SITES, INCLUDING STATE, COUNTY, GEOGRAPHICAL COORDINATES AND SITE CHARACTERIZATION

State	**City**	**Specie**	**Location (lat/lon/elevation)**	**Characterization**
RS	Bom Jesus	*Glossoscolex arnsi* n. sp.	-28,467102°S, -50,597372°W, 1003m	native vegetation in Atlantic Forest
RS	Bom Jesus	Glossoscolex *arnsi* n. sp.	-28,485754°S, -50,609524°W, 1043m	24 years under NTS
RS	Vacaria	Glossoscolex *arnsi* n.sp.	-28,391828°S, -51,082774°W, 879m	native vegetation in Atlantic Forest
SC	Faxinal dos Guedes	*Glossoscolex alessioi* n.sp.	-26,785627°S, -52,229168°W, 883m	28 years under no-tillage system
SC	Faxinal dos Guedes	*Glossoscolex alessioi* n.sp.	-26,807988°S, -52,238571°W, 924m	native vegetation in Atlantic Forest
PR	Mangueirinha	*Glossoscolex dallavecchiai* n.sp.	-26,05108°S, -52,235847°W, 966m	native vegetation in Atlantic Forest
RS	Cruz Alta	*Glossoscolex debortolii* n.sp.	-28,763297°S, -53,59013°W, 432m	native vegetation in Atlantic Forest
RS	Cruz Alta	*Glossoscolex debortolii* n.sp.	-28,76386°S, -53,583866°W, 419m	30 years under no-tillage system
RS	Panambi	*Glossoscolex strobeli* n.sp.	-28,348351°S, -53,41567°W, 514m	28 years under no-tillage system
RS	Cruz Alta	*Glossoscolex strobeli* n.sp.	-28,76386°S, -53,583866°W, 419m	30 years under no-tillage system
SP	Maracaí	*Glossoscolex* n. sp.77	-22,668361°S, -50,880278°W, 390m	native vegetation in Atlantic Forest

TABLE 6 continuation…

State	**City**	**Specie**	**Location (lat/lon/elevation)**	**Characterization**
MS	Maracaju	*Glossoscolex fuchsi* n.sp.	-21,70238°S, -55,632698°W, 610m	pasture over 10 years old, with no signs of degradation, with native plants growing
RS	Panambi	*Fimoscolex fridrichi* n.sp.	-28,375519°S, -53,445185°W, 494m	native vegetation in Atlantic Forest
RS	Panambi	*Fimoscolex fridrichi* n.sp.	-28,371324°S, -53,418971°W, 494m	no-tillage with crop succession (soybean and wheat or oats)
RS	Cruz Alta	*Fimoscolex fridrichi* n.sp.	-28,76386°S, -53,583866°W, 419m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* n.sp.	-28,167072°S, -53,814564°W, 419m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* n.sp.	-28,144014°S, -53,777835°W, 422m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* n.sp.	-28,149816°S, -53,774019°W, 385m	no-tillage with crop succession
PR	Mangueirinha	*Fimoscolex* n. sp.51	-26,051079°S, -52,237655°W, 981m	27 years under no-tillage system

Source: Author (2004)

3.5 RESULTS

3.5.1 Brief description of the type species of the genera *Glossoscolex* and *Fimoscolex*

Glossoscolecidae, Michaelsen, 1900

Genus *Glossoscolex* Leuckart, 1835

Type species *Glossoscolex giganteus* Leuckart, 1835

Diagnosis. Setae in 8 longitudinal lines, one or two male pores associated with a pair of copulatory bulbs. One pair of calciferous glands in segments XI or XII, of composite-tubular structure. Sexual system metandric and metagynic, frequently long seminal vesicles, but spermathecae absent.

***Glossoscolex* (*Glossoscolex*) *truncatus* species group**

Type species: *Glossoscolex truncatus* Rosa, 1895

Diagnosis. Intraclitellar male pores located in segment XVII, associated with two copulatory bulbs.

***Glossoscolex* (*Glossoscolex*) *bergi* species group**

Type species: *Glossoscolex bergi* Rosa, 1900

Diagnosis. Intraclitellar male pores located posterior to segment XX, associated with two copulatory bulbs.

Genus *Fimoscolex* Michaelsen, 1900

Type species. *Fimoscolex ohausi* Michaelsen, 1918

Diagnosis. Intraclitellar single male pore associated with a single (or none) copulatory bulb. Calciferous glands in segment XI or XII.

Remarks: The diagnosis of the genus has been changed to reflect that fact that copulatory bulbs may not necessarily be present in the genus, as observed in *F. fridrichi* n.sp.

3.5.2 Description of new species of *Glossoscolex* and *Fimoscolex*

***Glossoscolex arnsi* n. sp. Dudas, Brown & Bartz**

Holotype. one adult, native vegetation, in 0-20 cm soil, by qualitative method, Vacaria, Rio Grande do Sul, Brazil, 28°23” 30.581° S, 51°4”57.986’ W, 879 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0431 deposited at COFM.

Paratype. one adult, native vegetation, in 0-20 cm soil, by qualitative method, Bom Jesus, Rio Grande do Sul, Brazil, 28°28'01.6"S, 50°35'50.5"W, 1003 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0425 deposited at COFM.

Other materials. 41 individuals, same information as holotype, sample ID: BRRS0530; deposited at COFM. one adult, same information as paratype, sample ID: BRRS0531; deposited at COFM. two adults, same information as paratype, sample ID: BRRS0415 deposited at COFM. two adults, same information as paratype, sample ID: BRRS0419 deposited at COFM. 12 adults, same information as paratype, sample ID: BRRS0421 deposited at COFM. 37 individuals, same information as paratype, sample ID: BRRS0422 deposited at COFM.

Etymology. The species is named in honor of the farmer Ulfried Arns and his family, owners of Três Marias Farm, pioneers of no-tillage system in Rio Grande do Sul state.

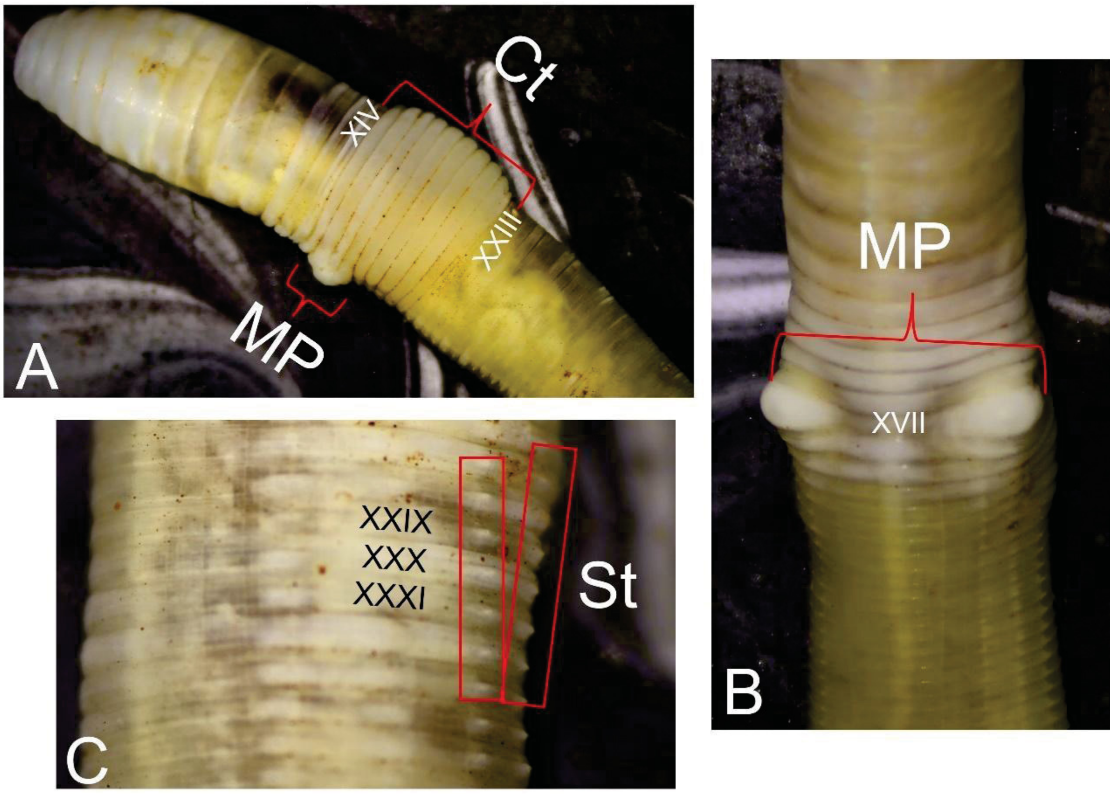
External morphology (FIGURE 15). Holotype: body length 59 mm after ethanol fixation. Body mass: 0.30 g fresh weight (alcohol preserved). Number of segments: 169. Diameter: 5 mm in the pre clitellar region (segment X), 6.3 mm in the clitellum (segment XVI) and 4 mm in the post clitellar region (segment XXX). Paratype: length 7.2 mm after ethanol fixation. Body mass: 0.34 g, number of segments: 159. Diameter: 3 mm in segment X, 4.3 mm in the clitellum at segment XVI and 2.9 mm in segment XXX. Body cylindrical, non-pigmented after fixation. Prostomium epilobic. Setae widely paired, visible throughout, setae *ab* visible from V onwards. Setal arrangement aa:ab:bc:cd:dd, 3:1.1:2.3:1:2.8 at segment XXX-L. Clitellum in XIV to XXIII, saddle shaped. Genital marks or tubercula pubertatis are absent. One pair of male pores, on *b* line setae, on a conical protuberance in XVII, 3.7 mm apart. Female pores not seen. Nephropores near *ab* line.

Internal morphology (FIGURE 16). Septa strong, thickened on VII, 7/8/9/10/11, strongly attached to the body wall, due to the thickness of the septa.

Gizzard in VI, with average size (width x length) of 1.3 x 1.7 mm. One pair of calciferous glands in XII, composite-tubular type, with dorsal blood vessels. Intestine begins in XV with typhlosole also beginning in XV. The last pair of hearts in XI, and dorsal blood vessels visible in segments VIII-X and above the calciferous glands. Nephridia starts pre-citellar, in segment IX/X, close to the body wall and intestine, one pair per segment. Unpaired testis sac, large, in XI, close to calciferous gland. Seminal vesicles in XIII, starting ventrally and ending dorsally above the intestine on segment LX, lobular shaped stretching in two lines. Ovarian funnels in segment 13. Spermathecae absent. One pair of copulatory pouches (bulbs, chambers) with oval shape in 1/3 XVI – 1/3 XX, 3 mm long.

Remarks. Regarding the clitellum, *Glossoscolex arnsi* n. sp. shares position (XIV-XIII) with *G. bondari*, *G. minor*, and *G. tocape*, although the first two have annular clitella, which differs from *G. arnsi* n. sp. The primary distinctions between *G. tocape* and *G. arnsi* n. sp. are the presence of ventrally fused testes sacs in XI in *G. tocape*, compared to the unpaired testis sac in the new species, and the presence of tubercula pubertaria (absent in the clitellum of *G. arnsi* n. sp.) associated with the internal copulatory bulbs in segments XVI-XVIII in *G. tocape* (shorter than in *G. arnsi* n. sp.). The configuration of the male pores in segment XVII of *G. arnsi* n. sp. is notably prominent, with large pores that are visible regardless of the specimen's position, relative to the specimen size. A unique characteristic observed in *G. arnsi* n. sp. is the position of the copulatory bulb, located from 1/3 XVI to 1/3 of XX, a feature not observed in other *Glossoscolex* species in the *truncatus* group

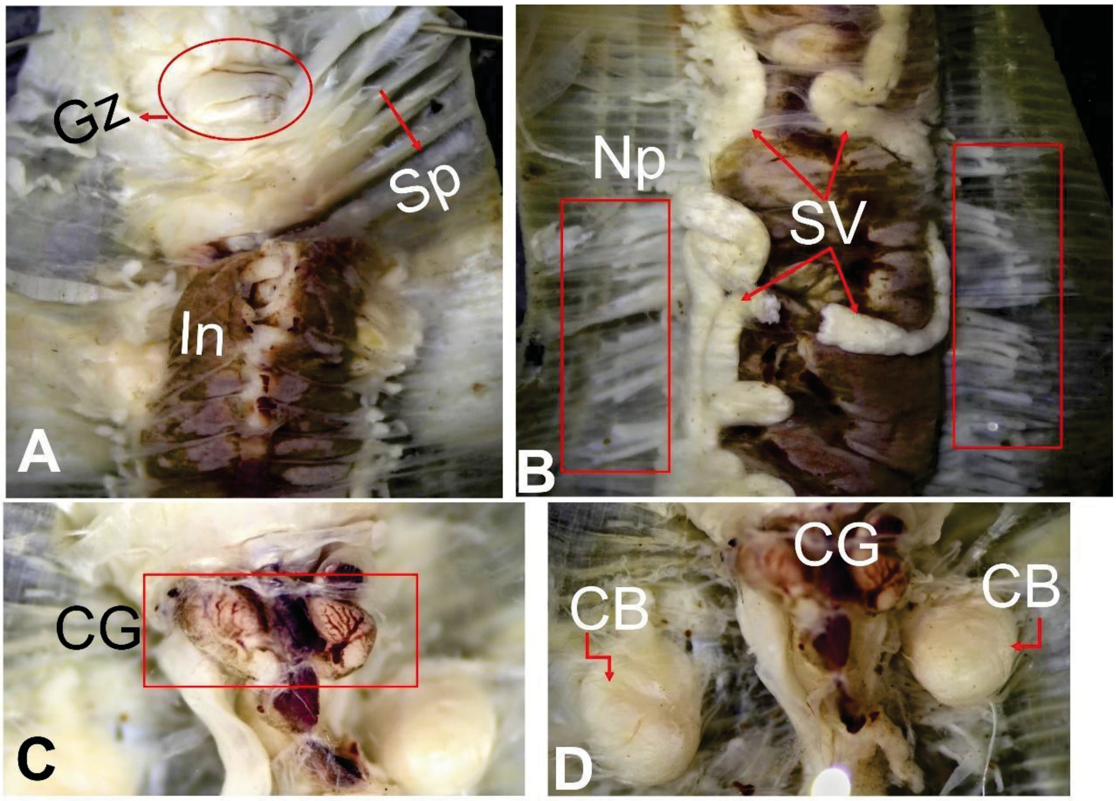
FIGURE 15 – EXTERNAL VIEW OF *GLOSSOSCOLEX ARNSI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex arnsi* n. sp. Holotype. A: Clitellum XIV-XIII, male pore in XVII. B: View of the pair of male pores in XVII. C: View with emphasis in the AB setal line. Ct: clitellum; MP: male pore; St: setae.

FIGURE 16 – INTERNAL VIEW OF *GLOSSOSCOLEX ARNSI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex arnsi* n. sp. Holotype. A: Gizzard in VI, septa in VII-IX, intestine, XV. B: View of seminal vesicle in XX, nephridia in the red rectangle. C: View with emphasis the calciferous gland, in XII. D: CG and highlighting the copulatory bulbs, in XVI. Gz: gizzard; Sp: septa; In: intestine; SV: seminal vesicle; Np: nephridia; CG: calciferous gland; CB: copulatory bulb.

***Glossoscolex alessioi* n. sp. Dudas, Brown & Bartz**

Holotype. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Faxinal dos Guedes, Santa Catarina, Brazil, 26°47'08.3"S, 52°13'45.0"W, 883 m asl, August 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRSC0296 deposited at COFM.

Paratype. Brazil, one adult, same information as the holotype. Sample ID: BRSC0337.

Other materials. Brazil, one adult, same information as the holotype. Sample ID: BRSC0338.

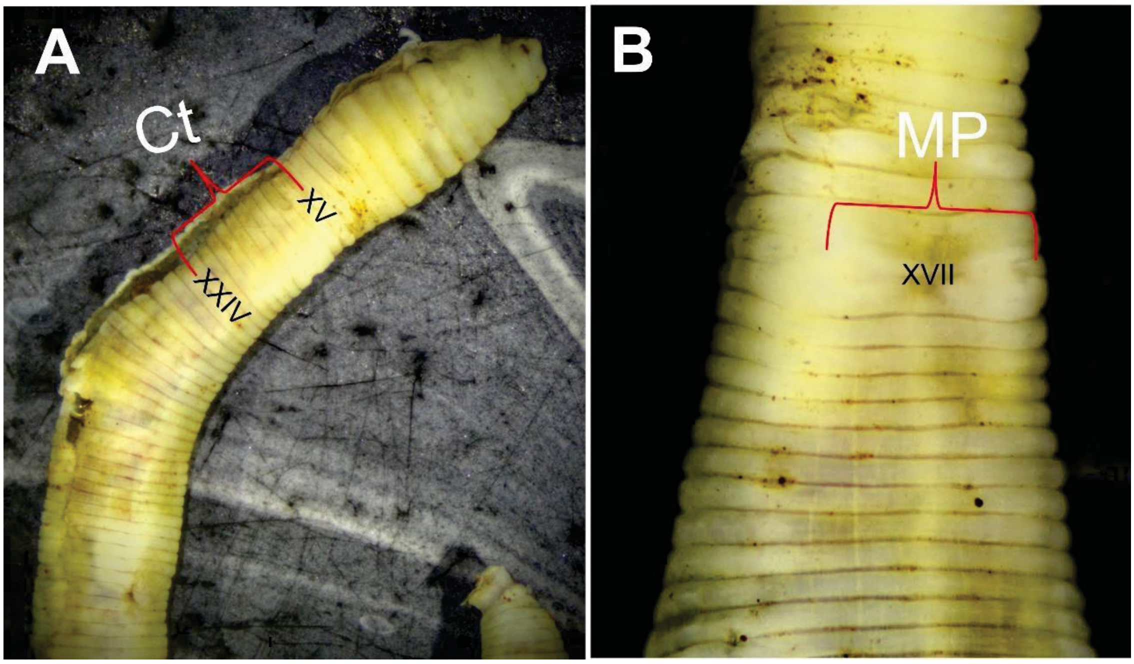
Etymology. The species was named in honor of the farmer Carlos Roberto Alessio and sons (Rodrigo and Diego), owners of the Banhado Verde Farm and pioneers of no-tillage system in Santa Catarina state.

External morphology (FIGURE 17). Holotype: body length 27 mm after ethanol fixation. Body mass: 0.04 g fresh weight (alcohol preserved). Number of segments: 129. Diameter: 2.2 mm in the pre clitellar region (segment X), 2.7 mm in the clitellum (segment XVI) and 1.8 mm in the post clitellar region (segment XXX). Paratype: length 42 mm after ethanol fixation. Body mass: 0.1 g, number of segments: 143. Diameter: 2.6 mm in segment X, 3.3 mm in the clitellum (segment XVI), and 2.4 mm in segment XXX. Body cylindrical, non-pigmentaded after fixation. Prostomium epilobic. Setae closely paired, visible throughout, setae *cd* visible from IV onwards. Setal arrangement aa:ab:bc:cd:dd, 3:1.1:1:1.9:3 at segment XXX-L. Clitellum in XV-XXIV saddle shaped. Genital marks or tubercula pubertatis absent. One pair of male pores, on *b* line setae, on XVI/XVII, 3.6 mm apart. Female pores not seen. Nephropores near *b* line.

Internal morphology (FIGURE 18). Septa strong, thickened on 7/8/9/10, strongly attached to the body wall, due to the thickness of the septa. Gizzard in VI. One pair of calciferous glands in XII, of composite-tubular type, with dorsal blood vessels. Intestine begins in XV. Last pair of hearts in XI, and dorsal blood vessel visible in segments VIII-X and above the calciferous glands. Nephridia starts pre-citellar, in segment IX-X, close to the body wall and intestine, one pair per segment. Paired testes sacs large in XII, behind the calciferous glands. Seminal vesicles long, starting in XII and going beyond LXX. They start coiled (spiral) and become two lines above the intestine. Ovaries not seen. Spermathecae absent. One pair of bean-shaped copulatory bulbs in XVII – 2/3 XX, 5 mm long.

Remarks. The saddle-shaped clitellum in XV-XXIV of *G. alessioi* n. sp. is similar to *G. bonariensis, G. catharinensis*, *G. embrapaensis*, and *G. dallavecchiai* n. sp. However, the only species that has similar testes sacs are the other new species *G. dallavecchiai* n. sp., both with a pair of testes in XII. The distinguishing feature between these species is the form and position of the copulatory bulbs. In *G. alessioi* sp. nov., the bulbs are bean-shaped in XVII-2/3 XX, whereas in *G. dallavecchiai* n. sp., the bulbs are more elongated occupying 2/3 XV-1/3 XIX.

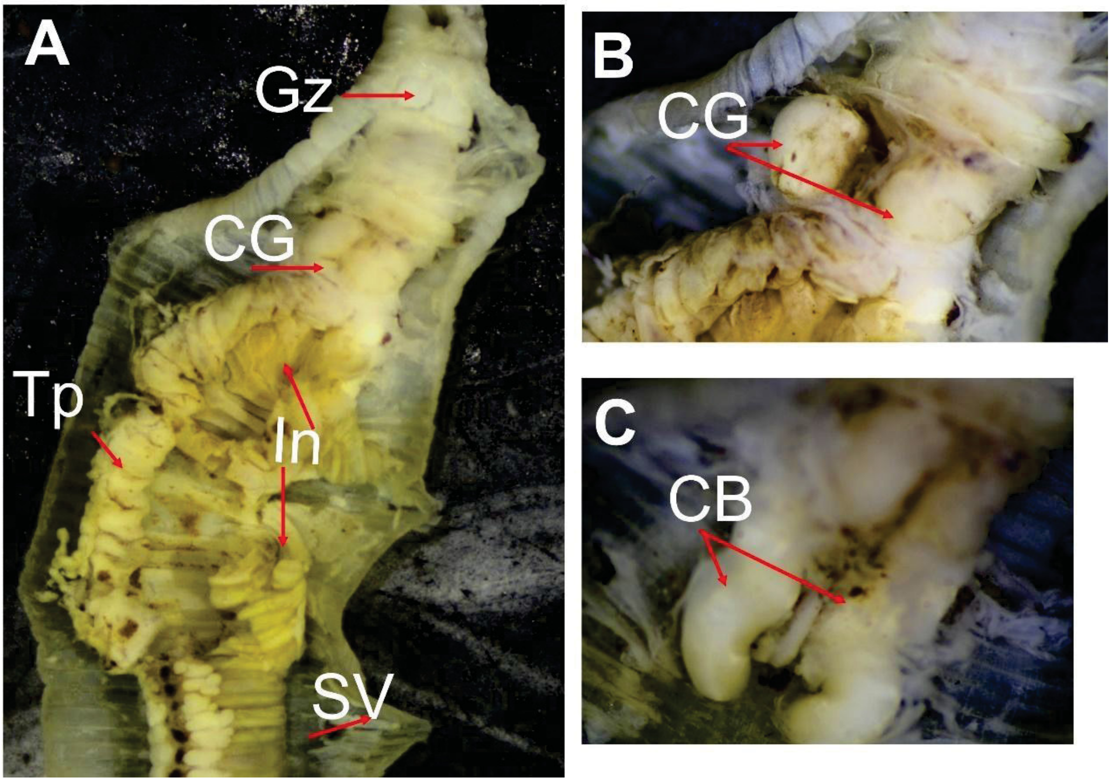
FIGURE 17 – EXTERNAL VIEW OF *GLOSSOSCOLEX ALESSIOI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex alessioi* n. sp. Holotype. A: Clitellum XV-XXIV. B: View of the pair of male pores in XVII. Ct: clitellum; MP: male pore.

FIGURE 18 - INTERNAL VIEW OF *GLOSSOSCOLEX ALESSIOI* n. sp..



SOURCE: Author (2024)

Legend: *Glossoscolex alessioi* n. sp. Holotype. A: View of gizzard in VI, calciferous glands, in XII, seminal vesicle, intestine and typhlosole, from XV. B: View with emphasis the calciferous gland, in XII. C: View highlighting the copulatory bulbs, in XX. Gz: gizzard; CG: calciferous gland; In: intestine; Tp: typhlosole; SV: seminal vesicle; CB: copulatory bulb.

***Glossoscolex dallavecchiai* n. sp. Dudas, Brown & Bartz**

Holotype. one subadult, native vegetation, in 0-20 cm soil, by qualitative method, Mangueirinha, Paraná, Brazil, 26°03'03.9"S, 52°14'09.1"W, 966 m asl, August 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRPR02167 deposited at COFM.

Paratype. one adult, same information as the holotype. Sample ID: BRPR02168.

Other materials. one juvenile, same information as the holotype. Sample ID: BRPR02359.

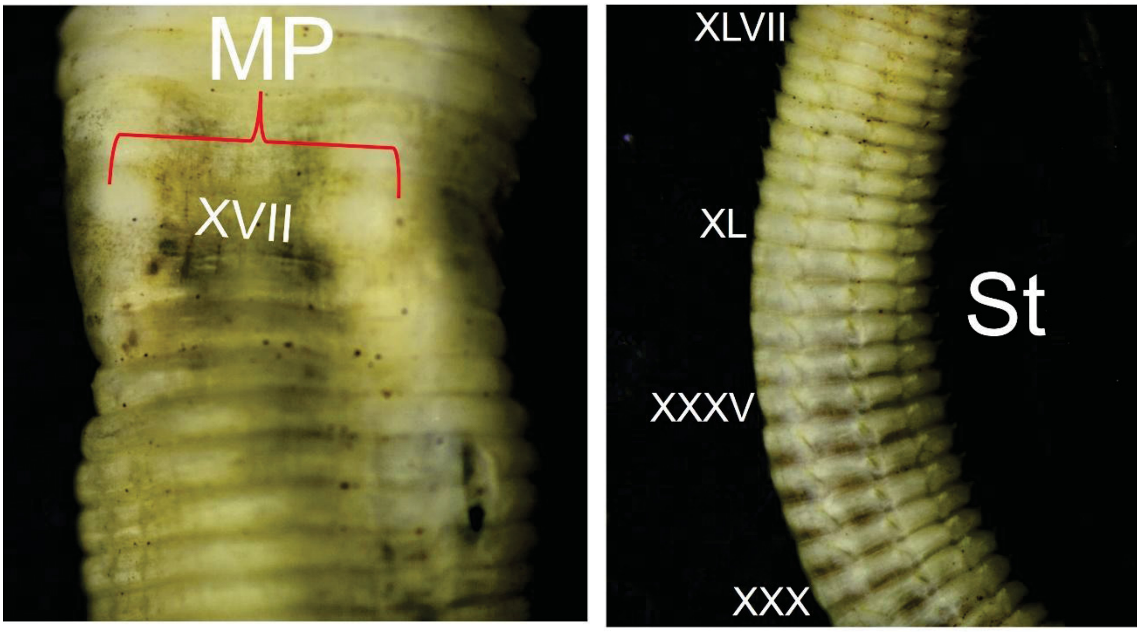
Etymology. The species was named in honor of the farmer Laércio Dalla Vecchia and his family, owners of the São Judas Tadeu Farm, and for his dedication to disseminating good soil management practices and the no-tillage system.

External morphology (FIGURE 19). Holotype: body length 7.3 mm after ethanol fixation. Body mass: 0.19 g fresh weight (alcohol preserved). Number of segments: 233. Diameter: 3.7 mm in the pre clitellar region (segment X), 4.3 mm in the clitellum (segment XVI) and 2.9 mm in the post clitellar region (segment XXX). Paratype: length 42 mm after ethanol fixation. Body mass: 0.14 g, number of segments: 198. Diameter: 2.9 mm in segment X, 3.4 mm in the clitellum (segment XVI), and 2.5 mm in segment XXX. Body cylindrical, non-pigmentaded after fixation. Prostomium open epilobic. Setae widely paired, visible throughout, setae *ab* visible from IV onwards. Setal arrangement aa:ab:bc:cd:dd, 2.1:1.1:1:1.8:2.1 at segment XXX-L. Clitellum in XV-XXIII saddle shaped. Genital marks or tubercula pubertatis absent. One pair of male pores, on *a* line setae, in XVII. Female pores not seen. Nephropores near *b* line.

Internal morphology (FIGURE 20). Septa weak in 7/8/9, and veil format in 10/11/12/13. Gizzard in VI, with average size (width x length) 1.9 mm x 2.1 mm. One pair of calciferous glands in XII, with dorsal blood vessels, of tubular composite type. Intestine begins in XV. Last pair of hearts in XI, and dorsal blood vessel visible in segments VIII-X, and above the calciferous glands. Nephridia starting post clitellar in segment XVI, close to the body wall and intestine, one pair per segment. Paired testes sacs large in XII, with very visible funnels going out of the sac towards seminal vesicles. Seminal vesicles elongated, in the shape of two parallel stripes. Ovaries not seen. Spermathecae absent. One pair of bean shaped copulatory bulbs in 2/3 XV – 1/3 XIX.

Remarks. *Glossoscolex dallavecchiai* n. sp. is similar to *G. alessioi* n. sp. as previously mentioned, although DNA analyses indicate that they are distinct species. The elongated form of the copulatory bulbs in this new species is a distinctive feature observed among the truncatus group, occupying segments 2/3 XV - 1/3 XIX. Furthermore, *G. dallavecchiai* n. sp. exhibits an uncommon type of anterior septa, weak and more fragile, resembling a veil-like structure that offers minimal resistance during dissection.

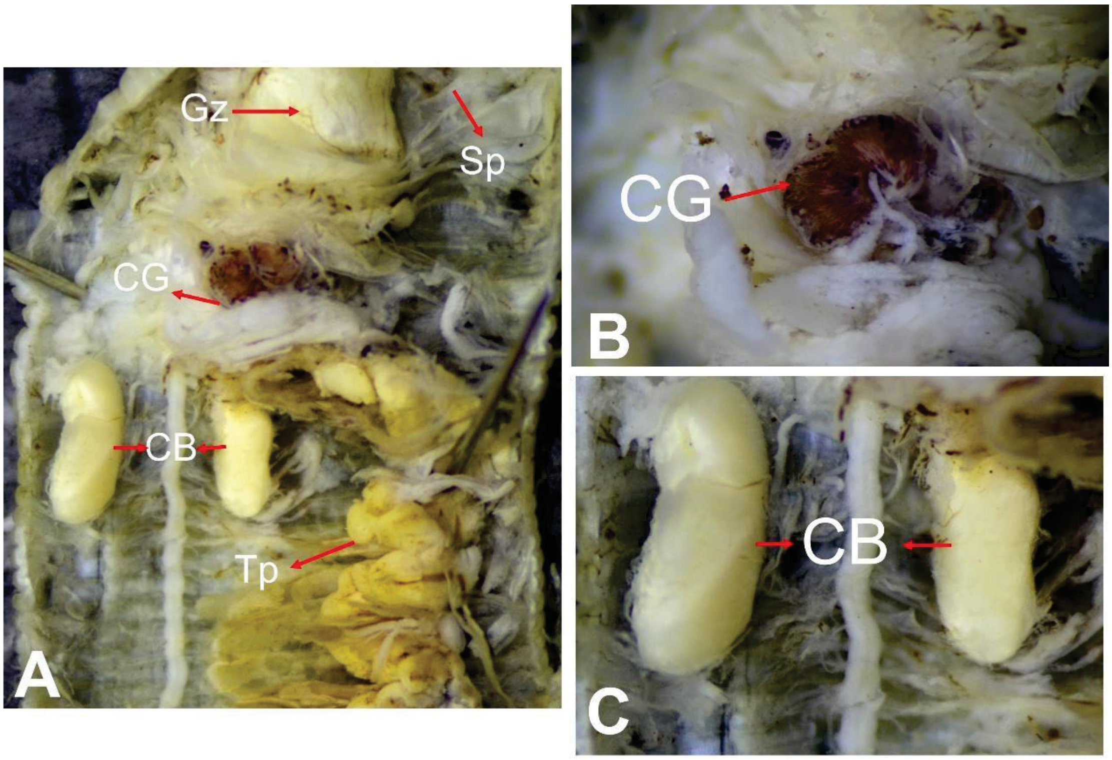
FIGURE 19 - EXTERNAL VIEW OF *GLOSSOSCOLEX DALLAVECCHIAI* n. sp



SOURCE: Author (2024)

Legend: *Glossoscolex dallavecchiai* n. sp. Holotype. A: View of the pair of male pores in XVII. B: Setal arrangement at XXX onwards. MP: male pore; St: setae.

