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New species of *Glossoscolex* and *Fimoscolex* earthworms (Oligochaeta: Glossoscolecidae) from the Brazilian Atlantic Forest and Cerrado biomes

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Abstract

Brazil exhibits remarkable biodiversity, with an estimated presence of over one thousand earthworm species, of which 71% remain undescribed. Here, we describe seven new species of earthworms belonging to the family Glossoscolecidae, discovered in areas of native vegetation, annual crops under no-tillage and no-tillage system and pastures across three regions of Brazil. Two additional possible new species were represented by too few specimens and, therefore, were not formally described. For these, we provide a diagnosis based on external and internal structures to be further evaluated when additional individuals are collected. The earthworm specimens were collected utilizing quantitative and qualitative methodologies. Among the species found, seven belong to the genus *Glossoscolex*: *Glossoscolex arnsi* sp. nov., *Glossoscolex alessioi* sp. nov., *Glossoscolex dallavecchiai* sp. nov., *Glossoscolex debortolii* sp. nov., *Glossoscolex strobeli* sp. nov., *Glossoscolex fuchsi* sp. nov., and *Glossoscolex* sp.77. The other two belong to the genus *Fimoscolex*: *Fimoscolex fridrichi* sp. nov., and *Fimoscolex* sp.51. With the exception of *G. strobeli* sp. nov., belonging to the *Glossoscolex bergi* group (characterized by intraclittelar male pores located in segments XXI/XXII), the remaining *Glossoscolex* species belong to the *G. truncatus* group, with intraclittelar male pores in segment XVII. The species *F. fridrichi* sp. nov. presents a notable characteristic in the absence of a copulatory bulb, despite possessing a male pore in segment XVII. A comparative table is provided, delineating similarities and differences between the newly described species and some previously documented species within the same genera.

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

Resumo

O Brasil apresenta notável biodiversidade, com uma presença estimada de mais de mil espécies de minhocas, das quais 71% permanecem não descritas. Aqui descrevemos sete novas espécies de minhocas pertencentes à família Glossoscolecidae, descobertas em áreas de vegetação nativa, sistemas de plantio direto e pastagem em três regiões do Brasil. Outras duas possíveis novas espécies foram representadas por um número insuficiente de espécimes e, portanto, não foram formalmente descritas. Para essas, apresentamos um diagnóstico baseado em estruturas externas e internas, que poderá ser reavaliado quando forem coletados novos materiais. Os espécimes de minhocas foram coletados utilizando as metodologias quantitativas e qualitativas. Entre as espécies encontradas, sete pertencem ao gênero *Glossoscolex*: *Glossoscolex arnsi* sp. nov., *Glossoscolex alessioi* sp. nov., *Glossoscolex dallavecchiai* sp. nov., *Glossoscolex debortolii* sp. nov., *Glossoscolex strobeli* sp. nov., *Glossoscolex fuchsi* sp. nov. As outras duas pertencem ao gênero *Fimoscolex*: *Fimoscolex fridrichi* sp. nov., e com exceção de *G. strobeli* sp. nov, que é classificada dentro do grupo *Glossoscolex bergi* (caracterizada por poros masculinos intraclitelares localizados nos segmentos XXI/XXII), as espécies restantes de *Glossoscolex* pertencem ao grupo *G.truncatus*, exibindo poros masculinos intraclitelares no segmento XVII. A espécie

F. fridrichi sp. nov. apresenta uma característica notável na 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 dentro dos mesmos gêneros. É imperativo agilizar a descrição das espécies de minhocas para facilitar a determinação dos padrões de distribuição do gênero e elucidar seus papéis ecológicos dentro dos ecossistemas edáficos.

Palavras chave: Biodiversity, Crassiclitellata, Megadrili, No-tillage, Forests

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 1.400 species have been estimated, so 71% 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 one masters. Also, there are difficulties in accessing earthworm communities in more remote areas in the country.

The most diverse earthworm family in Brazil is Glossoscolecidae, with 187 described species and subspecies worldwide (numbers updated from Brown *et al*. 2025, and including the species described here). Of these, 117 (63% of total) are found in Brazil, most of them (79 spp.) belonging to two similar genera commonly found in various land uses and soil management systems mainly in the Southern and Southeastern regions of the country: *Glossoscolex* and *Fimoscolex*. There are records of *Glossoscolex* in pastures (Bartz *et al.* 2018; Feijoo and Brown 2023), marshes (Bartz *et al.* 2012), native Atlantic Forest and highland grasslands (Demetrio *et al.* 2018; Dudas *et al.* 2023b, 2025), Pampa (Steffen *et al.* 2018), tree plantations (Brown and Gabriac 2021; Dudas *et al.* 2023b) and no-tillage systems (Santos *et al.* 2018; Demetrio *et al.* 2020; Dudas *et al.* 2023b). For *Fimoscolex*, there are records in marshes (Brown & James 2007), lawns and highland grassland (Dudas *et al.* 2023a, b, 2025), no-tillage systems (Bartz *et al.* 2023; Demetrio *et al.* 2020; Dudas *et al.* 2023b), native forests and tree plantations (Brown and Gabriac 2021; Demetrio *et al.* 2023; Dudas *et al.* 2023b). The distribution of these genera, therefore, includes diverse vegetation covers, and their interactions with the soil can vary depending on the species and the environment in which they are found (Righi 1999). Understanding the diversity, taxonomic characteristics, and ecological role of these species is important for the function of tropical soils and for the development of sustainable agricultural practices (Righi 1997). These genera are often found in the same location, in some cases together (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 quantity 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 needed.

Despite the recognized importance of some species of the Glossoscolecidae, there are significant gaps in the taxonomic knowledge of this family 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).

In the present study, we describe seven new species of the family Glossoscolecidae—six belonging to the genus *Glossoscolex* and one to *Fimoscolex*—as well as two additional possible new species for which an insufficient number of specimens were available. For these latter forms, we provide diagnostic descriptions to support their formal characterization in future studies. The specimens were collected in different municipalities across the Brazilian states of Rio Grande do Sul, Paraná, São Paulo, and Mato Grosso do Sul.

Material and methods

Earthworms were collected by removing nine soil monoliths 25 cm x 25 cm and 20 cm depth at each site, following an adaptation of Tropical Soil Biology and Fertility (TSBF) quantitative method (Anderson and Ingram 1993). Additionally, earthworms were collected using a qualitative method, by excavating at least nine additional monoliths of similar dimensions, and by digging under logs and areas with high content of organic matter. Earthworms collected

by 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 1). Sampling sites corresponded to two Brazilian biomes: Atlantic Forest and Cerrado. Selected environmental and soil characteristics of the sites where the new species were found are specified in Table 1.

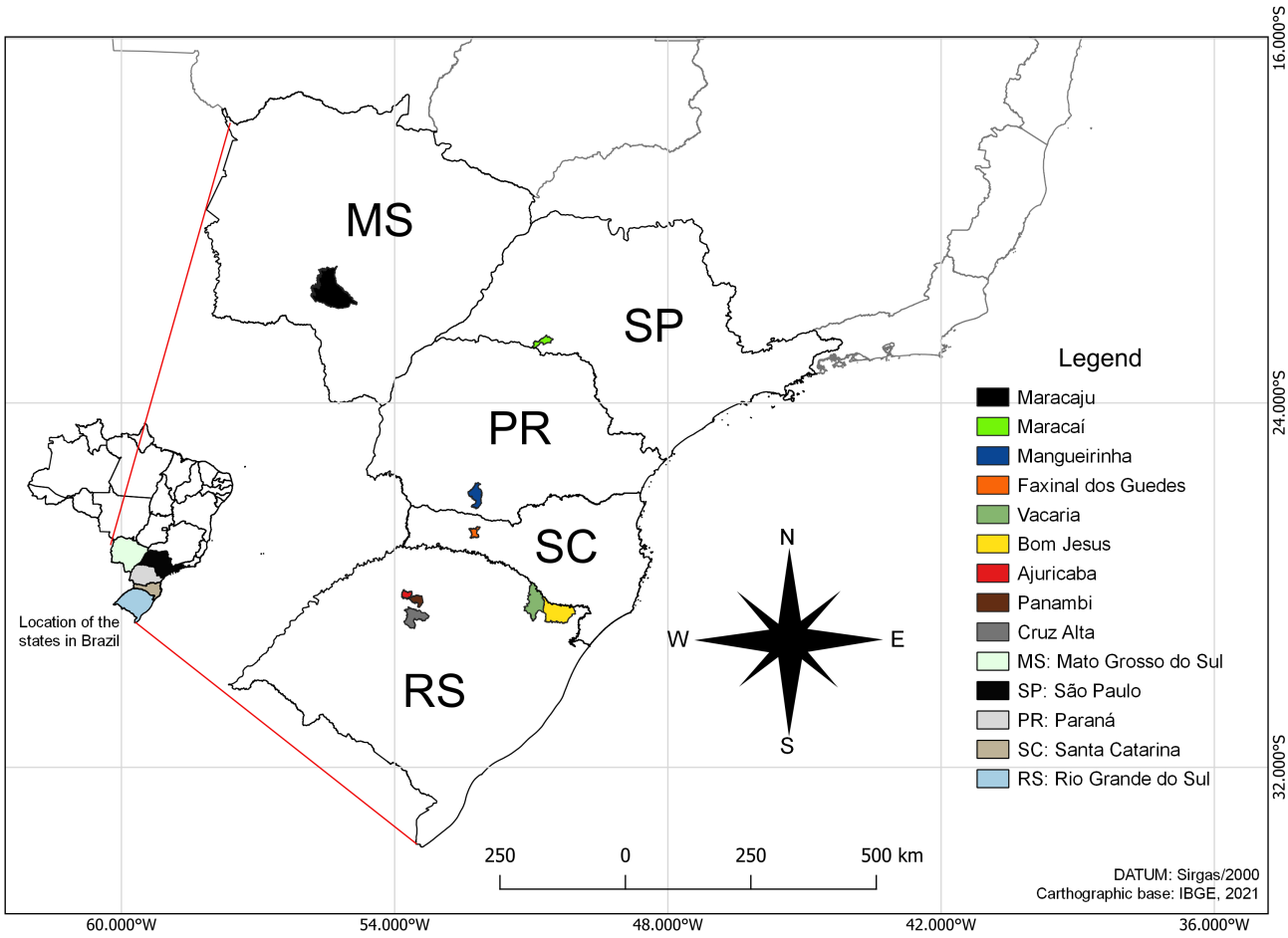

FIGURE 1. Map showing the locations of the municipalities in Brazil where earthworms were collected.

TABLE 1. Characterization of sampling sites, including state, city, geographic coordinates and site characterization, and the earthworm species found.

State	City	Species	Location (Lat/Lon/elevation)	Land use and soil management
RS	Bom Jesus	*Glossoscolex arnsi* sp. nov.	-28.467102°S, -50.597372°W, 1003m	Native vegetation in Atlantic Forest
RS	Bom Jesus	Glossoscolex *arnsi* sp. nov.	-28.485754°S, -50.609524°W, 1043m	24 years under NTS
RS	Vacaria	Glossoscolex *arnsi* sp. nov.	-28.391828°S, -51.082774°W, 879m	Native vegetation in Atlantic Forest
SC	Faxinal dos Guedes	*Glossoscolex alessioi* sp. nov.	-26785627°S, -52.229168°W, 883m	28 years under no-tillage system
SC	Faxinal dos Guedes	*Glossoscolex alessioi* sp. nov.	-26.807988°S, -52.238571°W, 924m	Native vegetation in Atlantic Forest
PR	Mangueirinha	*Glossoscolex dallavecchiai* sp. nov.	-26.05108°S, -52.235847°W, 966m	Native vegetation in Atlantic Forest
RS	Cruz Alta	*Glossoscolex debortolii* sp. nov.	-28.763297°S, -53.59013°W, 432m	Native vegetation in Atlantic Forest
RS	Cruz Alta	*Glossoscolex debortolii* sp. nov.	-28.76386°S, -53.583866°W, 419m	30 years under no-tillage system
RS	Panambi	*Glossoscolex strobeli* sp. nov.	-28.348351°S, -53.41567°W, 514m	28 years under no-tillage system

.....continued on the next page

TABLE 1. (Continued)

State	City	Species	Location (Lat/Lon/elevation)	Land use and soil management
RS	Cruz Alta	*Glossoscolex strobeli* sp. nov.	-28.76386°S, -53.583866°W, 419m	30 years under no-tillage system
SP	Maracaí	*Glossoscolex* sp.77	-22.668361°S, -50.880278°W, 390m	Native vegetation in Atlantic Forest
MS	Maracaju	*Glossoscolex fuchsi* sp. nov.	-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* sp. nov.	-28.375519°S, -53.445185°W, 494m	Native vegetation in Atlantic Forest
RS	Panambi	*Fimoscolex fridrichi* sp. nov.	-28.371324°S, -53.418971°W, 494m	No-tillage with crop succession (soybean and wheat or oats)
RS	Cruz Alta	*Fimoscolex fridrichi* sp. nov.	-28.76386°S, -53.583866°W, 419m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* sp. nov.	-28.167072°S, -53.814564°W, 419m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* sp. nov.	-28.144014°S, -53.777835°W, 422m	30 years under no-tillage system
RS	Ajuricaba	*Fimoscolex fridrichi* sp. nov.	-28.149816°S, -53.774019°W, 385m	No-tillage with crop succession
PR	Mangueirinha	*Fimoscolex* sp.51	-26.051079°S, -52.237655°W, 981m	27 years under no-tillage system

For the identification of the species, taxonomic keys and morphological characteristics of *Fimoscolex* and *Glossoscolex* were utilized following Righi (1972), Bartz *et al*. (2012; 2018) and Feijoo and Brown (2018). Initially, the external characters were observed, followed by dorsal dissection for examination of internal structures. All holotypes and other materials 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 was removed, and the extraction followed the Qiagen protocol—DNeasy® Blood & Tissue Handbook (Qiagen 2011). After extraction, the samples passed into the polymerase chain reaction (PCR) using the COI gene with the universal primers for metazoans of Folmer *et al.* (1994). For the final step of DNA purification the ExoSap-IT TM Express PCR Product Cleanup protocol was used. DNA sequencing was performed in Macrogen and Eurofins labs.

