

Two new species of *Atatina* earthworm (Rhinodrilidae, Clitellata) from the Gurupi Biological Reserve, Maranhão, Brazil

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Abstract

Only three species of the *Atatina* genus had been reported, both living distant one of another in the Amazon. Here we reported two new species for the genus living in remnant forest of the Gurupi Biological Reserve. *Atatina arimikuriaka* **sp. nov.** with three pairs of spermathecae, prostomium tentacular, and annular clitellum while *Atatina arimikuritea* **sp. nov.** has only one pair of spermathecae in IX, annular clitellum, and a smallest tubercula pubertatis. The two species are unique because the annular clitellum is contrasting with the saddle-shaped of other species.

Key words: Belém Endemism Area, Soil biota, Epigeic

Introduction

The *Atatina* genus has not been studied since Righi published his latest work related to *A. gatesi* (Righi *et al.* 1978). The original description of the genus, on the base of the single species *Atatina puba*, indicates species having one pair of testes in XI, ovaries in XIII and five pairs of lamellar calciferous glands in segments IX–XIII (Righi 1971). Thereafter, *Atatina gatesi* Righi and Ayres 1978 was described with calciferous glands in segments XI–XIV, indicating that both *A. puba* and *A. gatesi* have non-pedunculated calciferous glands. Recent research described *Atatina albida*, from French Guiana, with non-pedunculated calciferous glands located in segments IX–XII (Decaëns *et al.* 2024). This suggests that the main characteristic of the genus is not the quantity of calciferous glands, but rather the type and position, specifying that non-pedunculated and lamellar-type calciferous glands could be present in segments IX–XIV.

Individuals related to that genus have been found living in forests of eastern Amazon (Righi 1971) and creek banks in western Amazon (Righi *et al.* 1978), suggesting a potential diversity for the un-sampled regions of the Amazon biome. In the last decade, ten native species were added to the Maranhão's earthworm species list but, none of them belonging to the *Atatina* genus. To contribute to the taxonomic knowledge of earthworms in the Amazon region, here we describe two new species of *Atatina* and provide considerations to improve the genus description.

Material and method

Earthworms were collected in the Gurupi Biological Reserve (3°41'56.7"S, 46°45'57.8"W) in the state of Maranhão, Brazil. The method used was a combination of the standard Tropical Soil Biology and Fertility (Anderson & Ingram

1993) complemented by an active search as described in Decaëns *et al.* (2016). Most of the samples described in this research were found living in decaying trunk, litter, and epiphytic soil. The collected specimens were fixed in 99% alcohol, replaced three times, and labeled. Samples were analyzed by dorsal dissection and observed in a Coleman stereoscopic microscope. Setae were obtained after disintegration of tissue with KOH 10% and the slides were mounted in a water-glycerin solution. The holotypes and paratypes were deposited at the Laboratório de Biologia do Solo, Universidade Estadual do Maranhão, and the access number begins with LB.

Genus *Atatina* Righi, 1971

Diagnosis: after Righi (1971)

Setae in eight longitudinal lines. Intra-clitellar male pores. Non-pedunculated calciferous glands of lamellar structure between segments IX–XIV. Spermathecae present. One pair of testes in XI. Ovaries in XIII. A pair of long seminal vesicles in XII.

Type species. *Atatina puba* Righi, 1971.

Atatina arimikuriaka, sp. nov. Hernández-García & James (Figs 1A–G)

Holotype: LB00001, adult, complete, collected under trunk, Logged Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°37'21.09"S, 46°48'30.21"W, 135 masl, 25 March 2022, Hernández-García, L.M & Rousseau, G.X. colls.

Paratypes: LB00002, adult, amputee, collected on trunk, LB00003, adult, complete, collected in soil, same locality as holotype. LB00004, two adults, completes, collected in litter, Old-growth Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°37'12.54"S, 46°48'12.51"W, 139 masl, 24 March 2022, Hernández-García, L.M & Rousseau, G.X. colls. LB00005, adult, amputee, collected in soil, Burned Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°32'31.99"S, 46°47'43.93"W, 128 masl, 29 March 2022, Hernández-García, L.M & Rousseau, G.X. colls. LB00006, adult, amputee, collected in soil, Logged Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°37'16.21"S, 46°48'9.54"W, 133 masl, 24 March 2022, Hernández-García, L.M & Rousseau, G.X. colls.