FIGURE 20 - INTERNAL VIEW OF *GLOSSOSCOLEX DALLAVECCHIAI* n. sp



SOURCE: Author (2024)

Legend: *Glossoscolex dallavecchiai* n. sp. Holotype. A: view of gizzard in VI, calciferous glands, in XII, septa in VII/VIII, typhlosole, in XX, onwards and copulatory bulbs in XV-XIX. B View with emphasis the calciferous gland, in XII. C: View highlighting the copulatory bulbs, in XV-XIX. Gz: gizzard; Sp: septa; CG: calciferous gland; Tp: typhlosole; CB: copulatory bulb.

Glossoscolex debortolii n. sp. Dudas, Brown & Bartz

Holotype. one adult, native vegetation, in 0-20 cm soil, by qualitative method, Cruz Alta, Rio Grande do Sul, Brazil, 28°45'47.9"S, 53°35'24.5"W, 432 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0465 deposited at COFM.

Paratype. one adult, same information as holotype, sample ID: BRRS0532 deposited at COFM.

Other materials. three adults, same information as the holotype, sample ID: BRRS533. Brazil, two juveniles, same information as the holotype, sample ID: BRRS0534. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Cruz Alta, Rio Grande do Sul, Brazil, 28°45'49.9"S, 53°35'01.9"W, 419 m, August

2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0535 deposited at COFM.

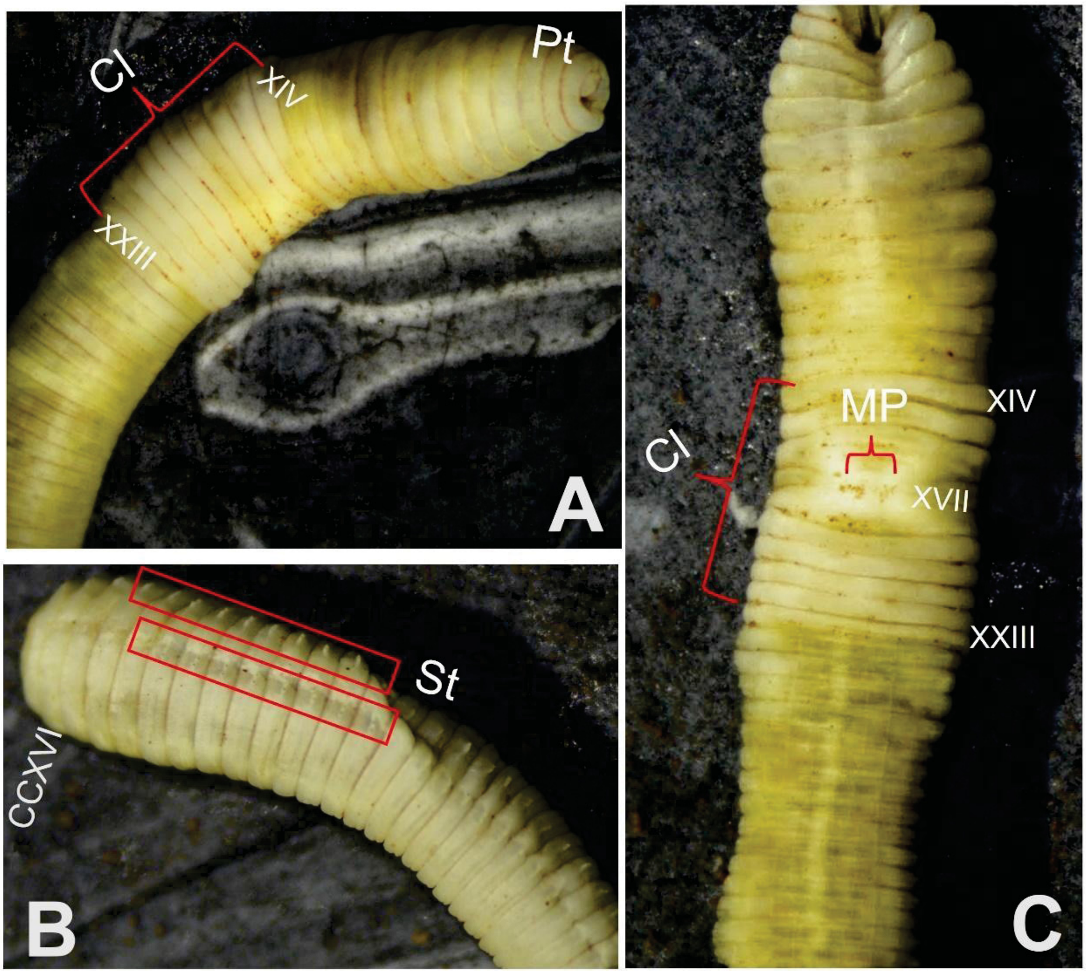
Etymology. The species was named in honor of the De Bortoli family, owners of the Santa Teresinha Farm, a reference in the practice of the no-tillage system in Rio Grande do Sul state.

External morphology (FIGURE 21). Holotype: body length 59 mm after ethanol fixation. Body mass: 0.14 g fresh weight (alcohol preserved). Number of segments: 216. Diameter: 2.5 mm in the pre clitellar region (segment X), 2.9 mm in the clitellum (segment XVI) and 2 mm in the post clitellar region (segment XXX). Paratype: length 49 mm after ethanol fixation. Body mass: 0.11 g, number of segments: 201. Diameter: 1.9 mm in segment X, 2.6 mm in the clitellum (segment XVI), and 1.6 mm in segment XXX. Body cylindrical, non-pigmentaded after fixation. Prostomium open prolobic. Setae closely paired, visible throughout, setae *cd* visible from V onwards. Setal arrangement aa:ab:bc:cd:dd, 6.9:1:3.9:1:6.6 at segment XXX-L. Annular clitellum in XIV-XXIII with no genital marks or tubercula pubertatis. One pair of male pores on *b* line setae in XVII, in the middle of the ventral region, in the segment. Female pores not seen. Nephropores near *ab* line.

Internal morphology (FIGURE 22). Septa weak in 7/8/9, with veil format in 10/11/12/13. Gizzard in VI, with average size (width x length) 2.7 mm x 2.6 mm. One pair of calciferous glands in XII of composite tubular type. Intestine begins in XV, with typhlosole commencing in the same segment. Last pair of hearts in XI, and dorsal blood vessel visible in segments VIII-X, and above the calciferous glands. Nephridia starting post clitellar, in segment XIX, close to the body wall and intestine, one pair per segment. Paired testes sacs in XIII, with iridescent color. Seminal vesicles above the intestine, in two thick stripes. Ovaries not seen. Spermathecae absent. One pair of copulatory pouches of butterfly shape in 1/3 XVI – 1/3 XIX.

Remarks. Regarding the shape and position of the clitellum (annular, XV-XXIII), *G. debortolii* n. sp. is similar to *G. bondari*, *G. mariae*, *G. terraopimus*, and *G. araucariaensis*. However, the first three differ from *G. debortolii* n. sp. in the position of the testes sacs. Although all of them present the sacs in pairs, *G. debortolii* n. sp. is the only one with sacs in XII. In contrast, *G. araucariensis* possesses only one testis sac in XI. In relation to the copulatory bulbs, the only species that shares the same position (XVI-XIX) as the new one is *G. bondari*, although it is of reniform (kidney) shape, while *G. debortolii* n.sp. has butterfly-shaped bulbs, a unique feature of this new species in the genus.

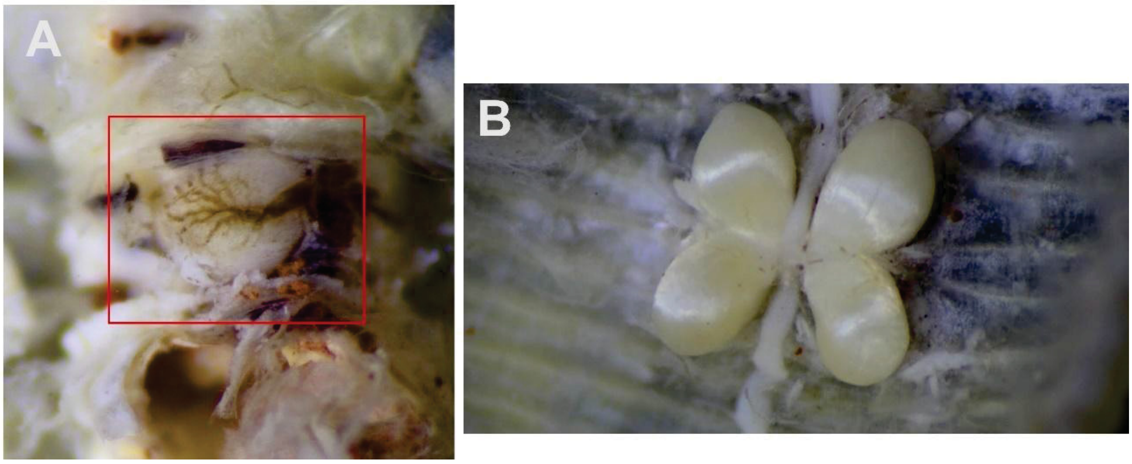
FIGURE 21 - EXTERNAL VIEW OF *GLOSSOSCOLEX DEBORTOLII* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex debortolii* n. sp. Holotype. A: View of the prostomium and clitellum in XIV-XIII. B: Setal arrangement, in the red rectangles, at the end of the worm, on the last segments. C: View of pair of male pores in XVII, in the midlle of the segment, and the ventral portion of clitellum. Pt: prostomium; Cl: clitellum, MP: male pore; St: setae

FIGURE 22 - INTERNAL VIEW OF *GLOSSOSCOLEX DEBORTOLII* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex debortolii* n. sp. Holotype. A: view with emphasis the calciferous gland, in XII. B: Internal view highlighting the copulatory bulbs, in XVI-XIX, with butterfly shape. CG: calciferous gland; CB: copulatory bulb.

Glossoscolex fuchsi **n. sp. Dudas, Brown & Bartz**

Holotype. one adult, in pasture over 10 years old, with no signs of degradation, with native plants growing, in 0-20 cm soil, by TSBF method, Maracaju, Mato Grosso do Sul, Brazil, 21°42'08.6"S 55°37'57.7"W, 610 m asl, April 2024, C. Rusch, F.M.O. Lourenço, F. Paixão, G.C. Francisco, J. Bortoluzzin, K.B.F. Pepe, M. Bartz colls. Sample ID: BRMS0158 deposited at COFM.

Paratype. one adult, same information as the holotype. Sample ID: BRMS0164.

Other materials. six adults and six juveniles, same information as the holotype, sample ID: BRMS0165. 17 adults, same information as holotype, sample ID: BRMS0159.

Etymology. The species was named in honor of the owner of the Retiro da Serra Farm, Richard Fuchs.

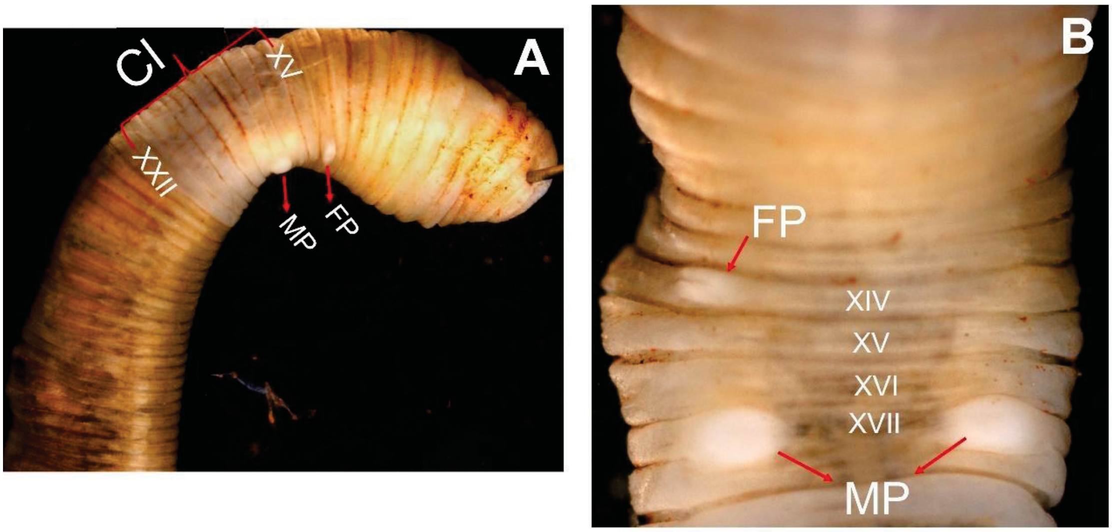
External morphology (FIGURE 23). Holotype: body length 54 mm after ethanol fixation. Body mass: 0.23 g fresh weight (alcohol preserved). Number of segments: 232 with intersegment. Diameter: 2.2 mm in the pre clitellar region (segment X), 2.4 mm in the clitellum (segment XVI) and 2 mm in the post clitellar region (segment XXX). Paratype: length 46 mm after ethanol fixation. Body mass:

0.11 g, number of segments: 182. Diameter: 2.2 mm in segment X, 2.7 mm in the clitellum (segment XVI) and 1.9 mm in segment XXX. Body cylindrical, non-pigmentated after fixation. Prostomium open epilobic. Setae closely paired, setae *cd* visible from V onwards. Setal arrangement aa:ab:bc:cd:dd, 22.5:1:12:1:21 at segment XXX-L. Clitellum in XV-XXII saddle shaped. Genital marks or tubercula pubertatis absent. One pair of female pores on XIII, 2.3 mm apart. One pair of male pores on *ab* line setae in XVII, in the middle of a shape resembling an eye, 2.1 mm apart. Nephropores near *ab* line.

Internal morphology (FIGURE 24). Septa membranous in 7/8/9/10. Gizzard in VI, with average size (width x length) 0.8 mm x 0.6 mm. One pair of calciferous glands in XII. Intestine begins in XV, with typhlosole commencing in the same segment. Last pair of hearts in XI. Paired testes sacs in XIII under the end of the calciferous glands. Seminal vesicles appear as thin parallel stripes, above the intestine. Ovaries in XIII. Spermathecae absent. One pair of copulatory bulbs of rounded shape in XV – XVII.

Remarks. *Glossoscolex fuchsi* n. sp. exhibits a similar clitellar position to *G. santarosaensis*, although they differ in shape (*G. santarosaensis* is annular). The female pores in *G. fuchsi* n. sp. are distinctly visible in XIV, a characteristic that is challenging to observe in *Glossoscolex* species.

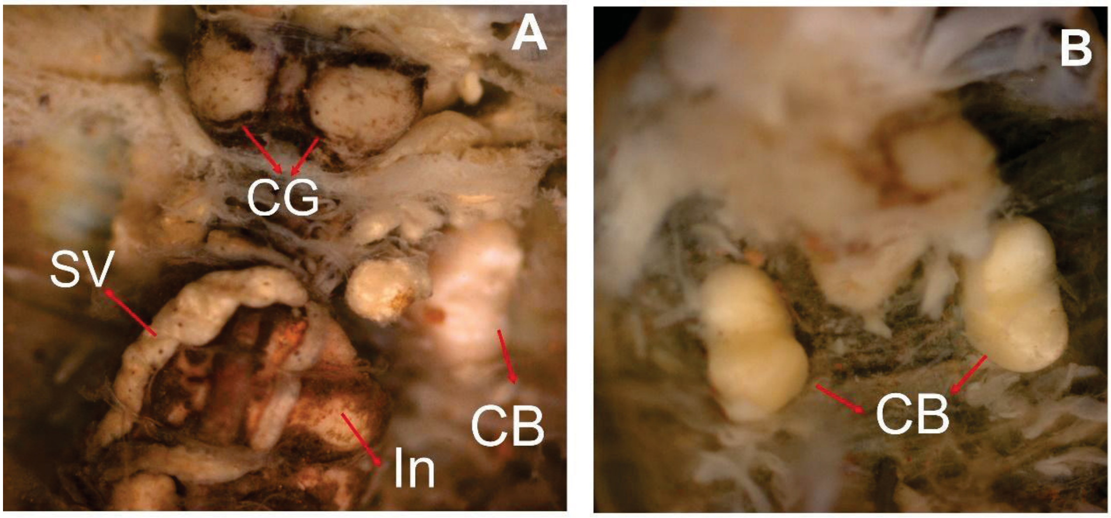
FIGURE 23 - EXTERNAL VIEW OF *GLOSSOSCOLEX FUCHSI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex fuchsi* n. sp. Holotype. A: clitellum in XV-XXII, female pore in XIV, and male pores in XVII. B: Ventral view of female and male pores. Cl: clitellum, MP: male pore; FP: female pore.

FIGURE 24 - INTERNAL VIEW OF *GLOSSOSCOLEX FUCHSI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex fuchsi* n. sp. Holotype. A: calciferous gland in XII, seminal vesicles above the intestine, copulatory bulbs and intestine in XV onwards. B: Copulatory bulbs in XIII-XVSV: CG: calciferous gland; SV: seminal vesicle; In: intestine; CB: copulatory bulb.

Glossoscolex strobeli **n. sp. Dudas, Brown & Bartz**

Holotype. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Cruz Alta, Rio Grande do Sul, Brazil, 28°45'49.9"S, 53°35'01.9"W, 419 m, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0471 deposited at COFM.

Paratype. one adult, native vegetation, in 0-20cm soil, by TSBF method, Panambi, Rio Grande do Sul, Brazil, 28°20'54.1"S, 53°24'56.4"W, 514 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0492 deposited at COFM.

Other materials. two adults and three juveniles, same information as the paratype. Sample ID: BRRS536.

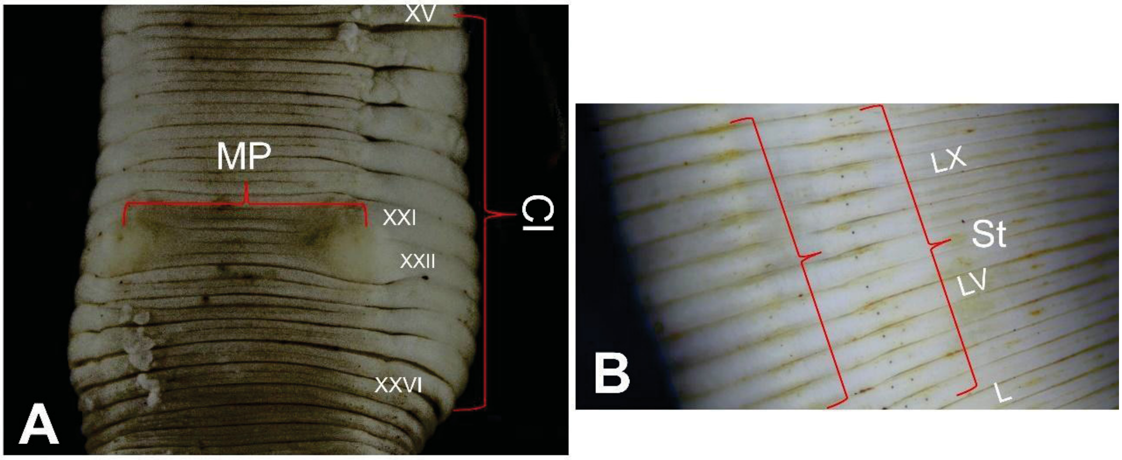
Etymology. The species was named in honor of Daniel Strobel and his family, owners of the Fazenda Condor, pioneers in the region and references in the practice of the no-tillage system.

External morphology (FIGURE 25). Holotype: body length 143 mm after ethanol fixation. Body mass: 5.99 g fresh weight (alcohol preserved). Number of segments: 341. Diameter: 7 mm in the pre clitellar region (segment X), 8 mm in the clitellum (segment XVI) and 6 mm in the post clitellar region (segment XXX). Paratype: length 102 mm after ethanol fixation. Body mass: 3.75 g, number of segments: 144. Diameter: 5.6 mm in segment X, 6.7 mm in the clitellum (segment XVI), and 5.1 mm in segment XXX. Body cylindrical, non-pigmented after fixation. Prostomium open prolobic. Setae closely paired, setae *ab* visible from IV onwards, but more visible beyond the clitellum. Setal arrangement aa:ab:bc:cd:dd, 3.7:1:2:1.3:3.7 at segment XXX-L. Clitellum in XV-XXVI saddle shaped. Genital marks or tubercula pubertatis absent. One pair of female pores in XIII. One pair of male pores on *b* line setae in XXI/XXII, 6 mm apart. Nephropores near *b* line.

Internal morphology (FIGURE 26). Septa was very strongly attached to the body wall and membranous in 7/8/9/10/11, after that becoming weaker and fragile. Gizzard in VI, with average size (width x length) 4 mm x 6 mm. One pair of calciferous glands in XII, under the seminal vesicles, of tubular composite type. Intestine begins in XV, with typhlosole in the same segment. Last pair of hearts in XI. Nephridia starting pre-clitellar in segment XIII close to the body wall and intestine, one pair per segment. Paired testes sacs in XII with visible funnels connected to the copulatory bulbs. Seminal vesicles have two white masses starting in XII and ending in XVI. Ovaries in segment XIV. Spermathecae absent. One pair of copulatory bulbs of rounded shape in XVI – XVIII.

Remarks. *Glossoscolex strobeli* n. sp. is the only species in this paper that belongs to the *bergi* group, characterized by worms that exhibit male pores after segment XX There are only two other species in this group: *G. bergi* and *G. grecoi*. *Glossoscolex strobeli* n. sp. has a longer clitellum (XV-XXVI) than the other species:XV-XXIV in *G. bergi* , and XV-XXIII in *G. grecoi*. The male pores are located in XXI/XXII, similar to *G. grecoi* but different from *G. bergi* (XX-XXII). In *G. grecoi* the testes sacs are in segment XI, while in the nes species they are inXII. Furthermore, the seminal vesicles in *G. strobeli* n.sp.. are shorter than in the other two species of this group, occupying only segments XII-XVI similar to *G. bergi (*XII-XV,XVI), but different from *G. grecoi* (XII-XVIII,XIX).

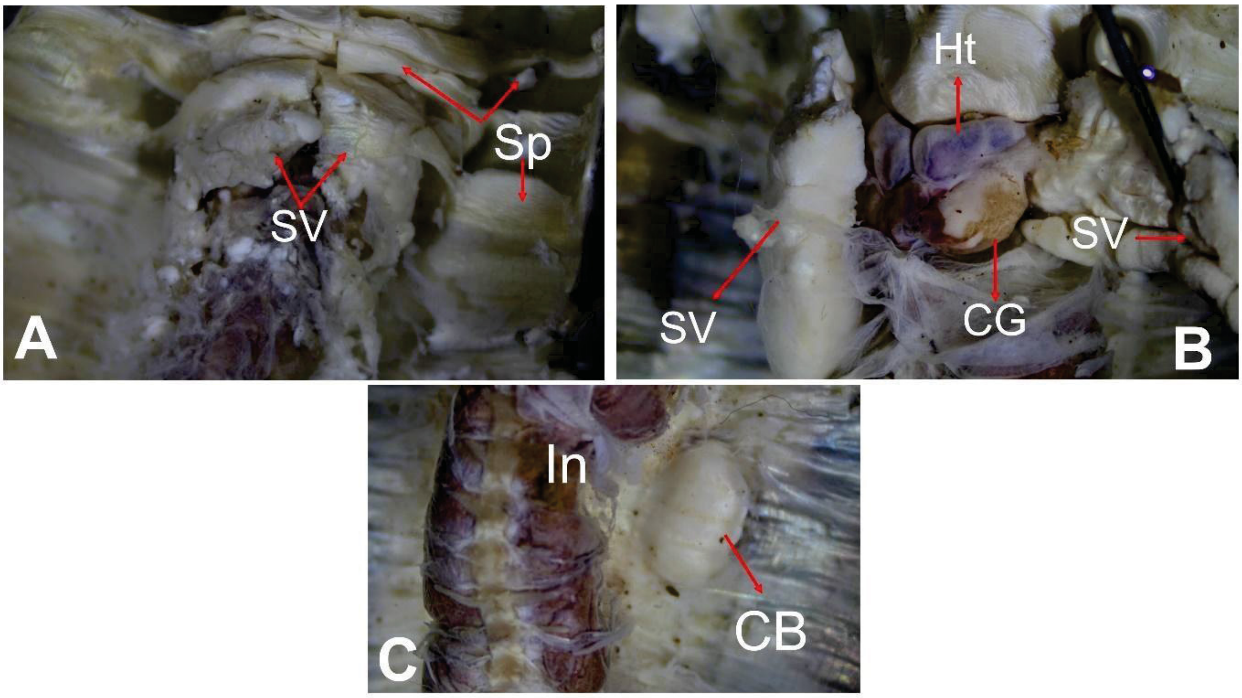
FIGURE 25 - EXTERNAL VIEW OF *GLOSSOSCOLEX STROBELI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex strobeli* n. sp. Holotype. A: clitellum in XV-XXVI, and male pores in XXI-XXII B: Setal arrangement, in L onwards. Cl: clitellum, MP: male pore; St: setae.

FIGURE 26 - INTERNAL VIEW OF *GLOSSOSCOLEX STROBELI* n. sp.



SOURCE: Author (2024)

Legend: *Glossoscolex strobeli* n.sp. Holotype. A: Strong septa, in XIX-X, seminal vesicle, in XI-XII. B: Last pair of hearths, in XI, seminal vesicles, and calciferous gland in XII. C: Intestine, in XV and the copulatory bulbs, in XVI-XVIII. Sp: septa; SV: seminal vesicle; Ht: hearth; CG: calciferous gland; In: intestine; CB: copulatory bulb.

Glossoscolex n. sp. 77 Dudas, Brown & Bartz

Holotype. one fragmented adult, native vegetation, in 0-20 cm soil, by TSBF method, Maracaí, São Paulo, Brazil, 22°40'06.1"S 50°52'49.0"W, 390 m, March 2024, F.M.O. Lourenço, G.A. Ramos, K.B.F. Pepe, M.L.C. Bartz, R.T. Dudas, T.M. Kinupp colls. Sample ID: BRSP0953 deposited at COFM.

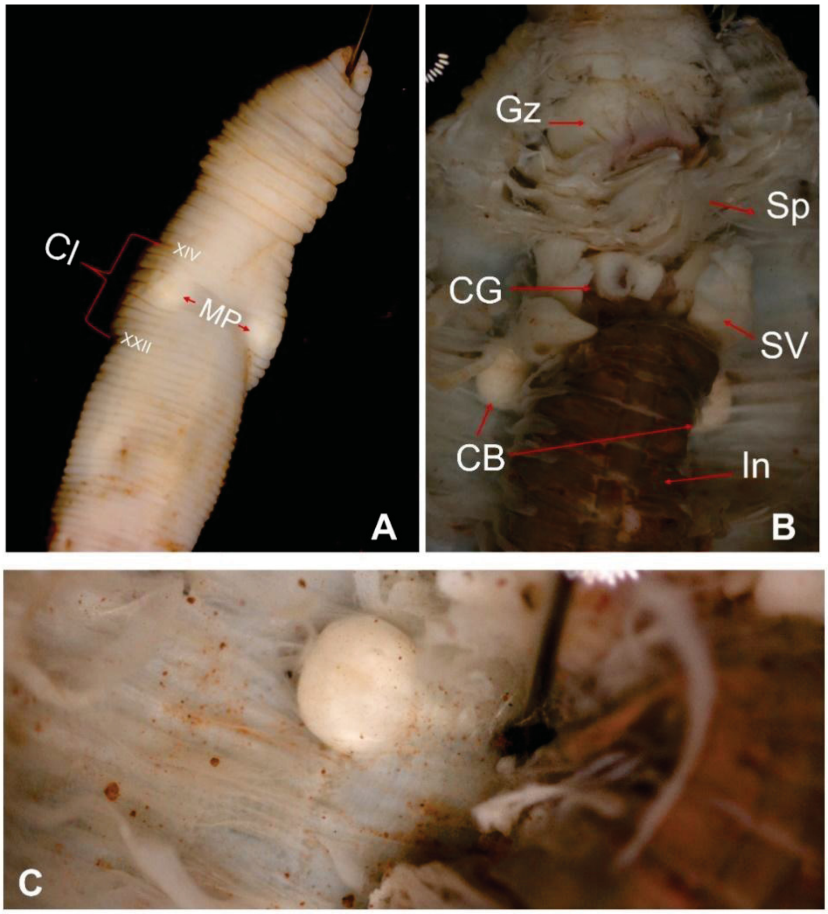
Etymology. As we only have one adult specimen, the number follows a sequence of potentially new species deposited in the Fritz Müller Oligochaete Collection (COFM). We provide information here so that it can be compared with other known species in the future, and in case further individuals are found with additional sampling efforts in the region.

External morphology (FIGURE 27 A). Holotype: body length 24 mm after ethanol fixation. Body mass: 0.11 g fresh weight (ethanol preserved). Number of segments: 47. Diameter: 3 mm in the pre clitellar region (segment X), 3.9 mm in the clitellum (segment XVI) and 2.9 mm in the post clitellar region (segment XXX). Body cylindrical, non-pigmentaded after fixation. Prostomium open prolobic. Setae closely paired, setae *ab* visible from IV/V onwards, but more visible after the clitellum. Setal arrangement aa:ab:bc:cd:dd, 3.3:1.2:2.1:1:3.5 at segment XXX-L Clitellum in XV-XXII saddle shaped. Genital marks or tubercula pubertatis absent. Female pores not seen. One pair of male pores, on *ab* setal line, in XVII, in a volcanic protuberance. Nephropores near *ab* line.

Internal morphology (FIGURE 27 B, C). Septa very strongly attached to the body wall and membranous in 7/8/9. Gizzard in VI, with average size (width x length) 4 mm x 6 mm. One pair of calciferous glands in XII. Intestine begins in XV, with typhlosole in the same segment. Last pair of hearts in XI. Nephridia starting pre clitellar, in segment XIII, close to the body wall and intestine, one pair per segment. Paired testes sacs in XII close to calciferous glands. Seminal vesicles starting in XII and ending in XV. Ovaries not seen. Spermathecae absent. One pair of copulatory bulbs of rounded shape in XVII – XVIII.