Analysis of DNA data was made by using the Molecular Evolutionary Genetics Analysis version 11—MEGA11 software (Temura *et al.* 2021). Estimates of evolutionary divergence between sequences was evaluated using the Kimura 2-parameter model (Kimura 1980; Temura *et al*. 2021). The evolutionary history was inferred using the Maximum Likelihood method with the Tamura-Nei model, which was selected because it accounts for unequal base frequencies and different rates of transitions and transversions. This model is particularly suitable for mitochondrial COI sequences, as it provides a more realistic representation of nucleotide substitution patterns, thereby improving the accuracy of phylogenetic inference. This analysis involved 11 nucleotide species sequences, and 500 replicates. We were unable to amplify the DNA from *Glossoscolex* sp.77 and *Glossoscolex fuchsi* sp. nov. Sequences from *Pontoscolex corethrurus* (code OQ222854.1), *Glossoscolex riograndensis* (code JX177853) and *Fimoscolex* sp. 25 (code OQ067384.1) were recovered from GenBank—NCBI. *P. corethrurus* was used as outgroup, while *G. riograndensis* and *Fimoscolex* sp.25 were included to illustrate genetic similarities among genera and divergence among species, thus supporting the phylogenetic analysis. All COI sequences obtained for the new specimens (holotypes) have been deposited in BOLD Systems under the following Process IDs: GLOBR001-25 (*Glossoscolex arnsi* sp. nov.); GLOBR002-25 (*Glossoscolex alessioi* sp. nov.); GLOBR003-25 (*Glossoscolex dallavecchiai* sp. nov.); GLOBR004-25 (*Glossoscolex debortolli* sp. nov.); GLOBR005-25 (*Glossoscolex strobeli* sp. nov.); GLOBR006-25 (*Fimoscolex fridrichi* sp. nov.); and GLOBR007-25 (*Fimoscolex* sp. 51)

Material examined

Glossoscolecidae, Michaelsen, 1900, Glossoscolecidae, James & Davidson, 2012

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)* Michaelsen, 1900**

Diagnosis. One pair of external male pores, associated with one pair of internal copulatory bulbs

***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 arnsi* Dudas, Brown & Bartz, sp. nov.**
(Figures 2 and 3)

Holotype. COFM BRRS0431, adult individual, native vegetation, in 0–20 cm soil, by qualitative method, Vacaria, Rio Grande do Sul, Brazil, -28.391473, -51.082457, 879 m asl, August 18, 2023, M.L.C. Bartz coll.

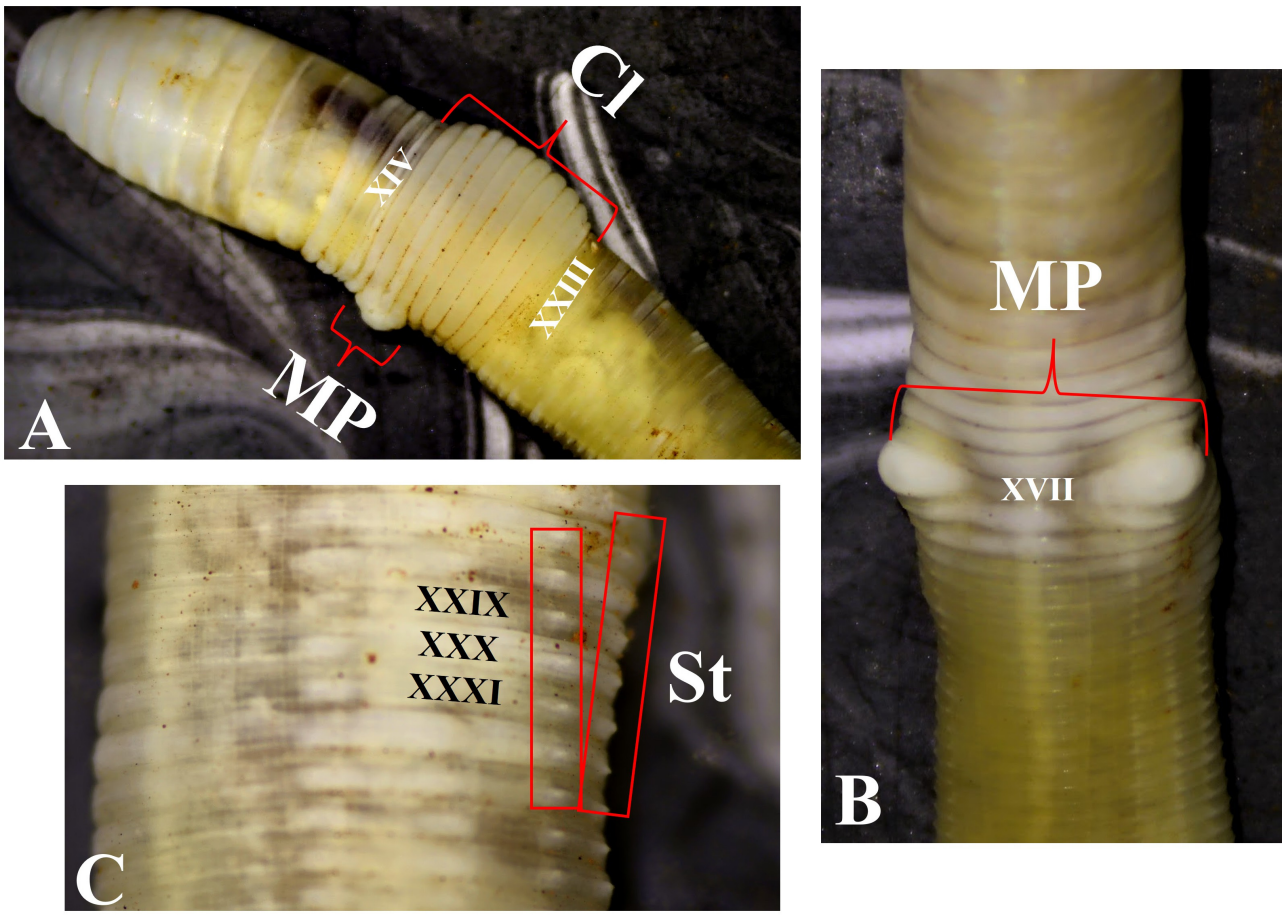

FIGURE 2. Holotype. *Glossoscolex arnsi* sp. nov. **A**: Clitellum XIV–XIII, male pore in XVII. **B.** View of the pair of male pores in XVII. **C.** View with emphasis in the *ab* setal line. Cl: clitellum; MP: male pore; St: setae.

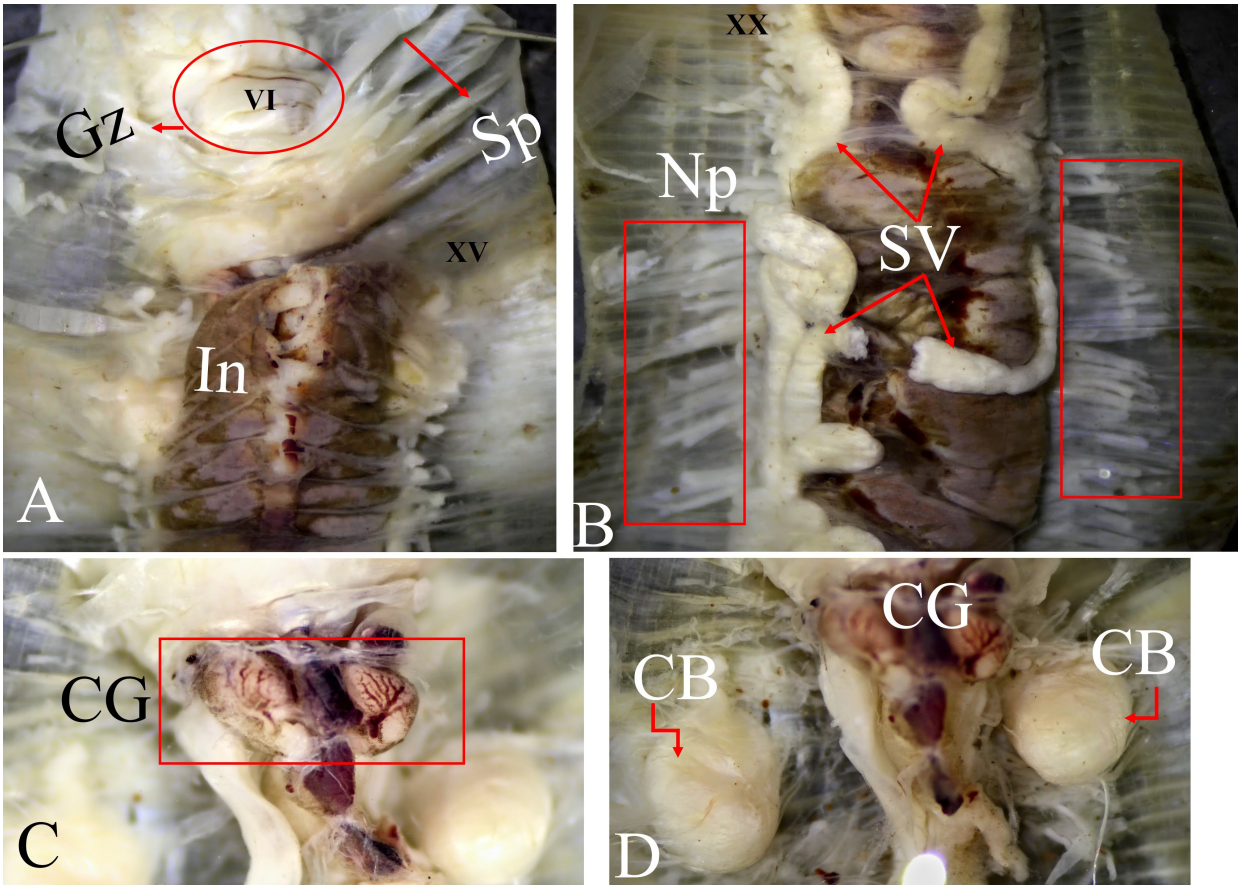

FIGURE 3. Holotype. Glossoscolex *arnsi* sp. nov. **A.** Gizzard in VI, septa in VII–IX, intestine, XV. **B.** View of seminal vesicle in XX, nephridia in the red rectangle. **C.** View with emphasis the calciferous gland, in XII. **D.** CG and highlighting the copulatory bulbs, in XVI. Gz: gizzard; Sp: septa; In: intestine; SV: seminal vesicle; Np: nephridia; CG: calciferous gland; CB: copulatory bulb.

Paratype. COFM BRRS0425, adult individual, native vegetation, in 0–20 cm soil, by qualitative method, Bom Jesus, Rio Grande do Sul, Brazil, -28.466743, -50.597859, 1003 m asl, 20 August 2023, M.L.C. Bartz coll.

Other materials. COFM BRRS0530, 41 individuals, same information as holotype. COFM BRRS531, one adult, same information as paratype. COFM BRRS0415, two adults, same information as paratype. COFM BRRS0419, two adults, No-Tillage System, in 0–20 cm soil, by TSBF method, Bom Jesus, Rio Grande do Sul, Brazil -28.485754, -50.609524, 1043 m asl, 20 August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. COFM BRRS 0421, 12 adults, No-Tillage System, in 0–20 cm soil, by qualitative method, Bom Jesus, Rio Grande do Sul, Brazil -28.485754, -50.609524, 1043 m asl, 20 August 2023, M.L.C. Bartz colls. COFM BRRS0422, 37 individuals, No-Tillage System, in 0–20 cm soil, by qualitative method, Bom Jesus, Rio Grande do Sul, Brazil -28.485754, -50.609524, 1043 m asl, 20 August 2023, M.L.C. Bartz and W.C. Demetrio colls.

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. 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 spacing uneven: *aa* and *dd* markedly larger than *ab* and *cd*, with *bc* intermediate. Setal arrangement *aa:ab:bc:cd:dd*, *3.0:1.1:2.3:1.0:2.8* mm, measured on segment XXX. This pattern departs from the typical widely paired arrangement, so we describe it as nearly widely paired. Setae *ab* visible from V onwards, and *cd* on VIII.

Clitellum in XIV to XXIII, saddle shaped. Genital marks or tubercula pubertatis 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 *B* line.

