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New earthworm species of *Righiodrilus* (Clitellata, Glossoscolecidae) from eastern Amazonia

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Abstract

Three new species of the glossoscolecid earthworm genus *Righiodrilus* are described from material collected in northern Pará and Maranhão States, Brazil. *Rhigiodrilus gurupi* **n. sp.** is characterized by four pairs of post-testicular spermathecae in xiv–xvii. *Rhigiodrilus viseuensis* **n. sp.** is distinguished by tubercula pubertatis in xix–xxiii and clitellum in xvi–xxiii. *Rhigiodrilus moju* **n. sp.** is the only species in the genus that lacks tubercula pubertatis. We provide an updated key and a distribution map for all species of *Righiodrilus*.

Key words: Haplotaxida, Oligochaeta, Annelida, taxonomy, Amazonia, geographic distribution

Introduction

Righiodrilus Zicsi, 1995 includes 27 species, which are distributed principally in South America's Amazonia region. An important compilation of species descriptions for this genus was made by Righi (1995) who placed all of them in *Glossodrilus*. The same year, in a revision of *Glossodrilus*, Zicsi (1995) erected the new genus *Righiodrilus* for species of *Glossodrilus* that possess a membranous reservoir on the surface of the calciferous gland, visible as a white area due to the calcium carbonate granules within. This reservoir is absent in *Glossodrilus*, and the structure of the calciferous gland is the only difference between the two genera (Zicsi 1995). *Righiodrilus* is distributed in Brazil, Ecuador, Colombia and Venezuela (Celis & Rangel 2015). Feijoo and Gil (2010) included three new species and presented a key for the species of *Righiodrilus*. In 2015, another new species was found in Colombia (Celis & Rangel 2015).

The Belém Center of Endemism (Da Silva *et al*. 2005) covers 243.000 km^2 in the western Brazilian Amazon, divided between eastern Pará State and western Maranhão State; it includes 147 municipalities, 62 in Pará and 85 in Maranhão (Almeida & Vieira 2010). Our work in the Belém Center of Endemism is to explore the earthworm diversity and to find new species. The many areas of endemism in the Amazon basin mean that biodiversity is geographically structured even in a region of relatively low topographic complexity (Da Silva *et al*. 2005). In earthworms, there are indications that their diversification is driven in part by low rates of dispersal and strong niche limitation (James & Brown 2006).

In this work we describe three new species of *Righiodrilus* collected from different locations within the Belém center of endemism and we provide an updated key and a distribution map for the species of the genus.

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Material and methods

Earthworms were collected using the TSBF method (Anderson & Ingram 1993); the monoliths had surface dimensions of 25x25 cm, a depth of 20 cm, and were subdivided into three layers, litter, 0–10 cm and 10–20 cm. Collected specimens were then fixed and conserved in 95% alcohol.

Thirty specimens were examined in this study. Descriptions and nomenclature follow Feijoo and Gil (2010). Measurements were taken with an eyepiece reticle attached to a Leica MZ 16 stereomicroscope and are presented in millimeters. Illustrations and photographs were made using a drawing tube and phototube, respectively, connected to theLeica MZ 16. Multiple focal plane images were stacked with Auto-Montage Pro 5.03.0061. The holotypes and twenty-seven paratypes are deposited at the Museu Paraense Emílio Goeldi, Belém, Pará, the accession numbers beginning with MPEG.

The map was established using the known records catalogued in Brown & James (2007), Feijoo & Gil (2010), and Celis & Rangel (2015).

Results and discussion

Genus *Righiodrilus* Zicsi, 1995

Type species. *Glossodrilus tico* Righi, 1982

Diagnosis (from Feijoo & Gil 2010). Setae in viii regular longitudinal lines, generally closely arranged and rarely paired, widely arranged. Gizzard in vi. One pair of intraclitellar male pores. Female pores in pairs or rarely single. One pair of calciferous glands in xi or xii with composite tubular structure and translucent membrane, which extends over two or three segments. Sexual system metandric and metagynic; seminal vesicles long in general. Spermathecae present, rarely absent, usually without seminal chamber or diverticula.

Righiodrilus gurupi Hernández-García, Rousseau & James, n. sp.
(Figure 1)

Holotype. MPEG.ANL 000286, adult. Centro Novo, PPBio Gurupi Norte, Maranhão, Brazil, primary forest, 3°41'S 46°45'W, 189 masl. 20 March 2015, Hernandez-Garcia, L.M. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000294 one adult, MPEG.ANL 000290 two adults, primary forest, MPEG.ANL 000287 two adults, MPEG.ANL 000288 three adults, MPEG.ANL 000289 one adult, MPEG.ANL 000291 one adult, MPEG.ANL 000292 one adult, primary forest disturbed, same locality as holotype. MPEG.ANL 000296 one adult, mature forest, MPEG.ANL 000295 two adults, secondary forest, Alcântara, Maranhão, Brazil, 2°21'56.3"S 44°27'10.5"W, 54 masl, 19 May 2015. Hernandez-Garcia, L.M., Burgos J.E & Rousseau G.X. colls.