Remarks. The shape and position of the clitellum in *Glossoscolex* n. sp. 77 are similar to those of *G. uruguayensis*, *G. corderoi*, *G. riograndesis*, *G. parvus*, *G. pastivus*, and *G. pampas*. The distinguishing feature of *Glossoscolex* n. sp. 77 are the testes sacs, with the new species being the only one with a pair located in XII, while the others exhibit variations in form (some in U form), quantity (paired/unpaired), and position (XI, XII-XIII). The size of the seminal vesicles is also unique to *Glossoscolex* n. sp. 77 when compared to those species, occupying XII-XV. Although *Glossoscolex* n. sp. 77 shows similarity in male pore form to *G. arnsi* n. sp., DNA analyses indicated that they are, in fact, distinct species.

FIGURE 27 - *GLOSSOSCOLEX* n. sp.77.



SOURCE: Author (2024)

Legend: *Glossoscolex* n. sp.77. Holotype. A: External ventral view, showing the clitellum, in XV-XXII, and the pair of male pores in XVII. B: Internal overview with gizzard in VI, septa, in VII-X, seminal vesicle, in XII-XV calciferous gland in XII. Intestine, in XV and the copulatory bulbs, in XVII-XVIII. C: emphasis in the copulatory bulb. Cl: clitellum; MP: Male pore; Gz: gizzard; Sp: septa; SV: seminal vesicle; CG: calciferous gland; In: intestine; CB: copulatory bulb.

Fimoscolex fridrichi n. sp. Dudas, Brown & Bartz

Holotype. one adult, in no tillage with crop succession (soybean and wheat or oats, in 0-20 cm soil, by TSBF method, Panambi, Rio Grande do Sul, Brazil, 28°22'16.8"S 53°25'08.3"W, 494 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0503.

Paratype. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, 28°08'38.5"S, 53°46'40.2"W, 422 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0452.

Other materials. two adults native vegetation, in 0-20cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, 28°10'01.5"S 53°48'52.4"W, 419 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0448. two adults in no-tillage with crop succession, in 0-20 cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, 28°08'59.3"S, 53°46'26.5"W, 385 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0455.five adults, same information as BRRS0455. Sample ID: BRRS0456. two adults, same information as BRRS0455. Sample ID: BRRS0460. one adult, same information as holotype, sample ID: BRRS0483. one adult same information as holotype, sample ID: BRRS0495. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Cruz Alta, Rio Grande do Sul, Brazil, 28°10'01.5"S 53°48'52.4"W, 419 m asl, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRRS0517. two adults, same information as holotype, sample ID: BRRS0529.

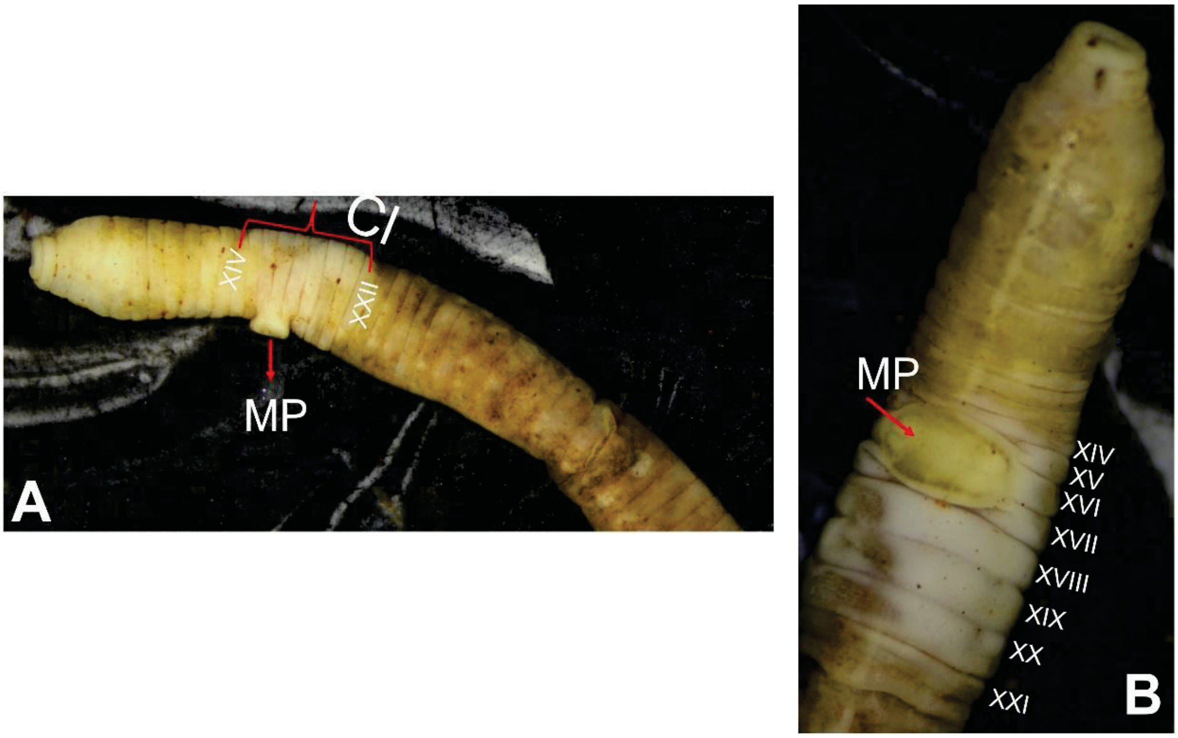
Etymology. The species was named in honor of farmer Marcos Fridrich, owner of the Faxinal Sul Farm, a reference in the management of the no-tillage system in his region.

External morphology (FIGURE 28). Holotype: body length 28 mm after ethanol fixation. Body mass: 0.05 g fresh weight (alcohol preserved). Number of segments: 104. Diameter: 1.8 mm in the pre clitellar region (segment X), 2.1 mm in the clitellum (segment XVI) and 1.5 mm in the post clitellar region (segment XXX). Paratype: length 48 mm after ethanol fixation. Body mass: 0.07 g, number of segments: 197. Diameter: 2.2 mm in segment X, 2.7 mm in the clitellum (segment XVI), and 1.9 mm in segment XXX. Body cylindrical, non-pigmented after fixation. Prostomium open epilobic. Setae closely paired, setae *ab* and *cd* barely visible from V onwards. Setal arrangement aa:ab:bc:cd:dd, 4.5:1.2:2.4:1:4.6 at segment XXX-L. Clitellum in XV-XXII saddle shaped. Genital marks or tubercula pubertatis absent. Female pore in XVIII. One male pore in XVI/XVII. Nephropores near *b* line.

Internal morphology (FIGURE 29). Septa thin and fragile in 7/8/9. Gizzard in VI, with average size (width x length) 0.8 mm x 0.6 mm. One pair of calciferous glands in XII. Intestine begins in XVI, with typhlosole commencing in the same segment. Last pair of hearts in XI. Paired testes sacs in XI under the beginning of the calciferous glands. Seminal vesicles thickened ventrally with iridescent color. Ovaries in XIII. Spermathecae absent. Copulatory bulb is absent, although in the segment XVII there's a lighter region that corresponds to the position of the external male pore.

Remarks. The position of the clitellum in *F. fridrichi* n. sp. is only similar to *F. nivae* (XIV-XXI), although in *F. nivae* it is saddle shaped, while in *F. fridrichi* n.sp. it is annular. The single male pore occurs in a swelling that occupies most of segment XVII, a distinctive characteristic. However, the most significant difference in *F. fridrichi* n. sp. is the absence of the copulatory bulb, a feature encountered in all the specimens collected. While all other described *Fimoscolex* species possess a single chamber, *F. fridrichi* n. sp. does not have any.

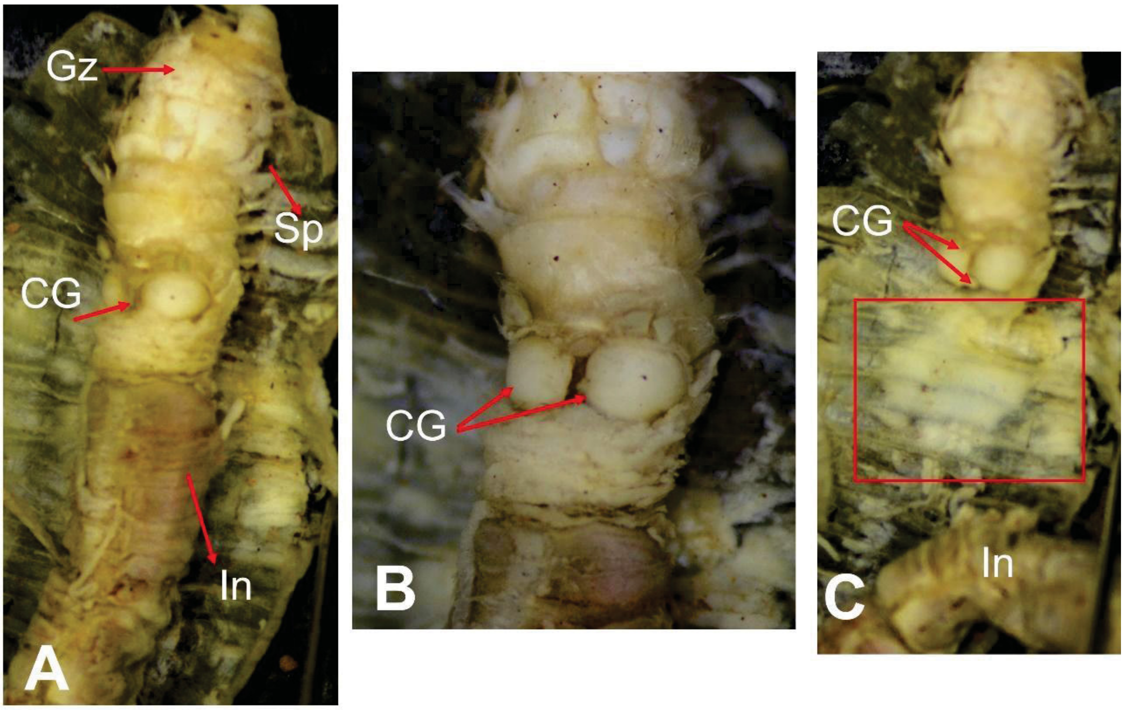
FIGURE 28 - EXTERNAL VIEW OF *FIMOSCOLEX FRIDRICHI* n. sp.



SOURCE: Author (2024)

Legend: *Fimoscolex fridrichi* n. sp. Holotype. A: lateral view of clitellum in XIV-XXII, and male pores in XVI-XVII. B: Ventral view male pore. Cl: clitellum, MP: male pore.

FIGURE 29 - INTERNAL VIEW OF *FIMOSCOLEX FRIDRICHI* n. sp.



SOURCE: Author (2024)

Legend: *Fimoscolex fridrichi* n. sp. Holotype. A: Gizzard, in VI, septa in VII-XIX, calciferous gland in XII, and intestine in XV onwards. B: Calciferous gland. C: Copulatory bulbs absent. Inside the red square is the region that is supposed to have a bulb, lighter due the external male pore. Gz: gizzard; Sp: septa; CG: calciferous gland; In: intestine.

Fimoscolex **n. sp.51 Dudas, Brown & Bartz**

Holotype. one adult, no-tillage system, in 0-20 cm soil, by TSBF method, Mangueirinha, Paraná, Brazil, 26°03'03.9"S, 52°14'15.6"W, 981 m asl, August 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRPR2149.

Paratype. one adult amputee, no-tillage system, in 0-20 cm soil, by TSBF method, Mangueirinha, Paraná, Brazil, 26,051079°S, 52,237655°W, 981 m asl, August 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. Sample ID: BRPR2151.

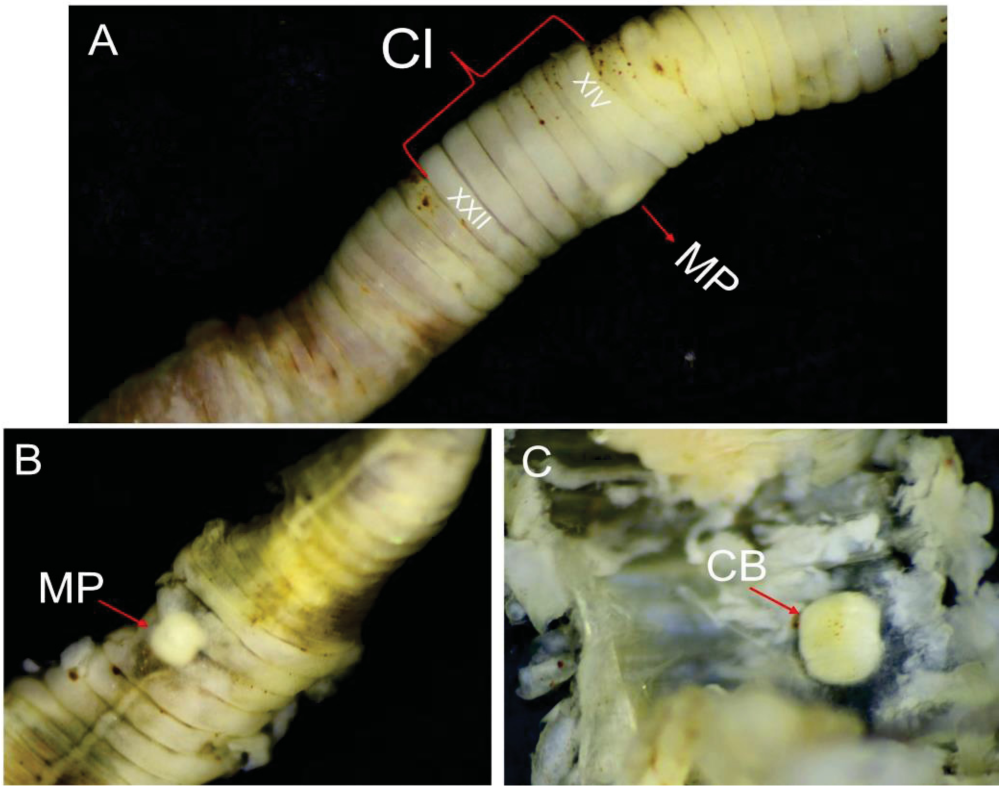
Etymology. The species name follows a sequential order of potentially new species deposited in the Fritz Muller Oligochaete Collection (COFM), until molecular data is available from *Fimoscolex bartzi* for comparative purposes.

External morphology (FIGURE 30 A). Holotype: body length 39 mm after ethanol fixation. Body mass: 0.11 g fresh weight (alcohol preserved). Number of segments: 164. Diameter: 1.4 mm in the pre clitellar region (segment X), 1.7 mm in the clitellum (segment XVI) and 1.3 mm in the post clitellar region (segment XXX). Paratype: length 33 mm after ethanol fixation. Body mass: 0.9 g, number of segments: 97. Diameter: 1.9 mm in segment X, 2.2 mm in the clitellum (segment XVI), and 1.4 mm in segment XXX. Body cylindrical, non-pigmented after fixation. Prostomium epilobic. Setae closely paired; setae *ab* visible from IV onwards. Setal arrangement aa:ab:bc:cd:dd, 17.7:1:2.5:1.1:17.2 at segment XXX-L. Clitellum in XV-XXII saddle shaped. Genital marks or tubercula pubertatis absent. Female pore not seen. One male pore in the middle of the segment XVII on a conical protuberance. Nephropores near *b* line.

Internal morphology (FIGURE 30 B, C). Septa strong and membranous in 7/8/9. Gizzard in VI with average size (width x length) 0.6 mm x 0.4 mm. One pair of calciferous glands in XII. Intestine begins in XV, with typhlosole commencing in the same segment. Last pair of hearts in XI. Paired testes sacs in XII under the beginning of the calciferous glands. Seminal vesicles in XII to XV, ventrally in two thin strips. Ovaries not seen. Spermathecae absent. Single oval copulatory bulb in XVII.

Remarks. *Fimoscolex* n. sp.51 exhibits morphological features similar to *F. bartzi*, however, due to the absence of an ovisac, the geographical distance between the species, and the lack of DNA sequences from both species, we are considering this as a separate *Fimoscolex* species for the meantime. Compared to the other *Fimoscolex* species, *Fimoscolex* n. sp.51 has similar clitellum location (XV-XXI) to *F. angai*, *F. inurus*, and *F. tairim*, although the latter has an annular shape instead of a saddle. The difference between *Fimoscolex* n. sp. 51 and *F. angai* is the position of the testes sacs; in the new species, they are paired only in segment XII, while in *F. angai*, they are paired occupying XI-XII. For *F. inurus*, the position of the sacs was not provided in the description, however the copulatory bulbs of *F. inurus* are in XVI-XVIII, while in *Fimoscolex* n.sp. 51 the bulb occupies only XVII.

FIGURE 30 - *FIMOSCOLEX* n. sp.51



Source: Author (2024)

Legend: *Fimoscolex* n. sp.51. Holotype. **A.** External lateral view of the clitellum (Cl) and the single male pore (MP). **B.** External ventral view with emphasis in the male pore. **C.** Internal view showing the copulatory bulb (CB).

3.6 DISCUSSION

The *Glossocolex* subgenus was initially categorized into four main groups based on the position of the male pore: when located in segment 17, the group is *G. truncatus,* in segment XVIII/XIX, *G. giganteus,* male pores on XIX/XX, *G. grandis,* and when presented in segments XX/XXI or XI/XXII, they belong to the *G. bergi* group (Righi and Lobo, 1979). Feijoo and Brown (2023) expanded the groups by adding a new one, *G. matogrossensis*, which has male pores intraclitellar in segments XV/XVI. The six new *Glossoscolex* described here belonging to the *G. truncatus* group, but certain distinctions were observed between the species described and other previously described *Glossoscolex* species, as shown in TABLE 7. Of the 58 known *Glossoscolex* species, 33 belong to the *truncatus* group, with a distribution range encompassing Argentina, Paraguay, and the Southeast and South regions of Brazil (Misirlioğlu *et al*. 2023).

The sole new species described here that belongs to the *G. bergi* group is *Glossoscolex strobeli* n. sp., which is an addition to the only other two species belonging to this group: *G. bergi* and *G. grecoi* (Feijoo and Brown, 2023). As these two species were described in 1900 (Rosa) and 1979 (Righi; Lobo, 1979) respectively, this new addition to the group extends the geographic distribution of this group to Rio Grande do Sul.

Regarding *Fimoscolex*, it is noteworthy to emphasize that the absence of a copulatory chamber in the newly identified species *F. fridrichi* n. sp. has changed the definition of the genus to include species without bulbs. The distinguishing feature from the *Glossoscolex* genus was the number of copulatory chambers. The evolutionary trajectory of this attribute remains uncertain, and it is yet to be determined whether the absence of the chamber represents an ancestral trait, indicating that the progenitor *Fimoscolex* lacked chambers which subsequently developed, or whether chambers were initially present and subsequently lost. Nonetheless, this feature appears to be an adaptation of the genus, whose relationship with *Glossoscolex* is becoming more and more intertwined as further samples are revealing more morphological variations

TABLE 7 – COMPARISON OF CHARACTERS OF THE NEW SPECIES *Glossoscolex arnsi* n. sp., *Glossoscolex alessioi* n. sp., *Glossoscolex dallavecchiai* n. sp., *Glossoscolex debortolii* n. sp., *Glossoscolex strobeli* n. sp., *Glossoscolex* n. sp.77, *Glossoscolex fu*chsi n. sp., *Fimoscolex fridrichi* n. sp. and *Fimoscolex* n. sp.51 (IN BOLD) WITH DIFFERENT SPECIES OF THE TRUNCATUS GROUP –*Glossocolex truncatus, Glossoscolex colonorum, Glossosocolex bonariensis, Glossoscolex bondari, Glossosocolex corderoi, Glossoscolex minor, Glossoscolex lacteus, Glossososcolex riograndensis, Glossoscolex nativus, Glossoscolex pollulos, Glossoscolex pampas,* and *Glossoscolex cardosoi* – the bergi group - *Glossoscolex bergi* and *Glossoscolex grecoi* – and the *Fimoscolex* genus – *Fimoscolex ohausi*, *Fimoscolex bartzi, Fimoscolex angai minor* and *Fimoscolex nivae.*

Genus	**Species**	**Author**	**Length (mm)**	**Segments (n°)**	**Setal ratio (mm)**	**Clitellum**	**Male pore**	**Calciferous gland**	**Test sacs**	**Septa**	**Last hearts**	**Seminal vesicle**	**Copulatory bulbs**
Glossoscolex	*truncatus**	Rosa, 1895	80-160	200-300	-	annular, 16-25	17	12	pair, U form, 11	-	enclosed	tubular long, 11,12-18	oval long, 17-18
Glossoscolex	*colonorum*	Michaelsen 1918	112-175	175-323	aa = 2 ½ – 3 1/2 bc, dd = ca.1/2 u	saddle, 15-20,21	-	oval, 11	unpaired, 11	-	-	band shaped, 12-16, 17	flattened globular
Glossoscolex	*bonariensis*	Cordero, 1942	57-65	130-145	aa=cd, aa=5bc, aa>dd	saddle, 15-24,25	17	ovoid, 12	1 pair united ventrally, 11	-	semi-enclosed	long slender, knoby, 11-32,52	ovoid, 16-18
Glossoscolex	*bondari*	Michaelsen, 1926	180-215	400-426	16.4:1:4.3:1:18.4	annular, 1/2 13,14,15-23	17	ovoid, 12	ventrally, 11	4/5, 6/7, 10/11	11	long strands with small irregular lobules, 12-67, 110	reniform, 16-19
Glossoscolex	*corderoi*	Righi, 1968	216-315	-	15.7:1:4.2:1:18:23	saddle, 15-22	17	piriform, 12	pair, 11	6/7, 10/11	11	irregularly lobed, 12-14	large ovoid, 17

TABLE 7 continuation…

Genus	Species	Author	Length (mm)	Segments (n°)	Setal ratio (mm)	Clitellum	Male pore	Calciferous gland	Test sacs	Septa	Last hearts	Seminal vesicle	Copulatory bulbs
Glossoscolex	*minor*	Zicsi & Csuzdi, 1999	24-45	198-231	30:1:10:1:50	annular, 14-23	17	11/12	unpaired, 11	-	-	band to club-shaped, 11-50	elongated, 14-19
Glossoscolex	*lacteus*	Zicsi & Csuzdi, 1999	40-72	123-178	12:1,4:6:1:18	annular, 16-1/n 24	17	11/12	1 pair united ventrally, ring form, 11	-	-	tubular elongate, 11,12-35,37	elongated, 15-20
Glossoscolex	*riograndensis*	Bartz & James, 2018	41-72	125-195	7,8:0,3:1:0,2:11,2	saddle, 15-18	17	heart shaped, 12	1 pai, U form, 11-12	simple	free	tubular with lobular edges, 12-62	round to oval, 11-13
Glossoscolex	*riograndensis nativus*	Bartz & James, 2018	48-53	169-180	6,8:0,2:1:0,1:9,8	saddle, 1/2 14-1/2 25	17	flat bean shaped, 12	1 pai, U form, 11-12	simple	free	tubular with lobular edges, 12-65	round, 16-18
Glossoscolex	*riograndensis pollulus*	Bartz & James, 2018	28-36	105-139	8:0,2:1:0,1:8,9	saddle, 15-26	17	small heart shaped, 12	1 pair, U form, 11-12	simple	free	tubular with lobular edges, 12-64	round, 16-18
Glossoscolex	*pampas*	Bartz & James, 2018	143-244	198-352	4,4:0,1:1,:0,1:6,3	saddle, 15 - 1/2 22, 22	17	egg shaped, 11	unpaired, 11	developed, united	enclosed	lobulated, like butterfly wings, 11-12, 13	short oval, 15-18
Glossoscolex	*cardosoi*	Feijoo & Brown, 2023	192-237	314-363	15.3:1:3.3:0.8:14.7	saddle, 15-23,24	17	ovoid, 12	unpaired, 11	6/7-9/10	11	12 only	bean-shaped, 18

TABLE 7 continuation…

Genus	Species	Author	Length (mm)	Segments (n°)	Setal ratio (mm)	Clitellum	Male pore	Calciferous gland	Test sacs	Septa	Last hearts	Seminal vesicle	Copulatory bulbs
Glossoscolex	***arnsi* n. sp.**	**Dudas, Brown & Bartz**	**59-72**	**159-169**	**3:1,1:2,3:1:2,8**	**saddle, 14-23**	**17**	**tubular-composite, 12**	**unpaired, 11**	**tickened, 7/8/9/10**	**11**	**lobulated, 13-60**	**pair, 1/3 16-1/3 20**
Glossoscolex	***alessioi* n. sp.**	**Dudas, Brown & Bartz**	**27-42**	**129-143**	**3:1,1:1,9:1:3**	**saddle, 15-24**	**17**	**composite-tubular, 12**	**paired, 12**	**tickened, 7/8/9/10**	**11**	**elongated stripes**	**pair, bean shaped, 17-2/3 20**
Glossoscolex	***dallavecchiai* n. sp.**	**Dudas, Brown & Bartz**	**61-73**	**198-233**	**2,1:1,1:1,8:1:2,1**	**saddle, 15-24**	**17**	**composite-tubular, 12**	**paired, 12**	**weak, veil format, 10/11/12/13**	**11**	**elongated parallel strips**	**pair, elongated, 2/3 15- 1/3 19**
Glossoscolex	***debortolii* n. sp.**	**Dudas, Brown & Bartz**	**49-59**	**201-216**	**6,9:1:3,9:1:6,6**	**annular, 15-23**	**17**	**composite-tubular, 12**	**paired, 12**	**simple, fragile**	**11**	**thickened stripes**	**pair, bean shaped, 1/3 16- 1/3 19**
Glossoscolex	**n. sp.77**	**Dudas, Brown & Bartz**	**24**	**47**	**3,3:1,2:2,1:1:3,5**	**saddle, 15-22**	**17**	**pair, 12**	**paired, 12**	**strong, 7/8/9**	**11**	**mass, 12-15**	**pair, round shape, 17-18**
Glossoscolex	***fuchsi* n. sp.**	**Dudas, Brown & Bartz**	**46-54**	**182-232**	**22,5:1:12:1:21**	**saddle, 14-22**	**17**	**pair, 12**	**paired, 12**	**membranous, 7/8/9/10**	**11**	**elongated stripes**	**pair, bean shape, 15-17**
Glossoscolex	*bergi**	Rosa, 1900	200	193	-	saddle, 15-24	20-22	12	-	thick, 7/8/9/10/11	free	12-15, 16	oval long, 20-21

TABLE 7 continuation…

Genus	Species	Author	Length (mm)	Segments (n°)	Setal ratio (mm)	Clitellum	Male pore	Calciferous gland	Test sacs	Septa	Last hearts	Seminal vesicle	Copulatory bulbs
Glossoscolex	*grecoi*	Righi & Lobo, 1979	170-264	263-351	4,2:5,6:5,4	saddle, 15-23	21/22	12	paired, 11	conical, thicked, 6/7,10/11	-	long, large, folded, 12-18,19	ovoid, 20-22
Glossoscolex	***strobeli* n. sp.**	**Dudas, Brown & Bartz**	**102-143**	**144-341**	**3,7:1:2:1,3:3,7**	**saddle, 15-26**	**21/22**	**composite-tubular, 12**	**paired, 12**	**membranous, 7/8/9/10/11**	**11**	**whitish mass, 12-16**	**pair, 16-18**
Fimoscolex	*ohausi**	Michaelsen 1918	120	191	–	saddle, 13,14–23	16/17	spherical, 12	single, tube form, 11	–	–	smooth grape shaped, 11–13,15	rounded
Fimoscolex	*bartzi*	Bartz & James, 2012	35-57	119-188	20:1:4:1:30	saddle, 15-21	17	bean shaped, 12	1 pair, united ventrally, ring form, 11	thickened united	enclosed	simple, elongate sacs, 11-14,15	oval, 2/3 16-1/13 18
Fimoscolex	*angai minor*	Zicsi & Csuzdi, 1987	77	189-201	ab=cd, aa=3bc	sadddle	17	oval, 12	single, ventral sac, cone form, 11	-	free	long strips, 11-23	oval, 16-18
Fimoscolex	*nivae*	Feijoo & Brown, 2018	39-66	156-173	17,1:1:2,9:0,9:28:6	saddle, 14-21	16/17	composite-tubular, 12	subesophageal sac, 11	6/7, 10/11	11	one long and whitish, satarting in 12	one bulb, 16-17

TABLE 7 continuation…

Genus	Species	Author	Length (mm)	Segments (n°)	Setal ratio (mm)	Clitellum	Male pore	Calciferous gland	Test sacs	Septa	Last hearts	Seminal vesicle	Copulatory bulbs
Fimoscolex	*fridrichi* n. sp.	Dudas, Brown & Bartz	28-47	104-197	4,5:1,2:2,4:1:4,6	annular, 14-21	16/17	pair, 12	paired, 11	thin and fragile, in 7/8/9	11	thickened, ventrally	none
Fimoscolex	n. sp.51	Dudas, Brown & Bartz	33-39	97-164	17,7:1:2,5:1,1:17,2	saddle, 15-21	17	pair, 12	paired, 12	strong, 7/8/9	11	thin, ventrally stripes	single, 17

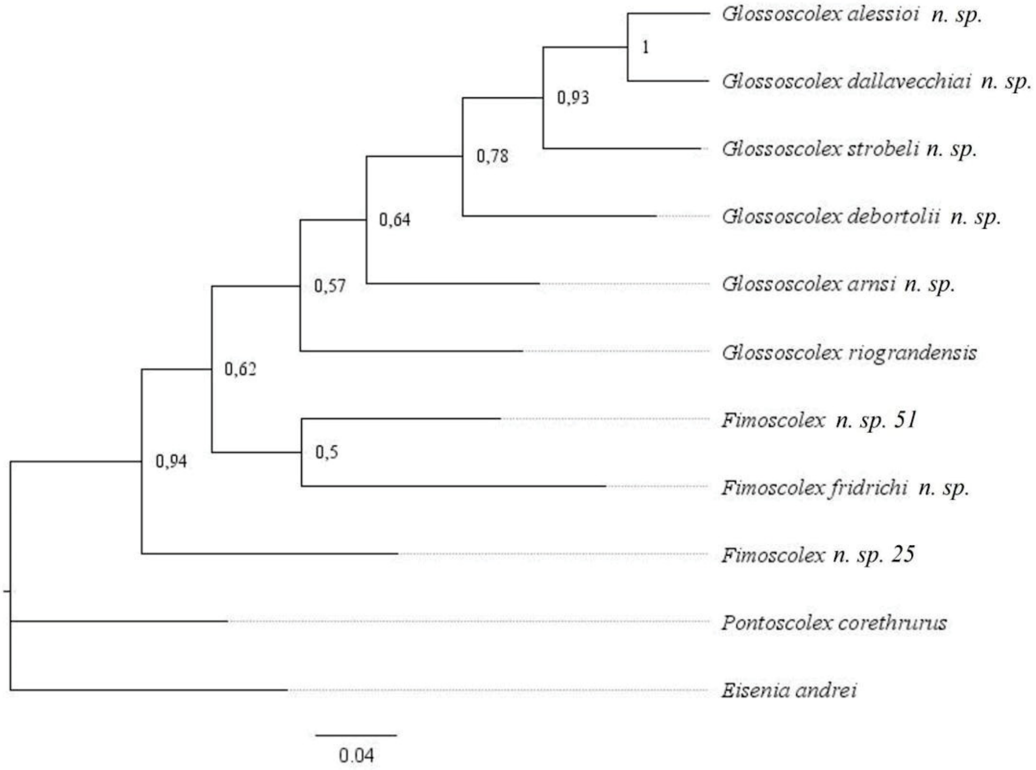
Source: Author (2024)

Legend: *type species of each group/genus

The DNA analysis shows that all the species described here are indeed new when compared with previously described species and with one another (FIGURE 31, TABLE 8). The pairwise distance (TABLE 8) analysis shows that they are closely related species, but the values suggest that they are all different. As for the phylogenetic tree, we can infer the proximity of *Glossoscolex* and *Fimoscolex*, despite clear distinctions between the new species *(*FIGURE 31). This analysis underscores the significance of integrating diverse methodologies for the accurate identification of new earthworm species, and the need for further molecular work in both genera, particularly including the type species of each genus, and of each of the subgenera and of the groups within *Glossoscolex* (*Glossoscolex*), to further elucidate phylogenetic relationships. Genetic information on *F. bartzi* is also urgently needed to adequately separate it (or not) from *Fimoscolex* n. sp.51.