Etymology: The species name comes from an adaptation of the Awa Guajá indigenous language, spoken by almost 600 people. These people were contacted 40 years ago and live in the indigenous territories Caru, Awa, Alto Turi-Açu and Arariboia in the state of Maranhão. They named this earthworm species “fat head” (jakyꞑ mete'eꞑnuhuꞑa) “earthworm” (arimikuri).

Description. Dimensions: holotype 57 mm in length by 2.35 mm at X, 2.76 mm at clitellum, 2.52 mm at XXX, 219 segments; complete paratypes 46–68 mm in length by 1.8–2.83 mm at X, 2.0–2.93 mm at clitellum and 1.90–2.7 mm at XXX, 218–302 segments. Body cylindrical, dorsally brown after fixed, light brown ventrally, mainly after clitellum. Setae *ab* and *cd* commence on II, closely paired type. Setal arrangement aa:ab:bc:cd:dd = 6.0:1.0:7.3:1.0:25.0 at XXX. Prostomium partially retracted, however, it can extend over more than one segment when stretched, approaching Righi's definition (Righi & Araujo 2000) of tentacular prostomium (Fig. 1A). Clitellum annular in XIII–XXII for all individuals collected. Genital markings absent. Tubercula pubertatis as thin puberal furrows in XVI–XXI next to C line (Fig. 1B). Post-clitellar common setae slightly sigmoidal, four individuals with truncated setae (cut off at apical part) in XXX–XL and 112–143 µm in length. Two complete extracted setae *a* measured 170 and 317 µm, smooth at the apical part (Fig. 1C). Intraclitellar genital setae at AB line, truncated, 200–300 µm in length; the unique complete seta obtained measured 312 µm, lacking ornamentation (Fig. 1D). Microscopical male and female pores not recognized externally. Three pairs of spermathecal pores are present just near the intersegmental lines 5/6–7/8, laterally in CD line. Nephropores visible from V in CD line.

Septa 6/7–8/9 absent, 9/10–14/15 membranous. Gizzard in segment VI, externally occupying four segments, highly muscular. Intestinal origin in XVI. Typhlosole started as thick simple folds at XVI that extends to LXVIII and occupy approximately 60% of the intestinal diameter. It gradually decreases in occupation and disappear after CLXXIX. Three pairs of intramural calciferous glands, lamellar structure, are present laterally in X–XII, as vertical folds of esophageal wall opening in the lumen, size depending on the esophagus development which is more dilated on XI (Fig. 1E). Holonephric, the nephrostome in postclitellar nephridia is connected to a tube (first loop), as long as

the bladder. A second loop, same length, connect to the bladder (Fig. 1F). Blood vessels of VII aborted, two pairs are visible in VIII–IX connected to dorsal and ventral vessel. Two pairs of big lateroesophageal hearts, kidney shaped, in X–XI. Supra-esophageal vessel runs over the dorsal side of the intestine.

Ovaries in XIII. A pair of female funnels are in 13/14; female pores open in 14/15 in *A* line. Three pairs of spermathecae in V–VII; Seminal chambers absent, spermatheca duct measuring 274–301 μm wide and 240–790 μm in length, saccular ampulla measuring 350–475 μm wide and 746–820 μm in length, the pores of the spermathecae open at the posterior region of the segment (Fig. 1G). One pair of testicles and male funnels enclosed in testes sacs in XI. A pair of long seminal vesicles in XII extending laterally to XLIII. A pair of intraparietal and iridescent deferent ducts, go parallel through *C* line and open on 16/17 on the tubercula pubertatis line.

Remarks. As some important characters, like male pore and seminal chambers, for *A. albida* Bartz & Decaëns 2024 were insufficiently described we focus comparisons with others *Atatina* species. *Atatina arimikuriaka* **sp. nov.** is similar to *A. puba* Righi 1971 by the position of male pore. Major differences are in the type and extension of clitellum (annular in XIII–XXII vs saddle-shaped in XIV–XIII), number of spermathecae (three vs one), and by the number and position of calciferous glands (X–XII vs IX–XIII).