Internal morphology. Septa strong, thickened on 7/8/9/10/11, strongly attached to the body wall, due thickness. 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, lamellar form. Five pair of hearts, the last pair in XI, and dorsal blood vessels visible in segments VIII–X, above the calciferous glands. Nephridia tubular, elongated, slightly curved distally (J-shaped), without evident vesiculation, astomate starting before clitellum, in segments IX,X, close to the body wall and intestine, one pair per segment, aligned with *C* line. Unpaired testis sac, large, in XI, close to the calciferous gland. Seminal vesicles in XII, starting ventrally, below calciferous gland, and ending dorsally above the intestine on segment LX, lobular shaped stretching in two lines. Ovarian funnels and ovaries free in segment 13. Spermathecae absent. One pair of copulatory pouches (bulbs, chambers) with oval shape covering 1/3 XVI–1/3 XX, 3 mm long in the holotype.

Remarks. Currently the *truncatus* group has 34 described species (Feijoo and Brown 2023, Dudas *et al.* 2025a,b), including the new species described here. Regarding the clitellum, *Glossoscolex arnsi* sp. nov. shares the extension and position (XIV–XIII) with *G. bondari* Michaelsen, 1926, *G. minor* Zicsi & Csuzdi, 1999, and *G. tocape* Righi, 1980, although the first two have annular clitella, which differs from *G. arnsi* sp. nov. The primary distinctions between *G. tocape* and *G. arnsi* sp. nov. 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 pubertatis (absent in the clitellum of *G. arnsi* sp. nov.) associated with the internal copulatory bulbs in segments XVI–XVIII in *G. tocape* (shorter than in *G. arnsi* sp. nov.). In *G. arnsi* sp. nov., the male pores are remarkably prominent for the body size, remaining conspicuous and easily observable from all perspectives, regardless of the worm's orientation. A unique characteristic observed in *G. arnsi* sp. nov. 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 (see table 2).

Glossoscolex alessioi Dudas, Brown & Bartz, sp. nov.

(Figures 4 and 5)

Holotype. COFM BRSC0296, adult specimen, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Faxinal dos Guedes, Santa Catarina, Brazil, -26.785627, -52.229168, 883 m asl, August 10, 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls.

Paratype. COFM BRSC0337, one adult, same information as the holotype.

Other materials. COFM BRSC0338, one adult, same information as the holotype.

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. 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-pigmented after fixation. Prostomium epilobic. Setae closely paired, visible throughout, setae *cd* visible from IV onwards and *ab* visible from VII. Setal arrangement *aa:ab:bc:cd:dd*, *3:1.1:1:1.9:3* at segment XXX. 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. Septa strong, thickened on 7/8/9/10, strongly attached to the body wall, due to thickness. Gizzard in VI. One pair of calciferous glands in XII, of composite-tubular type, with dorsal blood vessels. Intestine begins in segment XV, and the typhlosole starts in segment XX as cupped folds of the lamellar type. Five pair of hearts, being the last in XI, and dorsal blood vessel visible in segments VIII–X and above the calciferous glands. Holonephridial starting in IX–X and extending throughout the body; exonephric, stomate, positioned close to the body wall and intestine. Organs tubular, non-vesiculated, slender, and free in the coelom. Paired testes sacs large in XI, beginning just anterior and ventral to the calciferous gland. Seminal vesicles long, starting in XII and going until 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, in the holotype.

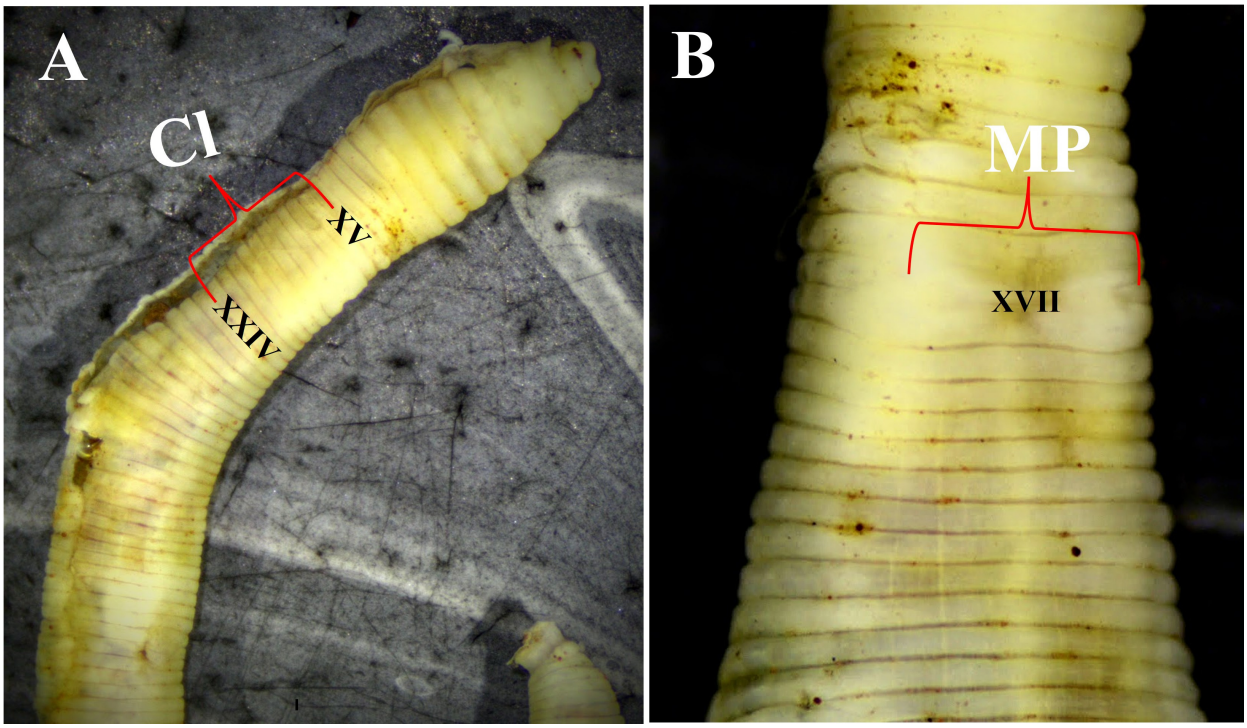

FIGURE 4. *Glossoscolex alessioi* sp. nov. Holotype. **A:** Clitellum XV–XXIV. **B:** View of the pair of male pores in XVII. Cl: clitellum; MP: male pore.

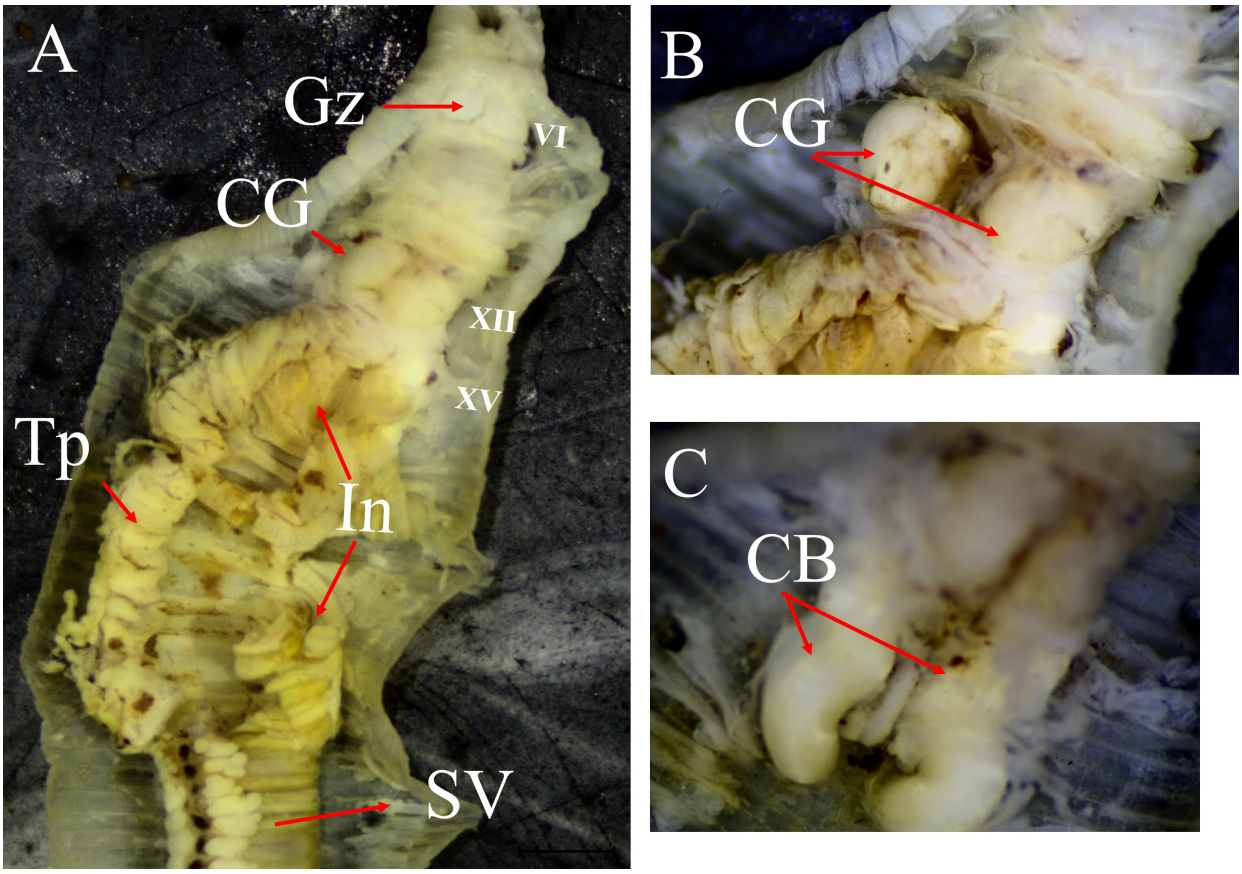

FIGURE 5. *Glossoscolex alessioi* sp. nov. Holotype. **A.** View of gizzard in VI, calciferous glands, in XII, seminal vesicle, intestine and typhlosole, from XV. **B:** View with emphasis the calciferous gland, in XII. **C:** View highlighting the copulatory bulbs, in XX. Gz: gizzard; CG: calciferous gland; In: intestine; Tp: typhlosole; SV: seminal vesicle; CB: copulatory bulb.

Remarks. The saddle-shaped clitellum in XV–XXIV of *G. alessioi* sp. nov. resembles that of *G. bonariensis* Cordero, 1942, *G. catharinensis* Michaelsen, 1918, *G. cardosoi* Feijoo & Brown, 2023, and *G. dallavecchiai* sp. nov. However, two distinct features differentiate *G. alessioi* sp. nov. from these species: the form and extension of the seminal vesicles (XII–LXX, spiral) and the copulatory bulbs (XVII-2/3 XX, bean-shaped). In *G. bonariensis*, the seminal vesicles extend from XI to LII and are straight, with the copulatory bulbs oval in XVI–XIX. In *G. catharinensis*, the vesicles are tubular, extending from XVII to CCXXX, and the bulbs may be reniform in XVI–XVII or oval in XVII–XIX. In *G. cardosoi*, the seminal vesicles are restricted to XII and the bulb occurs in XVIII.

***Glossoscolex dallavecchiai* Dudas, Brown & Bartz, sp. nov.**
(Figures 6 and 7)

Holotype. COFM BRPR2167, adult, native vegetation, in 0–20 cm soil, by qualitative method, Mangueirinha, Paraná, Brazil, -26.050652 -52.235993, 966 m asl, August 08, 2023, M.L.C. Bartz coll.

Paratype. one COFM BRPR2168, juvenile, same information as the holotype.

Other materials. COFM BRPR2359, one juvenile, same information as the holotype.

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. Holotype: body length 73 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-pigmented after fixation. Prostomium open epilobic. Setae widely paired, visible throughout, setae *ab* visible from IV onwards. Setal arrangement *aa:ab: bc:cd:dd*, *2.1:1.1:1:1:1.8:2.1* at segment XXX. 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.