Specimens of both *Glossoscolex* and *Fimoscolex* have been observed in forest plantations (da Silva *et al*. 2019; Demetrio *et al.* 2023a), native vegetation (Bartz *et al.* 2023), lawns (Dudas *et al.* 2023), and annual crops (Bartz *et al*. 2024). Nevertheless, as previously noted, the abundance of native species in the Cerrado and Atlantic Forest biomes tends to be lower in comparison to exotic species (Demetrio *et al*. 2023b). Further research is necessary to consolidate information regarding the distributions of these genera.

FIGURE 31 - EVOLUTIONARY ANALYSIS OF THE NEW SPECIES DESCRIBED



Source: Author (2024)

Legend: Evolutionary analysis of most of the new species described here using the cytochrome oxidase 1 genetic marker (barcode) and the maximum likelihood method. Barcode distance is shown in inset. *Glossoscolex riograndensis* and *Fimoscolex* n. sp.25 were used for comparison with the new species and *Eisenia andrei* and *Pontoscolex corethrurus* were used as an out-grouping species.

TABLE 8 – ESTIMATES OF EVOLUTIONARY DIVERGENCE BETWEEN THE SEQUENCES OF THE NEW SPECIES - *Glossoscolex arnsi* n. sp., *Glossoscolex alessioi* n. sp., *Glossoscolex dallavecchiai* n. sp., *Glossoscolex debortolii* n. sp., *Glossoscolex strobeli* n. sp., *Fimoscolex* n. sp.51 and *Fimoscolex fridrichi* n. sp. – THE COMPARISON – *Glossoscolex riograndensis* and *Fimoscolex* n. sp.25 – AND THE OUT-GROUPS – *Eisenia andrei* and *Pontoscolex corethrurus* - USING THE PAIRWISE DISTANCE ANALYSES

	Eisenia andrei	*Pontoscolex corethrurus*	*Glossoscolex arnsi*	*Glossoscolex alessioi*	*Glossoscolex dallavecchiai*	*Glossoscolex debortolii*	*Glossoscolex strobeli*	*Glossoscolex* n.sp.77	*Fimoscolex fridrichi*	*Glossoscolex riograndensis*	*Fimoscolex* n.sp.25
Eisenia andrei											
Pontoscolex corethrurus	0,24										
Glossoscolex arnsi	0,30	0,26									
Glossoscolex alessioi	0,29	0,28	0,21								
Glossoscolex dallavecchiai	0,30	0,28	0,20	0,08							
Glossoscolex debortolii	0,28	0,31	0,21	0,18	0,19						
Glossoscolex strobeli	0,29	0,32	0,21	0,15	0,15	0,18					
Glossoscolex n.sp.77	0,27	0,28	0,24	0,24	0,27	0,24	0,22				
Fimoscolex fridrichi	0,30	0,31	0,26	0,30	0,28	0,30	0,30	0,24			
Glossoscolex riograndensis	0,28	0,27	0,21	0,24	0,25	0,21	0,26	0,26	0,25		
*Fimoscolex n.*sp.25	0,29	0,27	0,27	0,30	0,30	0,26	0,28	0,25	0,30	0,26	

Source: Author (2024)

3.7 CONCLUSION

We describe seven new species and provide detailed morphological information on two potentially new species of the Glossoscolecidae family discovered during sampling campaigns conducted in the Cerrado and Atlantic Forest biomes. This finding confirms the substantial number of unknown earthworm species in these regions. In addition to taxonomic work, further biological and ecological work is needed in order to better understand the role of land use and soil management practices in maintaining native earthworm species. Furthermore, further information on the soil and environmental attributes is important to guarantee adequate preservation of these new species in their habitats.

Glossoscolex and *Fimoscolex* exhibit morphological similarities, but further integrated taxonomic methodologies, such as genetic sequencing, and ecological knowledge on the species, represent important approaches towards a more comprehensive knowledge on the taxonomic, biogeographic and evolutionary standing questions related to these genera.

3.8 REFERENCES

Anderson, J. M., & Ingram, J. S. (1994). Tropical soil biology and fertility: a handbook of methods. Soil Science, 157(4), 265.

Bartz, M. L. C., James, S. W., Pasini, A., & Brown, G. G. (2012). New earthworm species of *Glossoscolex* Leuckart, 1835 and *Fimoscolex* Michaelsen, 1900 (Clitellata: Glossoscolecidae) from Northern Paraná, Brazil. *Zootaxa*, 3458(1), 59-85. Recuperado de: htttp://doi.org/10.11646/zootaxa.3458.1.3

Bartz, M. L., James, S. W., Steffen, G. P., Antoniolli, Z., Steffen, R. B., & Brown, G. G. (2018). New species-group taxa of *Glossoscolex* (Clitellata: Glossoscolecidae) from Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 548-561. Recuperado de https://doi.org/10.11646/zootaxa.4496.1.42

Bartz, M. L., Barreto, J., Santos, A., Dudas, R. T., Ferreira, T., Maia, L. D. S., Demetrio, W.C., Smokanit, M., Schuster, P.A., Hernani, L.C. & Brown, G. G. (2023). Earthworm richness in no-tillage farming systems and riparian forests in Southern and Southeastern Brazil. *Zootaxa*, 5255(1), 362-376. Recuperado de: htttps://doi.org/10.11646/zootaxa.4496.1.42

Bartz, M. L. C., Dudas, R. T., Demetrio, W. C., & Brown, G. G. (2024). Earthworms as soil health indicators in no-tillage agroecosystems. *European Journal of Soil Biology*, 121.
Recuperado de: https://doi.org/10.1016/j.ejsobi.2024.103605

Britz, R., Hundsdörfer, A., & Fritz, U. (2020). Funding, training, permits—the three big challenges of taxonomy. *Megataxa,* 1(1), 49-52. Recuperado de: htttp://doi.org/10.11646/megataxa.1.1.10

Brown, G. G., Callaham Jr, M. A., Niva, C. C., Feijoo, A., Sautter, K. D., James, S. W., Fragoso, C., Pasini, A. & Schmelz, R. M. (2013). Terrestrial oligochaete research in Latin America: the importance of the Latin American meetings on oligochaete ecology and taxonomy. *Applied Soil Ecology*, 69, 2-12. Recuperado de https://doi.org/10.1016/j.apsoil.2012.12.006

Cardoso, G. B. X., Nadolny, H., Feijoo, A., Brown, G. G. (2014). Earthworm populations in an altitudinal gradient of the coastal Atlantic Rainforest in Paraná State, southern Brazil (Oligochaeta). In: Tomás Pavlíček; Patricia Cardet; Maria Teresa Almeida; Cláudia Pascoal; Fernanda Cássio (Org.): Advances in Earthworm Taxonomy VI (Annelida: Oligochaeta), Proceedings of the 6th International Oligochaete Taxonomy Meeting (6th IOTM). 1ed.Heidelberg: Kasparek Verlag, pp. 74-86.

da Silva, E., de Lima, O. G., de Andrade, D. P., & Brown, G. G. (2019). Earthworm populations in forestry plantations (*Araucaria angustifolia*, *Pinus elliottii*) and Native Atlantic Forest in Southern Brazil compared using two sampling methods. *Pedobiologia*, 72, 1-7.
Recuperado de: https://doi.org/10.1016/j.pedobi.2018.10.002

Demetrio, W. C., Santos, A., Ferreira, T., Nadolny, H., Cardoso, G. B., Torres, J. L., Dudas, R.T., de Oliveira, V., Barreto, J.O., James, S.W., da Silva, E., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in various land use systems in the Campos Gerais region of Lapa, Paraná, Brazil. *Zootaxa*, 4496(1), 503-516. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W. C., PM, D. F., Dudas, R., Zagatto, M. G., Feijoo, A., & Brown, G. G. (2023a). Earthworm species in native and planted forests in Brazil. *Zootaxa,* 5255(1), 304-323.
Recuperado de https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W., Brown, G., Pupin, B., Novo, R., Dudas, R., Baretta, D., Rombke, J., Bartz, M.L.C. & Borma, L. (2023b). Are exotic earthworms threatening soil biodiversity in the Brazilian Atlantic Forest?. *Applied Soil Ecology*, 182, 104693. Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104693

Dudas, R. T., Tavares, A. A., Ercole, C., BL, D. L., Carlos, E. D. S., Torres, J. L., Smokanit, M., Guaranha, R.M., Brown, G.G. & Bartz, M. L. (2023). Urban green areas as earthworm species maintainers in Curitiba, Paraná, Brazil. *Zootaxa*, 5255(1), 336-346.

Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.27

Feijoo, A.M. & Brown, G. G. (2018). New species of *Glossoscolex* and *Fimoscolex* earthworms (Oligochaeta: Glossoscolecidae) from Embrapa Forestry, Paraná, Brazil*. Zootaxa*, 4496(1), 492-502. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.38

Feijoo, A.M., & Brown, G. G. (2023). Three new *Glossoscolex* (Annelida: Crassiclitellata: Glossoscolecidae) in the truncatus group from the Brazilian Atlantic Forest. *Zootaxa,* 5255(1), 220-234. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.21

James, S. W., & Brown, G. G. (2006). Earthworm ecology and diversity in Brazil. *Soil biodiversity in Amazonian and other Brazilian ecosystems*, 56-116. Recuperado de: https://doi.org/10.1079/9781845930325.0056

Jouquet, P., Chaudhary, E., & Kumar, A. (2018). Sustainable use of termite activity in agro-ecosystems with reference to earthworms. A review. *Agronomy for Sustainable Development*, 38, 1-11.

Recuperado de: https://doi.org/10.1007/s13593-017-0483-1.

Kanianska, R., Jaďuďová, J., Makovníková, J., & Kizeková, M. (2016). Assessment of relationships between earthworms and soil abiotic and biotic factors as a tool in sustainable agricultural. *Sustainability*, 8(9), 906. Recuperado de: https://doi.org/10.3390/su8090906

Latif, R., Malek, M., Aminjan, A., Pasantes, J., Briones, M., & Csuzdi, C. (2020). Integrative taxonomy of some Iranian peregrine earthworm species using morphology and barcoding (Annelida: Megadrili). *Zootaxa*, 4877 1, 7. Recuperado de: https://doi.org/10.11646/zootaxa.4877.1.7.

Misirlioğlu, M., Reynolds, J., Stojanović, M., Trakić, T., Sekulić, J., James, S., Csuzdi, C., Decaëns, T., Lapied, E., Phillips, H.R.P., Camreon, E.K., & Brown, G. (2023). Earthworms (Clitellata, Megadrili) of the world: an updated checklist of valid species and families, with notes on their distribution. *Zootaxa*. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.33

Qiagen (2011). DNeasy Blood and Tissue Handbook. DNeasy Blood and Tissue Handbook.

Ratnasingham S., Hebert, P.D. bold: The Barcode of Life Data System (http://www.barcodinglife.org) (2007). Molecular Ecology Notes, 1;7(3):355-364. Recuperado de: https://doi.org/10.1111/j.1471-8286.2007.01678.x

Righi, G. (1972). Additions to the genus *glossoscolex* (oligochaeta, Glossoscolecidae). *Studies on Neotropical Fauna and Environment*, 7(1), 37-47. Recuperado de: https://doi.org/10.1080/01650527209360433

Righi, G., & Lobo, D. J. A. (1979). Nova contribuição ao gênero Glossoscolex (Oligochaeta) com sinopse do grupo giganteus. *Revista Brasileira de Biologia*, 39(4), 947-959.

Rosa, D. 1900. *Geoscolex bergi* n. sp. Comunicaciones del Museo Argentino de Ciencias Naturales, 1: 209–211.

Santos, A., Gorte, T., Demetrio, W. C., Ferreira, T., Nadolny, H., Cardoso, G. B., Tonetti, C., Ralisch, R., Nunes, A.P., Coqueiro, A.C.P., Leandro, H.C.L., Wandscheer, C.A.R., Bortoluzzi, J., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in no-tillage agroecosystems and native Atlantic forests in Western Paraná, Brazil. *Zootaxa*, 4496(1), 517-534. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.40

Steffen, G. P. K., Steffen, R. B., Bartz, M. L. C., James, S. W., Jacques, R. J. S., Brown, G. G., & Antoniolli, Z. I. (2018). Earthworm diversity in Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 562-575.

Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.43

Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.

Recuperado de: https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Recuperado de: https://doi.org/10.1073/pnas.04042061

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution.*

Recuperado de: https://doi.org/10.1093/molbev/msab120

Torrent, I. Use of ExoSAP-IT® PCR Product Cleanup Reagent in Next Generation Sequencing (NGS).

4 ARTICLE 3: LOOKS CAN BE DECEIVING: INCONGRUENCE BETWEEN GENETIC AND MORPHOLOGICAL DIVERSITY OF TWO EARTHWORM GENERA IN SOUTH AMERICA

4.1 RESUMO

As minhocas são reconhecidas como bioindicadores eficazes; no entanto, a integração de análises evolutivas e moleculares com a taxonomia clássica representa uma abordagem relativamente nova que ganhou destaque nas últimas décadas. Este capítulo visa empregar uma combinação de características morfológicas e sequenciamento genético utilizando a subunidade I do gene citocromo oxidase (COI) para destacar as relações entre os gêneros Glossoscolex e Fimoscolex dentro da família das minhocas Glossoscolecidae. As descobertas indicam que ambos os gêneros exibem convergências evolutivas e características parafiléticas, sugerindo uma história evolutiva complexa. Além disso, as características morfológicas relevantes usadas foram o número de poros masculinos e câmaras copulatórias, que eram anteriormente os únicos critérios taxonômicos para diferenciar os gêneros, sendo que Fimoscolex possuia um poro masculino e uma câmara copulatória. Novas descrições taxonômicas, no entanto, encontraram espécies de ambos os gêneros sem câmara copulatória. Análises moleculares demonstraram que essas características estão associadas a adaptações a fatores ambientais ou barreiras evolutivas, dado que a distribuição do gênero abrange uma extensão geográfica considerável. Foi identificada a presença de espécies crípticas, sendo a diferenciação possível apenas pela aplicação do barcode gap (4%-8%), validando assim a confiabilidade deste método para delimitação de espécies e identificação de linhagens ocultas. Os resultados obtidos ressaltam a importância da integração de múltiplas metodologias para garantir uma identificação mais precisa das minhocas, facilitando assim a correlação destes achados com as funções ecológicas desempenhadas no solo que contribuem para a melhoria do solo.

Palavras-chave: Citocromo oxidase I. Oligochaeta. Glossoscolecidae. Taxonomia. Barcode gap.

4.2 ABSTRACT

Earthworms are recognized as effective bioindicators; however, the integration of evolutionary and molecular analyses with classical taxonomy represents a relatively novel approach that has gained prominence in the past decades. This chapter aims to employ a combination of morphological traits and genetic sequencing utilizing the cytochrome oxidase subunit I (COI) gene to elucidate the relationships between the genera Glossoscolex and Fimoscolex within the earthworm family Glossoscolecidae. These findings indicated that both genera exhibit evolutionary convergences and paraphyletic characteristics, suggesting a complex evolutionary history. Furthermore, the relevant morphological features used were the number of male pores and copulatory bulbs, which were previously the only taxonomic criteria for differentiating the genera, with Fimoscolex having one male pore and one copulatory bulb. However, new taxonomic descriptions, found species of both genera without copulatory bulbs.

Molecular analyses have demonstrated that these characteristics are associated with adaptations to environmental factors or evolutionary barriers, given that the distribution of this genus spans a considerable geographical range. The presence of cryptic species was identified, with differentiation only possible through the application of the barcode gap (4%-8%), thus validating the reliability of this method for species delimitation and identification of hidden lineages. The results obtained underscore the importance of integrating multiple methodologies to ensure more accurate identification of earthworms, thereby facilitating the correlation of these findings with the ecological functions performed in the soil that contribute to soil improvement.

Keywords: Cytochrome oxidase I. Oligochaeta. Glossoscolecidae. Taxonomy. Barcode gap.

4.3 INTRODUCTION

Earthworms play a significant role in soil because of their burrowing and casting activities that affect soil aeration, improving structure and porosity, and altering nutrient cycling, and microbial activity (Le Bayon *et al*. 2021; Vidal *et al*. 2023). In addition to their ecological roles, earthworms also exhibit high diversity and wide distribution (Phillips *et al.* 2021). Worldwide over 5,700 species/subspecies of earthworms are known (Misirlioglu *et al*. 2023), but more than 30,000 are estimated to exist (Decaëns *et al*. 2024). Brazil hosts a substantial diversity of earthworms (James; Brown, 2006), with 10 of the 23 known families of earthworms, and approximately 336 known species (Brown *et al.* 2013), although the estimated total richness surpasses 2,100 species (Brown and James 2007). This positions Brazil as a key region for earthworm diversity and further taxonomic studies.

The complexity of earthworm taxonomy and evolution is due to the major deficit of fossil records and the number of characters that must be studied intricately through dissection for an adequate species description (Righi 1990, 1999; Marchán *et al.* 2022). Although classical morphological taxonomy has been used for decades, gaps remain to be addressed (Moreno; Borges, 2024). This is primarily because of the scarcity of taxonomists and reliance on morphological characteristics, which are sometimes insufficient for precise species classification. This may result in issues such as the underestimation of species richness and the inability to differentiate cryptic species that share morphological traits but are genetically distinct (Chang *et al.* 2018; Martinsson; Erséus, 2021).

In recent decades, significant advances have been made using integrative approaches to species description, combining traditional morphological methods with molecular techniques (Decaëns *et al.* 2016). Integrative taxonomy, which combines ecological, morphological and genetic data, has proven effective in identifying species and dealing with taxonomic uncertainties (Schlick-Steiner *et al*. 2010). Among the most utilized molecular tools is DNA barcoding, particularly the use of the gene cytochrome c oxidase subunit I (COI), a marker used in species delimitation (Hebert *et al.* 2003a). This gene is highly effective in differentiating closely related species and has been widely applied to aid in the identification and description of new earthworm species (Maggia *et al.* 2021, Golpeau *et al.* 2022, Decaëns *et al.* 2013, 2016, 2024a,b).

The use of specific primers for various organisms, targeting COI, has allowed the refinement of identification techniques, enabling not only the description of new species but also the filling of existing taxonomic gaps (Kvist, 2016). However, for earthworms, there are still no specific primers, so standard primers (Folmer *et al.* 1994) and/or primers, or cocktails of primers (Ivanova *et al.* 2007) from other organisms that have already shown positive results in DNA amplification are used.

One of the earthworm families whose morphological characteristics have led to the description of more than 150 species is Glossoscolecidae, widely distributed throughout the central-west, southeast, and southern regions of Brazil (Misirlioğlu *et al.* 2023). Within the family, one genus stands out as particularly widespread and diversified in Brazil: Glossoscolex Leuckart, 1835, also the first described from Latin America. This genus, together with its closely related neighbor Fimoscolex (Michaelsen, 1900), have shared morphological traits that are not yet well resolved. The main difference between the two genera is the number of male pores and copulatory pouches: Fimoscolex has one of each, while Glossoscolex always has two copulatory pouches, but either one or two male pores (Feijoo and Brown, 2018).

The use of molecular data may help unravel the phylogenetic relationships between these two genera and allow better understanding of their evolution. Furthermore, integrative taxonomy may also help answer standing questions related to their distribution, genetic and morphological diversity and evolutionary relationships. Combining DNA sequencing with ecological and morphological data, offers an unprecedented opportunity to fill these knowledge gaps and provide a more comprehensive view of earthworm biodiversity and evolution (Goulpeau *et al.* 2022, Marchán *et al*. 2022).

This study is therefore aimed at employing an integrative approach, incorporating both classical taxonomic description (morphological traits), ecological data (geographical distribution), and genetic sequencing, to address the existing knowledge gaps concerning the genera Glossoscolex and Fimoscolex in Brazil, with the objective of elucidating their evolutionary relationships. Furthermore, it sought to contribute to the resolution of the current deficiencies in earthworm taxonomy, thereby offering novel insights into their diversity and evolutionary patterns.

4.4 MATERIAL AND METHODS

4.4.1 Earthworms sampling and DNA processing

Earthworms were collected using two distinct methodologies: quantitative Tropical Soil Biology Fertility (TSBF) (Anderson; Ingram, 1993) and qualitative methods. Following collection, the specimens were preserved in 80-96% alcohol until laboratory manipulation for identification and DNA extraction. All specimens collected belonged to the Glossoscolecidae family, specifically from the genera *Glossoscolex* or *Fimoscolex*, with sampling dates ranging from 2004 to 2023. Appendix 1 presents some characteristics of the worms used as well as their place and date of collection.

For DNA extraction, tissue samples were removed from the specimens collected in 2023 and 2024 and subjected to an extraction process in accordance with the Qiagen protocol (DNeasy® Blood & Tissue Handbook, 2011). As for the worms collected before 2023, a piece of tissues was removed from the worms, and most of them were sent to BOLD Systems, for DNA amplification and sequencing (Ratnasingham; Hebert 2007). Some of them, however, were processed by Samuel James in the USA, Eliza Busch, Elodie da Silva or Márcio Gonçalves in Brazil (Embrapa Forestry), following methods described briefly in da Silva *et al.* (2017). Sequencing of these samples was performed in Sanger sequencers at the Federal University of Paraná, University of Kansas or Iowa State University (USA).

Following DNA extraction, the samples from 2023 and 2024 underwent polymerase chain reaction (PCR) using the NZYTaq II Master Mix and selected primers for DNA amplification. Subsequently, the samples were purified using the ExoSap-IT TM Express PCR Product Cleanup protocol. The samples were then sent to the Macrogen and Eurofins laboratories for the final step of DNA sequencing.

Initially, for earthworms collected prior to or during 2023 and 2024, the forward and reverse primers described by Folmer *et al.* (1994), which are considered universal primers for invertebrates, were utilized. For samples in which DNA amplification was unsuccessful with Folmer primers, we employed the methodology proposed by

Ivanova *et al.* (2007), in which several primer cocktails were developed for conserved regions in earthworm COI used to enhance amplification results (Simões, in preparation).

4.4.2 Data analysis

The sequences obtained were initially processed using FinchTV 1.4.0 software (Geospiza, 2004) and subsequently aligned using Molecular Evolutionary Genetics Analysis version 11 - MEGA11 (Temura *et al.* 2021). The pairwise distance and construction of the phylogenetic tree were also conducted using MEGA, employing the Maximum Composite Likelihood method and Neighbor-joining bootstrap method Tamura-Nei model with 1000 bootstrap replications, for each analysis. For the tree design the software FigTree 1.4.4 was used.

The method Assemble Species by Automatic Partitioning (ASAP; Puiliandre *et al.* 2021) was used for species delimitation and identification of the barcode gap, and R packages (ape and phangorn) were used for complementary analyses. The ASAP orders the clusters based on a reliability score forming different partitions according to the distance among samples (Puiliandre *et al.* 2021).

4.5 RESULTS AND DISCUSSION

4.5.2 Phylogenetic patterns in G*lossoscolex* and *Fimoscolex*

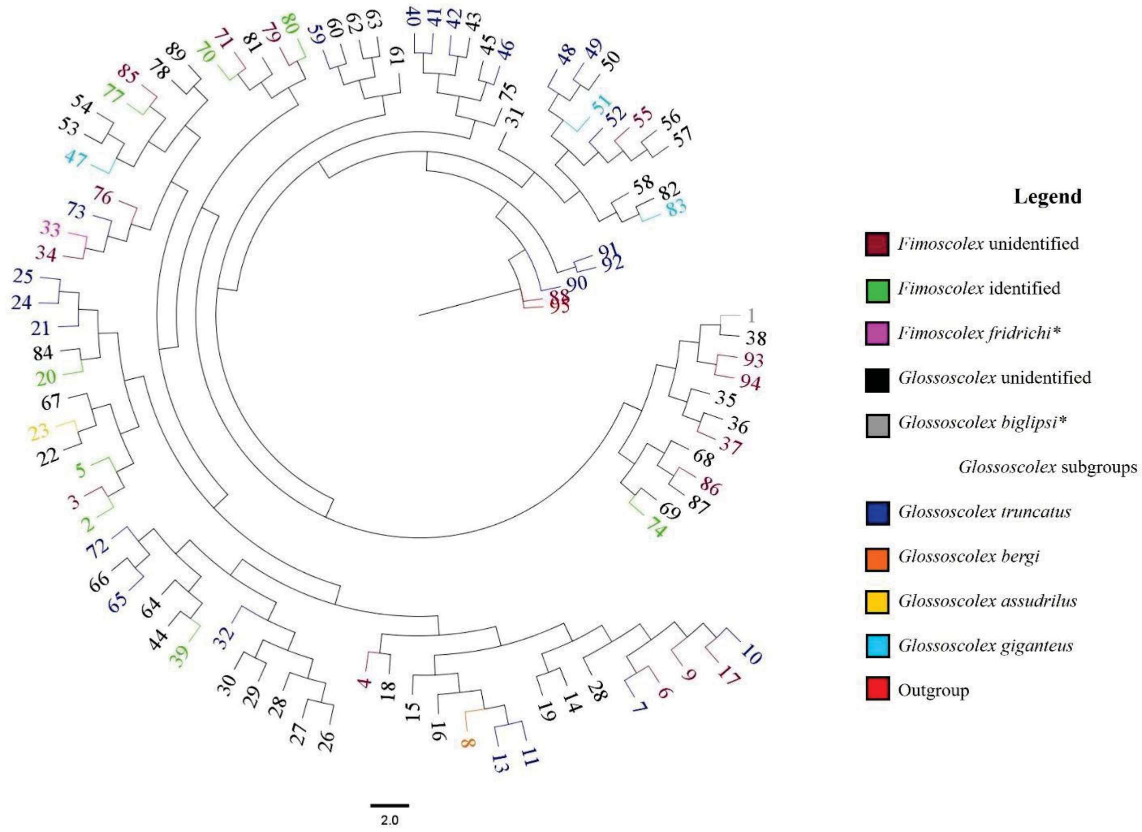
A total of 217 sequences were analyzed, comprising 158 from *Glossoscolex* and 59 from *Fimoscolex*. Among these, 24 species (59 sequences) of *Glossoscolex* and nine species (22 sequences) of *Fimoscolex* were previously described taxonomically (i.e., had been named). Using the ASAP method, the best partition resulted in a score of 8, and the 217 sequences formed 95 MOTUs. This partition corresponds to a threshold of 8%, with intraspecific divergence ranging from 0 to 4% and interspecific divergence from 8 to 36%. Previously Chang and James (2011) showed that, for earthworms, a threshold between 9% and 15% (Kimura 2-parameter distance) represents an ambiguous range when no morphological differences are observed between clades. They also stated that distances below 9% or above 15% can be unequivocally attributed to the same species or to different species, respectively (Chang and James, 2011). In our case the threshold based on the ASAP was lower (8%).

Some of the species had more than one specimen, for these cases a representative sequence was chosen based on the lowest genetic distance among samples. Figure 1 illustrates the Neighbor-joining tree derived from MOTU grouping. Table 9 provides information on date and location of the sequences of earthworms used for the Neighbor-Joining tree (Figure 32) and the appendix material provides detailed information on the DNA reference codes, sampling dates, and locations for each sequence.

The utilization of the outgroup (*Eisenia andrei*, family Lumbricidae) served as an evolutionary reference point to establish the ancestry of the focal genera, *Glossoscolex* and *Fimoscolex* (family Glossoscolecidae). The phylogenetic tree elucidated the evolutionary relationships between these genera, highlighting the complexity and ambiguity surrounding their evolutionary history. Notably, the tree revealed instances of rapid diversification, resulting in multiple species.

The clustering of *Fimoscolex* species with *Glossoscolex* further underscores the challenges in delineating these genera, suggesting that they are not monophyletic but exhibit close phylogenetic relationships. This observation indicates possible shared evolutionary pathways or incomplete lineage sorting, reflecting the complex history of these earthworms. Instances of multiple clusters within each genus suggest adaptive radiation, likely driven by ecological diversification across the different regions of Brazil. Hence, Feijoo and Brown (2018) called for the utilization of molecular tools to help distinguish these two genera, as their taxonomic separation is challenging due to apparent ecological plasticity (distribution of some species in various habitat and soil types) and overlapping morphological characteristics in both genera.

FIGURE 32 - NEIGHBOR-JOINING TREE OF 95 MOLECULAR OPERATIONAL TAXONOMIC UNIT (MOTU)



Source: Author (2024)

Legend: Neighbor-joining tree of 95 molecular operational taxonomic unit (MOTU) identified based on a genetic distance threshold of 8%, according to the Assemble Species by Automatic Partitioning (ASAP) results. The * represents species that lack copulatory bulb(s): *Fimoscolex fridrichi* (33) and *Glossoscolex biglipsi* (1). The outgroup (95) is represented by *Eisenia andrei* (Lumbricidae family).

TABLE 9 – CODES OF SEQUENCES, COLLECTION DATE AND LOCATION, AND THE CORRESPONDING MOTU RESULTED FROM ASAP, USED FOR THE NEIGHBOR-JOINING TREE.