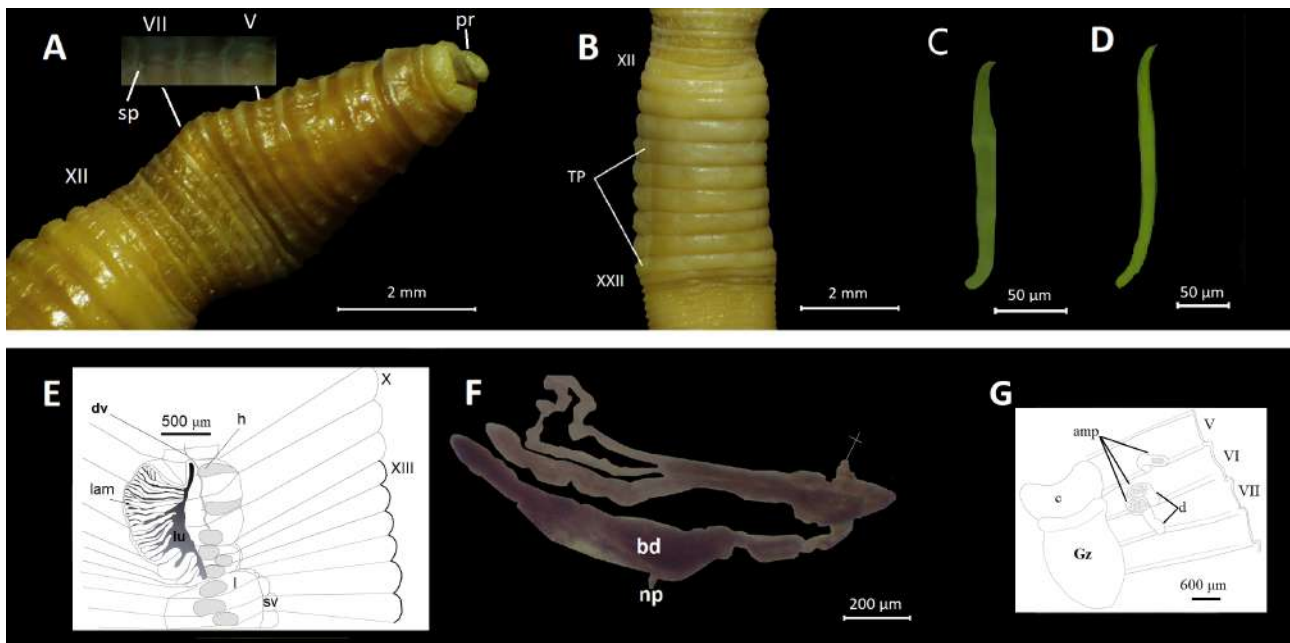


FIGURE 1. *Atatina arimikuriaka* **sp. nov.** **A.** Ventral view of first thirteen segments. **B.** Ventral view of clitellum region. **C.** Left common *a* seta of XXXVIII. **D.** Right genital *a* seta of XIX. **E.** Dorsal view in cross-section of the left calciferous glands of X–XII. **F.** Left post-clitellar nephridia of XXX. **G.** In situ dorsal view of right spermathecae of V–VII. **amp:** ampulla. **bd:** bladder. **c:** crop. **d:** duct. **dv:** dorsal blood vessel. **Gz:** gizzard. **h:** lateroesophageal heart. **I:** intestine. **lam:** lamella. **lu:** intestinal lumen. **np:** nephropore. **pr:** prostomium. **sv:** seminal vesicle. **sp:** spermathecal pores. **TP:** tubercula pubertatis.

Atatina arimikuritea, **sp. nov.** Hernández-García (Figs 2A–G)

Holotype: LB00007, adult, complete, collected in epiphytic soil, Old-growth Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°37'37.35"S, 46°48'32.44"W, 128 masl, 24 May 2023, Hernández-García, L.M & Rousseau, G.X. colls.

Paratype: LB00008, LB00009, two adults, one complete and one amputee, respectively, both collected in epiphytic soil, same locality as holotype. LB000010, adult, complete, collected in soil, Burned Forest, Gurupi Biological Reserve, Centro Novo do Maranhão, Maranhão, Brazil, 3°32'31.99"S, 46°47'43.93"W, 128 masl, 29 March 2022, Hernández-García, L.M & Rousseau, G.X. colls.

Etymology: The species name came from the Awa Guajá indigenous language for this species meaning “true” (tea) “earthworm” (arimirikuri).