Etymology. The species is named for the region where it was found, Gurupi.

Description. Holotype 65 mm by 3.0 mm at x, 3.4 mm at clitellum, 3.0 mm at xxx, 273 segments; paratypes 59–72 mm by 2.0–2.9 mm at x, 3.0–3.8 mm at clitellum and 2.0–2.9 mm at xxx, 205–267 segments. Body cylindrical and slightly flattened in clitellar region. Setae commence on ii. Setae widely paired throughout; setal formula *aa:ab:bc:cd*:*dd* = 0.6:0.6:0.5:0.4:0.8 at xxx. Prostomium prolobous open. Unpigmented. Clitellum saddle to just above b, xvi–xxiii, tubercula pubertatis xvii–xxi (Fig. 1A). Ovipores equatorial just medial to *a* setae and 0.9 mm apart on xiv; male pores 2.8 mm apart on xviii/xix within paired bands of tubercula pubertatis with slight long groove and associated to genital setae (Fig. 1C). Drawings of setae (Fig. 1B,C) show the size difference between somatic and genital setae, there is no difference in structure or their surface. Nephropores just above *b* setal line.

Septa 6/7 membranous, 7/8–10/11 equally thick and muscular, others membranous. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in chevron pattern vii–ix, valvular in xiv, intestinal origin xv; typhlosole origin xvi, xvii–xxix cupped folds, transformed to bifid lamina near xxx, completly flat bifid by xxxiv and non-bifid by cxxx. Calciferous glands paired in xii, under seminal vesicles, composite-tubular type, oval; supra-esophageal blood vessels running to the calciferous gland from basal to anterior part (Fig. 1D). Calcium carbonate reservoir conical and latero-dorsal (Fig. 1D: "M"). Holonephric, vesiculate (Fig. 1F); nephridial

ducts to body wall near level of *b* setae. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x–xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; four pairs of small lenticular spermathecae sacs are present near *b* setal line in xiv–xvii, 350x500 µm in size (Fig. 1E). Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; seminal vesicles expanded in xii, penetrate septa and range posteriorly along intestine to xiv; seminal vesicles lobulated occupying xi to xiv; vasa deferentia on body wall at *b* setal level en route to enter body wall in segment xviii/xix.

Remarks. *R. gurupi* **sp. nov.** differs from other members of the genus in the presence of four pairs of post-testicular spermathecae in xiv–xvii. Spermathecae are pre-testicular in species with 1, 2 or 3 pairs, or spermathecae are absent. The only other *Righiodrilus* species with more than 3 pairs of spermathecae is *R. sucunduris* (Righi, Ayres & Bittencourt, 1976) with six pairs in ix–xi, 2 pairs per segment.