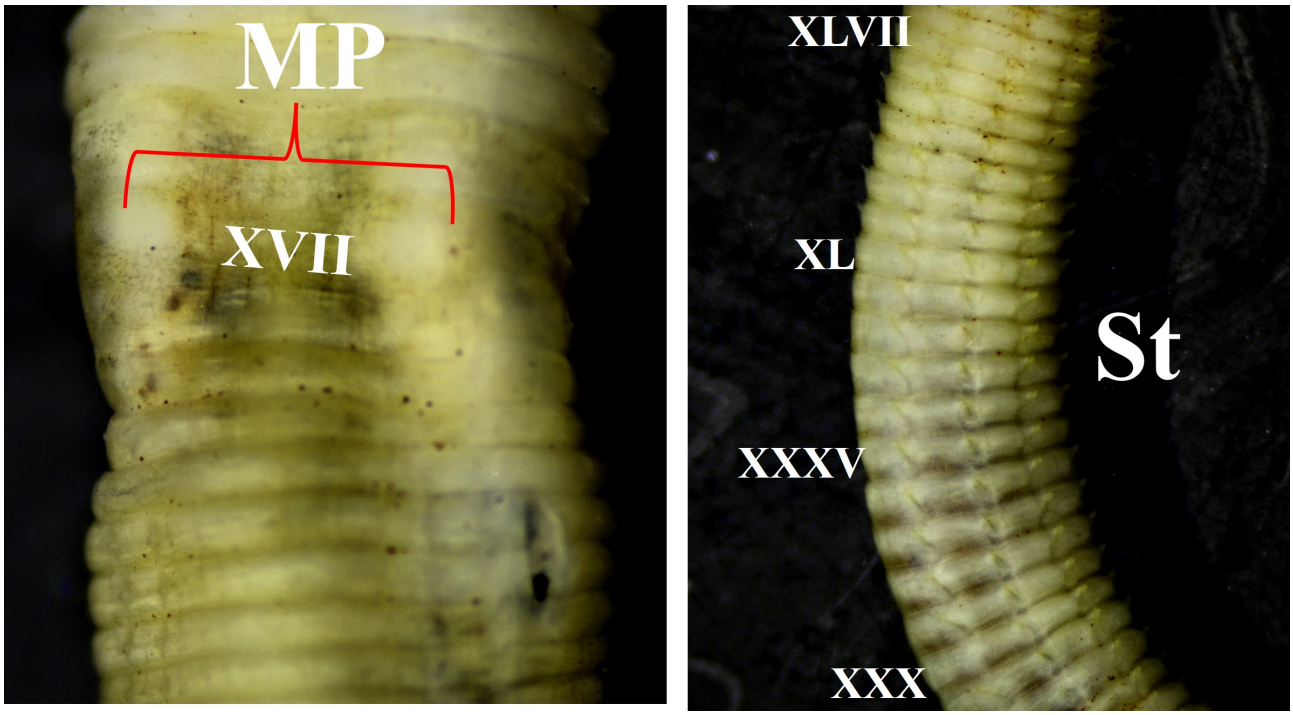

FIGURE 6. *Glossoscolex dallavecchiai* sp. nov. Holotype. **A.** View of the pair of male pores in XVII. **B.** Setal arrangement at XXX onwards. MP: male pore; St: setae.

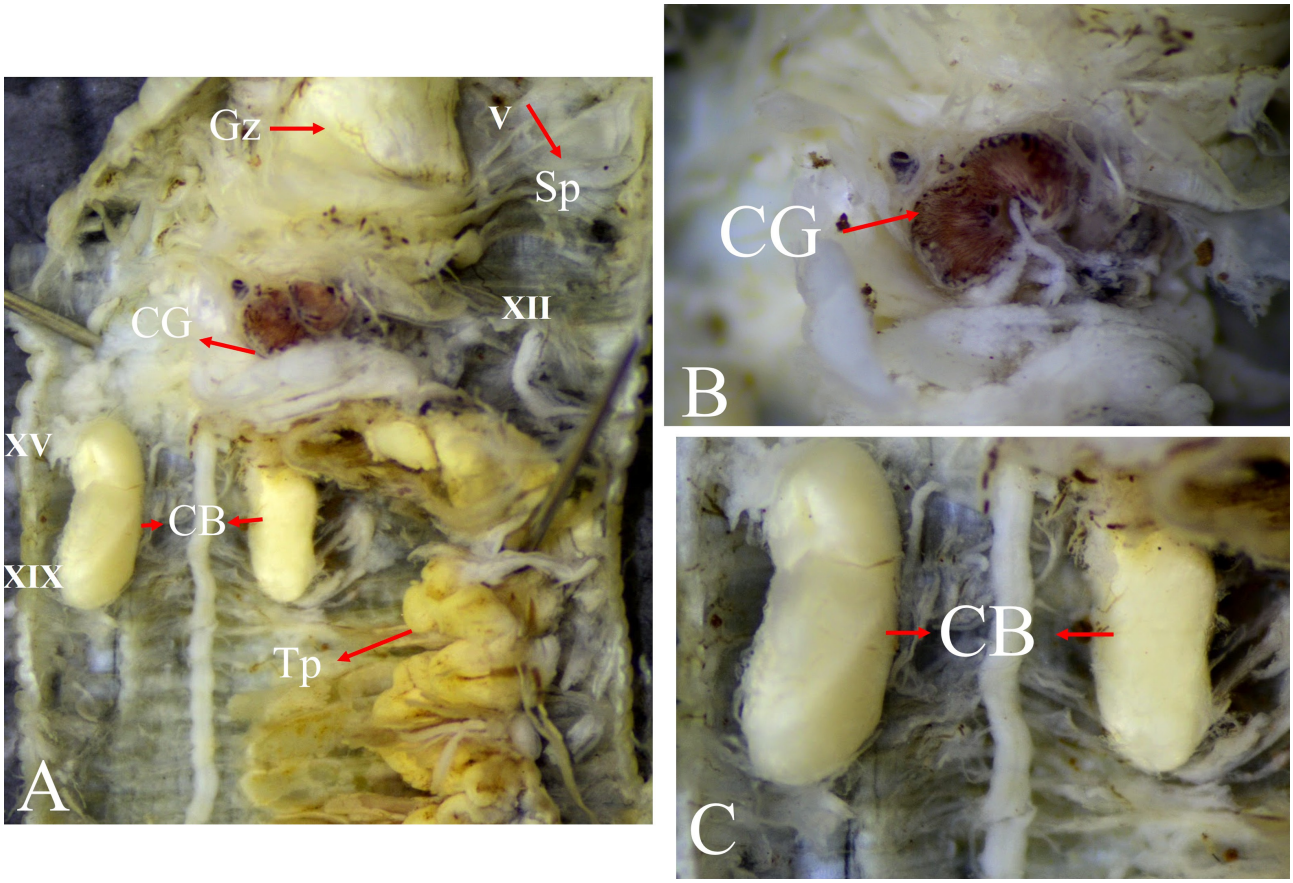

FIGURE 7. *Glossoscolex dallavecchiai* sp. nov. Holotype. **A.** view of gizzard in VI, calciferous glands, in XII, septa in VII/VIII, typhlosole, in XX, onwards and copulatory bulbs in XV–XIX. **B.** View with emphasis the calciferous gland, in XII. **C.** View highlighting the copulatory bulbs, in XV–XIX. Gz: gizzard; Sp: septa; CG: calciferous gland; Tp: typhlosole; CB: copulatory bulb.

Internal morphology. Septa weak in 7/8/9, and veil shaped 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, and together the typhlosole, with lamellar form. Five pairs of hearts, the last pair in XI, and dorsal blood vessel visible in segments VIII–X, and above the calciferous glands. Holonephridial, one pair per segment, starting post-clitellar in XVI and extending posteriorly; exonephric, stomate, positioned close to the body wall and intestine, non-vesiculated, slender, and free in the coelom. Paired testes sacs large in XI, with very visible funnels going out of the sac towards seminal vesicles. Seminal vesicles elongated, in the shape of two parallel strips, in XII–LXXIII. Ovaries and funnels not seen. Spermathecae absent. One pair of bean-shaped copulatory bulbs in 2/3 XV-1/3 XIX.

Remarks. *Glossoscolex dallavecchiai* sp. nov. is similar to *G. alessioi* sp. nov. and, consequently, to the other species cited in the previous remarks section. However, it differs in the form and extension of the seminal vesicles and copulatory bulbs. In *G. dallavecchiai* sp. nov., the vesicles form two parallel strips, and the bulbs are more elongated, occupying 2/3 XV-1/3 XIX. DNA analyses also support their separation as distinct species. The elongated copulatory bulbs are a distinctive feature within the *truncatus* group, extending through 2/3 XV to 1/3 XIX. Furthermore, *G. dallavecchiai* sp. nov. exhibits an uncommon type of anterior septa, weak and more fragile, resembling a veil-like structure that offers minimal resistance during dissection.

***Glossoscolex debortolii* Dudas, Brown & Bartz, sp. nov.**
(Figure 8 and 9)

Holotype. COFM BRRS0465, adult, native vegetation, in 0–20 cm soil, by qualitative method, Cruz Alta, Rio Grande do Sul, Brazil, -28.763181, -53.590796, 432 m asl, August 16, 2023, M.L.C. Bartz coll.

Paratype. COFM BRRS0532 one adult, same information as holotype.

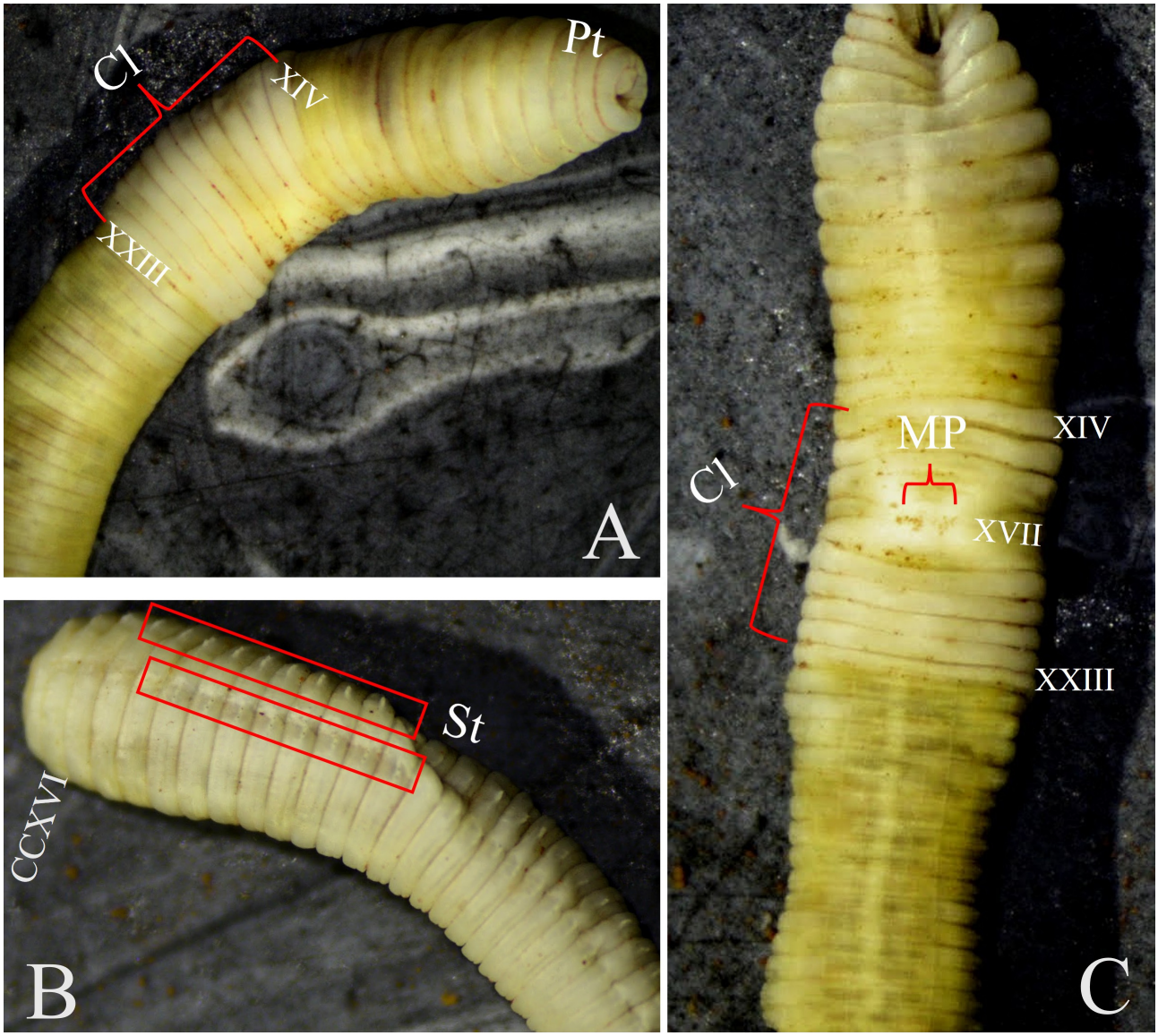

FIGURE 8. *Glossoscolex debortolii* sp. nov. Holotype. **A.** View of the prostomium and clitellum in XIV–XIII. **B.** Setal arrangement, in the red rectangles, at the end of the worm, on the last segments. **C.** View of pair of male pores in XVII, in the middle of the segment, and the ventrally portion of clitellum. Pt: prostomium; Cl: clitellum, MP: male pore; St: setae.

Other materials. COFM BRRS0533, three adults, same information as the holotype, COFM BRRS0534, two juveniles, same information as the holotype. COFM BRRS0535, one adult, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Cruz Alta, Rio Grande do Sul, Brazil, -28.763836, -53.58449, 419 m, August 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls.

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.