Description. Dimensions: holotype 18 mm in length by 1.8 mm at X, 2.0 mm at clitellum, 1.8 mm at XXX, 84 segments; complete paratypes 27–36 mm in length by 1.48–1.58 mm at X, 1.84–1.96 mm at clitellum and 1.73–1.74

mm at XXX, 120–135 segments. Body cylindrical, beige after fixed. Setae *ab* and *cd* commence on II, closely paired-type. Setal arrangement aa:ab:bc:cd:dd = 1.5:1.0:2.0:0.5:5.5 at XXX. Prostomium prolobic, invaginated. Clitellum annular in XIII–XXII. Genital markings absent (Fig. 2A,B). Tubercula pubertatis as puberal furrows in 2/3 XVIII–1/3 XX next to *C* line (Fig. 2B). Post-clitellar common setae sigmoidal, 0.123–0.136 µm, smooth at the apical part (Fig. 2C). Intraclitellar genital setae at *AB* line truncated, the unique seta recovered 192 µm in length, straight, and smooth at the apical part (Fig. 2D). Microscopical male and female pores not recognized externally. Spermathecae pores and associated marks not recognized. Nephropores visible from V in *CD* line.

Septa 6 /7–14/15 membranous. Gizzard in segment VI, highly muscular, occupying four segments. Intestinal origin in XVI. Beginning of typhlosole as simple folds at XVII. Gradually decreases in size and disappear after LVIII. Three pairs of intramural calciferous glands are present in X–XII, vertical lamellae open to esophagus lumen (Fig. 2E). Holonephric, the nephrostome in postclitellar nephridia is connected to a tube (first loop), as long as the bladder. A second loop, same length, connect to the bladder (Fig. 2F). Three pairs of fine blood vessels are laterally in VII–IX, all connected to dorsal and ventral vessels. Two pairs of lateroesophageal hearts, kidney shaped, in X–XI. Supra-esophageal vessel runs over the dorsal side of the intestine.

Ovaries in XIII. A pair of female funnels are in 13/14; female pores open in 14/15 in *A* line. One pair of spermathecae in IX; Seminal chambers absent, the duct of spermathecae measures 77–80 µm wide and 100–120 µm in length, the saccular ampulla measures 85–94 µm wide and 200–230 µm in length, spermathecae open posteriorly to the segment and laterally in *CD* line (Fig. 2G). One pair of testicles and male funnels are located ventrally, enclosed in testes sacs in XI. A pair of long seminal vesicles are in XII extended laterally to LXX. A pair of intraparietal, and iridescent deferent ducts, go parallel through *C* line and open on 17/18.