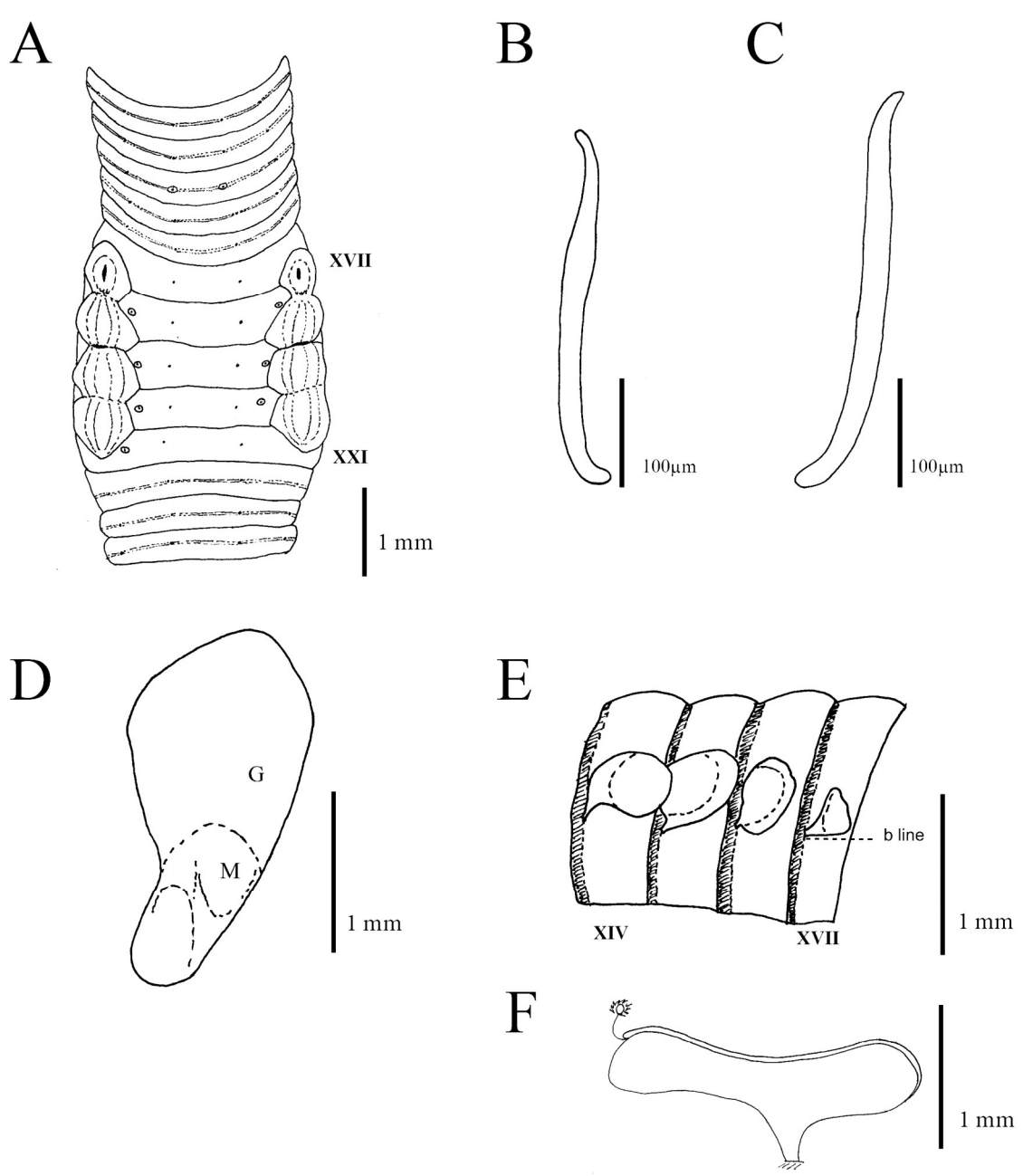

FIGURE 1. *Righiodrilus gurupi* **n. sp. A.** Ventral view of clitellar region. **B.** Somatic setae A of xxi. **C.** Genital setae A of xvii. **D.** Ventral view of calciferous gland of xii; m: membranous region; g: glandular region. **E.** Ventro-lateral view of spermathecae. **F.** Nephridium as seen in xiv–xvii.

***Righiodrilus viseuensis* Bartz, Santos & James, n. sp.**
(Figure 2)

Holotype. MPEG 000258, adult. Type locality. Viseu, Pará, tropical rain forest perturbed, 1°35'15.37" S, 46°16'40.18"W,47 masl. 14–18 August 2014, Santos, BTS; Aguiar-Neto, M & Barros, L colls.