Sequences	MOTU	Date	State	Municipality/Site
Glossoscolex biglipsi	1	02/2009	BR/MG	Itamonte
Fimoscolex dorivali	2	ND	BR/SP	Bananal
Fimoscolex sp.54	3	02/2009	BR/SP	Bocaína
Fimoscolex sp.56	4	02/2009	BR/SP	Bocaína
Fimoscolex elodieae	5	02/2009	BR/SP	Bananal
Fimoscolex sp.27	6	ND	BR/SC	Campo Belo do Sul
Glossoscolex alessioi	7	08/2023	BR/SC	Faxinal dos Guedes
Glossoscolex strobeli	8	08/2023	BR/RS	Panambi
Fimoscolex sp.60	9	01/2006	BR/PR	Ponta Grossa
Glossoscolex debortollii	10	08/2023	BR/RS	Cruz Alta
Glossoscolex terraopimus	11	01/2006	BR/PR	Faxinal
Glossoscolex SC	12	ND	BR/SC	Campo Belo do Sul
Glossoscolex terraopimus	13	11/2004	BR/PR	Ortigueira
Glossoscolex Ponta Grossa sp.315	14	01/2006	BR/PR	Ponta Grossa
Glossoscolex Três Barras	15	02/2010	BR/SC	Três Barras
Glossoscolex sp.33	16	ND	BR/SC	Santa Terezinha do Salto
Fimoscolex sp.3	17	01/2015	BR/PR	Lapa
Glossoscolex Irati	18	02/2024	BR/PR	Irati
Glossoscolex PicoPR	19	06/04	BR/PR	Campina Grande do Sul
Fimoscolex elkeae	20	02/2009	BR/SP	Bananal
Glossoscolex nativus	21	ND	BR/RS	Santana do Livramento
Glossoscolex Bocaina3	22	02/2009	BR/SP	Bocaína
Glossoscolex carvalhoi	23	02/2009	BR/SP	Bananal
Glossoscolex giocondoi	24	12/2004	BR/PR	ND
Glossoscolex mariarum OR *sanpedroensis*	25	04/2004	BR/PR	ND
Glossoscolex sp.31	26	ND	BR/SC	Otacílio Costa
Glossoscolex Campo Belo do Sul	27	ND	BR/SC	Campo Belo do Sul
Glossoscolex sp.7	28	ND	BR/RS	Estrela
Glossoscolecidae sp.32	29	ND	BR/SC	Otacílio Costa
Glossoscolecidae sp.3	30	ND	BR/SC	Otacílio Costa
Glossoscolex Bocaina 1	31	02/2009	BR/SP	Bocaína
Glossoscolex arnsi	32	08/2023	BR/RS	Bom Jesus
Fimoscolex fridrichi	33	08/2023	BR/RS	Panambi
Fimoscolex sp.3	34	ND	BR/SC	São Miguel do Oeste
Glossoscolex CMA	35	01/2006	BR/SP	Campinas do Monte Alegre

Table 9 continuation

Sequences	MOTU	Date	State	Municipality/Site
Glossoscolex GS	36		BR/RS	Santa Maria
Fimoscolex n.sp.1 GS	37	ND	BR/RS	Maçambara
Glossoscolex Assistencia	38	02/2006	BR/SP	Assistência
Fimoscolex feijooi	39	02/2009	BR/RJ	N.P. Itatiaia
Glossoscolex palus	40	02/2006	BR/PR	Bandeirantes
Glossoscolex palus	41	04/2004	BR/PR	Bandeirantes
Glossoscolex lutocolus	42	03/2005	BR/PR	Jaguapitã
Glossoscolex Boituva	43	05/2012	BR/SP	Boituva
Glossoscolecidae Lauro Muller	44	ND	BR/SC	Lauro Muller
Glossoscolex	45	02/2009	BR/SP	Itabéra
Glossoscolex uliginosus	46	05/2004	BR/PR	Sao Jerônimo
Glossoscolex demeijeri	47	04/2005	BR/PR	Guaraqueçaba
Glossoscolex maschio	48	01/2010	BR/PR	Colombo
Glossoscolex sp.77	49	03/2024	BR/SP	Maracaí
Glossoscolex sp.7	50	ND	BR/SC	Chapecó
Glossoscolex paulistus	51	02/2006	BR/SP	Assistência
Glossoscolex Bananal	52	ND	BR/SP	Bananal
Glossoscolex Bocaina3	53	02/2009	BR/SP	Bocaina
Glossoscolex Intervales	54	02/2009	BR/SP	Intervales
Fimoscolex sp.56	55	02/2009	BR/SP	Bocaina
Glossoscolex redgiant	56	02/2009	BR/RJ	N.P.* Itatiaia
Glossoscolex redgiant	57	02/2009	BR/RJ	N.P Itatiaia
Glossoscolex Ilha Bela	58	04/2006	BR/SP	Ilha Bela
Glossoscolex mariebartzae	59	07/1905	BR/MG	Itamonte
Glossoscolex Itamonte Small	60	02/2009	BR/MG	Itamonte
Glossoscolex Bela vista	61	06/2004	ND	ND
Glossoscolex	62	09/2004	BR/MS	Fatima do Sul
Glossoscolex	63	ND	BR/MS	ND
Glossoscolex Bocaina 5	64	02/2009	BR/SP	Bocaína
Glossoscolex lutocolus	65	05/2005	BR/PR	Primeiro de Maio
Glossoscolex CampGrandedoSul	66	03/2012	BR/PR	Campina Grande do Sul
Glossoscolex sp.37	67	12/2011	BR/PR	Campina Grande do Sul
Glossoscolex Campina Grande	68	06/2004	BR/PR	ND
Glossoscolex sp.317	69	02/2006	BR/SP	Buri
Fimoscolex barettai	70	07/1905	BR/MG	Itamonte
Fimoscolex sp.52	71	02/2009	BR/RJ	Itatiaia

Table 9 continuation...

Sequences	MOTU	Date	State	Municipality/Site
Glossoscolex pampas	72	ND	BR/RS	Uruguaiana
Glossoscolex riograndensis	73	ND	BR/RS	São Vicente do Sul
Fimoscolex nivae	74	09/2009	BR/PR	Colombo
Glossoscolex Lavras Novas	75	07/2006	BR/MG	ND
Fimoscolex sp.52	76	08/2023	BR/RS	Ajuricaba
Fimoscolex pereirai	77	07/1905	BR/RJ	N.P. Itatiaia
Glossoscolex Campos Jordao	78	05/2006	BR/SP	Campos Jordão
Fimoscolex sp.58	79	05/2006	BR/SP	Campos Jordão
Fimoscolex sporadochaetus	80	07/2006	BR/MG	Lavras Novas
Glossoscolex sp.	81	ND	BR/ES	Linhares
Glossoscolex Seropedica	82	ND	BR/RJ	Seropédica
Glossoscolex giganteus	83	ND	BR/PR	Caratuva
Glossoscolex sp. Xanxere	84	08/2011	BR/SC	Xanxerê
Fimoscolex	85	ND	BR/RJ	Rebio
Fimoscolex sp.61	86	04/2004	BR/SP	Boracéia
Glossoscolex Salesopolis	87	04/2004	BR/SP	Salesópolis
Fimoscolex sp.28	88	ND	BR/SC	São Miguel do Oeste
Glossoscolex BAN5	89	02/2009	BR/SP	Bananal
Glossoscolex lutocolus	90	04/2004	BR/PR	Paiquerê
Glossoscolex lutocolus	91	05/2005	BR/PR	Primeiro de Maio
Glossoscolex uliginosus	92	05/2004	BR/PR	São Jerônimo da Serra
Fimoscolex sp.59	93	10/2008	BR/PR	Piraquara
Fimoscolex sp.n.25	94	2018/2019	ND	ND
Eisenia andrei	95	01/2024	Portugal	Coimbra

*N.P = National Park.

In this study, the mitochondrial cytochrome oxidase I (COI) gene was employed as a molecular marker because of its widespread use in DNA barcoding and ability to provide insights into species-level differentiation. COI is particularly effective in identifying species and exploring cryptic diversity because of its relatively rapid mutation rate, which captures recent evolutionary events (Hebert *et al*. 2004; Huang *et al.* 2007). However, its utility in resolving deeper phylogenetic relationships, such as those at the genus or family level, is often limited, because COI primarily reflects maternal inheritance and does not capture recombination or nuclear genomic information (Conrado *et al.* 2017; Tiwari *et al*. 2021). Besides, COI presents a saturation in the third base codon that results in a loss of phylogenetics signal above genetic distances typical of closely related species (Breinholt and Kawahara 2013)

The use of a multi-marker approach, incorporating more than one gene besides COI, such as ribosomal 16S and 18S, or additional nuclear genes like ITS or histone H3, could provide greater resolution for phylogenetic relationships. This strategy is particularly effective for filling gaps in integrative identification and improving bootstrap support in deeper phylogenetic and evolutionary analyses (James and Davidson 2012; Dupont *et al.* 2016). Additionally, leveraging next-generation sequencing (NGS) technologies, such as restriction site-associated DNA sequencing (RADseq), ultra conserved elements (UCEs) and Anchored Hybrid Enrichment (Lemmon *et al.* 2012), offers a broader genomic perspective (Harvey *et al*. 2016). These approaches enable researchers to capture whole-genome representations and thousands of loci, providing high-resolution data suitable for resolving complex phylogenies (McCormack *et al*. 2013). RADseq has been successfully used to uncover fine-scale genetic structure and evolutionary relationships in non-model organisms (Andrews *et al*. 2016; Malinsky *et al*. 2018), while UCEs have demonstrated utility in delineating phylogenies across varying evolutionary depths (Faircloth *et al.* 2012). Whole-genome sequencing and metagenomics approaches can also contribute to elucidating cryptic diversity, uncovering adaptive traits, and identifying lineage-specific markers (Taheri *et al.* 2018).

Despite these challenges, the COI marker remains a powerful tool for exploring genetic diversity and delineating species boundaries, particularly in the context of understudied genera such as *Glossoscolex* and *Fimoscolex* (Pérez-Losada et al. 2012; Martinsson and Erséus, 2021). By integrating traditional markers like COI with genome-wide approaches, we may be able to enhance the resolution of phylogenetic studies and foster a deeper understanding of evolutionary processes in these genera.

These findings are consistent with those of previous studies (Huang *et al*. 2007; Martinsson & Erséus 2021) that reported high genetic divergence within and among earthworm genera, often reflecting cryptic diversity or adaptive radiation. Furthermore, Kvist *et al*. (2014) emphasized that annelids frequently exhibit high genetic divergence, which is attributed to historical isolation or ecological specialization. These results highlight the necessity of combining genetic and morphological data to resolve taxonomic ambiguities (Dupont *et al*. 2016; Decaëns *et al*. 2013).

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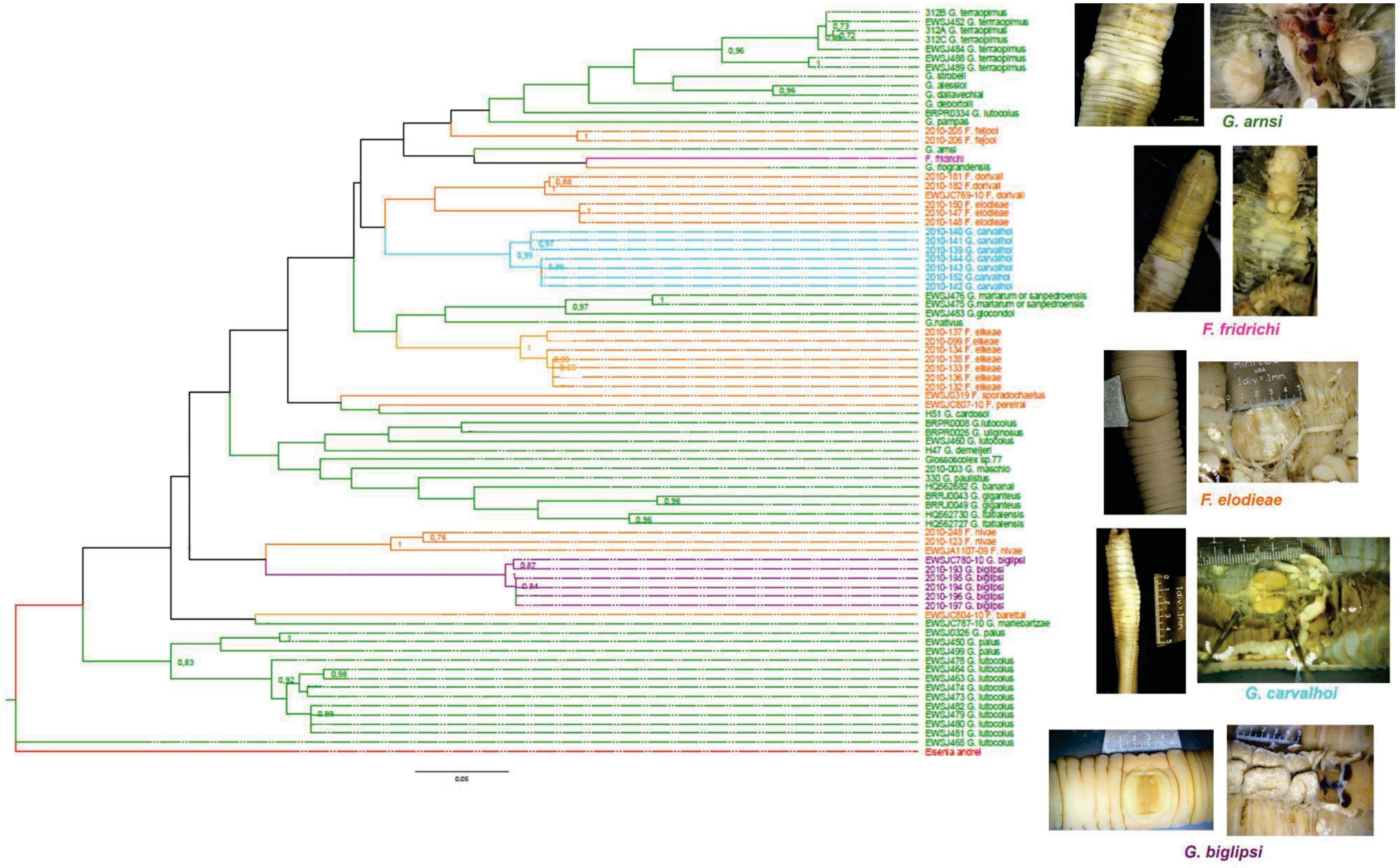
4.5.3 Relationship between morphological traits and molecular analyses

To analyze evolutionary relationship between the genera *Glossoscolex* and *Fimoscolex* and their distinct morphological characteristics, a Maximum Likelihood tree was constructed using already described species. This analysis facilitated the identification of two primary characteristics that suggested variations between species and genera: the quantity and combination of male pores and copulatory chambers. Figure 33 employs color coding to highlight the occurrence of these characteristics. Species in green have two male pores and two copulatory bulbs; blue indicates one pore and one bulb; orange represents one pore and one bulb; purple denotes two pores and no bulb; and pink signifies one pore and no bulb. The number of male pores and copulatory bulbs was until now the main distinguishing feature between the *Glossoscolex* and *Fimoscolex*, with *Fimoscolex* exhibiting a single male pore and copulatory bulb (Feijoo and Brown 2018). However, new findings showed more variations in these structures for the two genera.

FIGURE 33 - PHYLOGENETIC TREE WITH DNA SEQUENCES OF *GLOSSOSCOLEX* AND *FIMOSCOLEX* GENUS AND MORPHOLOGIC TRAITS



Source: Author (2024)

Legend: The phylogenetic tree displays the evolutionary relationships between species in the genera *Glossoscolex* and *Fimoscolex*, with *Eisenia andrei* serving as the outgroup. The numbers at each node represent bootstrap support values, indicating robustness of the respective clades. Values lower than 0,65 were omitted from the tree. Colors represent different morphological traits. Green: two male pores and two copulatory bulbs; Blue: One male pore, two copulatory bulbs; Orange: One male pore, one copulatory bulb; Purple: two male pores, no copulatory bulb; Pink: One male pore, no copulatory bulb. Red: outgroup *E. andrei*.

Species of *Glossoscolex* exhibiting two pores and two chambers (in green) as well as *Fimoscolex* with one pore and one chamber (in orange) in several lineages can indicate polyphyletic evolution, with bootstrap values approaching one (Figure 2). The presence of stable characteristics suggests that these lineages are genetically well-defined (James *et al.* 2012; Feijoo; Brown, 2023). *Glossoscolex carvalhoi*, the only species with a genetic sequence possessing one pore and two chambers (Dudas *et al.* 2025, in press), appear positioned between *Glossoscolex* and *Fimoscolex* groups (Figure 2). This placement suggests the possibility of independent evolution within a distinct lineage, potentially indicating evolutionary convergence, wherein different lineages develop similar but non-identical characteristics owing to external factors. Dupont *et al.* (2016) proposed that this phenomenon validates the use of molecular techniques to interpret convergent morphological characteristics. As it is the sole species in our sequence dataset that possesses one pore and two copulatory bulbs, further efforts should be made to obtain DNA on the other species with this trait: *G. sacii* a very large earthworm occurring in São Paulo state (Righi 1971) and *G. jimi* from Santa Catarina (Righi 1972).

Within the tree, these groups exhibited greater homogeneity, with varied bootstrap values, potentially indicating an intermediate characteristic and illustrating the process of transition and/or adaptation to specific niches. Consequently, intermediate characteristics typically signify lineages undergoing diversification (Martisson; Erséus 2021).

Recently described species, however, have revealed new morphological characteristics, including the absence of copulatory bulbs. This characteristic was observed in one species from each genus. *Glossoscolex biglipsi* (Dudas *et al.* 2025 in preparation), represented in purple, constituting a genetically distinct lineage with lower bootstrap values, potentially indicative of recent evolution or instability. And

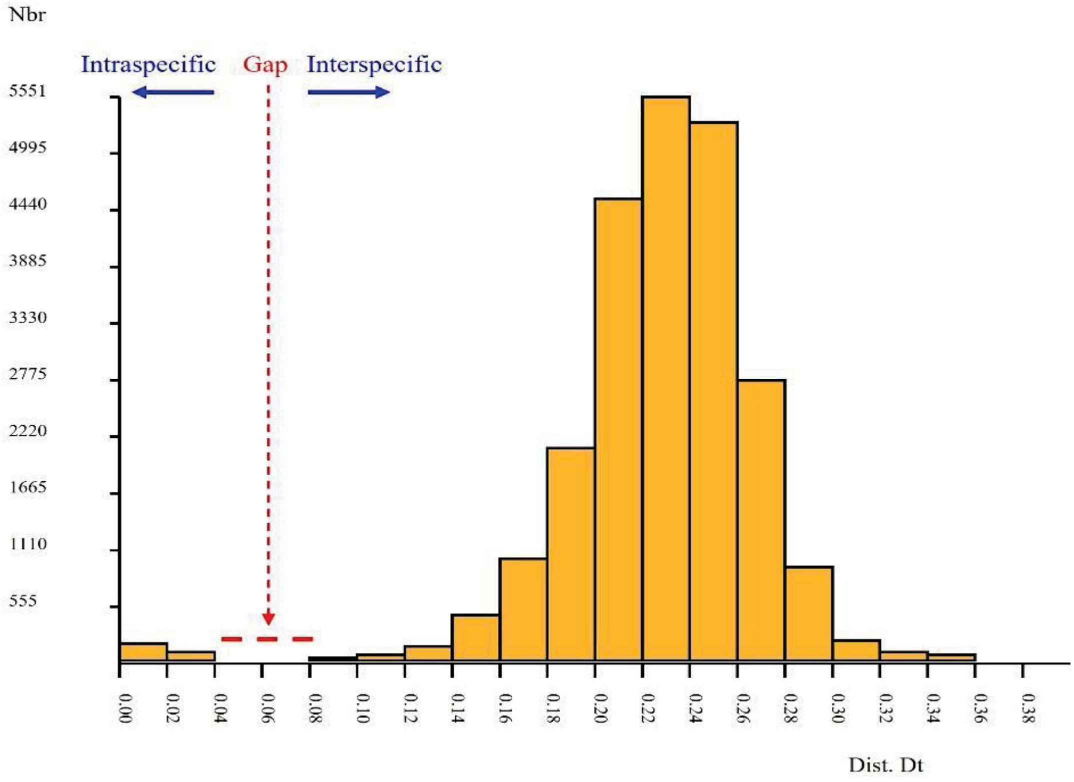
Fimoscolex fridrichi, exhibiting high genetic diversity, potentially signifying greater morphological plasticity or environmental adaptation. Decaëns *et al*. (2013) suggested that lineages with low morphological differentiation might have greater genetic variability. Furthermore, Hueng *et al.* (2007) showed that increased variability often correlates with high levels of genetic divergence.

Additional highlights patterns in the tree (Figure 2): species with one male pore and one copulatory bulb (represented in orange) and *G. biglipsi*, which has two male pores but no copulatory bulbs, tend to exhibit greater genetic distances. This may indicate fewer shared characteristics and greater divergence between these taxa. According to Marchan *et al.* (2018), the use of DNA barcoding helps detect cryptic diversity in genera/species that are morphologically similar.

As for the ASAP result considering only the described species, the grouping made was consistent with what had already been observed in other studies, in which the dataset presented high genetic diversity, but with some distinct morphological characteristics (Goupeau *et al.* 2022), as is the case of the samples in this study, in which genera that have an evolutionary history occurring simultaneously and with morphological variations with small changes within the genera and between them, and species with unique traits (like *G. biglipsi* and *F. fridrichi*) can be used as examples of cryptic diversity.

A histogram of genetic distances with density curves was constructed using the sequences of *Glossoscolex* and *Fimoscolex* (figure 34). The objective of the analysis was to elucidate the delimitation of species, emphasizing groups separated by substantial genetic distances, which may represent independent lineages, evaluating the barcode gap to analyze intra and interspecific distances for species delimitation (Hebert *et al.* 2004), and/or indicate the presence of cryptic species. Cryptic species exhibit high morphological similarity but possess genetic differences that may be associated with adaptations or reproductive barriers. According to Marchan *et al*. (2018), the use of integrative methodologies facilitates the identification of diversity that is not detected morphologically.

FIGURE 34 - HISTOGRAM OF GENERAL DISTRIBUTION OF GENETIC DISTANCES BETWEEN ANALYZED SPECIES



Source: Author (2024)

Legend: The Y axis represents the number of comparisons between pairs of sequences. The intraspecific distance ranges from 0% to 4%. And the interspecific distance ranges from 8% to 36%. The barcode gap corresponds to 4% to 8%.

Elevated genetic distances within morphologically similar groups suggest the presence of species that are not visually distinguishable. FIGURE 41 provides an overview of the genetic distances between the analyzed species. Most genetic distances were concentrated around 20%, with values below 4% indicating intraspecific comparisons and values above 8% indicating interspecific comparisons (between species and genera). Meyer and Paulay (2005) asserted that the gap is significant for species delimitation; however, in some instances, it may be subtle, which can be attributed to recent evolution or the presence of cryptic species.

1.1.1. Cryptic diversity among *Glossoscolex* genera

Some of the *Glossoscolex* species already taxonomically described were grouped in different MOTUs on the ASAP and in the phylogenetic trees (Suplementary material 1, Figures 1, 2). For *Glossoscolex lutocolus*, had the firsts described specimens from Paraná state, Brazil, in alluvial mud of swamps (hence the name) and were identified with two male pores in segment XVI and two copulatory bulbs (Bartz and James, 2012). In the ASAP the MOTUs for this species were 42 (with 10 individuals), 65, 90 and 91 (all with one individual). All the specimens were collected in Paraná, in five different municipalities in the years 2004 and 2005, in wetlands/swamps. The genetic distance among these MOTUs ranged from 20% to 24%, and considering the threshold of 8%, this indicates that we have intraspecific divergence for *G. lutocolus.*

For *Glossoscolex uliginosus,* we had two separate MOTUs, 46 and 92, both with only one individual. This species was first encountered in São Jerônimo da Serra, in Paraná state, Brazil, and as *G. lutocolus,* in areas of swamps and wetlands. Regarding the morphology, the pair of male pores are in the papillae on intersegmental line of XVI/XVII, with a pair of copulatory bulbs (Bartz and James, 2012). The genetic distance between the MOTUs is 21%, higher than the threshold. In the case of *G. uliginosus*, both specimens were collected in the same place and same date, which can indicate a few things, like two different lineages in the same location, taxonomical misconception, or DNA contamination.

Similar to *G. uliginosus, Glossoscolex palus*, also have two MOTUs, 40 (with two specimens) and 41 (one specimen), both collected in Bandeirantes municipality, in Paraná state, Brazil, in the same date and location. This species is characterized by a pair of male pores in segment XVII and a pair of copulatory bulbs (Bartz and James, 2012). The genetic distance between the MOTUs is 11%. And the last species that was grouped in different MOTUs was *Glossoscolex terraopimus*, 11 (with five specimens) and 13 (with two specimens). This species was first collected in municipalities of Faxinal and Ortigueira, Paraná state, Brazil, near forest sites. Characterized by male pores in segment XVII and two copulatory bulbs (Bartz and James, 2012). The genetic distance between the MOTUs is 10%. For these last two species, the genetic distance is shortly higher than the resultant ASAP threshold 8%, but nonetheless this may indicate the intraspecific divergence among the collected material, as well as other possibilities previously mentioned. Further research, sampling and sequencing are needed to confirm or discard these assumptions.

The use of genetic distance, morphological traits and molecular software’s helps to infer evolutionary and adaptive patterns of speciation processes, and the combination of morphological and molecular description methodologies reveals that the division of the *Glossoscolex* and *Fimoscolex* is more complex than just determining morphological differences or similarities. The presence of a barcode gap highlights the reliability of the dataset for exploring species delimitation and identifying cryptic species, emphasizing the distinct evolutionary trajectories of these taxa.

The combination of ASAP and other methods is supported by the demonstrated efficiency in detecting cryptic species and the ability to align genetics with morphology (Goupeaul *et al.* 2022). Dupont *et al.* (2023) emphasized the use of DNA barcoding to identify cryptic species in tropical areas, highlighting the relevance of molecular methods with the aim of protecting unique lineages and regions with high diversity, such as Brazil. Studies in the genus *Glossoscolex* have shown that differences in the position of male pores and copulatory chambers are associated with genetic distinctions between species (Feijoo and Brown, 2023). Furthermore, they substantiate the need for an integrative approach in the description of new species, incorporating classical taxonomy together with molecular analyses from the outset.

4.6 CONCLUSION

Based on 217 sequences of described and undescribed species of the genera *Glossoscolex* and *Fimoscolex*, morphological and molecular analyses were performed to identify evolutionary relationships between these genera. The results indicate challenges in classifying these genera as monophyletic, as there are indications of evolutionary convergence among the samples. The presence of cryptic species within the genera was observed, which may be attributed to ecological adaptations or evolutionary barriers, given that these genera are found in diverse locations across Brazil with significant geographical distances.

In this study, the evaluated sequences were derived from the COI gene. While this approach is effective, various studies have emphasized the advantages of utilizing multiple genes for molecular analyses to enhance the results and their accuracy, thereby exploring different phylogenetic approaches. Furthermore, the results underscore the importance of conducting classical taxonomy together with DNA analysis to refine species delimitation and rectify potential taxonomic inconsistencies, thereby ensuring the most accurate identification of earthworm species.

Future research focusing on the genera *Glossoscolex* and *Fimoscolex*, integrating taxonomy with molecular analyses, including COI, 16S, and 18S genes, and correlating these data with ecological and environmental variables, will yield more robust insights into all aspects of this group of earthworms. These organisms serve as excellent environmental indicators and play a crucial role in improving soil quality.

4.7 REFERENCES

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, 17(2), 81-92. Recuperado de: https://doi.org/10.1038/nrg.2015.28

Anderson, J. M., & Ingram, J. S. (1994). Tropical soil biology and fertility: a handbook of methods. Soil Science, 157(4), 265.

Anderson, F. E., Williams, B. W., Horn, K. M., Erséus, C., Halanych, K. M., Santos, S. R., & James, S. W. (2017). Phylogenomic analyses of Crassiclitellata support major Northern and Southern Hemisphere clades and a Pangaean origin for earthworms. BMC *Evolutionary Biology*, 17, 1-18. Recuperado de: https://doi.org/10.1186/s12862-017-0973-4

Barraux, A., Noël, S., Roy, V., & Dupont, L. (2024). Challenges of molecular barcode-based identification of earthworm specimens for biodiversity assessment. *Frontiers in Ecology and Evolution*, 12, 1358984. Recuperado de: https://doi.org/10.3389/fevo.2024.1358984

Breinholt, J. W., & Kawahara, A. Y. (2013). Phylotranscriptomics: saturated third codon positions radically influence the estimation of trees based on next-gen data. *Genome Biology and Evolution*, *5*(11), 2082-2092. Recuperado de: https://doi.org/10.1093/gbe/evt157

Brown, G. G., James, S. W., Csuzdi, C., Lapied, E., Decäens, T., Reynolds, J. W., Misirlioglu, M., Mirjana, S., Tanja, T., Jovana, S., Phillips, H.R.P. & Cameron, E. (2024). A checklist of megadrile earthworm (Annelida: Clitellata) species and subspecies of the world. Available from: *Zenodo*. Recuperado de: https://doi.org/10.5281/zenodo.7301848

Chang, C. H., Rougerie, R., & Chen, J. H. (2018). DNA barcoding reveals cryptic diversity and distinct population genetic structure in the earthworm Amynthas corticis. *Molecular Ecology Resources*, 18(5), 952-965.

Conrado, A. C., Arruda, H., Stanton, D. W., James, S. W., Kille, P., Brown, G.,Silva, E., Dupont, L., Taheri, S., Morgan, A.J., Simões, N., Rodrigues, A., Montiel, R & Cunha, L. (2017). The complete mitochondrial DNA sequence of the pantropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Clitellata): Mitogenome characterization and phylogenetic positioning. *ZooKeys*, (688), 1. Recuperado de: https://doi.org/10.3897/zookeys.688.13721

Decaëns, T., Porco, D., Rougerie, R., Brown, G. G., & James, S. W. (2013). Potential of DNA barcoding for earthworm research in taxonomy and ecology. *Applied Soil Ecology*, 65, 35-42. Recuperado de: https://doi.org/10.1016/j.apsoil.2013.01.001

Decaëns, T., Porco, D., James, S. W., Brown, G. G., Chassany, V., Dubs, F., Dupont, L., Lapied, E., Rougeri, R., Rossi, J.P., & Roy, V. (2016). DNA barcoding reveals diversity patterns of earthworm communities in remote tropical forests of French Guiana. *Soil Biology and Biochemistry*, 92, 171-183. Recuperado de: https://doi.org/10.1016/j.soilbio.2015.10.009

Decaëns, T., Brown, G.G., Cameron, E.K., Csuzdi, C., Eisenhauer, N., Gérard, S., Goulpeau, A., et al., 2024a. A can of worms: estimating the global number of earthworm species. https://doi.org/10.1101/2024.09.08.611896

Decaëns, T., Carolina Bartz, M.L., Feijoo-Martínez†, A., Goulpeau, A., Lapied, E., Marchán, D.F., Maggia, M.-E., et al., 2024b. Earthworms (Oligochaeta, Clitellata) of the Mitaraka range (French Guiana): commented checklist with description of one genus and eighteen species new to science. Zoosystema 46. https://doi.org/10.5252/zoosystema2024v46a9

Demetrio, W. C., Ribeiro, R. H., Nadolny, H., Bartz, M. L., & Brown, G. G. (2020). Earthworms in Brazilian no-tillage agriculture: Current status and future challenges. *European Journal of Soil Science*, 71(6), 988-1005. Recuperado de: https://doi.org/10.1111/ejss.12918

Dudas, R.T., James, S.W., Bartz M.L.C. & Brown, G.G. (2025) New earthworm species of *Glossoscolex* Leuckart, 1835 and *Fimoscolex* Michaelsen, 1900 (Clitellata: Glossoscolecidae) from southeastern Brazil. Zootaxa, in press.

Dupont, L., Porco, D., Symondson, W. O. C., & Roy, V. (2016). Hybridization relics complicate barcode-based identification of species in earthworms. Molecular Ecology Resources, 16(4), 883-894. Recuperado de: 0.1111/1755-0998.12517

Dupont, L., Brunet, C. M., Fourcade, Y., James, S., Gabriac, Q., & Coulis, M. (2023) Recording earthworm diversity on the tropical island of Martinique using DNA barcoding unveiled endemic species in bromeliad plants. *Soil Biology and Biochemistry*, 182, 1-10. Recuperado de: https://doi.org/10.1016/j.soilbio.2023.109038

Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic biology*, 61(5), 717-726. Recuperado de: https://doi.org/10.1093/sysbio/sys004

Feijoo, A.M. & Brown, G. G. (2018). New species of *Glossoscolex* and *Fimoscolex* earthworms (Oligochaeta: Glossoscolecidae) from Embrapa Forestry, Paraná, Brazil. *Zootaxa*, 4496(1), 492-502. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.38

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology Biotechnology*, 3, 294-299.