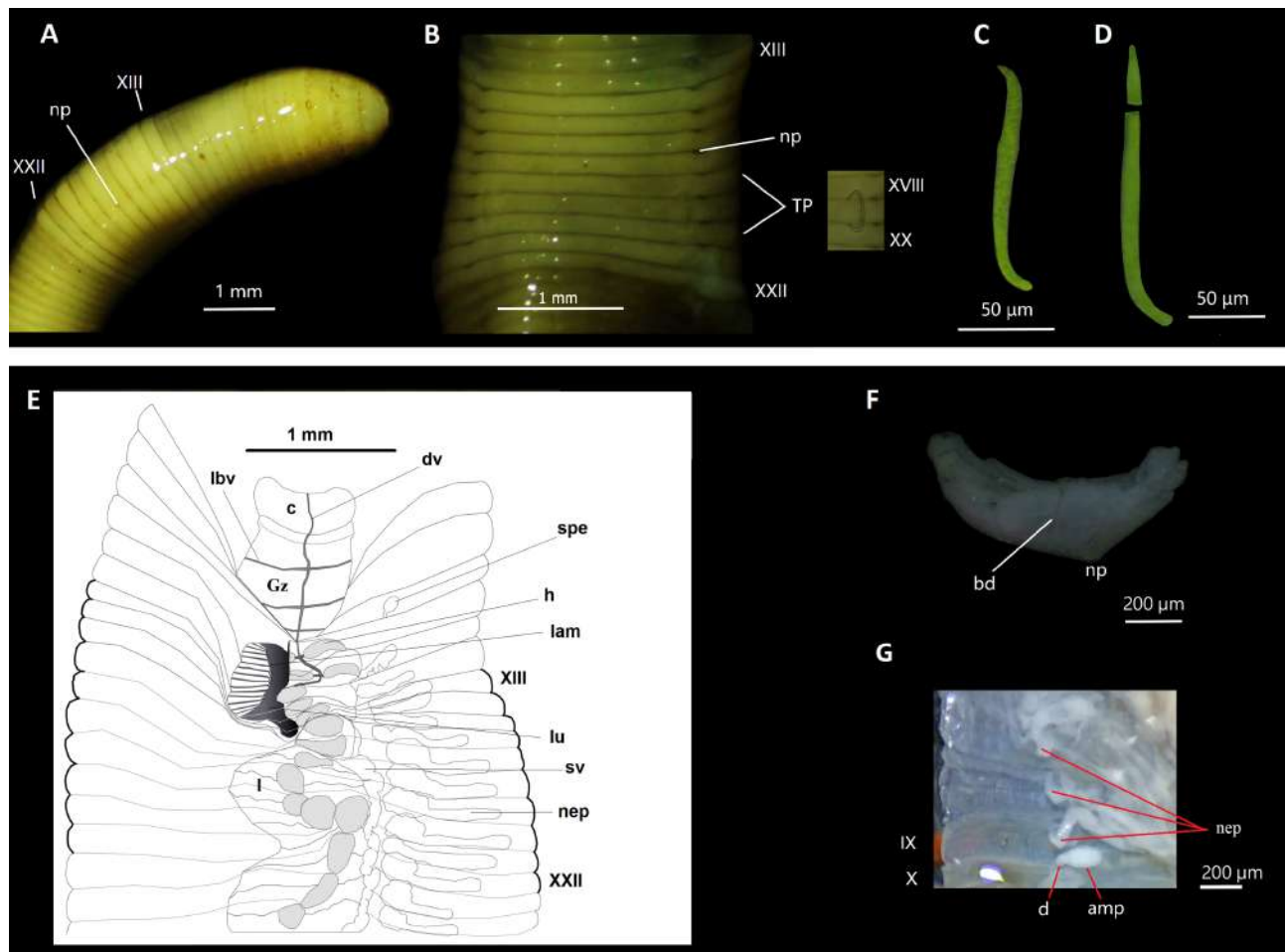


FIGURE 2. *Atatina arimikuritea* sp. nov. **A.** Lateral view of anterior region showing the clitellum. **B.** Ventral view of clitellum region after dissection. **C.** Left common *a* seta of XXXV. **D.** Right genital *a* seta of XVIII. **E.** Dorsal view in cross-section of the left calciferous gland of X–XII. **F.** Left post-clitellar nephridia of XXX. **G.** Dorsal view of left spermathecae of IX. **amp:** ampulla. **bd:** bladder. **c:** crop. **d:** duct. **dv:** dorsal blood vessel. **Gz:** gizzard. **h:** lateroesophageal heart. **I:** intestine. **lam:** lamella. **lbv:** lateral blood vessel. **lu:** intestinal lumen. **nep:** nephridia. **np:** nephropore. **sv:** seminal vesicle. **TP:** tubercula pubertatis.