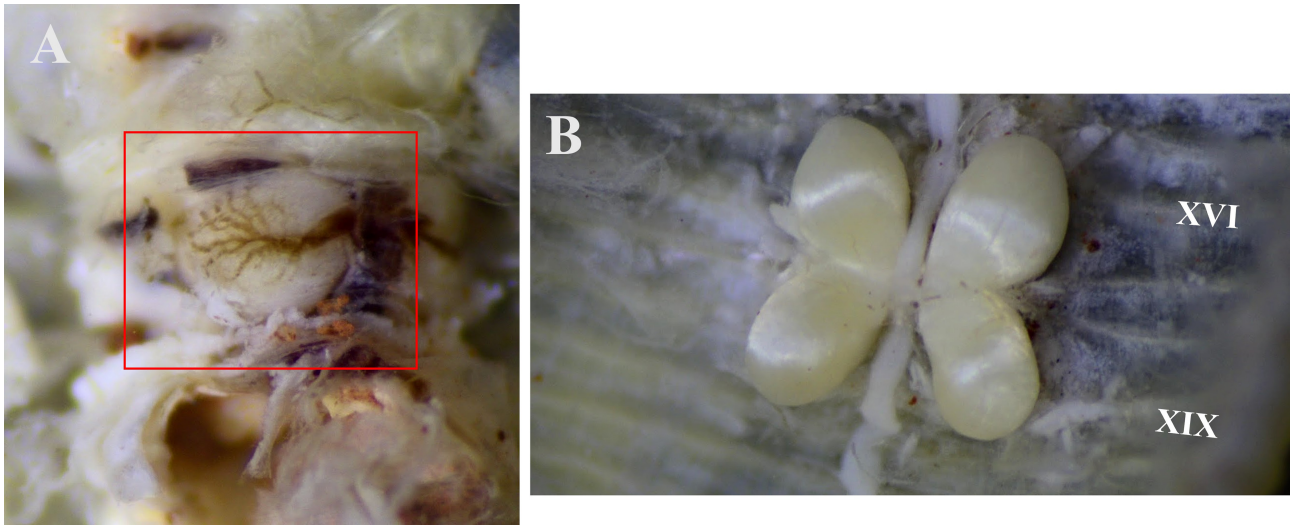

FIGURE 9. *Glossoscolex debortolii* sp. nov. Holotype. **A.** view with emphasis the calciferous gland, in XII. **B.** Internal view highlighting the copulatory bulbs, in XVI–XIX, with butterfly shape.

External morphology. 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-pigmented 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. 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. 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, in a total of five pairs, and dorsal blood vessel visible in segments VIII–X, and above the calciferous glands. Holonephridial, starting post-clitellar in XIX and extending posteriorly; exonephric, stomate, positioned close to the body wall and intestine. Tubular, non-vesiculated, whitish, and free in the coelom. Paired testes sacs in XI with iridescent color, deep below septa and followed by calciferous gland in XII, with the beginning of the gland above the end of the sacs. Seminal vesicles starting in XVIII, extending above the intestine until XLVI, arranged as two thick strips from XIII to XXVII, then becoming intermingled, and after segment XXXV forming a disorganized white mass that continues posteriorly. Ovaries and funnels 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* sp. nov. is similar to *G. bondari* Michaelen, 1926, *G. mariarum* Bartz & James, 2012, *G. terraopimus* Bartz & James, 2012, and *G. araucariaensis* Feijoo & Brown, 2023. The most distinctive feature of *G. debortolii* sp. nov. is the copulatory bulbs, which display a well-defined butterfly-like shape. Although *G. bondari* shares a similar extension (XVI–XIX), its bulbs are reniform. In the other species, the bulbs differ both in shape and extension: fusiform, XV–XIX in *G. mariarum*; reniform, XV–XVII, in *G. terraopimus*; cylindrical, XVIII–XXI in *G. embrapaensis*; and bean-shaped, XVII–XVIII in *G. araucariaensis*.

***Glossoscolex fuchsi* Dudas, Brown & Bartz, sp. nov.**
(Figures 10 and 11)

Holotype. COFM BRMS0158, 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, -21.701826, -55.632806, 610 m asl, April 29, 2024, C. Rusch, F.M.O. Lourenço, F. Paixão, G.C. Francisco, J. Bortoluzzi, K.B.F. Pepe, M. Bartz colls.

Paratype. COFM BRMS0164, one adult, same information as the holotype.

Other materials. COFM BRMS 0165, six adults and six juveniles, same information as the holotype. COFM BRMS0159, 17 adults, in pasture over 10 years old, with no signs of degradation, with native plants growing, in 0–20 cm soil, by qualitative method, Maracaju, Mato Grosso do Sul, Brazil, -21.701826, -55.632806, 610 m asl, April 30, 2024, M.C.L. Bartz coll.

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

External morphology. 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-pigmented 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. Clitellum in XV–XXII saddle shaped. Genital marks or tubercula pubertatis absent. One pair of female pores on XIV, 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. 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, tubular-composite, in XII. Intestine begins in XV, with typhlosole commencing in the same segment. Five pairs of hearts, with last pair in XI. Holonephridial, exonephric, stomate, positioned close to the body wall and intestine, tubular, more robust than in related species, non-vesiculated, whitish, and free in the coelom. Paired testes sacs in XI under the beginning of the calciferous glands. Seminal vesicles appear as thin parallels strips, above the intestine, starting in XII until XIX, then becoming zig-zag in format until the end in LIX. Ovaries in XIII. Spermathecae absent. One pair of copulatory bulbs of rounded shape in XV–XVII.

Remarks. *Glossoscolex fuchsi* sp. nov. exhibits a similar clitellar position to *G. santarosaensis* Feijoo & Brown, 2023, although they differ in shape (*G. santarosaensis* is annular). The female pores in *G. fuchsi* sp. nov. are distinctly visible in XIV, a characteristic that is challenging to observe in *Glossoscolex* species.

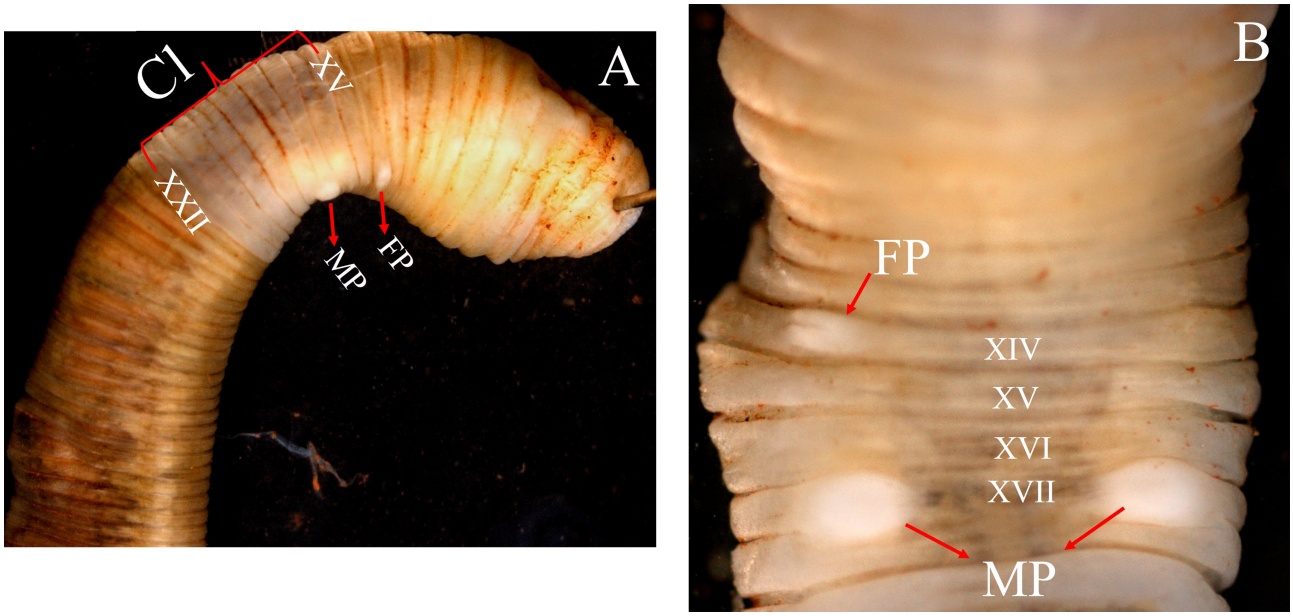

FIGURE 10. *Glossoscolex fuchsi* sp. nov. Holotype. **A.** clitellum in XV–XXII, female pore in XIV, and male pores in XVII. **B.** Ventral view of female and male pores. Cl: clitellum, MP: male pore; FP: female pore.

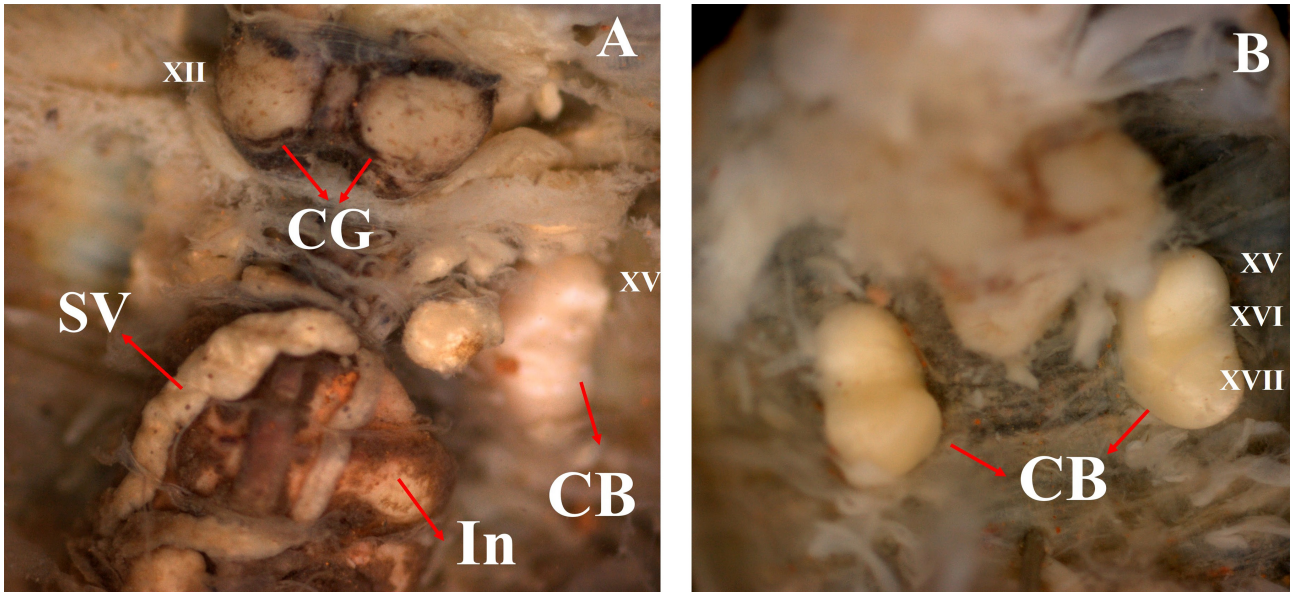

FIGURE 11. *Glossoscolex fuchsi* sp. nov. Holotype. **A.** calciferous gland in XII, seminal vesicles above the intestine, copulatory bulbs and intestine in XV onwards. **B.** Copulatory bulbs in XIII–XV: CG: calciferous gland; SV: seminal vesicle; In: intestine; CB: copulatory bulb.

Glossoscolex (*Glossoscolex*) *bergi* species group (Rosa, 1900)

Type species: *Glossoscolex bergi* (Rosa, 1900).

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

Glossoscolex strobeli Dudas, Brown & Bartz, sp. nov.

(Figures 12 and 13)

Holotype. COFM BRRS0471, adult, annaul crops under no-tillage system, in 0–20 cm soil, by qualitative method, Cruz Alta, Rio Grande do Sul, Brazil, -28.76386, -53.583866, 419 m, 16 August 2023, M.L.C. Bartz coll.

Paratype. COFM BRRS0492, one adult, annual crops under no-tillage system, in 0–20cm soil, by qualitative method, Panambi, Rio Grande do Sul, Brazil, -28,348659, -53,41603, 515 m asl, 12 August 2023, M.L.C. Bartz, W.C. Demetrio colls.

Other materials. COFM BRRS536, two adults and three juveniles, same information as the paratype.

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

External morphology. 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. 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.

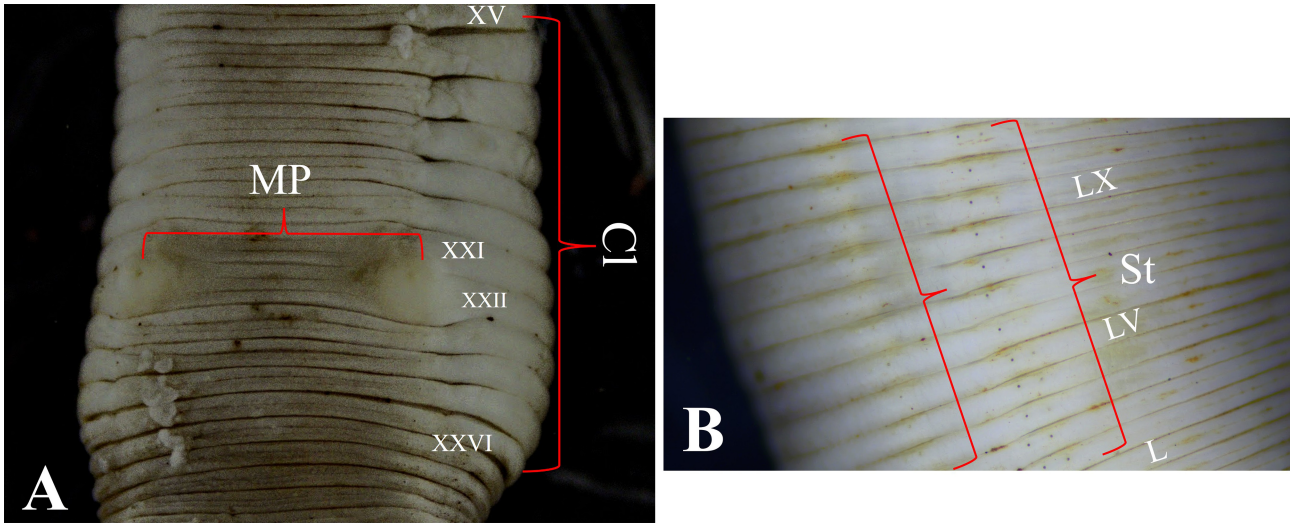

FIGURE 12. *Glossoscolex strobeli* sp. nov. Holotype. **A.** clitellum in XV–XXVI, and male pores in XXI–XXII **B.** Setal arrangement, in L onwards. Cl: clitellum, MP: male pore; St: setae.