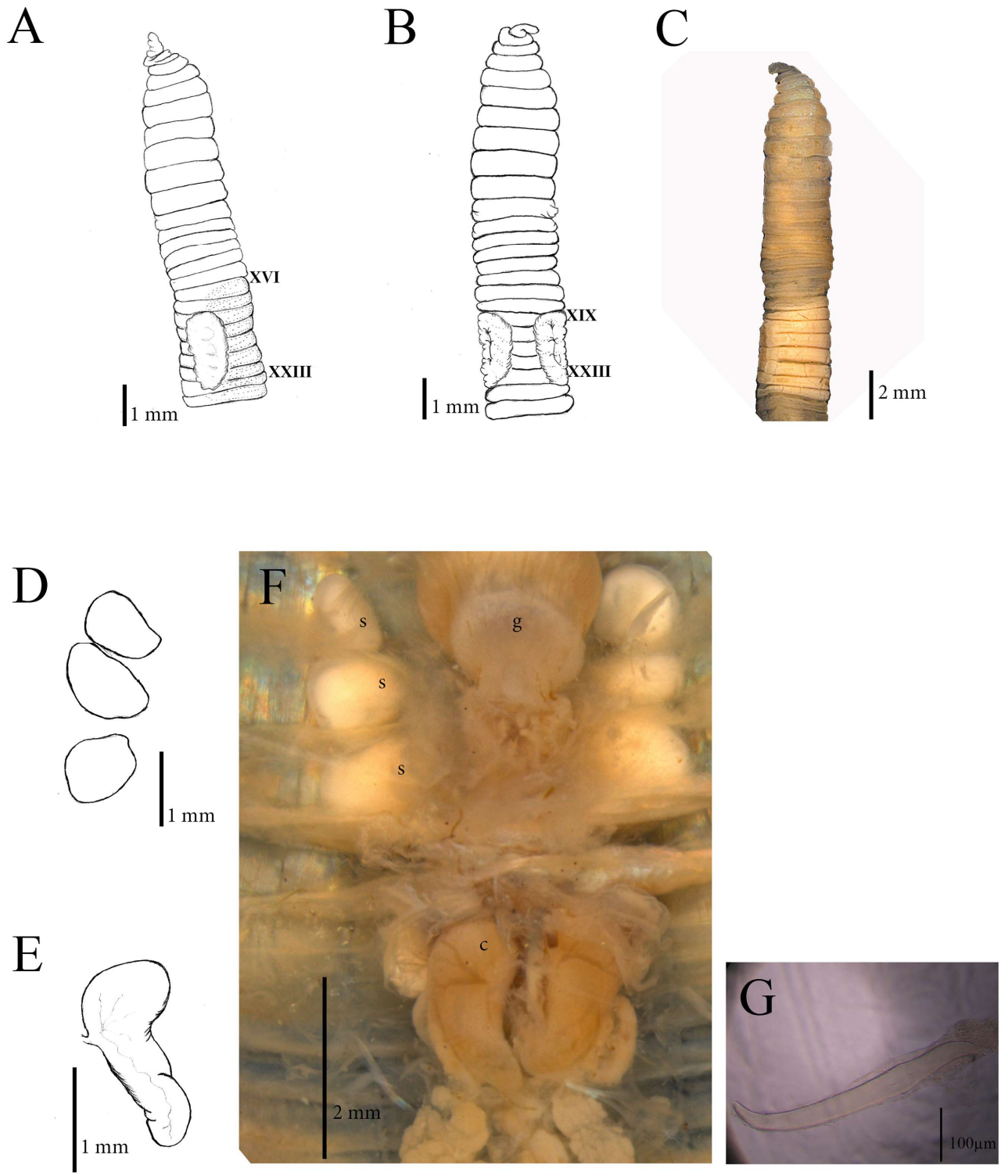

FIGURE 2. *Righiodrilus viseuensis* **n. sp**. **A.** Lateral view of anterior body with clitellum. **B.** Ventral view of anterior body with clitellum. **C.** Photography of lateral view of anterior body with clitellum. **D.** Spermathecae of vi–viii. **E.** Dorsal view of calciferous gland of xii. **F.** Photography of dorsal view showing gizzard (segment vi), spermathecae (segments vi, vii and viii) and calciferous gland (segment xii); g=gizzard, s=spermathecae, c=calciferous gland. **G.** Somatic seta A of xv.

Paratypes. MPEG 000248 one adult, MPEG 000252 three adults, MPEG 000260 two adults. Same locality as holotype.

Etymology. The species is named for the region where it was found.

Description. Holotype 95 mm by 3 mm at x, 4 mm at clitellum, 4 mm at xxx, 284 segments; paratypes 92.25 mm by 4.5 mm at x, 4.5 mm at clitellum and 4.25 mm at xxx, 241 segments. Body cylindrical and slightly flattened. Setae *ab* commence on iii. Setae closely paired throughout; setal formula *aa:ab:bc:cd:dd* = 0.7:0.7:0.5:0.6:2 at xxx. Prostomium tentacular, post-setal secondary annulations present viii–xiv and after clitellum (Fig. 2C). Unpigmented, clitellum whitish. Ovipores in *b* 0.7 mm apart on xiv; male pores 3 mm apart on xix within paired oval dimpled porophores; clitellum saddle to just above *b*, xvi–xxiii (Fig. 2A); tubercula pubertatis in xix–xxiii (Fig. 2A,B). Nephropores within *bc*.

Septa 9/10, 10/11 and 11/12 thin; 6/7, 7/8 and 8/9 equally thick and muscular. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in chevron pattern vii–xi, valvular in xiv, intestinal origin xv; typhlosole origin xvi, xvii–xxxiii simple lamina in zig-zag with ventral edge bent over to form pockets, simple lamina xxxiv–xliv, after xliv becoming triple lamina. Calciferous glands paired xii, under seminal vesicles, composite-tubular type, comma-shaped, pedunculated (Fig. 2E); blood vessels to gland include large branch of dorsal vessel to approximate center of each gland. Calcium carbonate reservoir shape conical. Holonephric, vesiculate; nephridial ducts to body wall near level of *b* setae.

Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x–xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard, esophagus back to calciferous glands; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; three pairs of spermathecae in segments vi, vii and viii; spermathecae rounded and convex (Fig. 2D,F), size 1.24x0.23mm to 0.90x0.12mm. Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; medial to hearts of xi pass narrow tubes to seminal vesicles; seminal vesicles expanded in xii, penetrate septa and range posteriorly along intestine to xx; seminal vesicles lobulated occupying xii to xx; vasa deferentia on body wall at *b* en route to enter body wall in segment xix.

Remarks. The new species is unique among members of the genus for having the tubercula pubertatis in xix–xxiii and clitellum in xvi–xxiii. *Righiodrilus viseuensis* **n. sp.** is similar to *R. tinga* (Righi, 1971) and *R. schubarti* (Righi, Ayres & Bittencourt, 1978) in the number and position of the spermathecae. *R. schubarti* differs from the new species by the position of the clitellum at xv–xxiv, and *R. tinga* differs from the new species by male pores in xvii/xviii. *R. viseuensis* **n. sp**. is also similar to *R. tico* (Righi, 1982) by having the male pores in 1/2 xix; the latter species differs by the position of tubercula pubertatis in 1/2 xix–1/2 xxi.

Righiodrilus moju Bartz, Santos & James, n. sp.

(Figure 3)

Holotype. MPEG 000278, adult. Type locality. Moju-Pará, habitat tropical rain forest perturbed, 2°10”40.76”S, 48°47'36.72”W, 31 masl. 14–18 August 2014, Santos, BTS & team colls.

Paratypes. MPEG 000285, four adults. Same locality as holotype.

Etymology. The species is named for the region where it was found.

Description. Holotype 86 mm by 5 mm at x, 6 mm at clitellum, 4 mm at xxx, 230 segments; paratypes 67 mm by 4.2 mm at x, 4.3 mm at clitellum and 3.5 mm at xxx, 196 segments. Body cylindrical and slightly flattened. Setae *ab* commence on iii. Setae closely paired throughout; setal formula *aa:ab:bc:cd:dd* = 0.7:0.6:0.6:0.6:1 at xxx. Prostomium tentacular, post-setal secondary annulations present viii–xiv and after clitellum. Unpigmented, clitellum whitish. Ovipores in *b* setal lines 0.7 mm apart on xiv; male pores 3.5 mm apart on xx within paired oval dimpled porophores; clitellum saddle to just above *b*, xvi–xxiii (Fig. 3B); Four pairs of intumescences on seta c in segments xviii–xxi (Fig. 3A,B). Tubercula pubertatis absent. Nephropores between *bc*.

Septa 6/7,7/8, 8/9, 9/10, 10/11 equally thick and muscular, septum 11/12 absent. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in radial pattern vii–xii, valvular in xiv, intestinal origin xv; typhlosole origin xvi–xxx simple lamina, xix–xxviii, zig-zag with ventral edge bent over to form pockets, after xxix becoming triple lamina. Calciferous glands paired xii (Fig. 3E,F), under seminal vesicles, composite-tubular type, comma-shaped, not pedunculated; blood vessels to gland include large branch of dorsal vessel to approximate

center of each gland. Calcium carbonate reservoir shape conical. Holonephric, vesiculate; ducts to body wall near level of *b* setae. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x–xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard, esophagus back to calciferous glands; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; two pairs of spermathecae in segments ix and x (Fig. 3C,D); spermathecae cylindrical and elongate, ca. 3 mm long. Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; medial to hearts of xi pass narrow tubes to seminal vesicles; seminal vesicles lobulated expanded in xii, penetrate septa and range posteriorly along intestine to xii–xvi; vasa deferentia on body wall between *bc* en route to enter body wall in segment xx.