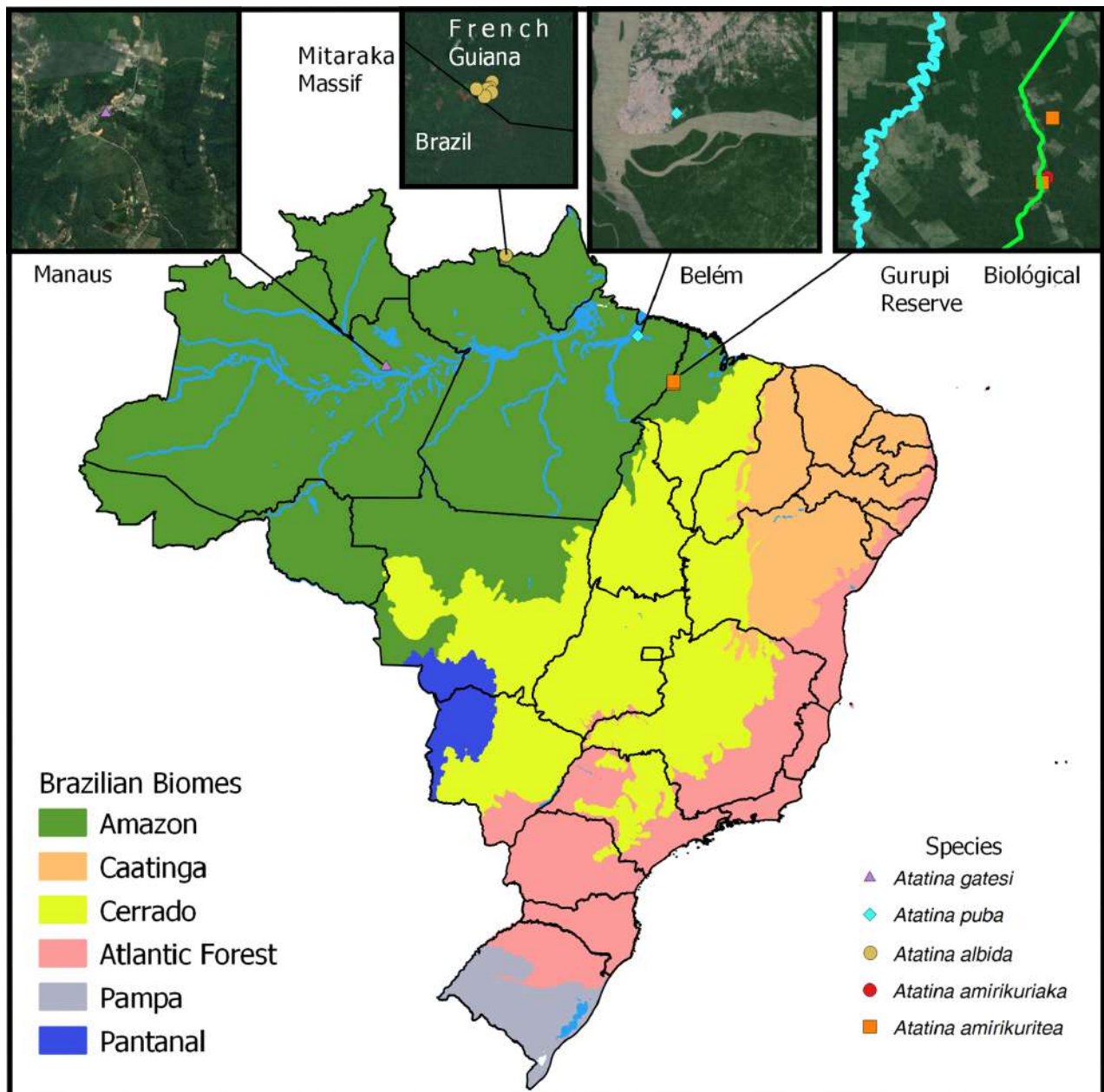


FIGURE 3. Geographical distribution of the five species of *Atatina* in south America and potential gaps in the Amazon biome to find new species.

Remarks. The new species *Atatina arimikuritea* **sp. nov.** is similar to *A. puba* Righi 1971 and *A. gatesi* Righi and Ayres 1978 by the presence of one pair of spermathecae and the prolobic prostomium. The main similarity found with *Atatina arimikuriaka* **sp. nov.** is the type and extension of the clitellum. Differences with *A. puba* and *A. gatesi* include the clitellum type (annular vs saddle-shaped) and extension (XIII–XXII vs XIV–XXIII or XIV–XXI), and position of first calciferous glands (X vs IX or XI). Difference with *Atatina arimikuriaka* **sp. nov.** includes the number of spermathecae (1 vs 3), the prostomium type (prolobic vs tentacular) and size (*Atatina arimikuritea* **sp. nov.** is a half of size). More details for comparison between species are in table 1.

TABLE 1: List of main characters of the earthworm species belonging to the *Atatina* genus. eli: Clitellum. *Amputee. GBR: Gurupi Biological Reserve