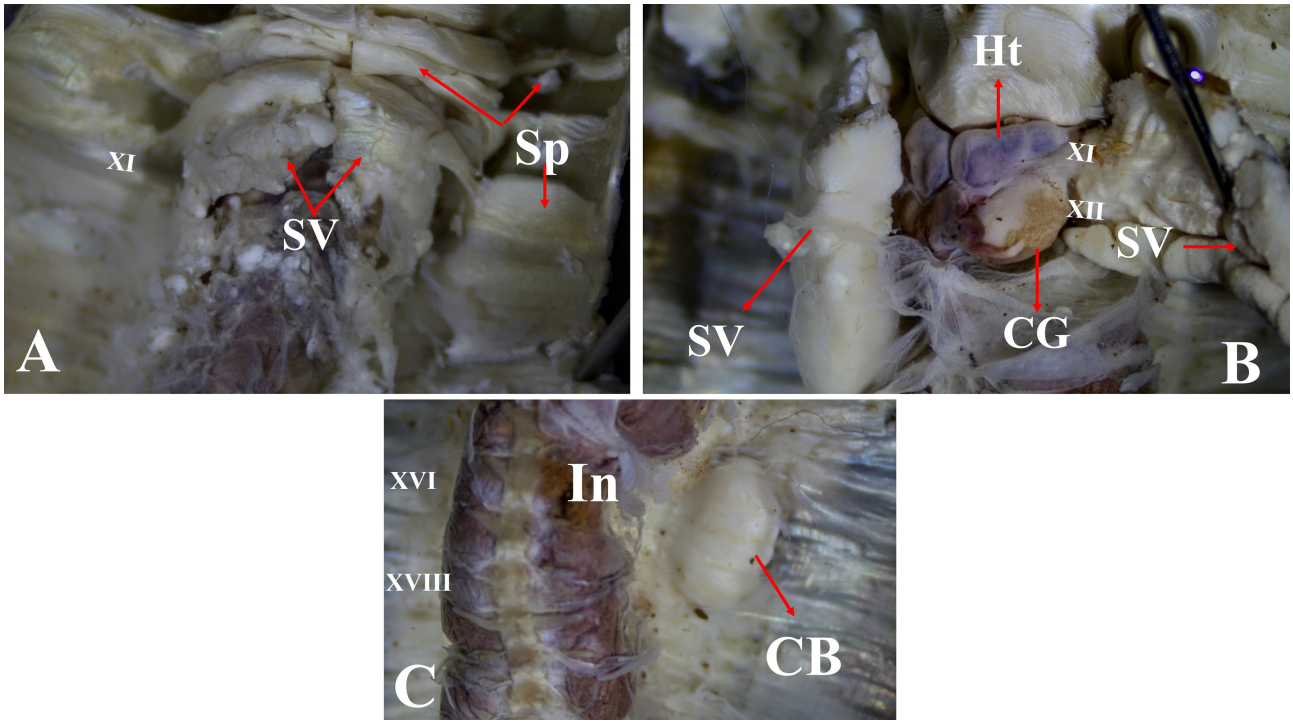

FIGURE 13. *Glossoscolex strobeli* sp. nov. Holotype. **A.** Strong septa, in XI–X, seminal vesicle, in XI–XII. B: Last pair of hearths, in XI, seminal vesicles, and calciferous gland in XII. **C.** Intestine, in XV and the copulatory bulbs, in XVI–XVIII. Sp: septa; SV: seminal vesicle; Ht: hearth; CG: calciferous gland; In: intestine; CB: copulatory bulb.

Internal morphology. Septa membranous 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. Five pairs of hearts, last pair in XI. Holonephridial, starting pre-clitellar in XIII and extending posteriorly; exonephric, stomate, positioned close to the body wall and intestine, tubular, non-vesiculated, whitish, and free in the coelom. Paired testes sacs in XI with visible funnels connected to the seminal vesicles. Seminal vesicles are two white masses starting in XII and ending in XVI. Ovaries in segment XIII, more visible on the right side. Spermathecae absent. One pair of copulatory bulbs of rounded shape in XX–XXIII.

Remarks. *Glossoscolex strobeli* sp. nov. 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* sp. nov. 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). Furthermore, the seminal vesicles in *G. strobeli* sp. nov. occupy only segments XII–XVI similar to *G. bergi* (XII–XV, XVI), but shorter than in *G. grecoi* (XII–XVIII, XIX).

Glossoscolex sp. 77

(Figure 14)

Material. COFM BRRS 0471, one amputated adult, native vegetation, in 0–20 cm soil, by TSBF method, Maracaí, São Paulo, Brazil, -22.668361, -50.880278, 390 m, March 25, 2024, F.M.O. Lourenço, G.A. Ramos, K.B.F. Pepe, M.L.C. Bartz, R.T. Dudas, R. Ralisch, T.M. Kinupp colls.

External morphology diagnosis. 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-pigmented 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. 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 diagnosis. 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, with composite tubular structure. Intestine begining in XV, with typhlosole in the same segment. Five pairs of hearts, with last pair in XI. Holonephridial, starting pre-clitellar in XIII and extending posteriorly; exonephric, stomate, positioned close to the body wall and intestine, tubular, slender, non-vesiculated, whitish, and slightly coiled distally, free in the coelom. Paired testes sacs in XI, previous 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* sp.77 are similar to those of *G. uruguayensis* Cordero, 1943, *G. corderoi* Righi, 1968, *G. riograndesis*, *G. parvus*, *G. pastivus*, and *G. pampas* Bartz & James, 2018. As for the paired testes sacs, in *Glossoscolex* sp.77 they are located only in XI, while the others exhibit variations in form (some in U form), quantity (paired/unpaired), and position (starting in XI and going to XII–XIII). The size of the seminal vesicles is also unique to *Glossoscolex* sp.77 when compared to these other species, occupying XII–XV. Although *Glossoscolex* sp.77 shows similarity in male pore form to *G. arnsi* sp. nov., DNA analyses indicated that they are, in fact, distinct species.

The species name follows a sequential order of potentially new species deposited in the Fritz Muller Oligochaete Collection (COFM). The formal description of this species is awaiting collection of additional material. We provide information on its morphology and genetics here so that it can be compared with other known species in the future.

Genus *Fimoscolex* Michaelsen, 1900

Type species. *Fimoscolex ohausi* Michaelsen, 1900.

Diagnosis. Intraclitellar single male pore associated with or without a single copulatory bulb. Calciferous glands in segment XI or XII.

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

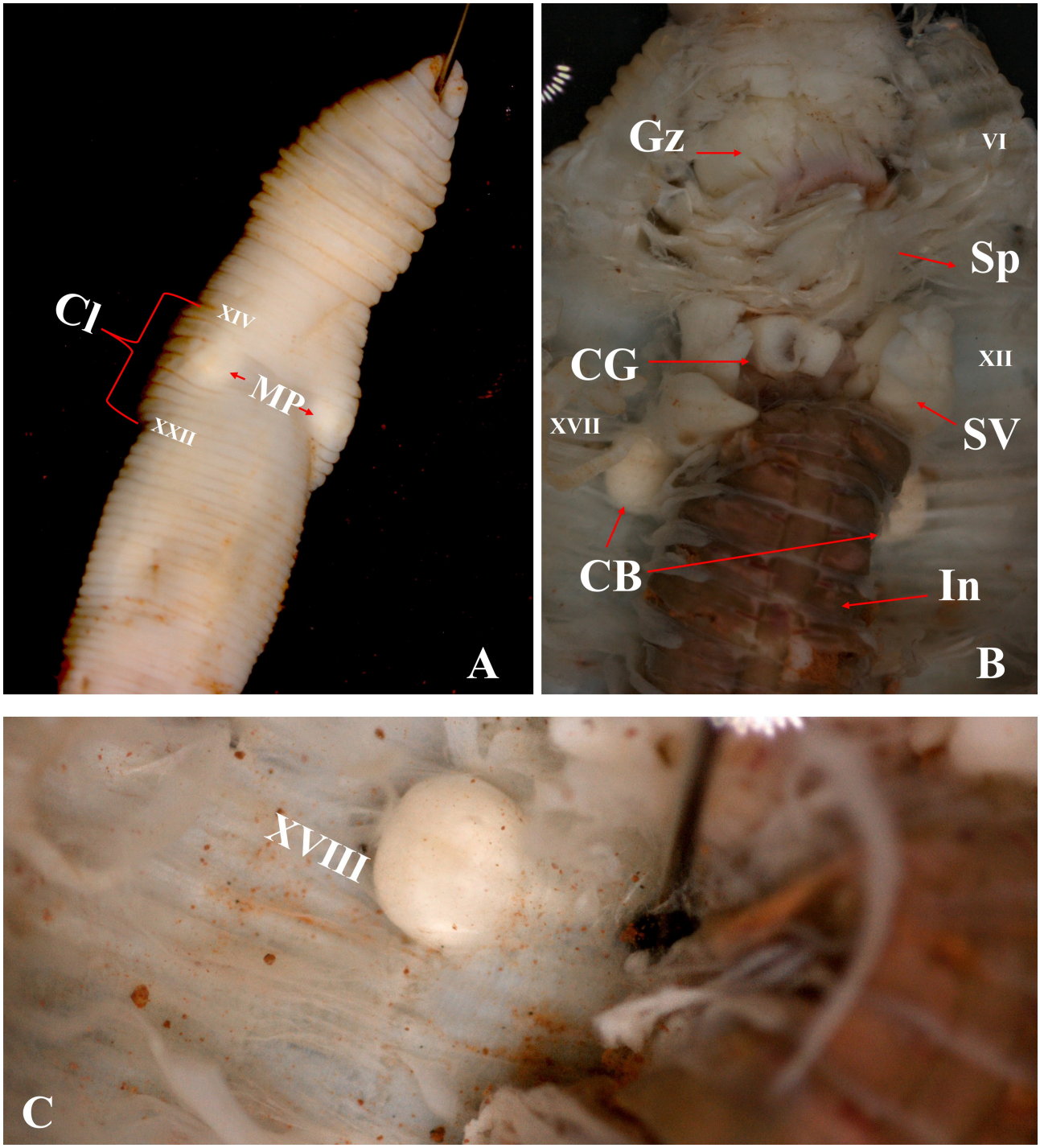

FIGURE 14. *Glossoscolex* sp.77 sp. nov. Holotype. **A**. External ventral view, showing the clitellum, in XV–XXII, and the pair of male pores in XVII. **B**. Internal overview with gizzard in VI, septa, in VII–X, seminal vesicle, in XII–XV calciferous gland in XII. Intestine, in XV and the copulatory 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* Dudas, Brown & Bartz, sp. nov.**
(Figure 15 and 16)

Holotype. COFM BRRS0503, adult, in annual crops under no-tillage, in 0–20 cm soil, by qualitative method, Panambi, Rio Grande do Sul, Brazil, -28.371324, -53.418971, 494 m asl, August 12, 2023, M.L.C. Bartz coll.