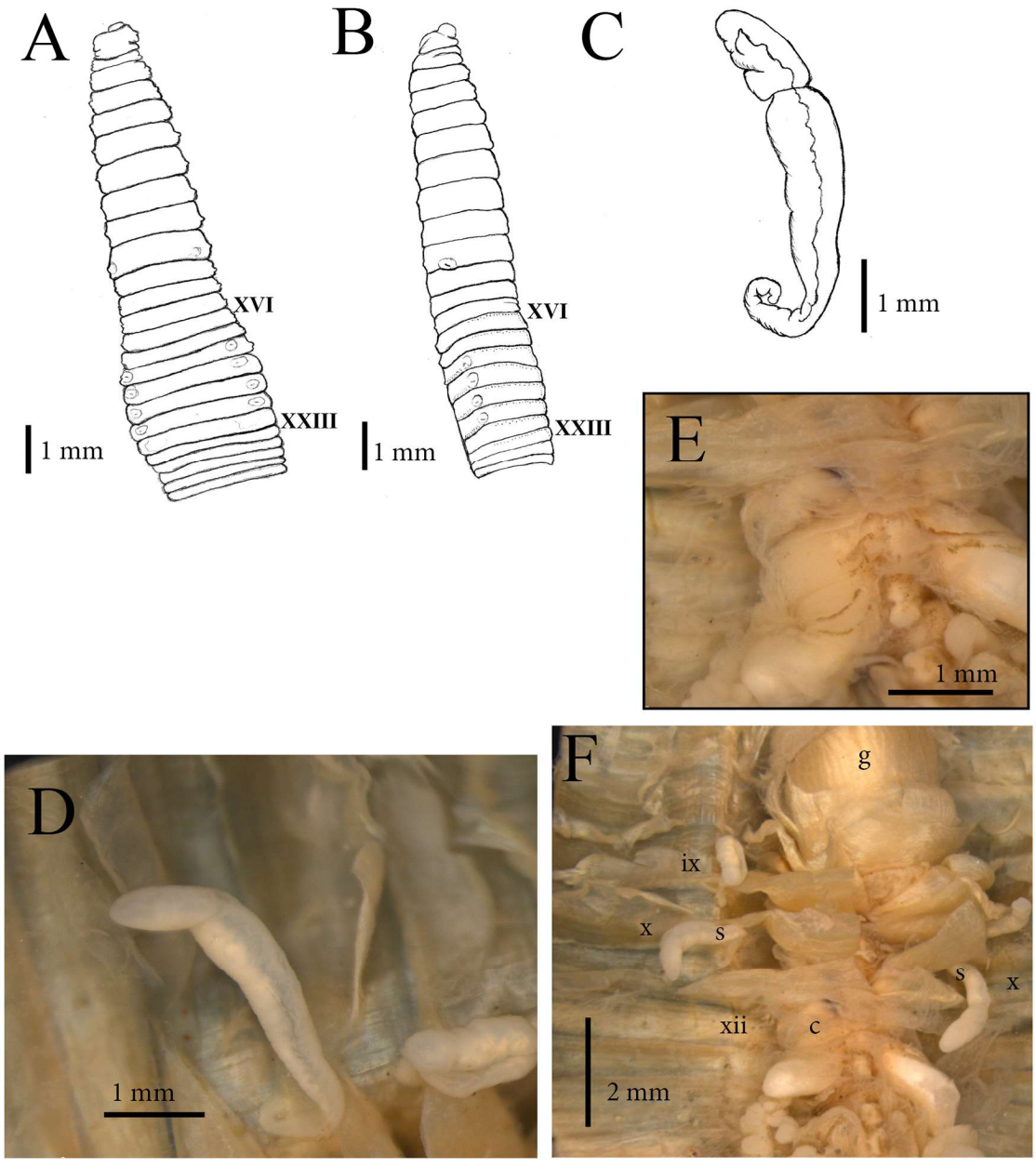

FIGURE 3. *Righiodrilus moju* **n. sp. A.** Ventral view of anterior body with clitellum. **B.** Lateral view of anterior body with clitellum. **C.** Spermatheca. **D.** Photography of the spermathecae (partly in segment ix and completely in segment x). **E.** Dorsal view of calciferous gland of xii. **F.** Photography of dorsal view showing internal structures: gizzard (segment vi), spermathecae (segments ix and x) and calciferous gland (segment xii); g=gizzard, s=spermathecae, c=calciferous gland.