Goulpeau, A., Penel, B., Maggia, M. E., Marchán, D. F., Steinke, D., Hedde, M., & Decaëns, T. (2022). OTU delimitation with earthworm DNA barcodes: A comparison of methods. *Diversity*, 14(10), 866. Recuperado de: https://doi.org/10.3390/d14100866

Harvey, M. G., Smith, B. T., Glenn, T. C., Faircloth, B. C., & Brumfield, R. T. (2016). Sequence capture versus restriction site associated DNA sequencing for shallow systematics. *Systematic biology*, 65(5), 910-924. Recuperado de: https://doi.org/10.1093/sysbio/syw036

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003a). Biological identifications through DNA barcodes*. Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1512), 313-321. Recuperado de: https://doi.org/10.1098/rspb.2002.2218

Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(suppl_1), S96-S99.

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, 101(41), 14812-14817. Recuperado de: https://doi.org/10.1073/pnas.0406166101

Huang, J., Xu, Q., Sun, Z. J., Tang, G. L., & Su, Z. Y. (2007). Identifying earthworms through DNA barcodes. *Pedobiologia*, 51(4), 301-309. Recuperado de: https://doi.org/10.1016/j.pedobi.2007.05.003

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7(4), 544-548. Recuperado de: https://doi.org/10.1111/j.1471-8286.2007.01748.x

James, S. W., & Brown, G. G. (2006).

James SW, Porco D, Decaens T, Richard B, Rougerie R, Erseus C (2010) DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): Resurrection of *L. herculeus* (Savigny, 1826). *PLoS ONE* 5:15629

James, S. W., & Davidson, S. K. (2012). Molecular phylogeny of earthworms (Annelida: Crassiclitellata) based on 28S, 18S and 16S gene sequences. *Invertebrate Systematics*, 26(2), 213-229. Recuperado de: https://doi.org/10.1071/IS11012

King, R. A., Tibble, A. L., & Symondson, W. O. (2008). Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular ecology*, 17(21), 4684-4698. Recuperado de: https://doi.org/10.1111/j.1365-294X.2008.03931.xv

Kvist, S. (2016). Does a global DNA barcoding gap exist in Annelida?. *Mitochondrial DNA Part a*, 27(3), 2241-2252. Recuperado de: https://doi.org/10.3109/19401736.2014.984166

Lemmon, A. R., Emme, S. A., & Lemmon, E. M. (2012). Anchored hybrid enrichment for massively high-throughput phylogenomics. Systematic biology, 61(5), 727-744. Recuperado de: https://doi.org/10.1093/sysbio/sys049

Le Bayon, R. C., Bullinger, G., Schomburg, A., Turberg, P., Brunner, P., Schlaepfer, R., & Guenat, C. (2021). Earthworms, plants, and soils. *Hydrogeology, chemical weathering, and soil formation*, 81-103. Recuperado de: https://doi.org/10.1002/9781119563952.ch4

Le Cadre, J., Klemp, F. L., Bálint, M., Scheu, S., & Schaefer, I. (2024). Applicability and perspectives for DNA barcoding of soil invertebrates. *PeerJ*, 12, e17709. Recuperado de: https://doi.org/10.7717/peerj.17709

Maggia, M.-E., Decaëns, T., Lapied, E., Dupont, L., Roy, V., Schimann, H., Orivel, J., et al. (2021). At each site its diversity: DNA barcoding reveals remarkable earthworm diversity in neotropical rainforests of French Guiana. Applied Soil Ecology, 164, 103932. https://doi.org/10.1016/j.apsoil.2021.103932

Malinsky, M., Trucchi, E., Lawson, D. J., & Falush, D. (2018). RADpainter and fineRADstructure: population inference from RADseq data. *Molecular biology and evolution*, 35(5), 1284-1290. Recuperado de: https://doi.org/10.1093/molbev/msy023

Marchán, D. F., Cosín, D. J. D., & Novo, M. (2018). Why are we blind to cryptic species? Lessons from the eyeless. *European Journal of Soil Biology*, 86, 49-51. Recuperado de: https://doi.org/10.1016/j.ejsobi.2018.03.004

Marchán, D. F., Decaëns, T., Domínguez, J., & Novo, M. (2022). Perspectives in earthworm molecular phylogeny: recent advances in lumbricoidea and standing questions. *Diversity*, 14(1), 30. Recuperado de: https://doi.org/10.3390/d14010030

Martinsson, S., & Erséus, C. (2021). Cryptic Clitellata: Molecular species delimitation of clitellate worms (Annelida): An overview. Diversity, 13(2), 36. Recuperado de: https://doi.org/10.3390/d13020036

McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular phylogenetics and evolution*, 66(2), 526-538. Recuperado de: https://doi.org/10.1016/j.ympev.2011.12.007

Meyer, C. P., & Paulay, G. (2005). DNA barcoding: error rates based on comprehensive sampling. *PLoS biology*, 3(12), e422. Recuperado de: https://doi.org/10.1371/journal.pbio.0030422

Misirlioğlu, M., Reynolds, J., Stojanović, M., Trakić, T., Sekulić, J., James, S., Csuzdi, C., Decaëns, T., Lapied, E., Phillips, H.R.P., Camreon, E.K., & Brown, G. (2023). Earthworms (Clitellata, Megadrili) of the world: an updated checklist of valid species and families, with notes on their distribution. *Zootaxa*. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.33.

Moreno, A.,G., Borges, S. (2004). Advances in earthworm taxonomy. Editorial Complutense.

Pérez-Losada, M., Bloch, R., Breinholt, J. W., Pfenninger, M., & Domínguez, J. (2012). Taxonomic assessment of Lumbricidae (Oligochaeta) earthworm genera using DNA barcodes. *European Journal of Soil Biology*, 48, 41-47. Recuperado de: https://doi.org/10.1016/j.ejsobi.2011.10.003

Phillips, H. R. P., C. A. Guerra, M. L. C. Bartz, M. J. I. Briones, G. Brown, T. W. Crowther, O. Ferlian, K. B. Gongalsky, J. Van Den Hoogen, J. Krebs, A. Orgiazzi, D. Routh, B. Schwarz, E. M. Bach, J.Bennett, U. Brose, T. Decaëns, B. König-Ries, M. Loreau, J. Mathieu, C. Mulder, W. H. Van Der Putten, K. S. Ramirez, M. C. Rillig, D. Russell, M. Rutgers, M. P. Thakur, F. T. De Vries, D. H. Wall, D.A. Wardle, M. Arai, F. O. Ayuke, G. H. Baker, R. Beauséjour, J. C. Bedano, K. Birkhofer, E. Blanchart, B. Blossey, T. Bolger, R. L. Bradley, M. A. Callaham, Y. Capowiez, M. E. Caulfield, A. Choi, F. V. Crotty, A. Dávalos, D. J. Diaz Cosin, A. Dominguez, A. Esteban Duhour, N. Van Eekeren, C. Emmerling, L. B. Falco, R.Fernández, S.J. Fonte, C. Fragoso, A. L. C. Franco, M. Fugère, A. T. Fusilero, S. Gholami, M. J. Gundale, M. Gutiérrez López, D. K. Hackenberger, L. M. Hernández, T. Hishi, A. R. Holdsworth, M. Holmstrup, K. N. Hopfensperger, E. Huerta Lwanga, V. Huhta, T. T. Hurisso, B. V. Iannone, M. Iordache, M. Joschko, N. Kaneko, R. Kanianska, A. M. Keith, C. A. Kelly, M. L. Kernecker, J. Klaminder, A. W. Koné, Y. Kooch76, S. T. Kukkonen, H. Lalthanzara, D. R. Lamme, I. M. Lebedev, Y. Li, J. B. Jesus Lidon, N. K. Lincoln, S. R. Loss, R. Maricha, R. Matula, J. Hendrik Moos, G. Moreno, A. Morón-Ríos, B. Muys, J. Neirynck, L. Norgrove, M. Novo, V. Nuutinen, V. Nuzzo, M. Rahman, J. Pansu, S. Paudel, G. Pérès, L. Pérez-Camacho, R. Piñeiro, J.-F. Ponge, M. Imtiaz Rashid, S. Rebollo, J. Rodeiro-Iglesias, M. Á. Rodríguez, A. M. Roth, G. X. Rousseau, A. Rozen, E. Sayad, L. Van Schaik, B. C. Scharenbroch, M. Schirrmann, O. Schmidt, B. Schröder, J. Seeber, M. P. Shashkov, J. Singh, S. M. Smith, M. Steinwandter, J. A. Talavera, D. Trigo, J. Tsukamoto, A. W. De Valença, S. J. Vanek, I. Virto, A. A. Wackett, M. W. Warren, N. H. Wehr, J. K. Whalen, M. B. Wironen, V. Wolters, I. V. Zenkova, W. Zhang, E. K. Cameron & N. Eisenhauer (2021) Global distribution of earthworm diversity. *Science* v. 366, p. 480–485.

Pinadero, S. J., Marchán, D. F., Navarro, A. M., Tilikj, N., Novo, M., Domínguez, J., & Trigo, D. (2023). Comparative phylogeography and integrative systematic revision of Iberian endemic earthworms (Crassiclitellata, Lumbricidae). Zoologica Scripta, 52(4), 345-357. Recuperado de: https://doi.org/10.1111/zsc.12586

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology,* 21(8), 1864-1877.

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2), 609-620.

Qiagen (2011). DNeasy Blood and Tissue Handbook. DNeasy Blood and Tissue Handbook.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular ecology notes*, 7(3), 355-364.

Richard, B., Decaëns, T., Rougerie, R., James, S. W., Porco, D., & Hebert, P. D. N. (2010). Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. *Molecular ecology resources*, 10(4), 606-614. Recuperado de: https://doi.org/10.1111/j.1755-0998.2009.02822.x

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual review of entomology*, 55(1), 421-438. Recuperado de: https://doi.org/10.1146/annurev-ento-112408-085432

Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America*

Taheri, S., James, S., Roy, V., Decaëns, T., Williams, B. W., Anderson, F., Rougerie, R., Chang, C.H., Brown, G., Cunha, L. and Stanton, D.W.G. & Dupont, L. (2018). Complex taxonomy of the ‘brush tail’peregrine earthworm P*ontoscolex corethrurus*. *Molecular Phylogenetics and Evolution, 124*, 60-70. Recuperado de: https://doi.org/10.1016/j.ympev.2018.02.021

Torrent, I. Use of ExoSAP-IT® PCR Product Cleanup Reagent in Next Generation Sequencing (NGS).

Vidal, A., Blouin, M., Lubbers, I., Capowiez, Y., Sanchez-Hernandez, J. C., Calogiuri, T., & van Groenigen, J. W. (2023). The role of earthworms in agronomy: Consensus, novel insights and remaining challenges. *Advances in agronomy*, 181, 1-78. Recuperado de: https://doi.org/10.1016/bs.agron.2023.05.001

5 GENERAL CONCLUSION

Earthworms, despite their widespread recognition, are complex organisms in numerous aspects. This study aimed to integrate three crucial dimensions – ecology, taxonomy, and genetics – to clarify the role of earthworms in soil ecosystems and to examine how their evolutionary history can illuminate pertinent morphological issues.

The ecological research, conducted across diverse Land Use Systems and biomes, yielded 1,769 earthworm specimens, representing seven distinct families distributed among 24 species. Of the 13 native species identified, 11 are novel to science. While exotic species predominated, the occurrence of native and previously undescribed species in conservation systems demonstrates the impact of management practices on earthworm populations. Among the 11 newly discovered species, only one was found in areas of bad management (BM), while the others were recorded in native vegetation (NV) and/or in No-Tillage system (NTS). Through the application of the indicative value (IndVal), it was possible to assign bioindication values to the species. The results corroborate previous studies, indicating that exotic species are indicative of more degraded environments, while native species are associated with more natural or preserved areas. Furthermore, the association with soil characteristics reinforces the utility of these species as bioindicators.

Using classical taxonomy and DNA barcodes, nine new native species were described, belonging to the genera *Glossoscolex* and *Fimoscolex*, which are known for their association with high-quality soils. Taxonomic description is essential for relating ecology to the biology of earthworms, as understanding their morphological characteristics enables the interpretation of their environmental indications. However, species description continues to progress slowly due to the scarcity of specialized professionals. Although more than 300 species are known in Brazil, it is estimated that this number may exceed 1,000, underscoring the necessity to train specialists to address this gap and strengthen the relationship between ecology and taxonomy.

As a tool to accelerate identification processes, genetic sequencing was employed. The focus was on the genetic relationships of the genera *Glossoscolex* and *Fimoscolex*, utilizing the cytochrome oxidase subunit I (COI) gene, which has demonstrated efficacy in revealing genetic and evolutionary relationships. The complexity of these genera was observed, characterized by evolutionary convergences and distinct lineages. Phylogenetic analysis, together with morphological characteristics such as the number of male pores and copulatory bulbs, revealed that species with two pores and copulatory bulbs exhibit greater genetic stability, while species without bulbs, recently observed, have an uncertain evolutionary history. The absence of this structure may reflect adaptations or evolutionary barriers. Furthermore, barcode gap analyses highlighted the presence of cryptic species and substantial genetic variation within and between genera, indicating that species of the same genus may be genetically disparate.

The utilization of integrative methodologies, combining morphology, ecology, and genetics, proved highly effective. Morphological characteristics facilitate the understanding of evolutionary relationships, while molecular analyses elucidate species-specific aspects. This approach provides more robust inferences regarding the ecology and environments in which these species are found.

Subsequent investigations should explore novel combinations of methodologies, such as the utilization of multiple genetic markers, to enhance understanding of the significance of earthworms as crucial organisms for soil quality. Through the integration of ecology, taxonomy, and genetics, this research emphasizes not only biodiversity but also the potential of earthworms as bioindicators and conservation agents, thereby contributing to the sustainability of Brazilian ecosystems.

6 REFERENCES

Akhila, A., & Entoori, K. (2022). Role of earthworms in soil fertility and its impact on agriculture: A review. Int. J. Fauna Biol. Stud, 9(3), 55-63. Recuperado de: https://doi.org/10.22271/23940522.2022.v9.i3a.907

Anderson, J. M., & Ingram, J. S. (1994). Tropical soil biology and fertility: a handbook of methods. *Soil Science*, 157(4), 265.

Anderson, F. E., Williams, B. W., Horn, K. M., Erséus, C., Halanych, K. M., Santos, S. R., & James, S. W. (2017). Phylogenomic analyses of Crassiclitellata support major Northern and Southern Hemisphere clades and a Pangaean origin for earthworms. *BMC Evolutionary Biology*, 17, 1-18. Recuperado de: https://doi.org/10.1186/s12862-017-0973-4

Barraux, A., Noël, S., Roy, V., & Dupont, L. (2024). Challenges of molecular barcode-based identification of earthworm specimens for biodiversity assessment. *Frontiers in Ecology and Evolution*, 12, 1358984. Recuperado de: | https://doi.org/10.3389/fevo.2024.1358984

Bartz, M. L., Brown, G. G., Rosa, M. G., Locatelli, M., James, S. W., & Baretta, D. M. (2011). Urobenus sp: das matas para as áreas sob plantio direto. *Revista Plantio Direto*, 124, 6-7.

Bartz, M. L. C., James, S. W., Pasini, A., & Brown, G. G. (2012). New earthworm species of Glossoscolex Leuckart, 1835 and Fimoscolex Michaelsen, 1900 (Clitellata: Glossoscolecidae) from Northern Paraná, Brazil. *Zootaxa*, 3458(1), 59-85. Recuperado de: htttp://doi.org/10.11646/zootaxa.3458.1.3

Bartz, M.L.C., Pasini, A, Brown, G.G. (2013a) Earthworms as soil quality indicators in Brazilian No-Tillage systems, *Applied Soil Ecology* 69, 39–48. Recuperado de: https://doi.org/ 10.1016/j.apsoil.2013.01.011.

Bartz, M., James, S., Steffen, G., Antoniolli, Z., & Brown, G. (2013b). New species of Glossoscolex (Clitellata: Glossoscolecidae) from Rio Grande do Sul, Brazil. In: INTERNATIONAL OLIGOCHAETE TAXONOMY MEETING, 6., 2013, Palmeira de Faro, Portugal. Book of abstracts.[Braga]: University of Minho, CBMA, 2013..

Bartz, M. L. C., Brown, G. G., da Rosa, M. G., Klauberg Filho, O., James, S. W., Decaëns, T., & Baretta, D. (2014a). Earthworm richness in land-use systems in Santa Catarina, Brazil. *Applied Soil Ecology*, 83, 59-70. Recuperado de: https://doi.org/10.1016/j.apsoil.2014.03.003

Bartz, M. L., Pasini, A., & Brown, G. G. (2014b). Earthworm richness, abundance and biomass in different land use systems in northern Paraná, Brazil (Oligochaeta). Advances in Earthworm Taxonomy VI (Annelida: Oligochaeta). Heidelberg: Kasparek Verlag, 59-73.

Bartz, M. L., James, S. W., Steffen, G. P., Antoniolli, Z., Steffen, R. B., & Brown, G. G. (2018). New species-group taxa of Glossoscolex (Clitellata: Glossoscolecidae) from Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 548-561. Recuperado de https://doi.org/ 10.11646/zootaxa.4496.1.42

Bartz, M. L., Barreto, J., Santos, A., Dudas, R. T., Ferreira, T., Maia, L. D. S., Demetrio, W.C., Smokanit, M., Schuster, P.A., Hernani, L.C. & Brown, G. G. (2023). Earthworm richness in no-tillage farming systems and riparian forests in Southern and Southeastern Brazil. *Zootaxa*, 5255(1), 362-376. Recuperado de: htttps://doi.org/10.11646/zootaxa.4496.1.42

Bartz, M. L. C., Dudas, R. T., Demetrio, W. C., & Brown, G. G. (2024). Earthworms as soil health indicators in no-tillage agroecosystems. *European Journal of Soil Biology*, 121, 103605. Recuperado de: https://doi.org/10.1016/j.ejsobi.2024.103605

Blakemore, R. J., Ito, M. T., & Kaneko, N. (2006). Alien earthworms in the Asia/Pacific region with a checklist of species and the first records of Eukerria saltensis (Oligochaeta: Ocnerodrilidae) and Eiseniella tetraedra (Lumbricidae) from Japan, and Pontoscolex corethrurus (Glossoscolecidae) from Okinawa. *Assessment and control of biological invasion risks*, 173-181.

Bonilla-Bedoya, S., Valencia, K., Herrera, M. Á., López-Ulloa, M., Donoso, D. A., & Pezzopane, J. E. M. (2023). Mapping 50 years of contribution to the development of soil quality biological indicators. *Ecological Indicators*, 148, 110091. Recuperado de: https://doi.org/10.1016/j.ecolind.2023.110091

Breinholt, J. W., & Kawahara, A. Y. (2013). Phylotranscriptomics: saturated third codon positions radically influence the estimation of trees based on next-gen data. *Genome Biology and Evolution*, *5*(11), 2082-2092. Recuperado de: https://doi.org/10.1093/gbe/evt157

Briones, M. J. I., & Schmidt, O. (2017). Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. *Global change biology*, 23(10), 4396-4419. Recuperado de: https://doi.org/10.1111/gcb.13744

Britz, R., Hundsdörfer, A., & Fritz, U. (2020). Funding, training, permits—the three big challenges of taxonomy. Megataxa, 1(1), 49-52. Recuperado de: htttp://doi.org/10.11646/megataxa.1.1.10

Brown, G. G., Benito, N. P., Pasini, A., Sautter, K. D., de F Guimarães, M., & Torres, E. (2003). No-tillage greatly increases earthworm populations in Paraná state, Brazil: The 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. *Pedobiologia*, 47(5-6), 764-771. Recuperado de: https://doi.org/10.1078/0031-4056-00256

Brown, G. G., & James, S. W. (2007). Ecologia, biodiversidade e biogeografia das minhocas no Brasil. Minhocas na América Latina: biodiversidade e ecologia. Londrina: Embrapa Soja, 297-381.

Brown, G. G., & Domínguez, J. (2010). Uso das minhocas como bioindicadoras ambientais: princípios e práticas - o 3° Encontro Latino Americano de Ecologia e Taxonomia de Oligoquetas (ELAETAO3). *Acta zoológica mexicana*, 26(spe2), 1-18. Recuperado de: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0065-17372010000500001

Brown, G. G., Callaham Jr, M. A., Niva, C. C., Feijoo, A., Sautter, K. D., James, S. W., Fragoso, C., Pasini, A. & Schmelz, R. M. (2013). Terrestrial oligochaete research in Latin America: the importance of the Latin American meetings on oligochaete ecology and taxonomy. *Applied Soil Ecology*, 69, 2-12. Recuperado de: https://doi.org/10.1016/j.apsoil.2012.12.006

Brown, G. G., Silva, E. D., Thomazini, M. J., Niva, C. C., Decaëns, T., Cunha, L. F., Nadolny, H.S., Demetrio, W.C., Santos, A., Ferreira, T., Maia, L.S., Conrado, A.C., Segalla, R.F., Feerreira, A.C., Pasini, A., Bartz, M.L.C., Sautter, K.D., James, S.W., Baretta, D., Antoniolli, Z.I., Briones, M.J.I., Sousa, J.P., Römbke, J. & Lavelle, P. (2018). The role of soil fauna in soil health and delivery of ecosystem services (pp. 197-241). Recuperado de: https://doi.org/10.19103/AS.2017.0033.11

Brown, G. G., James, S. W., Csuzdi, C., Lapied, E., Decäens, T., Reynolds, J. W., Misirlioglu, M., Mirjana, S., Tanja, T., Jovana, S., Phillips, H.R.P. & Cameron, E. (2023). A checklist of megadrile earthworm (Annelida: Clitellata) species and subspecies of the world. Available from: *Zenodo*. recuperado de: https://doi.org/10.5281/zenodo.7301848

Bruz, L. D. S. M., Santos, A., Demetrio, W. C., Feliciano, L. P., Fernandes, C. H., Bartz, M. L. C.,Bernardi, A.C. de C., Pezzopane, J.R.M. & Brown, G. G. (2023). Earthworms in various agricultural and forest ecosystems in São Carlos-SP, Brazil. *Zootaxa*, 5255(1), 324-335. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.26

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., De Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groeningen, J.W. & Brussaard, L. (2018). Soil quality–A critical review. *Soil biology and biochemistry*, 120, 105-125. Recuperado de: https://doi.org/10.1016/j.soilbio.2018.01.030

Callaham, M. A., Blair, J. M., & Hendrix, P. F. (2001). Different behavioral patterns of the earthworms Octolasion tyrtaeum and Diplocardia spp. in tallgrass prairie soils: potential influences on plant growth. *Biology and Fertility of Soils*, 34, 49-56. Recuperado de: https://doi.org/10.1007/s003740100370

Capowiez, Y., Marchán, D., Decaëns, T., Hedde, M., & Bottinelli, N. (2024). Let earthworms be functional-Definition of new functional groups based on their bioturbation behavior. *Soil Biology and Biochemistry*, *188*, 109209.

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., Santos, C. A. D., Alves, P. R. L., de Paula, Al.M.,, Nakatani, A.S., Pereiria, J de M. & Nogueira, M. A. (2013). Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, 70, 274-289. Recuperado de: https://doi.org/10.1590/S0103-90162013000400009

Chang, C. H., Rougerie, R., & Chen, J. H. (2018). DNA barcoding reveals cryptic diversity and distinct population genetic structure in the earthworm Amynthas corticis. *Molecular Ecology Resources*, 18(5), 952-965.

Chang, C. H., Bartz, M. L., Brown, G., Callaham, M. A., Cameron, E. K., Dávalos, A., Dobson, A., Görres, J.H, Herrick, B.M., Ikeda, H., James, S.W., Johnston, M.R., McCay, T.S., McHugh, D., Minamiya, Y., Nouri-Aii, M., Novo, M., Ortiz-Pachar, J., Pinder, R.A., Ransom, T., Richardson, J.B., Snyder, B.A. & Szlavecz, K. (2021). The second wave of earthworm invasions in North America: biology, environmental impacts, management and control of invasive jumping worms. *Biological Invasions*, 23, 3291-3322. Recuperado de: https://doi.org/10.1007/s10530-021-02598-1

Conrado, A. C., Arruda, H., Stanton, D. W., James, S. W., Kille, P., Brown, G.,Silva, E., Dupont, L., Taheri, S., Morgan, A.J., Simões, N., Rodrigues, A., Montiel, R & Cunha, L. (2017). The complete mitochondrial DNA sequence of the pantropical earthworm Pontoscolex corethrurus (Rhinodrilidae, Clitellata): Mitogenome characterization and phylogenetic positioning. *ZooKeys*, (688), 1. Recuperado de: https://doi.org/10.3897/zookeys.688.13721

Costa, A. Da; Albuquerque, J.A.; Costa, A. Da; Pértile, P.; Silva, F.R. da. Water retention and availability in soils of the State of Santa Catarina-Brazil: effect of textural classes, soil classes and lithology. *Revista Brasileira de Ciência do Solo*, v.37, p.1535-1548, 2013. Recuperado de: https://doi.org/10.1590/ s0100-06832013000600010.

Csuzdi, C., Chang, C. H., Pavlícek, T., Szederjesi, T., Esopi, D., & Szlávecz, K. (2017). Molecular phylogeny and systematics of native North American lumbricid earthworms (Clitellata: Megadrili). *PloS one*, 12(8), e0181504. Recuperado de: https://doi.org/10.1371/journal.pone.0181504

da Silva, E., de Lima, O. G., de Andrade, D. P., & Brown, G. G. (2019). Earthworm populations in forestry plantations (Araucaria angustifolia, Pinus elliottii) and Native Atlantic Forest in Southern Brazil compared using two sampling methods. *Pedobiologia*, 72, 1-7. Recuperado de: https://doi.org/10.1016/j.pedobi.2018.10.002

de Faria Godoi, R., Rodrigues, D. B., Borrelli, P., & Oliveira, P. T. S. (2021). High-resolution soil erodibility map of Brazil. *Science of The Total Environment*, 781, 146673. Recuperado de: https://doi.org/10.1016/j.scitotenv.2021.146673

Decaëns, T., Porco, D., Rougerie, R., Brown, G. G., & James, S. W. (2013). Potential of DNA barcoding for earthworm research in taxonomy and ecology. *Applied Soil Ecology*, 65, 35-42. Recuperado de: https://doi.org/10.1016/j.apsoil.2013.01.001

Decaëns, T., Porco, D., James, S. W., Brown, G. G., Chassany, V., Dubs, F., Dupont, L., Lapied, E., Rougeri, R., Rossi, J.P., & Roy, V. (2016). DNA barcoding reveals diversity patterns of earthworm communities in remote tropical forests of French Guiana. *Soil Biology and Biochemistry*, 92, 171-183. Recuperado de: https://doi.org/10.1016/j.soilbio.2015.10.009

Dekemati, I., Simon, B., Bogunovic, I., Kisic, I., Kassai, K., Kende, Z., & Birkás, M. (2020). Long term effects of ploughing and conservation tillage methods on earthworm abundance and crumb ratio. *Agronomy*, 10(10), 1552. Recuperado de: https://doi.org/10.3390/agronomy10101552

Demetrio, W. C., Santos, A., Ferreira, T., Nadolny, H., Cardoso, G. B., Torres, J. L., Dudas, R.T., de Oliveira, V., Barreto, J.O., James, S.W., da Silva, E., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in various land use systems in the Campos Gerais region of Lapa, Paraná, Brazil. *Zootaxa*, 4496(1), 503-516. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W. C., Ribeiro, R. H., Nadolny, H., Bartz, M. L., & Brown, G. G. (2020). Earthworms in Brazilian no-tillage agriculture: Current status and future challenges. *European Journal of Soil Science*, 71(6), 988-1005. Recuperado de: https://doi.org/10.1111/ejss.12918

Demetrio, W. C., PM, D. F., Dudas, R., Zagatto, M. G., Feijoo, A., & Brown, G. G. (2023). Earthworm species in native and planted forests in Brazil. *Zootaxa*, 5255(1), 304-323. Recuperado de https://doi.org/10.11646/zootaxa.4496.1.39

Demetrio, W., Brown, G., Pupin, B., Novo, R., Dudas, R., Baretta, D., Römbke, J. Bartz, M.L.C. & Borma, L. (2023). Are exotic earthworms threatening soil biodiversity in the Brazilian Atlantic Forest?. *Applied Soil Ecology*, 182, 104693. Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104693

Dewi, W. S., & Senge, M. (2015). Earthworm diversity and ecosystem services under threat. *Reviews in Agricultural Scienc*e, 3, 25-35. Recuperado de: https://doi.org/10.7831/ras.3.0_25

Dudas, R. T., Demetrio, W. C., Nadolny, H. S., Brown, G. G., & Bartz, M. L. C. (2023a). Earthworms in the state of Paraná, Brazil: State of the art. *Revista Brasileira de Ciência do Solo*, 47, e0220159. Recuperado de: https://doi.org/10.36783/18069657rbcs20220159

Dudas, R. T., Tavares, A. A., Ercole, C., BL, D. L., Carlos, E. D. S., Torres, J. L., Smokanit, M., Guaranha, R.M., Brown, G.G. & Bartz, M. L. (2023b). Urban green areas as earthworm species maintainers in Curitiba, Paraná, Brazil. *Zootaxa*, 5255(1), 336-346. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.27

Dudas, R.T., James, S.W., Bartz M.L.C. & Brown, G.G. (2025) New earthworm species of Glossoscolex Leuckart, 1835 and Fimoscolex Michaelsen, 1900 (Clitellata: Glossoscolecidae) from southeastern Brazil. *Zootaxa*, in press.

Dupont, L., Porco, D., Symondson, W. O. C., & Roy, V. (2016). Hybridization relics complicate barcode-based identification of species in earthworms. *Molecular Ecology Resources*, 16(4), 883-894. Recuperado de: 0.1111/1755-0998.12517

Dupont, L., Brunet, C. M., Fourcade, Y., James, S., Gabriac, Q., & Coulis, M. (2023) Recording earthworm diversity on the tropical island of Martinique using DNA barcoding unveiled endemic species in bromeliad plants. *Soil Biology and Biochemistry*, 182, 1-10. Recuperado de: https://doi.org/10.1016/j.soilbio.2023.109038

Edwards, C. A., & Arancon, N. Q. (2022). The role of earthworms in organic matter and nutrient cycles. In *Biology and ecology of earthworms* (pp. 233-274). New York, NY: Springer US.

Feijoo, A.M. & Brown, G. G. (2018). New species of Glossoscolex and Fimoscolex earthworms (Oligochaeta: Glossoscolecidae) from Embrapa Forestry, Paraná, Brazil. *Zootaxa*, 4496(1), 492-502. Recuperado de: https://doi.org/ 10.11646/zootaxa.4496.1.38

Feijoo, A.M., & Brown, G. G. (2023). Three new Glossoscolex (Annelida: Crassiclitellata: Glossoscolecidae) in the truncatus group from the Brazilian Atlantic Forest. *Zootaxa*, 5255(1), 220-234. Recuperado de: https://doi.org/ 10.11646/zootaxa.5255.1.21

Ferreira, T., Santos, A., Demetrio, W. C., Cardoso, G. B., Moraes, R., Assis, O., Niva, C.C., Smokanit, M., Knopik, J., Sautter, K.D., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in public parks in Curitiba, Paraná, Brazil. *Zootaxa*, 4496(1), 535-547. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.41.