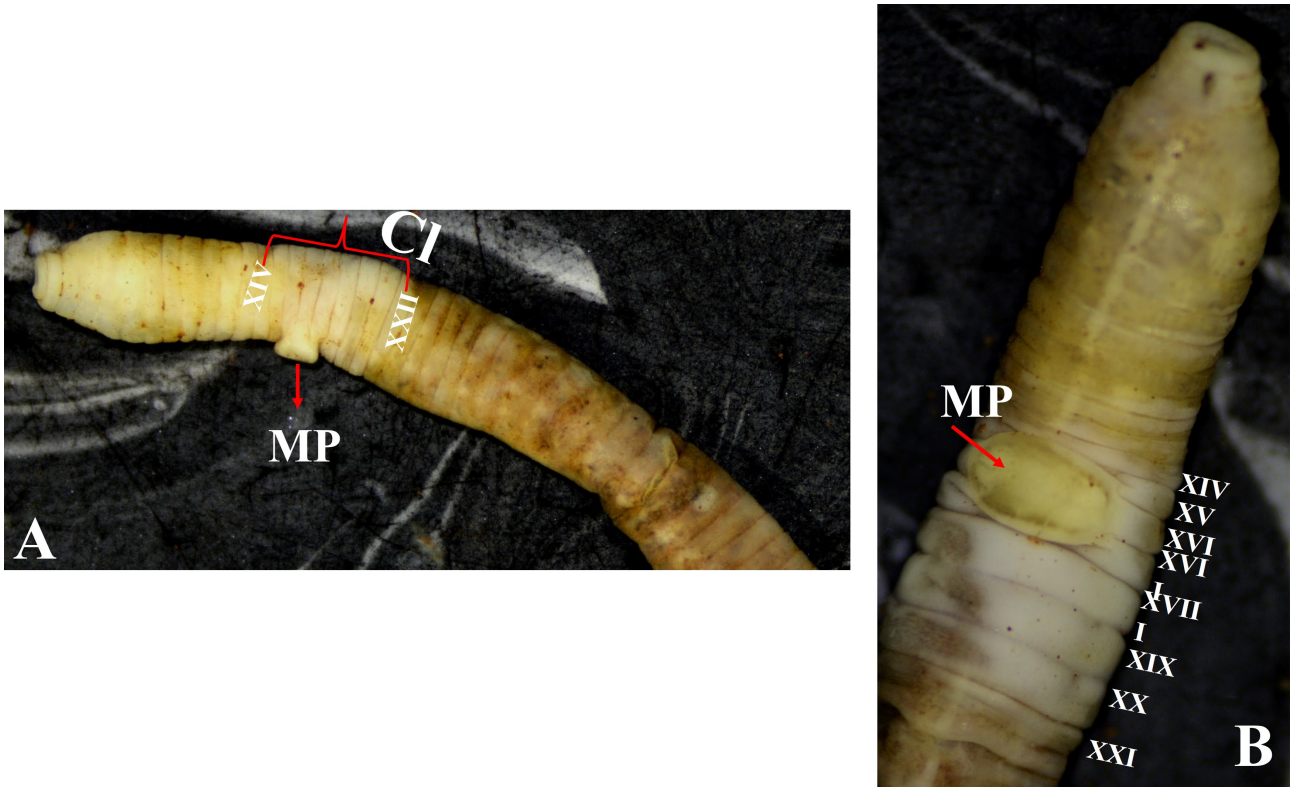

FIGURE 15. *Fimoscolex fridrichi* sp. nov. Holotype. **A.** lateral view of clitellum in XIV–XXII, and male pores in XVI–XVII. **B.** Ventral view male pore. Cl: clitellum, MP: male pore.

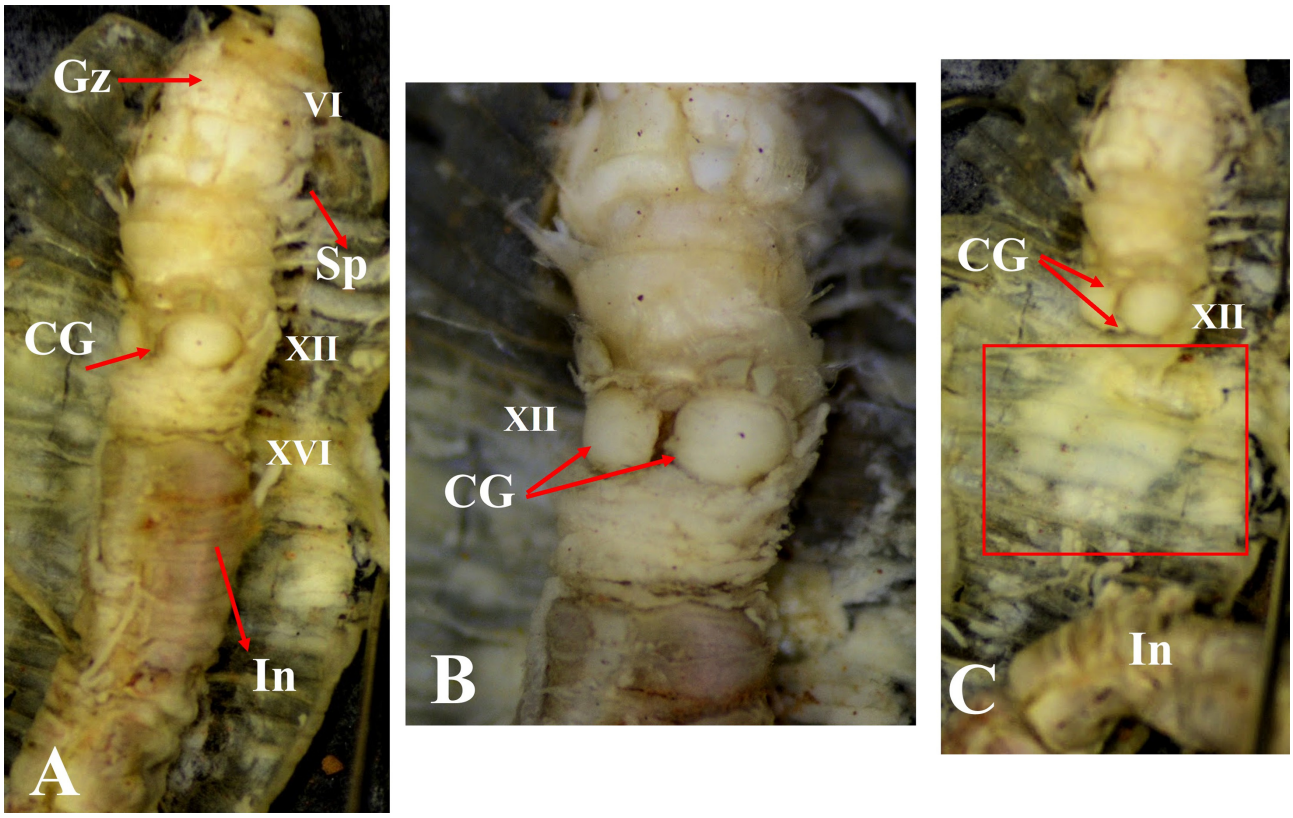

FIGURE 16. *Fimoscolex fridrichi* sp. nov. Holotype. A: Gizzard, in VI, septa in VII–XIX, calciferous gland in XII, and intestine in XV onwards. B: Calciferous gland. C: Copulatory bulbs absent. Inside the red square is the region that is supposed to have a bulb, lighter due the external male pore. Gz: gizzard; Sp: septa; CG: calciferous gland; In: intestine.

Paratype. COFM BRRS0452, one adult, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, -28.144014, -53.777835, 422 m asl, August 14, 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls.

Other materials. COFM BRRS0448, two adults, native vegetation, in 0–20cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, -28.167072, -53.814564, 419 m asl, August 14, 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. COFM BRRS0455, two adults, annual crops under no-tillage, in 0–20 cm soil, by TSBF method, Ajuricaba, Rio Grande do Sul, Brazil, -28.149816, -53.774019, 385 m asl, August 14, 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. COFM BRRS0456, five adults, same information as holotype. COFM BRRS0460, two adults, same information as BRRS0455. COFM BRRS 0483, one adult, same information as holotype. COFM BRRS0495, one adult same information as holotype. COFM BRRS0517, one adult, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Cruz Alta, Rio Grande do Sul, Brazil, -28.76386, -53.583866, 419 m asl, August 16 2023, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls. COFM BRRS0529, two adults, same information as holotype.

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. 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. Clitellum in XV–XXII saddle shaped. Genital marks or tubercula pubertatis absent. Female pore not seen. One male pore in XVI/XVII. Nephropores near *B* line.

Internal morphology. 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 of composite tubular internal shape. Intestine begins in XVI, with typhlosole commencing in the same segment. Five pairs of hearts, with last pair in XI. Paired testes sacs in XI under the beginning of the calciferous glands. Seminal vesicles from XII–CXXVII 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 *Fimoscolex fridrichi* sp. nov. is only similar to *F. nivae* Feijoo & Brown, 2018—XIV–XXI–, although in *F. nivae* it is saddle shaped, while in *F. fridrichi* sp. nov. 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* sp. nov. 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* sp. nov. does not have any.

Fimoscolex sp.51 Dudas, Brown & Bartz

(Figure 17)

Material. COFM BRPR2149, one adult, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Mangueirinha, Paraná, Brazil, -26.050852, -52.237767, 981 m asl, August 08, 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls.

COFM BRPR2151, one adult amputee, annual crops under no-tillage system, in 0–20 cm soil, by TSBF method, Mangueirinha, Paraná, Brazil, -26.051079, -52.237655, 981 m asl, August 08, 2023, G.G. Brown, G.C. Francisco, M.L.C. Bartz, N. Durães, R.T. Dudas, R. Roani, T. Ferreira, W.C. Demetrio colls.

External morphology diagnosis. Body length 33 in one individual and 39 mm in the other, after ethanol fixation. Number of segments: 97 in one individual and 164 in the other. Diameter: 1.3 mm in one individual and 1.4 mm in the other, in the pre clitellar region (segment X). 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. 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.

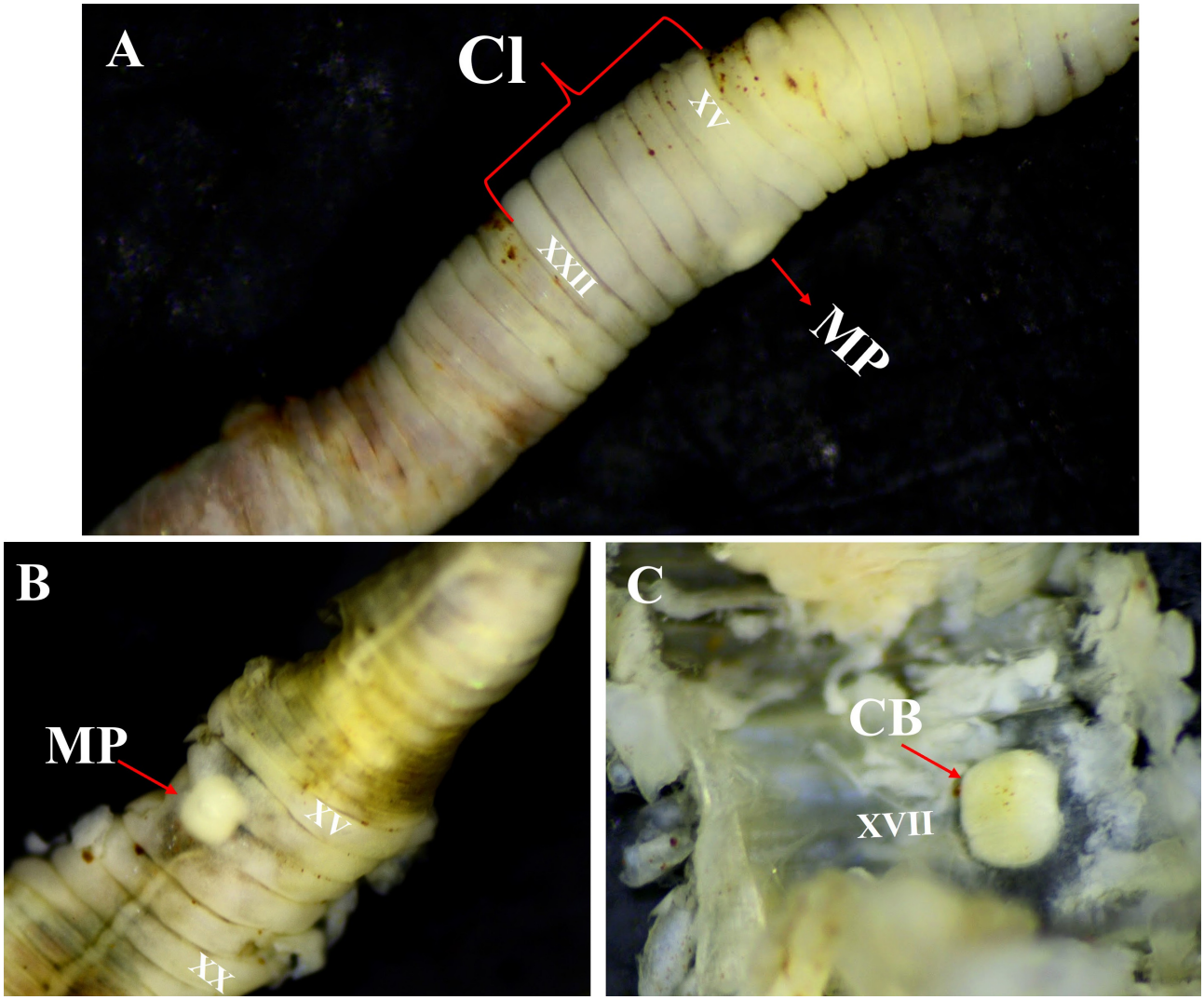

FIGURE 17. *Fimoscolex* sp.51. Holotype. **A.** External lateral view of the clitellum (Cl) and the single male pore (MP). **B.** External ventral view with emphasis in the male pore. **C.** Internal view showing the copulatory bulb (CB).