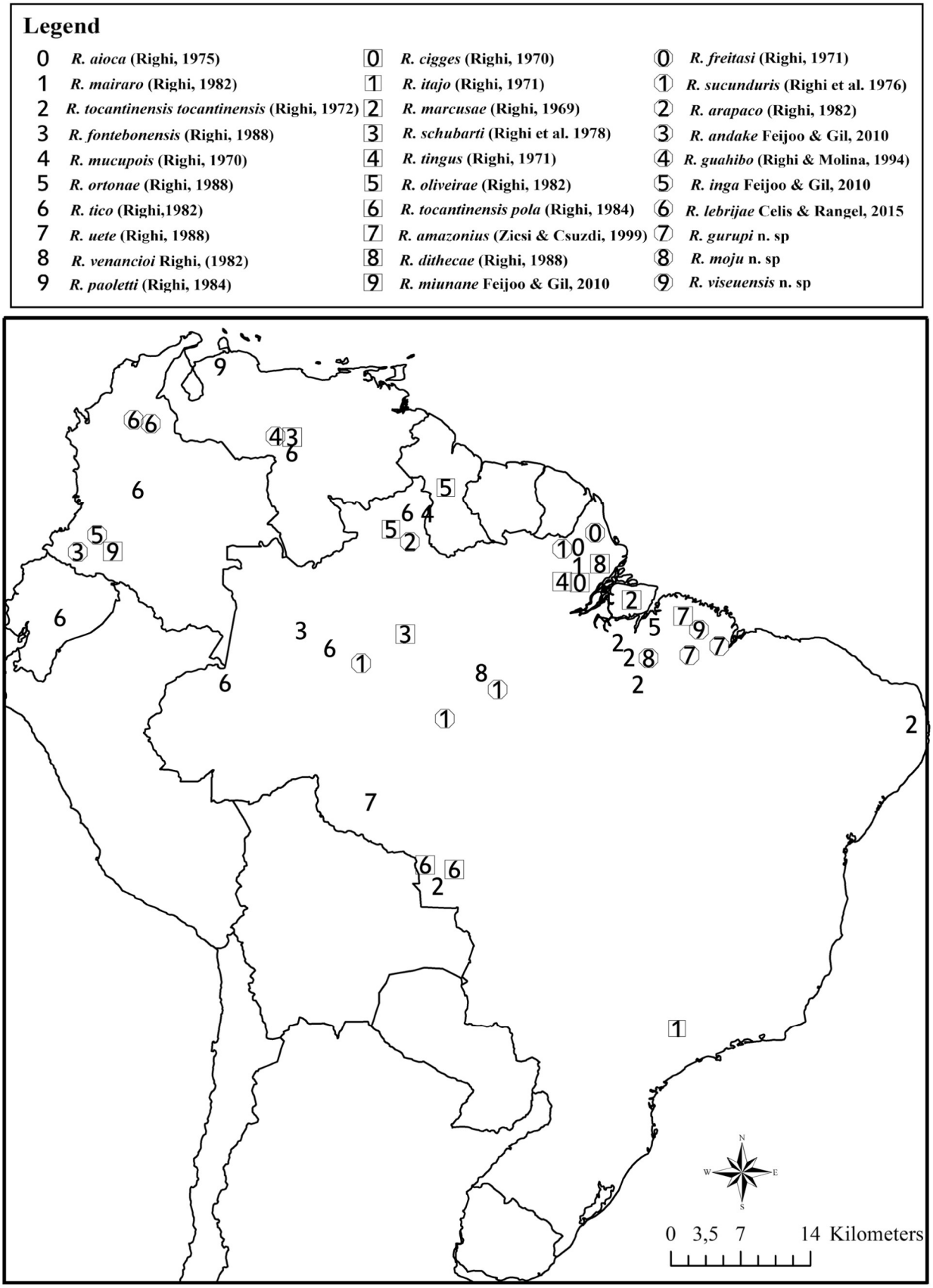

FIGURE 4. Records of species of the genus *Righiodrilus*, with data from Brown and James (2007), Feijoo and Gil (2010), and Celis and Rangel (2015).

Remarks. The difference between the new species and all other members of the genus is the absence of tubercula pubertatis, unique combination the form and quantity of spermathecae and with pair of intumescences the ventral region of body. *Righiodrilus moju* **n. sp.** is similar to *R. mairaro* (Righi, 1982) and *R. arapaco* (Righi, 1982) with regard to the shape, number and position of the spermathecae, and the characters of the clitellum.

Conclusion

With the three new species described in this paper, there are now 30 species of *Righiodrilus*. A map with finds of all species of this genus is presented in Figure 4. A key to species of *Righiodrilus* is presented below.

Key to species of *Righiodrilus* (updated and modified from Feijoo & Gil 2010)