Ferreira, T., James, S. W., Bartz, M. L. C., ACR, D. L., Dudas, R., & Brown, G. G. (2023a). Distribution and diversity of earthworms in different land use systems in Rio Grande do Sul, Brazil. *Zootaxa*, 5255(1), 399-416. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.32.

Ferreira, T., Niva, C. C., Dudas, R., Roani, R., Durães, N., Marchão, R. L., James, S.W., Bartz, M.L.C., & Brown, G. G. (2023b). Earthworm species in different land use systems in the state of Goiás and the Federal District of Brazil. *Zootaxa*, 5255(1), 283-303. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.24

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology Biotechnology*, 3, 294-299.

Fonte, S. J., Barrios, E., & Six, J. (2010). Earthworms, soil fertility and aggregate-associated soil organic matter dynamics in the Quesungual agroforestry system. *Geoderma*, 155(3-4), 320-328. Recuperado de: https://doi.org/10.1016/j.geoderma.2009.12.016

Fonte, S. J., Hsieh, M., & Mueller, N. D. (2023). Earthworms contribute significantly to global food production. *Nature Communications*, 14(1), 5713. Recuperado de: https://doi.org/10.1016/j.geoderma.2009.12.016

Food Agriculture Organizatio - FAO (2015) - Montanarella, L., Badraoui, M., Chude, V., Costa, I. D. S. B., Mamo, T., Yemefack, M., & MCKENZIE, N. (2015). Status of the world's soil resources: main report.

Fragoso, C., Brown, G. G., Patrón, J. C., Blanchart, E., Lavelle, P., Pashanasi, B., Senapati, B. & Kumar, T. (1997). Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Applied soil ecology*, 6(1), 17-35. Recuperado de: https://doi.org/10.1016/S0929-1393(96)00154-0

Gates, G. E. Introduction to the Systematics and Biology of Megadrile Oligochaetes with Special Reference to Southeast Asia: *Transactions, American Philosophical Society* (vol. 62, part 7).

Gong, X., Wang, S., Wang, Z., Jiang, Y., Hu, Z., Zheng, Y., Chen, X., Li, H., Liu, M. & Scheu, S. (2019). Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. *Geoderma*, 347, 59-69. Recuperado de: https://doi.org/10.1016/j.geoderma.2019.03.043

Goulpeau, A., Penel, B., Maggia, M. E., Marchán, D. F., Steinke, D., Hedde, M., & Decaëns, T. (2022). OTU delimitation with earthworm DNA barcodes: A comparison of methods. *Diversity*, 14(10), 866. Recuperado de: https://doi.org/10.3390/d14100866

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003a). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: *Biological Sciences*, 270(1512), 313-321. Recuperado de: https://doi.org/10.1098/rspb.2002.2218

Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B: *Biological Sciences*, 270(suppl_1), S96-S99.

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of *Sciences,* 101(41), 14812-14817. Recuperado de: https://doi.org/10.1073/pnas.0406166101

Huang, J., Xu, Q., Sun, Z. J., Tang, G. L., & Su, Z. Y. (2007). Identifying earthworms through DNA barcodes. *Pedobiologia*, 51(4), 301-309. Recuperado de: https://doi.org/10.1016/j.pedobi.2007.05.003

Hughes, M. S., Bull, C. M., & Doube, B. M. (1994). The effects of sheep manure on the survival and growth of the earthworm Microscolex dubius (Annelida: Acanthodrilidae). *Applied Soil Ecology*, 1(4), 291-298. Recuperado de: https://doi.org/10.1016/0929-1393(94)90006-X

ICMBIO - Centro Nacional de Pesquisa e Conservação da Biodiversidade do Cerrado e Caatinga - Biodiversidade. (s.d.). ICMBio — Instituto Chico Mendes de Conservação da Biodiversidade.

Instituto Agrônomico (IAC) (2001) Método de análise química para avaliação de solos tropicais. Campinas. 2001.Availabe from: https://lab.iac.sp.gov.br/
https://www.icmbio.gov.br/cbc/conservacao-da-biodiversidade/biodiversidade.html

Instituto Brasileiro de Florestas - IBF. Bioma Mata Atlântica:
https://www.ibflorestas.org.br/bioma-mata-atlantica

Instituto Brasileiro de Geografia e Estatística (IBGE). (2004): “Mapa de biomas e de vegetação”. Available from:
https://ww2.ibge.gov.br/home/presidencia/noticias/21052004biomashtml.shtm.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. Molecular *Ecology Notes*, 7(4), 544-548. Recuperado de: https://doi.org/10.1111/j.1471-8286.2007.01748.x

James, S. W., & Brown, G. G. (2006). Diversity and biogeography of earthworms in tropical rainforests. In P. Lavelle, A. Spain, & E. Blanchart (Eds.), *Soil Ecology in Tropical Ecosystems* (pp. 56-77). Cambridge University Press.

James, S. W., & Brown, G. G. (2006). Earthworm ecology and diversity in Brazil. Soil biodiversity in Amazonian and other Brazilian ecosystems, 56-116. Recuperado de: https://doi.org/10.1079/9781845930325.0056

James SW, Porco D, Decaens T, Richard B, Rougerie R, Erseus C (2010) DNA barcoding reveals cryptic diversity in Lumbricus terrestris L., 1758 (Clitellata): Resurrection of L. herculeus (Savigny, 1826). *PLoS ONE* 5:15629

James, S. W., & Davidson, S. K. (2012). Molecular phylogeny of earthworms (Annelida: Crassiclitellata) based on 28S, 18S and 16S gene sequences. Invertebrate Systematics, 26(2), 213-229. Recuperado de: https://doi.org/10.1071/IS11012

James, S. W., Bartz, M. L., & Brown, G. G. (2023). New Ocnerodrilidae genera, species and records from Brazil (Annelida: Crassiclitellata). *Zootaxa.* Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.22

Jouquet, P., Chaudhary, E., & Kumar, A. (2018). Sustainable use of termite activity in agro-ecosystems with reference to earthworms. A review. *Agronomy for Sustainable Development*, 38, 1-11. Recuperado de: https://doi.org/10.1007/s13593-017-0483-1.

Kanianska, R., Jaďuďová, J., Makovníková, J., & Kizeková, M. (2016). Assessment of relationships between earthworms and soil abiotic and biotic factors as a tool in sustainable agricultural. *Sustainability*, 8(9), 906. Recuperado de: https://doi.org/10.3390/su8090906

Kassam, A., Friedrich, T., Shaxson, F., & Pretty, J. (2009). The spread of conservation agriculture: justification, sustainability and uptake. *International journal of agricultural sustainability*, 7(4), 292-320. Recuperado de: https://doi.org/10.3763/ijas.2009.0477

King, R. A., Tibble, A. L., & Symondson, W. O. (2008). Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular ecology*, 17(21), 4684-4698. Recuperado de: https://doi.org/10.1111/j.1365-294X.2008.03931.xv

Kvist, S. (2016). Does a global DNA barcoding gap exist in Annelida?. *Mitochondrial DNA* Part a, 27(3), 2241-2252. Recuperado de: https://doi.org/10.3109/19401736.2014.984166

Lal, R. (2019). Rights-of-Soil. *Journal of Soil and Water Conservation*. 74. 81A-86A. Recuperado de: https://doi.org/10.2489/jswc.74.4.81A

Latif, R., Malek, M., Aminjan, A., Pasantes, J., Briones, M., & Csuzdi, C. (2020). Integrative taxonomy of some Iranian peregrine earthworm species using morphology and barcoding (Annelida: Megadrili).. Zootaxa, 4877 1, *zootaxa*.4877.1.7 Recuperado de: https://doi.org/10.11646/zootaxa.4877.1.7

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P.O.W.H. & Dhillion, S. (1997). Soil function in a changing world: the role of invertebrate ecosystem engineers.

Lemmon, A. R., Emme, S. A., & Lemmon, E. M. (2012). Anchored hybrid enrichment for massively high-throughput phylogenomics. Systematic biology, 61(5), 727-744. Recuperado de: https://doi.org/10.1093/sysbio/sys049

Le Bayon, R. C., Bullinger, G., Schomburg, A., Turberg, P., Brunner, P., Schlaepfer, R., & Guenat, C. (2021). Earthworms, plants, and soils. Hydrogeology, chemical weathering, and soil formation, 81-103. Recuperado de: https://doi.org/10.1002/9781119563952.ch4

Le Cadre, J., Klemp, F. L., Bálint, M., Scheu, S., & Schaefer, I. (2024). Applicability and perspectives for DNA barcoding of soil invertebrates. *PeerJ*, 12, e17709. Recuperado de: https://doi.org/10.7717/peerj.17709

Lees, K. J., McKenzie, A, Newell Price, J., Critchley, C. N., Rhymer, C. M., Chambers, B. J., & Whittingham, M. J. (2016). The effects of soil compaction mitigation on below-ground fauna: How earthworms respond to mechanical loosening and power harrow cultivation. *Agriculture, Ecosystems & Environment*, 232, 273–282. Recuperado de: doi:10.1016/j.agee.2016.07.026

Luederwaldt, H. (1927). A coleção de minhocas (Oligochaeta) no Museu Paulista. Revista do Museu Paulista, 15, 545-556.

Marchán, D. F., Cosín, D. J. D., & Novo, M. (2018). Why are we blind to cryptic species? Lessons from the eyeless. *European Journal of Soil Biology*, 86, 49-51. Recuperado de: https://doi.org/10.1016/j.ejsobi.2018.03.004

Marchán, D. F., Fernández, R., Domínguez, J., Díaz Cosín, D. J., & Novo, M. (2020). Genome-informed integrative taxonomic description of three cryptic species in the earthworm genus Carpetania (Oligochaeta, Hormogastridae). *Systematics and biodiversity*, 18(3), 203-215. Recuperado de: https://doi.org/10.1080/14772000.2020.1730474

Marchán, D. F., Decaëns, T., Domínguez, J., & Novo, M. (2022). Perspectives in earthworm molecular phylogeny: recent advances in lumbricoidea and standing questions. *Diversity,* 14(1), 30. Recuperado de: https://doi.org/10.3390/d14010030

Martinsson, S., & Erséus, C. (2021). Cryptic Clitellata: Molecular species delimitation of clitellate worms (Annelida): An overview. *Diversity*, 13(2), 36. Recuperado de: https://doi.org/10.3390/d13020036

Meyer, C. P., & Paulay, G. (2005). DNA barcoding: error rates based on comprehensive sampling. *PLoS biology,* 3(12), e422. Recuperado de: https://doi.org/10.1371/journal.pbio.0030422

Misirlioğlu, M., Reynolds, J., Stojanović, M., Trakić, T., Sekulić, J., James, S., Csuzdi, C., Decaëns, T., Lapied, E., Phillips, H.R.P., Camreon, E.K., & Brown, G. (2023). Earthworms (Clitellata, Megadrili) of the world: an updated checklist of valid species and families, with notes on their distribution. *Zootaxa*. Recuperado de: https://doi.org/10.11646/zootaxa.5255.1.33.

Moreno, A.G., Borges, S. (2004). Advances in earthworm taxonomy. Editorial Complutense.

Nadolny, H. S. (2017). Estado da arte das minhocas como bioindicadoras da qualidade dos solos brasileiros (Doctoral dissertation, PhD Thesis, Federal University of Paraná, Soils Department, Curitiba).

Paoletti, M. G. (1999). The role of earthworms for assessment of sustainability and as bioindicators. *Agriculture, Ecosystems & Environment*, 74(1-3), 137-155. Recuperado de: https://doi.org/10.1016/S0167-8809(99)00034-1

Pelosi, C., Gavinelli, F., Petit-Dit-Grézériat, L., Serbource, C., Schoffer, J. T., Ginocchio, R., & van Gestel, C. A. M. (2024). Copper toxicity to earthworms: A comprehensive review and meta-analysis. *Chemosphere*, 362, 142765. Recuperado de: https://doi.org/10.1016/j.chemosphere.2024.142765

Pérez-Losada, M., Bloch, R., Breinholt, J. W., Pfenninger, M., & Domínguez, J. (2012). Taxonomic assessment of Lumbricidae (Oligochaeta) earthworm genera using DNA barcodes. *European Journal of Soil Biology*, 48, 41-47. Recuperado de: https://doi.org/10.1016/j.ejsobi.2011.10.003

Phillips, H.R.P.; Guerra, C.A.; Bartz, M.L.C.; Briones, M.J.I.; Brown, G.; Crowther, T.W.; Ferlian, O.; Gongalsky, K.B.; Hoogen, J. Van Den; Krebs, J.; Orgiazzi, A.; Routh, D.; Schwarz, B.; Bach, E.M.; Bennett, J.; Brose, U.; Decaëns, T.; König-Ries, B.; Loreau, M.; Mathieu, J.; Mulder, C.; Van Der Putten, W.H.; Ramirez, K.S.; Rillig, M.C.; Russell, D.; Rutgers, M.; Thakur, M.P.; De Vries, F.T.; Wall, D.H.; Wardle, D.A.; Arai, M.; Ayuke, F.O.; Baker, G.H.; Beauséjour, R.; Bedano, J.C.; Birkhofer, K.; Blanchart, E.; Blossey, B.; Bolger, T.; Bradley, R.L.; Callaham, M.A.; Capowiez, Y.; Caulfield, M.E.; Choi, A.; Crotty, F.V.; Dávalos, A.; Cosin, D.J.D.; Dominguez, A.; Duhour, A.E.; Eekeren, N. Van; Christoph Emmerling, C.; Falco, L.B.; Fernández, R.; Fonte, S.J.;

Fragoso, C.; Gutiérrez López, M.G.; Hackenberger, D.K.; Hernández, L.M.; Hishi, T.; Holdsworth, A.R.; Holmstrup, M.; Hopfensperger, K.N.; Huerta Lwanga, E.; Huhta, V.; Hurisso, T.T.; Iannone Iii, B.V.; Iordache, M.; Joschko, M.; Kaneko, N.; Kanianska, R.; Aidan M. Keith, A.M.; Kelly, C.A.; Kernecker, M.L.; Klaminder, J.; Koné, A.W.; Kooch, Y.; Kukkonen, S.T.; Lalthanzara, H.; Lammel, D.R.; Lebedev, I.M.; Li, Y.; Jesus Lidon, J.B.; Lincoln, N.K.; Loss, S.R.; Marichal, R.; Matula, R.; Moos, J.H.; Moreno, G.; Morón-Ríos, A.Muys, B.; Neirynck, J.; Norgrove, L.; Novo, M.; Nuutinen, V.; Victoria Nuzzo, V.; Rahman P, M.; Pansu, J.; Shishir Paudel, S.; Pérès, G.; Pérez-Camacho, L.; Piñeiro, R.; Ponge, J.-F.; Rashid, M.I.; Rebollo, S.; Javier Rodeiro-Iglesias, J.; Rodríguez, M.Á.; Roth, A.M.; Rousseau, G.X.; Rozen, A.; Sayad, E.; Van Schaik, L.; Scharenbroch, B.C.; Schirrmann, M.; Schmidt, O.; Schröder, B.; Seeber, J.; Shashkov, M.P.; Singh, J.; Smith, S.M.; Steinwandter, M.; Talavera, J.A.; Trigo, D.; Tsukamoto, J.; Valença, A.W. De; Vanek, S.J.; Virto, I.; Wackett, A.A.; Warren, M.W.; Wehr, N.H.; Whalen, J.K.; Wironen, M.B.; Wolters, V.; Zenkova, I.V.; Zhang, W.; Cameron, E.K.; Eisenhauer, N. Global distribution of earthworm diversity. *Science*, v.366, p.480-485, 2019. Recuperado de: https://doi.org/ 10.1126/science.aax4851

Pinadero, S. J., Marchán, D. F., Navarro, A. M., Tilikj, N., Novo, M., Domínguez, J., ... & Trigo, D. (2023). Comparative phylogeography and integrative systematic revision of Iberian endemic earthworms (Crassiclitellata, Lumbricidae). *Zoologica Scripta*, 52(4), 345-357. Recuperado de: https://doi.org/10.1111/zsc.12586

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology,* 21(8), 1864-1877.

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2), 609-620.

Qiagen (2011). DNeasy Blood and Tissue Handbook. DNeasy Blood and Tissue Handbook.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular ecology notes*, 7(3), 355-364.

Reynolds, J. W., & MISIRLIOĞLU, Ý. M. (2018). Preliminary key to Tunisian megadriles (Annelida, Clitellata, Oligochaeta), based on external characters, insofar as possible. *Megadrilogica*, 23(11).

Richard, B., Decaëns, T., Rougerie, R., James, S. W., Porco, D., & Hebert, P. D. N. (2010). Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. *Molecular ecology resources*, 10(4), 606-614. Recuperado de: https://doi.org/10.1111/j.1755-0998.2009.02822.x

Righi, G. (1972). Additions to the genus Glossoscolex (oligochaeta, Glossoscolecidae). *Studies on Neotropical Fauna and Environment*, 7(1), 37-47. Recuperado de: https://doi.org/10.1080/01650527209360433

Righi, G., & Lobo, D. J. A. (1979). Nova contribuição ao gênero Glossoscolex (Oligochaeta) com sinopse do grupo giganteus. Revista Brasileira de Biologia, 39(4), 947-959.

Rodríguez, M. P., Domínguez, A., Moreira Ferroni, M., Wall, L. G., & Bedano, J. C. (2020). The diversification and intensification of crop rotations under no-till promote earthworm abundance and biomass. *Agronomy*, 10(7), 919. Recuperado de: https://doi.org/10.3390/agronomy10070919

Rosa, D. 1900. Geoscolex bergi n. sp. Comunicaciones del Museo Argentino de Ciencias Naturales, 1: 209–211.

Rota, E., & de Jong, Y. (2015). Fauna Europaea: Annelida-Terrestrial Oligochaeta (Enchytraeidae and Megadrili), Aphanoneura and Polychaeta. *Biodiversity Data Journal,* (3). Recuperado de: https://doi.org/10.3897/BDJ.3.e5737

Rutgers, M.; Orgiazzi, A.; Gardi, C.; Römbke, J.; Jänsch, S.; Keith, A.M.; Neilson, R.; Boag, B.; Schmidt, O.; Murchie, A.K.; Blackshaw, R.P.; Pérès, G.; Cluzeau, D.; Guernion, M.; Briones, M.J.I.; Rodeiro, J.; Piñeiro, R.; Cosín, D.J.D.; Sousa, J.P.; Suhadolc, M.; Kos, I.; Krogh, P.-H.; Faber, J.H.; Mulder, C.; Bogte, J.J.; Wijnen, H.J. Van; Schouten, A.J.; Zwart, D. De. Mapping Earthworm Communities in Europe. *Applied Soil Ecology*, V.97, P.9 Recuperado de: https://doi.org/10.1016/j.apsoil.2015.08.015

Santos, A., Gorte, T., Demetrio, W. C., Ferreira, T., Nadolny, H., Cardoso, G. B., Tonetti, C., Ralisch, R., Nunes, A.P., Coqueiro, A.C.P., Leandro, H.C.L., Wandscheer, C.A.R., Bortoluzzi, J., Brown, G.G. & Bartz, M. L. (2018). Earthworm species in no-tillage agroecosystems and native Atlantic forests in Western Paraná, Brazil. *Zootaxa*, 4496(1), 517-534. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.40

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual review of entomology*, 55(1), 421-438. Recuperado de: https://doi.org/10.1146/annurev-ento-112408-085432

Schwert, D.P. (1991). Chapter 11: Oligochaeta: Lumbricidae. In: Dindal, D. L. (Ed.). (1991). *Soil biology guide*. John Wiley & Sons.

Silva, R. D., Rodrigues, I., Guimarães, A., & Drumond, M. (2015). Comportamento de construção de câmara de estivação por Rhinodrilus alatus e sua relação com a pluviosidade. Encontro Latino-Americano De Ecologia E Taxonomia De Oligoquetas, 5.

Singh, J., Singh, S., & Vig, A. P. (2016). Extraction of earthworm from soil by different sampling methods: a review. *Environment, Development and Sustainability*, 18, 1521-1539. Recuperado de: https://doi.org/10.1007/s10668-015-9703-5

Singh, J., Schädler, M., Demetrio, W., Brown, G. G., & Eisenhauer, N. (2019). Climate change effects on earthworms-a review. *Soil organisms*, 91(3), 114. Recuperado de: https://doi.org/10.25674/so91iss3pp114

Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America*

Smith, R. G., McSwiney, C. P., Grandy, A. S., Suwanwaree, P., Snider, R. M., & Robertson, G. P. (2008). Diversity and abundance of earthworms across an agricultural land-use intensity gradient. *Soil and Tillage Research*, 100(1-2), 83-88. Recuperado de: https://doi.org/10.1016/j.still.2008.04.009

Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A., McDowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I., Sobocká, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya, T., Scholes, R. J., and Scholes, M. C. (2015) Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils, *Soil,* 1, 665–685. Recuperado de: https://doi.org/10.5194/soil-1-665-2015, 2015.

Steffen, G. P. K., Steffen, R. B., Bartz, M. L. C., James, S. W., Jacques, R. J. S., Brown, G. G., & Antoniolli, Z. I. (2018). Earthworm diversity in Rio Grande do Sul, Brazil. *Zootaxa*, 4496(1), 562-575. Recuperado de: https://doi.org/10.11646/zootaxa.4496.1.43

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). Manual de métodos de análise de solo.

Torppa, K. A., & Taylor, A. R. (2022). Alternative combinations of tillage practices and crop rotations can foster earthworm density and bioturbation. *Applied Soil Ecology*, 175, 104460. Recuperado de: https://doi.org/10.1016/j.apsoil.2022.104460

Torrent, I. Use of ExoSAP-IT® PCR Product Cleanup Reagent in Next Generation Sequencing (NGS).

Vidal, A., Blouin, M., Lubbers, I., Capowiez, Y., Sanchez-Hernandez, J. C., Calogiuri, T., & van Groenigen, J. W. (2023). The role of earthworms in agronomy: Consensus, novel insights and remaining challenges. *Advances in agronomy*, 181, 1-78. Recuperado de: https://doi.org/10.1016/bs.agron.2023.05.001

Vršič, S., Breznik, M., Pulko, B., & Rodrigo-Comino, J. (2021). Earthworm abundance changes depending on soil management practices in slovenian vineyards. *Agronomy*, 11(6), 1241. Recuperado de: https://doi.org/10.3390/agronomy11061241

Yang, Y., Ding, J., Zhang, Y., Wu, J., Zhang, J., Pan, X., & He, F. (2018). Effects of tillage and mulching measures on soil moisture and temperature, photosynthetic characteristics and yield of winter wheat. *Agricultural Water Management*, 201, 299-308. Recuperado de: https://doi.org/10.1016/j.agwat.2017.11.003

Zicsi, A., Römbke, J. & Garcia, M. (2001) Regenwürmer (Oligochaeta) aus der Umgebung von Manaus (Amazonien). Regenwürmer aus Südamerika 32. *Revue Suisse de Zoologie,* 108 (1), 153–164. Recuperado de: https://doi.org/10.5962/bhl.part.79624

APPENDIX

EARTHWORM TOTAL ABUNDANCE AND BIOMASS FOR EACH COUNTY AND LAND USE SYSTEM SAMPLED, CONSIDERING QUANTITATIVE (TSBF) AND QUALITATIVE METHODS

Biome: Atlantic Forest City: Mangueirinha - Paraná	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Lumbricidae family						
Bimastos parvus	-	4 / -	-	-	0.05 / -	-
Megascolecidae family	-	-	-	-	-	-
Amynthas corticis	-	26 / -	-	-	3.1 / -	-
Amynthas gracilis	-	- / 41	1	-	- / 13.4	-
Juveniles	-	18 / -	1	-	0.6 / -	0.14 / -
Ocnerodrilidae sp.1	4 / -	-	- / 4	0.01/-	-	- / 0.94
Native species						
Glossoscolecidae family						
Fimoscolex n.sp. 51	-	2 / -	-	-	0.06 / -	-
Glossoscolex dallavechiai	-	-	- / 2	-	-	- / 0.3
Juveniles	-	- / 3	-	-	-	-
Rhinodrilidae family						
Andiorhinus duseni	-	- / 1	-	-	-	-
Urobenus brasiliensis	-	-	- / 1	-	-	- / 0.9
Juveniles	-	-	- / 23	-	-	- / 5.8
Unidentifiable juveniles	-	-	2/-	-	-	0.519 / -

Biome: Atlantic Forest City: Faxinal dos Guedes - Santa Catarina	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Megascolecidae family						
Amynthas gracilis	11 / 10	-	-	4.5 / 3.9	-	-
Juveniles	2 / -	-	-	0.4 / -	-	-
Ocnerodrilidae family						
Ocnerodrilidae sp.1	1 / -	- / 1	-	0.01 / -	- / 0.01	-
Native species						
Glossoscolecidae family						
Glossoscolex alessioi	-	3 / -	- / 2	-	0.4 / -	-
Juveniles	-	-	1 / -	-	-	- / 0.1
Rhinodrilidae family						
Urobenus brasiliensis	-	2 / -	- / 2	-	1.2 / -	- / 0.5
Unidentifiable juveniles	2 / -	-	- / 2	0.04 / -	-	- / 0.2

Biome: Atlantic Forest City: Panambi - Rio Grande do Sul	Total abundance (TSBF/Quali)			Total biomass (TSBF/Quali)		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Acanthodrilidae family						
Microscolex dubius	-	5 / 2	-	-	1.06 / 0.22	-
Lumbricidae family						
Bimastos parvus	-	3 / -	-	-	0.09 / -	-
Megascolecidae family						
Amynthas corticis	-	8 / -	-	-	4.4 / -	-
Amynthas gracilis	-	5 / 8	-	-	3.6 / 9.1	-
Ocnerodrilidae family						
Eukerria saltensis	12 / 2	1 / -	2 / -	0.27 / 0.16	0.01 / -	0.02 / -
Juveniles	19 / -	- / 2	2 / -	0.2 / -	0.02 / -	0.04 / -
Native species						
Glossoscolecidae family						
Fimoscolex fridrichi	3 / -	-	1 / -	0.09 / -	-	0.07 / -
Glossoscolex strobeli	-	- / 6	-	-	- / 22.5	-
Juveniles	-	-	- / 1	-	-	- / 0.08
Rhinodrilidae family						
Urobenus brasiliensis	-	-	- / 1	-	-	- / 0.05
Unidentifiable juveniles	- / 1	-	-	- / 0.02	-	-

Biome: Atlantic Forest City: Ajuricaba - Rio Grande do Sul	Total abundance (TSBF/Quali)			Total biomass (TSBF/Quali)		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Acanthodrilidae family						
Microscolex dubius	- / 1	-	-	- / 0,07	-	-
Ocnerodrilidae family	-	-	-	-	-	-
Eukerria saltensis	-	- / 1	-	-	- / 0.01	-
Ocnerodrilidae sp.1[2]	-	-	-	-	-	-
Juveniles	-	-	-	-	-	-
Native species						
Glossoscolecidae family						
Fimoscolex fridrichi	8 / 2	1 / 1	2/	0.32 / 0.08	0.05/0.07	0.15 /
Fimoscolex n.sp. 51[3]	-	-	- / 1	-	-	- / 0.14
Juveniles	-	1 / 1	-	-	0.03 / 0.05	-
Rhinodrilidae family						
Juvelines	-	-	2/5	-	-	0.11 / 0.95
Unidentifiable juveniles	3 / 4	1 / -	1 / -	0.03 / 0.02	0.004 / -	0.007 / -

Biome: Atlantic Forest City: Cruz Alta - Rio Grande do Sul	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Megascolecidae family						
Amynthas corticis	-	20 / -	-	-	2.1 / -	-
Ocnerodrilidae family						
Eukerria saltensis	6 / 7	7 / 1	1/-	0.1 / 0.8	0.07 / 0.02	0.08 /
Ocnerodrilidae sp.1	-	-	-	-	-	-
Juveniles	4 / -	9 / -	-	0.6 / -	0.8 / -	-
Native species						
Glossoscolecidae family						
Fimoscolex fridrichi	-	1 / -	-	-	0.08 / -	-
Glossoscolex debortoli	-	1 / -	1 / 6	-	0.14 / -	- / 0.85
Glossoscolex strobeli	-	- / 1	-	-	- / 5.9	-
Juveniles	-	6 / -	- / 1	-	-	-
Unidentifiable juveniles	2 / -	1 / -	-	0.01 / -	0.001 / -	-

Biome: Atlantic Forest City: Vacaria- Rio Grande do Sul	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Lumbricidae family						
Bimastos parvus	-	-	1 / -	-	-	0.06 / -
Octolasion tyrtaeum	19 / 13	14 / 17	-	4.2 / 3.1	2.3 / 3.2	-
Megascolecidae family						
Amynthas corticis	-	5/1	-	-	3.0 / 0.51	-
Ocnerodrilidae family						
Eukerria saltensis	1 / -	-	6/-	0.009 / -	-	0.09 / -
Native species	-	-	-	-	-	-
Glossoscolecidae family						
Glossoscolex arnsi	-	-	- / 44	-	-	- / 12.7
Rhinodrilidae family						
Urobenus brasiliensis	-	-	1 / 2	-	-	0.16 / 0.1
Unidentifiable juveniles	-	-	3 / -	-	-	0.002 / -

Biome: Atlantic Forest City: Bom Jesus - Rio Grande do Sul	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Megascolecidae family						
Amynthas corticis	- / 1	-	-	- / 0.36	-	-
Ocnerodrilidae family						
Eukerria saltensis	- / 5	- / 38	-	- / 0.07	- / 0.53	-
Ocnerodrilidae sp.1	- / 1	-	-	- / 0.001	-	-
Juveniles	1 / -	-	-	0.003 / -	-	-
Native species						
Glossoscolecidae family						
Glossoscolex arnsi	-	3 / 49	-	-	0.5 / 6.06	-
Glossoscolex alessioi	-	-	-/2	-	-	- / 0.7
Juveniles	- / 1	-	-	-/0.12	-	-
Rhinodrilidae family						
Urobenus brasiliensis	- / 2	2 / -	1 / -	- / 1.4	0.5 / -	1.03 / -
Unidentifiable juveniles	4 / -	-	4 / -	0.2 / -	-	0.7 / -

Biome: Cerrado City: Sinop - Mato Grosso	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster affinis	5 / -	-/2	-	0.4 / -	- / 0.06	-
Juveniles	-	-	-	-	-	-
Megascolecidae family						
Juveniles	7 / -	-	-	1.7 / -	-	-
Native species						
Rhinodrilidae family						
Andiorrhinus (Turedrilus) sp. 1	- / 4	-	1 / -	-	- / 3.01	0.52 / -
Unidentifiable juveniles	24 / -	3 / -	-	0.7 / -	0.05 / -	-