Internal morphology diagnosis. Septa strong and membranous in 7/8/9. Gizzard in VI. One pair of calciferous glands in XII of composite tubular structure. Holonephridial, exonephric, stomate, positioned close to the body wall and intestine, non-vesiculated, whitish, and slightly recurved, free in the coelom. Intestine begining in XV, with typhlosole commencing in the same segment. Five pairs of hearts, with last pair in XI. Paired testes sacs in XI under the end 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* sp.51 exhibits morphological features similar to *F. bartzi* Bartz & James, 2012, however, due to the absence of an ovisac, the geographical distance between the species, and the lack of DNA sequences from both species, we consider this as a separate *Fimoscolex* species for the meantime. *Fimoscolex* sp.51 has similar clitellum location (XV–XXI) to *F. angai* Righi, 1971, *F. inurus* Cognetti, 1913, and *F. tairim* Righi, 1974, although the latter has an annular shape instead of a saddle. The difference between *Fimoscolex* sp.51 and *F. angai* is the position of the seminal vesicles (XII–XV in the former species), while in *F. angai*, they are long strips in XII–XIX, XXI. 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* sp.51 the bulb occupies only XVII.

The species name follows a sequential order of potentially new species deposited in the Fritz Muller Oligochaete Collection (COFM). The formal description of this species is awaiting availability of molecular data from *Fimoscolex bartzi* for comparative purposes.

Discussion

The subgenus *Glossoscolex (Glossoscolex)* was initially divided into four main species groups based on the position of the male pores (Righi and Lobo 1979). Species with pores in segment XVII belong to the *G. truncatus* group, while those with pores in XVIII/XIX form the *G. giganteus* group. Pores in XIX/XX characterize the *G. grandis* group, while pores in XX/XXI or XXI/XXII define the *G. bergi* group. More recently, Feijoo and Brown (2023) proposed an additional group, *G. matogrossensis*, characterized by intraclitellar male pores in XV/XVI.

Five of the six new *Glossoscolex* species described here belong to the *G. truncatus* group. However, they are clearly distinguished from previously described species of the genus by a combination of morphological and molecular characters, as summarized in Table 2. Of the 58 known *Glossoscolex* species, 33 belong to the *truncatus* group (including the new species described here), with a distribution range encompassing Argentina, Paraguay, and the Southeast and South regions of Brazil (Feijoo and Brown 2023). The first author to define the *truncatus* group was Michaelsen (1918) and this was later refined by Righi (1978) and Righi and Lobo (1979).

Glossoscolex strobeli sp. nov., is a new addition to the only two other species of the *G. bergi* group: *G. bergi* and *G. grecoi* (Feijoo and Brown 2023). This new addition extends the geographic distribution of this group to Rio Grande do Sul. Previously, *G. bergi* had been recorded in the western region of Paraná near Foz do Iguaçu in Brazil and Puerto Presidente Franco in Paraguay (Zicsi and Csuzdi 1987), and Misiones Province in Argentina (Rosa 1900; Genise *et al.* 2013). On the other hand *G. grecoi* is known only from three closely-located sites in the central-eastern region of São Paulo state (Righi and Lobo 1979).

Regarding *Fimoscolex*, it is noteworthy to emphasize that the absence of a copulatory chamber in the newly identified species *F. fridrichi* sp. nov. 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.

The DNA analyses confirm that all species described here represent distinct taxa when compared both with each other and with previously described ones (Figure 18, Table 3). Pairwise distances were calculated using the Kimura 2-parameter (K2P) model, which is the standard for barcoding comparisons. The lowest interspecific distance was 0.08 between *G. alessioi* sp. nov. and *G. dallavecchiai* sp. nov., a value that lies on the lower end of typical interspecific divergence in earthworms. This result supports their close relationship but also validates their separation, consistent with the morphological differences observed in the form and extension of the seminal vesicles and copulatory bulbs. Distances among the remaining new species were higher (0.14–0.31; Table 3), further supporting their recognition as distinct species. In earthworms, interspecific COI divergences are usually much higher than in other invertebrates, with thresholds around 9–14% being commonly reported (Decaëns *et al*. 2013; Marchán *et al*. 2022; Goulpeau *et al*. 2022).

Sequences from *E. andrei* (Lumbricidae) and *P. corethrurus* (Rhinodrilidae) included as outgroups, provided external reference points for tree rooting and illustrating divergence across families. In addition, inclusion of *G. riograndensis* and *Fimoscolex* sp.25 (GenBank sequences) highlighted genetic differences between the newly described taxa and previously available congeners. Comparisons showed that divergences between the new *Glossoscolex* species and *G. riograndensis* were consistently high (0.20–0.24), while divergences with *Fimoscolex* sp.25 exceeded 0.22, in line with the morphological distinctions in the genera. The phylogenetic tree also indicates the close affinity between *Glossoscolex* and *Fimoscolex*, while still resolving clear distinctions among the new taxa (Figure 18). This confirms the value of combining molecular and morphological evidence for accurate species delimitation in these two genera. Nonetheless, broader molecular studies remain necessary, including additional markers beyond COI and the incorporation of the type species of each genus, subgenera, and species groups within *Glossoscolex* (*Glossoscolex*). In particular, genetic information for *F. bartzi* is urgently needed to clarify its relationship with *Fimoscolex* sp.51.

TABLE 2. Comparison of characters of the new species *Glossoscolex arnsi* n.sp., *Glossoscolex alessioi* n.sp., *Glossoscolex dallavecchiai* n.sp., *Glossoscolex debortoli* n.sp. *i, Glossoscolex strobeli* n.sp., *Glossoscolex* sp.77, *Glossoscolex fuchsi* n.sp., *Fimoscolex fridrichi* n.sp. and *Fimoscolex* 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*—in the bergi group—*Glossoscolex bergi* and *Glossoscolex grecoi*—and in 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 1/2–3 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
Glossoscolex	*angai 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
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 strips**	**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**

.....continued on the next page

TABLE 2. (Continued)

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	***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 strips**	**pair, bean shaped, 1/3 16–1/3 19**
Glossoscolex	**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 strips**	**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
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, starting in 12	one bulb, 16–17
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	**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, ventral strips**	**single, 17**

*type specie of each group/genus

TABLE 3. Estimates of pairwise genetic distances Kimura 2-parameter model between the sequences of *Glossoscolex arnsi* n.sp., *Glossoscolex alessioi* n.sp., *Glossoscolex dallavecchiai* n.sp., *Glossoscolex debortolii* n.sp., *Glossoscolex strobeli* n.sp., *Fimoscolex* sp.51 and *Fimoscolex fridrichi* n.sp., the already described *Glossoscolex riograndensis,* the undescribed *Fimoscolex* sp.25 and the out-groups *Eisenia andrei* and *Pontoscolex corethrurus*.

	Eisenia andrei	*Pontoscolex corethrurus*	*Glossoscolex arnsi*	*Glossoscolex alessioi*	*Glossoscolex dallavechiai*	*Glossoscolex debortolii*	*Glossoscolex strobeli*	*Fimoscolex* sp.51	*Fimoscolex fridrichi*	*Glossoscolex riograndensis*	*Fimoscolex* sp.25
Eisenia andrei											
Pontoscolex corethrurus	0.24										
Glossoscolex arnsi	0.30	0.27									
Glossoscolex alessioi	0.29	0.28	0.22								
Glossoscolex dallavechiai	0.30	0.27	0.20	0.08							
Glossoscolex debortolii	0.27	0.30	0.22	0.17	0.18						
Glossoscolex strobeli	0.30	0.32	0.20	0.15	0.14	0.17					
Fimoscolex sp.51	0.31	0.30	0.23	0.22	0.25	0.25	0.25				
Fimoscolex fridrichi	0.29	0.31	0.25	0.31	0.28	0.31	0.30	0.27			
Glossoscolex riograndensis	0.26	0.29	0.20	0.23	0.23	0.19	0.24	0.22	0.26		
Fimoscolex sp.25	0.29	0.29	0.27	0.29	0.29	0.25	0.27	0.25	0.29	0.27	

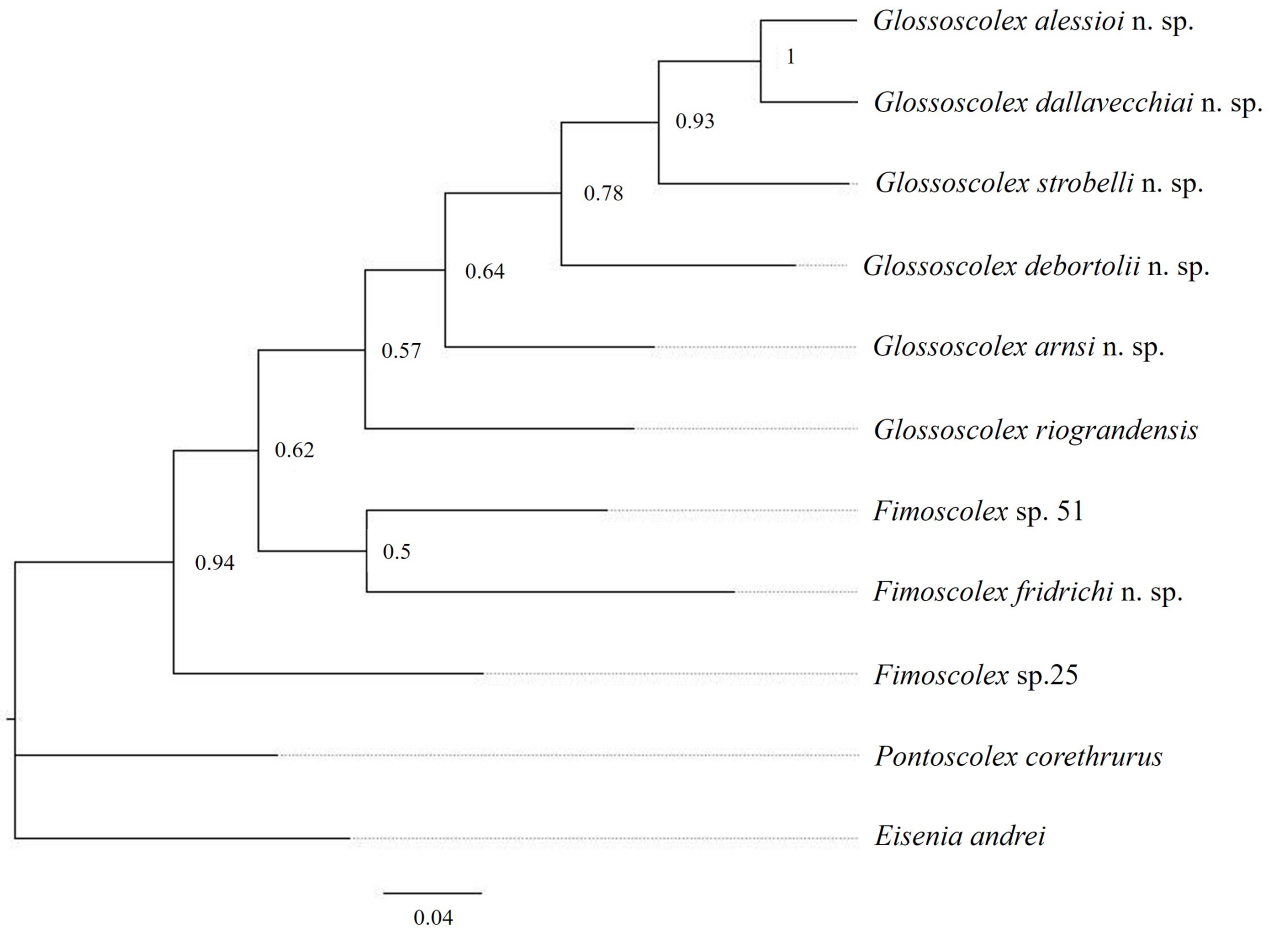

FIGURE 18. Evolutionary analysis of most of the new species described here using the citochrome oxidase 1 (COI) genetic marker (barcode) and the maximum likelihood method. Barcode distances are shown inset. *Glossoscolex riograndensis* and *Fimoscolex* sp.25 were used for comparison with the new species; *Eisenia andrei* and *Pontoscolex corethrurus* were used as out-grouping species.

Specimens of both *Glossoscolex* and *Fimoscolex* have been observed in planted forest areas (da Silva *et al*. 2019), native vegetation (Bartz *et al.* 2023), lawns (Dudas *et al.* 2023a,b), and annual crops like the many no-tillage farm fields where the new native species were collected here and by others previously (Demetrio *et al.* 2020; Bartz *et al*. 2012; 2013; 2014; 2024). Here, we reported these species also from a pasture in Mato Grosso do Sul and native vegetation in four Brazilian states (São Paulo, Paraná, Santa Catarina and Rio Grande do Sul). 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).

Conclusion

We describe seven new species of the Glossoscolecidae family discovered during three sampling campaigns conducted in the Cerrado and Atlantic Forest biomes. This finding confirms the substantial number of unknown earthworm species in these regions, and their presence in many no-tilled agricultural fields, especially in Southern Brazil. 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, additional information on soil and environmental attributes is important to guarantee adequate preservation of these new species in their habitats.

Glossoscolex and *Fimoscolex* exhibit morphological and genetic similarities, but further integrated taxonomic methodologies, such as sequencing of multiple loci, and ecological knowledge on these genera represent important

approaches towards a more comprehensive knowledge on the taxonomic, biogeographic and evolutionary standing questions related to these earthworms commonly found in Southern and Southeastern Brazil.

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