1. Spermathecae absent 2
- Spermathecae present 3
2. Male pores in xvii ..... ***R. muinane*** Feijoo & Gil, 2010
- Male pores with tubercula pubertatis in anterior region of xvii or middle ½ 16 – ½ 17 ..... ***R. venancioi*** (Righi, 1982)
3. One pair of spermathecae 4
- More than one pair of spermathecae 5
4. Spermathecae in viii–xix ..... ***R. dithecae*** (Righi, 1988)
- Spermathecae in xix–x ..... ***R. guahibo*** (Righi & Molina, 1994)
5. Four or six pairs of spermathecae 6
- Two or three pairs of spermathecae 7
6. Six pairs of spermathecae in ix–xi, 2 pairs per segment; clitellum in xvi–xxi; male pores in xvii/xviii ..... ***R. sucunduris*** (Righi, Ayres & Bittencourt, 1976)
- Four pairs of spermathecae, in xiv–xvii; clitellum in xvi–xxiii; male pores in xviii/xix ..... ***R. gurupi*** **n. sp.**
7. Two pairs of spermathecae 8
- Three pairs of spermathecae 9
8. Spermathecae in 7/8 and 8/9 10
- Not as above 11
9. Spermathecae in 7/8, 8/9, 9/10 24
- Spermathecae in vi, vii and viii ..... ***R. viseuensis*** **n. sp.**
10. Clitellum in xv–xxi 12
- Clitellum in another position 13
11. Spermathecae in 9/10 and 10/11 ..... ***R. amazonius*** (Zicsi & Csuzdi, 1999)
- Spermathecae in another position 16
12. Tubercula pubertatis in 1/2 xix–1/2 xx. Male pores in 19/20 ..... ***R. uete*** (Righi, 1988)
- Tubercula pubertatis in xvii–xix. Male pores in 17/18 ..... ***R. cigges*** (Righi, 1970)
13. Male pores in 20/21 14
- Not as above 15
14. Clitellum in xvii–xxiii; tubercula pubertatis in xix–xxiii ..... ***R. freitasi*** (Righi, 1971)
- Clitellum in xvi–xiv; tubercula pubertatis in 1/2 xix–xxii ..... ***R. aioca*** (Righi, 1975)
15. Male pores in 1/2 xix; clitellum in xvi–xxii; tubercula pubertatis in 1/2 xix–1/2 xxi ..... ***R. tico*** (Righi, 1982)
- Male pores in xvii; clitellum in 1/2 xv–1/2 xxiii; tubercula pubertatis in xvii – xx ..... ***R. andake*** Feijoo & Gil, 2010
16. Spermathecae in 8/9and 9/10 17
- Spermathecae in ix and x 18
17. Clitellum in xv–xxi 19
- Clitellum in 1/2 xvi–1/2 xxii or xvi–xxii ..... ***R. arapaco*** (Righi, 1982)
18. Male pores microscopic ..... ***R. oliveirae*** (Righi, 1982)
- Male pores not microscopic 21
19. Male pores in xvii 20
- Male pores in 19/20 ..... ***R. mairaro*** (Righi, 1982)
20. Tubercula pubertatis in xviii–xix ..... ***R. paolettii*** (Righi, 1984)
- Tubercula pubertatis in 1/2 xvi–1/2 xix ..... ***R. lebrijae*** Celis & Rangel, 2015
21. Clitellum in xv–xxii; tubercula pubertatis present 22
- Clitellum in xvi–xxiii; tubercula pubertatis absent ..... ***R. moju*** **n. sp**
22. Male pores in xvi/xvii; tubercula pubertatis in 1/2 xvi–1/2 xviii 23
- Male pores in 17/18; tubercula pubertatis in 1/2 xvii–xx ..... ***R. ortonae*** (Righi, 1988)
23. Spermathecae in line with setae b ..... ***R. itajo*** (Righi, 1971)
- Spermathecae in line with setae d ..... ***R. fontebonensis*** (Righi, 1988)
24. Male pores in xviii 25
- Not as above 26

25. Clitellum in xv–xxii; tubercula pubertatis in 1/2 xvii–1/2 xx 27
- Clitellum and tubercula pubertatis in another position 28
26. Clitellum in xv–xxi; tubercula pubertatis in 1/2 xvi–1/2 xix 29
- Clitellum in xiv–1/2 xxii; tubercula pubertatis in 1/2 xvii–1/2 xx ..... ***R. inga*** Feijoo & Gil, 2010
27. Papillae pubertatis absent in xxi; tubercula as grooves with dark color in xviii, xix–xx ... ***R. tocantinensis pola*** (Righi, 1984)
- Papillae pubertatis present in genital field 1/2 xvii–1/2 xx; papilla midventral in xxi. ..... ***R. tocantinensis tocantinensis*** (Righi, 1972)
28. Clitellum in 1/2 xiii–xxii; tubercula pubertatis in xvii–xx ..... ***R. marcusae*** (Righi, 1969)
- Clitellum in xv–xxiv; tubercula pubertatis in xvii–xxiii ..... ***R. schubarti*** (Righi *et al*., 1978)
29. Male pores in 16/17 ..... ***R. mucupois*** (Righi, 1970)
- Male pores in 17/18 ..... ***R. tingus*** (Righi, 1971)

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