Biome: Cerrado City: Sorriso - Mato Grosso	Total abundance (TSBF/Quali)			Total biomass (TSBF/Quali)		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Benhamidae family						
Dichogaster affinis	13 / 14	21 / 20	-	0.17 / 0.7	0.21 / 0.32	-
Dichogaster saliens	-	-	-	-	-	-
Juveniles	-	9 / -	-	-	0.11 / -	-
Rhinodrilidae family						
Pontoscolex corethrurus	10 / 24	-	-	1.19 / 3.0	-	-
Unidentifiable juveniles	5 / -	-	-	0.03 / -	-	-

Biome: Cerrado City: Lucas do Rio Verde- Mato Grosso	Total abundance (TSBF/Quali)			Total biomass (TSBF/Quali)		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Benhamidae family						
Dichogaster affinis	8 / 17	24 / 17	-	0.1 / 0.22	0.34 / 0.17	-
Dichogaster saliens	4 / -	-	-	0.15 / -	-	-
Rhinodrilidae family						
Pontoscolex corethrurus	1 / -	-	-	0.27 / -	-	-
Native species						
Glossoscolecidae family						
Juveniles	-	- / 1	-	-	- / 1.6	-
Rhinodrilidae family						
Juveniles	-	- / 2	1 / -	-	- / 3.4	0.07 / -
Unidentifiable juveniles	3 / 1	9 / 1	-	0.03 / 0.34	0.05 / 0.03	-

Biome: Cerrado City: Conquista- Minas Gerais	Total abundance (TSBF/Quali)			Total biomass (TSBF/Quali)		
	BM	NTS	NV	BM	NTS	NV
Exotic species						
Benhamidae family						
Dichogaster affinis	9 / -	4 / 16	-	0.07 / -	0.13 / 0.12	-
Dichogaster saliens	4 / -	-	-	0.04 / -	-	-
Unidentifiable juveniles	1 / 3	-	1 / -	0.01 / 0.05	-	0.01 / -

Biome: Atlantic Forest City: Carambeí - Paraná	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster saliens	1 / -	-	-	0.04 / -	-	-
Megascolecidae family						
Amynthas corticis	-	-	6 / -	-	-	1.1 / -
Amynthas gracilis	-	5 / 18	-	-	1.4 / 5.9	-
Metaphire californica	-	6 / 12	-	-	1.7 / 4.3	-
Juveniles	-	8 / 9	1 / -	-	1.5 / 1.4	0.01 / -
Native species						
Glossoscolecidae family						
Juveniles	-	-	2 / 1	-	-	0.04 / 0.09
Rhinodrilidae family						
Urobenus brasiliensis	-	-	1 / -	-	-	2.01 / -

Biome: Atlantic Forest City: Maracaí - São Paulo	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster affinis	10 / 3	21/70	1 / -	0.23 / 0.07	0.41 / 1.05	0.07 / -
Dichogaster saliens	1/ 2	9/-	-	0.02 / 0.03	0.14 / -	-
Native species						
Glossoscolecidae family						
Glossoscolex *fuchsi*	-	-	1 / -	-	-	0.11 / -
Rhinodrilidae family						
Juveniles	-	-	- / 1	-	-	- / 0.001
Unidentifiable juveniles	- / 6	4 / -	1 / -	- / 0.09	0.07 / -	0.05 / -

Biome: Cerrado City: Rio Brilhante - Mato Grosso do Sul	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster affinis	8 / 12	- / 13	2 / 1	0.02 / 0.24	- / 3.0	0.05 / 0.02
Juveniles	3 / -	-	-	0.04 / -	-	-
Ocnerodrilidae family						
Eukerria saltensis	-	3 / -	-	-	0.03 / -	-
Ocnerodrilidae sp.1	-	-	-	-	-	-
Juveniles	3 / -	56 / -	9 / -	0.07 / -	0.63 / -	0.08 / -
Rhinodrilidae family						
Pontoscolex corethrurus	4 / 4	-	-	0.09 / 0.6	-	-
Unidentifiable juveniles	5 / -	- / 3	-	0.021 / -	- / 0.31	-

Biome: Cerrado City: Maracaju - Mato Grosso do Sul	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster affinis	5 / 4	12 / 7	-	0.09 / 0.06	0.18 / 0.013	-
Dichogaster saliens	7 / 9	15 / 3	-	0.15 / 0.16	0.12 / 0.03	-
Juveniles	11 / 11	18 / 12	-	0.14 / 0.12	0.16 / 0.013	-
Ocnerodrilidae family						
Eukerria saltensis	-	12 / 16	2 / -	-	0.12 / 0.18	0.03 / -
Juveniles	-	11 / -	-	-	0.08 / -	-
Rhinodrilidae family						
Pontoscolex corethrurus	3 / -	-	96 / 28	0.21 / -	-	24.1 / 10.5
Native species						
Glossoscolecidae family	-	-	-	-	-	-
Glossoscolex *fuchsi*	17 / 17	-	-	2.22 / 2.8	-	-
Glossoscolex n.sp. 77	-	-	-	-	-	-
Juveniles	- / 8	-	-	- / 0.92	-	-
Unidentifiable juveniles	17 / -	13 / 7	3 / -	0.23 / -	0.12 / 0.009	0.03/-

Biome: Cerrado City: Formosa do Rio Pardo - Bahia	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Exotic species						
Benhamidae family						
Dichogaster affinis	-	4 / 5	-	-	0.04 / 0.06	-
Juveniles	-	2 / 11	-	-	0.02 / 0.05	-
Unidentifiable juveniles	2 / -	-	-	0.03 / -	-	-

Biome: Cerrado City: Balsas - Maranhão	**Total abundance (TSBF/Quali)**			**Total biomass (TSBF/Quali)**		
	BM	**NTS**	**NV**	**BM**	**NTS**	**NV**
Unidentifiable juveniles	-	- / 1	-	-	- / 2.43	-

Source: Author (2024)

Legend: BM: Bad management, NTS: No-Tillage System, NV: Native vegetation. The methods are separated by /, being TSBF / Qualitative.

EARTHWORM SEQUENCES CODES, CORRESPONDING MOTU, COLLECTION DATE, MUNICIPALITY AND CHARACTERIZANTION OF THE SITE THE EARTHWORMS WAS COLECTED. IN BOLD, THE REPRESENTATIVE SEQUENCES FOR FIGURE 31.

Sequence code	MOTU	Collection Date	Country/State	Municipality	Site
2010-193 G. biglipsi	1	11/02/2009	BR/MG	Itamonte	Forest soil, with Araucaria trees
2010-194 G. biglipsi	1	11/02/2009	BR/MG	Itamonte	Pasture soil, with Araucaria trees
2010-195 G. biglipsi	1	11/02/2009	BR/MG	Itamonte	Pasture soil, with Araucaria trees
2010-196 G. biglipsi	1	11/02/2009	BR/MG	Itamonte	Pasture soil, with Araucaria trees
2010-197 G. biglipsi	1	11/02/2009	BR/MG	Itamonte	Pasture soil, with Araucaria trees
EWSJC780-10 G. biglipsi	**1**	**11/02/2009**	**BR/MG**	**Itamonte**	**Pasture soil, with Araucaria trees**
2010-181 F. dorivali	2	ND	BR/SP	Bocaina	Secondary forest along small trail near park entrance gate
2010-182 F. dorivali	2	10/02/2009	BR/SP	Bocaina	Secondary forest along small trail near park entrance gate
EWSJC769-10 F. dorivali	**2**	**ND**	**BR/SP**	**Bananal**	**Ecological Research Station of Bananal, in alluvial soil near small stream**
2010-163 Fimoscolex sp.54	**3**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria plantation**
2010-164 Fimoscolex sp.54	3	10/02/2009	BR/SP	Bocaina	Araucaria plantation
COFMBRSP0934 Fimoscolex sp.2	3	ND	BR/RJ	Bocaina	National Park Serra da Bocaina
2010-167 Fimoscolex sp.56	4	10/02/2009	BR/SP	Bocaina	Araucaria plantation
2010-168 Fimoscolex sp.56	**4**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria plantation**
2010-169 Fimoscolex sp.56	4	10/02/2009	BR/SP	Bocaina	Araucaria plantation
2010-170 Fimoscolex sp.56	4	10/02/2009	BR/SP	Bocaina	Araucaria plantation
2010-147 F. elodieae	**5**	**09/02/2009**	**BR/SP**	**Bananal**	**Ecological Research Station of Bananal, in alluvial soil near small stream**
2010-148 F. elodieae	5	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in alluvial soil near small stream
2010-150 F. elodieae	5	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in alluvial soil near small stream
Fimoscolex sp.49	6	ND	BR/SC	ND	Santa Catarina Federal University campus
EWSC2.23 Fimoscolex sp.27	6	ND	BR/SC	Campo Belo do Sul	Pasture
EWSC2.24 Fimoscolex sp.27	**6**	**ND**	**BR/SC**	**Campo Belo do Sul**	**Pasture**
EWSC2.86 Glossocolex sp.33	6	ND	BR/SC	Santa Terezinha do Salto	No-tillage
G. alessioi	**7**	**08/2023**	**BR/SC**	**Faxinal dos Guedes**	**No tillage System**

G. dallavechiaia	7	August 2023	BR/PR	Mangueirinha	Native vegetation
EWSC1.11 Glossoscolex sp.5	7	ND	BR/SC	Xanxerê	Permanent pasture
EWSC1.14 Glossoscolex sp.5 Xanxere	7	ND	BR/SC	Xanxerê	Integration livestock-crop
G. strobeli	**8**	**08/2023**	**BR/RS**	**Panambi**	**No tillage System**
315A Fimoscolex large315	9	30/01/2006	BR/PR	Ponta Grossa	Wet grassland above small stream
315B Fimoscolex sp.60	**9**	**30/01/2006**	**BR/PR**	**Ponta Grossa**	**Wet grassland above small stream**
G. debortollii	**10**	**08/2023**	**BR/RS**	**Cruz Alta**	**Native vegetation**
312A G. terraopimus	11	30/01/2006	BR/PR	Faxinal	Roadside forest and ditch
312B G. terraopimus	**11**	**30/01/2006**	**BR/PR**	**Faxinal**	**Roadside forest and ditch**
312C G. terraopimus	11	30/01/2006	BR/PR	Faxinal	Roadside forest and ditch
EWSJ452 G. terraopimus	11	05/11/2004	BR/PR	Faxinal	ND
EWSJ484 G. terraopimus	11	05/11/2004	BR/PR	Londrina	Forest reserve
Glossoscolex sp. FazBartz	11	14/07/1905	BR/PR	Londrina/Faxinal	Annual crop under NT
EWSC2.13 GlossoscolexSC	**12**	**ND**	**BR/SC**	**Campo Belo do Sul**	**Eucaliptus reforestation**
EWSJ488 G. terraopimus	**13**	**05/11/2004**	**BR/PR**	**Ortigueira**	**Telepar tower**
EWSJ489 G. terraopimus	13	05/11/2004	BR/PR	Ortigueira	Telepar tower
315A Glossoscolex Ponta Grossa sp315	14	30/01/2006	BR/PR	Ponta Grossa	Wet grassland above small stream
315B Glossoscolex Ponta Grossa sp315	14	30/01/2006	BR/PR	Ponta Grossa	Wet grassland above small stream
315C Glossoscolex Ponta Grossa sp.315	**14**	**30/01/2006**	**BR/PR**	**Ponta Grossa**	**Wet grassland above small stream**
315D Glossoscolex Ponta Grossa sp.315	14	30/01/2006	BR/PR	Ponta Grossa	Wet grassland above small stream
315E Glossoscolex Ponta Grossa sp315	14	30/01/2006	BR/PR	Ponta Grossa	Wet grassland above small stream
2010-011 Glossoscolex tres barras SC	**15**	**24/02/2010**	**BR/SC**	**Três Barras**	**Araucaria plantation**
EWSC2.64 Glossoscolex sp.4 SC	16	ND	BR/SC	Santa Terezinha do Salto	Eucaliptus reforestation
EWSC2.65 Glossoscolex sp.33	**16**	**ND**	**BR/SC**	**Santa Terezinha do Salto**	**Eucaliptus reforestation**
EWSC2.66 Glossoscolex	16	ND	BR/SC	Santa Terezinha do Salto	Pasture
EWSC2.67 Glossoscolex	16	ND	BR/SC	Santa Terezinha do Salto	Pasture
EWSC2.81 Glossoscolecidae SC	16	ND	BR/SC	Santa Terezinha do Salto	No-tillage
EWSC2.82 GlossoscolecidaeSC	16	ND	BR/SC	Santa Terezinha do Salto	No-tillage

COFMBRPDDNA0527 Fimoscolex sp.3 Uru	**17**	**08/01/2015**	**BR/PR**	**Lapa**	**Mata do Uru - grass lawn**
COFMBRPRDNA0529 Fimoscolex sp.3 Uru	17	08/01/2015	BR/PR	Lapa	Mata do Uru - grass lawn
2010-008 Glossoscolex Irati	**18**	**23/02/2024**	**BR/PR**	**Irati**	**Old growth forest**
EWSJ471 Glossoscolex PicoPR	19	19/6/04	BR/PR	ND	Pico Parana trail in mossy forest
EWSJ472 Glossoscolex PicoPR	**19**	**19/6/04**	**BR/PR**	**Pico Panará**	**Pico Parana trail in mossy forest**
2010-099 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in Araucaria reforestation
2010-132 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-133 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-134 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-135 F. elkeae	**20**	**09/02/2009**	**BR/SP**	**Bananal**	**Ecological Research Station of Bananal, in forest soil**
2010-136 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-137 F. elkeae	20	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-227 Glossoscolex Santa Maria GS	21	09/09/2009	BR/RS	Santa Maria	Federal University Santa Maria - Pasture 2
2010-229 Glossoscolex Santa Maria GS	21	09/09/2009	BR/RS	Santa Maria	Federal University Santa Maria - Taquareira 5
G. nativus	**21**	**ND**	**BR/RS**	**Santana do Livramento**	**Native field**
2010-173 Glossoscolex Bocaina3	**22**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria - grass**
2010-174 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Araucaria - grass
2010-175 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Araucaria plantation P23?
2010-176 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Araucaria plantation P23?
2010-177 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Araucaria plantation P23?
2010-184 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Secondary forest along small trail near park entrance gate
2010-185 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Secondary forest along small trail near park entrance gate
2010-186 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Secondary forest along small trail near park entrance gate
2010-187 Glossoscolex Bocaina3	22	10/02/2009	BR/SP	Bocaina	Bocaina National Park Secondary forest along small trail near park entrance gate
2010-139 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-140 G. carvalhoi	**23**	**09/02/2009**	**BR/SP**	**Bananal**	**Ecological Research Station of Bananal, in forest soil**
2010-141 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-142 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil

2010-143 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-144 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
2010-152 G. carvalhoi	23	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in alluvial soil near small stream
BartzMT2009-3-2a Glossoscolex Arapongas	24	ND	BR/PR	Arapongas	Pasture
EWSJ483 G. giocondoi	**24**	**05/12/2004**	**BR/PR**	**ND**	**Mata de Godoy**
EWSJ475 Glossoscolex mariarum OR sanpedroensis	25	29/04/2004	BR/PR	ND	RPPN Sao Pedro Lupionopolis forest
EWSJ476 Glossoscolex mariarum OR sanpedroensis	**25**	**29/04/2004**	**BR/PR**	**ND**	**RPPN Sao Pedro Lupionopolis forest**
EWSC2.95 Glossoscolex sp.31	**26**	**ND**	**BR/SC**	**Otacílio Costa**	**Native forest**
EWSC2.15 Glossoscolex SC Campo Belo do Sul	**27**	**ND**	**BR/SC**	**Campo Belo do Sul**	**Eucaliptus reforestation**
H52 Glossoscolex sp.7 Estrela 2A RS	**28**	**ND**	**BR/RS**	**Estrela**	**ND**
H54 Glossoscolex sp.5 Estrela 1A RS	28	ND	BR/RS	Estrela	ND
EWSC3.01 Glossoscolecidae sp.32	**29**	**ND**	**BR/SC**	**Otacílio Costa**	**Native forest**
EWSC3.12 Glossoscolecidae sp.3 Otacilio Costa	**30**	**ND**	**BR/SC**	**Otacílio Costa**	**Integration livestock-crop**
2010-160|Glossoscolex Bocaina1	31	10/02/2009	BR/SP	Bocaina	Dense old forest, in bromeliads
2010-162 Glossoscolex Bocaina 1	**31**	**10/02/2009**	**BR/SP**	**Bocaina**	**Dense old forest, in bromeliads**
G. arnsi	**32**	**August 2023**	**BR/RS**	**Bom Jesus**	**Native vegetation**
F. fridrichi	**33**	**August 2023**	**BR/RS**	**Panambi**	**Native vegetation**
EWSC1.27 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	Forest
EWSC1.38 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	Eucaliptus reforestation
EWSC1.39 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	Eucaliptus reforestation
EWSC1.40 Fimoscolex sp.3 Sao Miguel do Oeste	**34**	**ND**	**BR/SC**	**São Miguel do Oeste**	**Permanent pasture**
EWSC1.49 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	Integration livestock-crop
EWSC1.60 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	No-tillage
EWSC1.61 Fimoscolex sp.3 Sao Miguel do Oeste	34	ND	BR/SC	São Miguel do Oeste	No-tillage

324 Glossoscolex Campina do Monte Alegre	**35**	**02/01/2006**	**BR/SP**	**Campinas do Monte Alegre**	**ND**
2010-221|Glossoscolex Santa Maria GS	**36**		**BR/RS**	**Santa Maria**	**ND**
Fimoscolex nsp1 GS	**37**	**ND**	**BR/RS**	**Maçambara**	**Native vegetation fragment**
321 Glossoscolex Assistencia	**38**	**02/02/2006**	**BR/SP**	**Assistência**	**pasture in farm SE of town**
2010-205 F. feijooi	**39**	**12/02/2009**	**BR/RJ**	**Itatiaia**	**National Park Itatiaia, Abrigo Reboucas in high plateau grassland**
2010-206 F. feijooi	39	12/02/2009	BR/RJ	Itatiaia	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
EWSJ0326 G. palus	**40**	**12/02/2006**	**BR/PR**	**Bandeirantes**	**Wet pasture**
EWSJ450 G. palus	40	17/04/2004	BR/PR	Bandeirantes	ND
EWSJ499 G. palus	**41**	**17/04/2004**	**BR/PR**	**Bandeirantes**	**Bandeirantes wetland**
EWSJ462 G. lutocolus	42	30/04/2004	BR/PR	Londrina (Paiquerê)	Wetland
EWSJ463 G. lutocolus	42	14/03/2005	BR/PR	Jaguapitã	Wetland
EWSJ464 G. lutocolus	**42**	**14/03/2005**	**BR/PR**	**Jaguapitã**	**Wetland**
EWSJ473 G. lutocolus	42	30/04/2004	BR/PR	Londrina (Paiquerê)	Wetland
EWSJ474 G. lutocolus	42	30/04/2004	BR/PR	Londrina (Paiquerê)	Wetland
EWSJ478 G. lutocolus	42	29/04/2004	BR/PR	Lupionópolis	Swamp
EWSJ479 G. lutocolus	42	03/05/2005	BR/PR	Ibiaci	Riparian wetland
EWSJ480 G. lutocolus	42	03/05/2005	BR/PR	Ibiaci	Riparian wetland
EWSJ481 G. lutocolus	42	03/05/2005	BR/PR	Ibiaci	Riparian wetland
EWSJ482 G. lutocolus	42	03/05/2005	BR/PR	Ibiaci	Riparian wetland
BRSPDNA004 Glossoscolex Boituva	**43**	**5/22/2012**	**BR/SP**	**Boituva**	**Bought from Sr José Carlos**
EWSC4.38 Glossoscolecidae Lauro Muller	**44**	**ND**	**BR/SC**	**Lauro Muller**	**Native forest**
2010-114 Glossoscolex Itabera	45	06/02/2009	BR/SP	Itabera	Wetland on edge of Itabera town
2010-115 Glossoscolex	**45**	**06/02/2009**	**BR/SP**	**Itabera**	**Wetland on edge of Itabera town**
2010-116 Glossoscolex Itabera	45	06/02/2009	BR/SP	Itabera	Wetland on edge of Itabera town
EWSJ465 G. uliginosus	**46**	**22/05/2004**	**BR/PR**	**Sao Jerônimo**	**Wetland**
H47 G. demeijeri	**47**	**17/04/2005**	**BR/PR**	**Guaraqueçaba**	**On the road**
2010-003 G. maschio	**48**	**22/01/2010**	**BR/PR**	**Colombo**	**Old growth forest at Embrapa Forest**
BRPRDNA0019 Glossoscolex sp.39	48	4/20/2012	BR/PR	Campina grande do Sul	ND

EWSJ491 Glossoscolex Boraceia	48	21/04/2004	BR/SP	Boracéia	Biological Station forest
Glossoscolex sp.77	**49**	**03/2024**	**BR/SP**	**Maracaí**	**Native vegetation**
EWSC1.73 Glossoscolex sp.7 Chapeco	50	ND	BR/SC	Chapecó	Eucaliptus reforestation
EWSC1.74 Glossoscolex sp.7 Chapeco	**50**	**ND**	**BR/SC**	**Chapecó**	**Eucaliptus reforestation**
330 G. paulistus	**51**	**02/02/2006**	**BR/SP**	**Assistência**	**pasture in farm SE of town**
333 Glossoscolex Assistencia	51	02/02/2006	BR/SP	Assistência	Pasture in farm SE of town
HQ562682 Glossoscolex Bananal	**52**	**ND**	**BR/SP**	**Bananal**	**Ecological Research Station of Bananal, on road near HQ**
2010-117 Glossoscolex Bocaina3	**53**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria plantation**
2010-125 Glossoscolex Intervales	54	07/02/2009	BR/SP	Intervales	Gruta Colorada trail, secondary forest
2010-126 Glossoscolex Intervales	54	07/02/2009	BR/SP	Intervales	Gruta Colorada trail, secondary forest
2010-127|Glossoscolex Intervales	**54**	**07/02/2009**	**BR/SP**	**Intervales**	**Gruta Colorada trail, secondary forest**
2010-128 Glossoscolex Intervales	54	07/02/2009	BR/SP	Intervales	Gruta Colorada trail, secondary forest
2010-129 Glossoscolex Intervales	54	07/02/2009	BR/SP	Intervales	Gruta Colorada trail, secondary forest
2010-171 Fimoscolex sp.56	**55**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria plantation**
2010-201 Glossoscolex redgiant	**56**	**12/02/2009**	**BR/RJ**	**Itatiaia**	**National Park Itatiaia, Abrigo Reboucas in high plateau grassland**
2010-202 Glossoscolex redgiant	56	12/02/2009	BR/RJ	Itatiaia	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
2010-203 Glossoscolex redgiant	56	12/02/2009	BR/RJ	Itatiaia	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
HQ2727 G. itatiaiaensis	56	12/02/2009	BR/RJ	Itatiaia	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
2010-200 Glossoscolex redgiant	**57**	**12/02/2009**	**BR/RJ**	**Itatiaia**	**National Park Itatiaia, Abrigo Reboucas in high plateau grassland**
HQ562730 G. itatiaiaensis	57	ND	BR/RJ	Itatiaia	Itatiaia National Park, Abrigo Reboucas
335 Glossoscolex Ilha Bela	**58**	**02/04/2006**	**BR/SP**	**Ilha Bela**	**forest near Pacuiba**
2010-198 Glossoscolex Itamonte2	59	11/02/2009	BR/MG	Itamonte	Pasture soil, with Araucaria trees
EWSJC787-10 G. mariebartzae	**59**	**01/07/1905**	**BR/MG**	**Itamonte**	**Pasture soil, with Araucaria trees**
2010-190 Glossoscolex ItamonteSmall	**60**	**11/02/2009**	**BR/MG**	**Itamonte**	**Forest soil, with Araucaria trees**
COFMBRMG0094 Glossoscolex sp.	60	02/07/2019	MG	Aimorés	Pasture, Integrated system
2010-249 Glossoscolex Colombo	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest

2010-250 Glossoscolex Colombo	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
2010-251 Glossoscolex Embrapa	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
2010-252 Glossoscolex Embrapa	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
2010-253 Glossoscolex Colombo	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
2010-254 Glossocolex Embrapa	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
2010-255 Glossoscolex Embrapa	61	28/09/2009	BR/PR	Colombo	Embrapa Florestas property, native forest
EWSJ467 Glossoscolex Bela Vista	61	**18/06/2004**	BR/PR	ND	Graciosa Bela Vista forest
EWSJ468 Glossoscolex Bela vista	**61**	**18/06/2004**	**ND**	**ND**	**Graciosa Bela Vista forest**
EWSJ487 Glossoscolex Fatima do Sul	**62**	**20/09/2004**	**ND**	**ND**	**Bait shop**
JX177850.1 Fimoscolex sp. GPKS-2012	62	ND	ND	ND	ND
EWSJ139 Glossoscolex	**63**	**ND**	**BR/MS**	**ND**	**ND**
2010-165 Glossoscolex Bocaina 5	**64**	**10/02/2009**	**BR/SP**	**Bocaina**	**Araucaria plantation**
2010-166 Glossoscolex Bocaina5	64	10/02/2009	BR/SP	Bocaina	Araucaria plantation
BRPR0334 G. lutocolus	**65**	**03/05/2005**	**BR/PR**	**Primeiro de Maio**	**Spring seepage area outskirts of Ibiaci district**
BRPRDNA0066 Glossoscolex CampGrandedoSul	**66**	**3/18/2012**	**BR/PR**	**Campina grande do Sul**	**Base 3**
BRPRDNA0078 Glossoscolexsp.37	**67**	**12/14/2011**	**BR/PR**	**Campina grande do Sul**	**Swamp**
H51 G. cardosoi	67	ND	BR/PR	Campina grande do Sul	ND
EWSJ496 Glossoscolex Campina Grande	68	17/06/2004	BR/PR	Campina grande do Sul	ND
EWSJ497 Glossoscolex Campina Grande	**68**	17/06/2004	**BR/PR**	**ND**	**Campina Grande so Sul, Serendipity Farm**
317A Glossoscolex Buri SP	69	01/02/2006	BR/SP	Buri	Swamp and river banks
EWSJA1122-09 Glossoscolex Buri sp.317	**69**	**01/02/2006**	**BR/SP**	**Buri**	**Swamp and river banks**
2010-216 Fimoscolex sp.53	70	11/02/2009	BR/RJ	Rio de Janeiro	Parque Nacional Itatiaia, Maua old growth forest,
EWSJC804-10 F. barettai	**70**	**01/07/1905**	**BR/MG**	**Itamonte**	**Pasture soil, with Araucaria trees**
2010-207 Fimoscolex sp.52	**71**	**12/02/2009**	**BR/RJ**	**Itatiaia**	**National Park Itatiaia, Abrigo Reboucas in high plateau grassland**
2010-208 Fimoscolex sp.52	71	12/02/2009	BR/RJ	Rio de Janeiro	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
2010-209|Fimoscolex sp.52	71	12/02/2009	BR/RJ	Rio de Janeiro	National Park Itatiaia, Abrigo Reboucas in high plateau grassland

G. pampas	**72**	**ND**	**BR/RS**	**Uruguaiana**	**Native field**
2010-230 Glossoscolex Santa Maria GS	73	09/09/2009	BR/RS	Santa Maria	Universidade Federal de Santa Maria, Taquareira 5
G. riograndensis	**73**	**ND**	**BR/RS**	**São Vicente do Sul**	**Eucaliptus reforestation**
2010-123 F. nivae	74	06/02/2009	BR/SP	Barra do Chapeu	Forest soil
2010-248 F. nivae	**74**	**28/09/2009**	**BR/PR**	**Colombo**	**Embrapa Florestas property, native forest**
EWSC1.07 Fimoscolex sp.29	74	ND	BR/SC	Xanxerê	Eucaliptus reforestation
EWSC1.08 Fimoscolex sp.29	74	ND	BR/SC	Xanxerê	Eucaliptus reforestation
EWSJA1107-09 F. nivae	74	30/01/2006	ND	ND	wet grassland above small stream
H65 F. albidus	74	ND	BR/SP	Capão Bonito	ND
336 Glossoscolex Lavras Novas MG	**75**	**02/07/2006**	**BR/MG**	**ND**	**Forest above Bacio Custodio**
Fimoscolex sp.52	**76**	**08/2023**	**BR/RS**	**Ajuricaba**	**Native vegetation**
2010-219 Fimoscolex Maromba	77	11/02/2009	BR/RJ	Itatiaia	National Park Itatiaia, Abrigo Reboucas in high plateau grassland
EWSJC807-10 F. pereirai	**77**	**01/07/1905**	**BR/RJ**	**Parque Nacional Itatiaia, Abrigo Reboucas**	**High plateau grassland**
337 Glossoscolex Campos Jordao	**78**	**02/05/2006**	**BR/SP**	**Campos Jordão**	**Grassland and forest**
334 Fimoscolex sp.58	**79**	**02/05/2006**	**BR/SP**	**Campos Jordão**	**Grassland and forest**
EWSJ0319 F. sporadochaetus	**80**	**02/07/2006**	**BR/MG**	**Lavras Novas**	**Forest above Bacio Custodio**
COFMBRES0537 Glossoscolex sp.	**81**	**ND**	**BR/ES**	**Linhares**	**ND**
COFMBRES0544 Glossoscolex sp.	81	ND	BR/ES	Linhares	ND
BRRJ0043 G. giganteus	82	ND	BR/RJ	Seropédica	Embrapa Agroecologia
H48 GlossoscolexRJ Seropedica	**82**	**ND**	**BR/RJ**	**Seropédica**	**Embrapa Agroecologia**
BRRJ0049 G. giganteus	**83**	**ND**	**BR/PR**	**Caratuva**	**Cloud Forest - Dense high montane rainforest**
BRSC0037 Glossoscolex sp. Xanxere	**84**	**08/2011**	**BR/SC**	**Xanxerê**	**ND**
COFMBRRJDNA0055 Fimoscolex	**85**	**ND**	**BR/RJ**	**Rebio**	**Rebio 6**
EWSJ493 Fimoscolex sp.61	**86**	**21/04/2004**	**BR/SP**	**Boracéia**	**Biological Station forest**
EWSJ469 Glossoscolex Salesopolis	**87**	**21/04/2004**	**BR/SP**	**Salesópolis**	**Wetland**
EWSJ470 Glossoscolex Salesopolis	87	21/04/2004	BR/SP	ND	Wetland
EWSC1.28 Fimoscolex sp.28	**88**	**ND**	**BR/SC**	**São Miguel do Oeste**	**Forest**

2010-145 Glossoscolex BAN5	89	09/02/2009	BR/SP	Bananal	Ecological Research Station of Bananal, in forest soil
EWSJ460 G. lutocolus	90	30/04/2004	BR/PR	Londrina (Paiquerê)	Wetland
BRPR0008 G. lutocolus	91	03/05/2005	BR/PR	Primeiro de Maio	Spring seepage area outskirts of Ibiaci district
BRPR0026 G. uliginosus	92	22/05/2004	BR/PR	São Jerônimo da Serra	Grassland, swamp
2010-040 Fimoscolex sp.59	93	02/10/2008	BR/PR	Piraquara	ND
OQ067383.1 Fimoscolex sp.n.25	94	2018/2019	ND	ND	ND
Eisenia andrei	95	15/01/2024	Portugal	Coimbra	Laboratory

Legend: Not Determined data (ND).