Character	<i>A. puba</i>	<i>A. gatesi</i>	<i>A. albida</i>	<i>A. arimikuriaka</i> n. sp	<i>A. arimikuritea</i> n. sp
Reference	Righi 1971	Righi 1978	Bartz & Decaëns	This study	This study
Length (mm)	30–31	26*	24–41	55–68	27–36
Segments (Number)	130–144	108	119–299	219–302	120–135
Diameter (mm) X/eli/XXX	1.2–1.3/1–1.04/0.7–0.76	0.88/0.97/0.70	1.5–2/1.5–2.1/1.5–2.4	1.44–2.83/1.6–2.93/1.28–2.7	1.48–1.58/1.86–1.94/1.73–1.74
Prostomium	prolobic	prolobic	Proepilobic	tentacular	prolobic
First setae	II	III	no reported	II	II
Setae ratio in XXX	1.2:1.2:0.4:5.8	2.9:1.4:6.3:1:13.36	2:1:3:1:12	4.8:1.6:2:0.6:15.8	1.5:1.2:0.5:5.5
Setae arrange	regular	regular	regular	regular	regular
Common Setae of XXX Excavation	ornamented	smooth	not reported	smooth	smooth
Common Setae of XXX Length (mm)	not reported	not reported	not reported	0.17–0.317	0.123–0.136
Clitellar Genital Setae Excavation	not reported	not reported	not reported	smooth	smooth
Clitellar Genital Setae Length (mm)	not reported	not reported	not reported	0.312	0.192
Clitellum	XIV–XXIII	XIV–XXI	XV–XXIV	XIII–XXII	XIII–XXII
Clitellum shape	saddle-shaped	saddle-shaped	saddle-shaped	annular	annular
Tubercula pubertatis	1/3 XIX–2/3 XXI	absent	XVII–XXIV	XVI–XXI	2/3 XVIII–1/3 XX
Calciferous Gland	IX–XIII	XI–XIV	IX–XII	X–XII	X–XIV
Esophageal hearts	X–XII	X–XIII	IX–X	X–XI	X–XI
Seminal vessel length	extended to XXIV	extended to XXI	extended to LX	extended to XLIII	extended to LXX
Male pore	16/17 in <i>c</i> line	not reported	not recognized	16/17 near <i>c</i> line	17/18 near <i>c</i> line
Ovary	XIII	XIII	not recognized	XIII	XIII
Female pore	not recognized	not recognized	not recognized	14/15 in <i>a</i> line	14/15 in <i>a</i> line
Spermathecae pores	9/10 in <i>d</i> line	10/11 in <i>cd</i> line	8/9, 9/10	5/6, 6/7, 7/8 in <i>cd</i> line	9/10 in <i>cd</i> line
Seminal Chambers	present	absent	not recognized	absent	absent
Locality	Pará: Belém	Amazonas: Manaus	French Guiana: Mitaraka Massif	Maranhão: GBR	Maranhão: GBR

Discussion

The last 50 years only five species of the *Atatina* genus have been described, four of them found in the Amazon biome in Brazil (Fig. 3). Seven adults of *A. puba* were collected specifically in Belém (Righi, 1971), and just one individual adult was used to describe *A. gatesi* collected near Manaus (Righi *et al.* 1978). Despite exhaustive earthworm sampling in the Gurupi Biological Reserve (Garcia 2019) we captured some specimens after intensively sampling more than 60 plots, with a large portion of individuals collected in epiphytic soil, as also was found in decaying trunk in forest of Mitaraka Massif, French Guiana (Decaëns *et al.* 2024). Therefore, the species of the *Atatina* genus are rare and probably inhabiting only forest ecosystems.

The Amazon biome is a large potential habitat for the *Atatina* species as they already occur from East (Maranhão) to West (Manaus) and North (French Guiana). Consequently, the probability to find new species in unsampled forests is high as many geographical barriers limit the dispersion of earthworms.

Some morphological differences were found as species are geographically distant and the phenotypic characteristic diverged. The intramural calciferous glands are more common, present in four of the five species described, and only *A. puba* presents extramural calciferous gland as pouches widely opened in the oesophagus (Righi, 1971).

The lamellar structure of the calciferous glands was described in both in *A. puba* (Righi, 1971) and *A. gatesi* (Righi *et al.* 1978, Righi, 1995). Several species within the Rhinodrilidae family have calciferous gland with lamellar structures, including those in the *Andiorrhinus*, *Andiodrilus*, *Eudevosclex* and *Taironina* genera. However, the calciferous glands in those genera are extramural and are present in the pretesticular segments.

Atatina species also differ of those other species due to the small size, less than 6cm in length. They are likely a group of epigeic earthworms, important in litter decomposition but vulnerable to land use changes. For this reason, more attention should be focused on future conservation efforts.

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