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The first troglobitic *Pseudonannolene* from Brazilian iron ore caves (Spirostreptida: Pseudonannolenidae)

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Abstract

Pseudonannolene spelaea **n. sp**. is the first strictly cave-dwelling species described for the family Pseudonannolenidae. It is found in iron ore caves in the Brazilian Amazon. The family Pseudonannolenidae is exclusively Neotropical and frequently found in caves of Brazil, from which 20 species are known. The new species is compared with its congeners and with related cave-dwelling species. The family Pseudonannolenidae is discussed, and comments are presented on the conservation status of the caves where the species is found, which potentially may be the target of anthropogenic impacts resulting from iron ore extraction.

Key words: Neotropics, Brazil, troglobitic, conservation, iron ore

Introduction

The family Pseudonannolenidae Silvestri, 1895 has a wide distribution in South America, with species known from Argentina to the Guianas (Pocock 1910; Mauriès 1987; Adis 2002). In Brazil, the genus *Pseudonannolene* Silvestri, 1895 is the most representative, having 20 described species, 8 of which are considered troglophiles (populations established in the subterranean environment as well as the externalon the surface) (Brölemann 1909; Trajano *et al*. 2000; Fontanetti 2000). The distribution of the genus covers a large part of the national territory, including the states of Amazonas, Mato Grosso, Mato Grosso do Sul, Tocantins, Goiás, Ceará, Sergipe, Bahia, Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Silvestri 1902; Pinto-da-Rocha 1995; Trajano *et al.* 2000; Pinto-da-Rocha *et al*. 2001; Golovatch *et al.* 2005; Souza-Silva & Ferreira 2009; Zampaulo & Ferreira 2009; Bento 2011; Donato 2011). Although it is a genus of wide distribution, its relationships still remain obscure. The family is, usually allocated placed in the suborder Epinannolenidea of the order Spirostreptida, which is distributed in part of Central America, southern Africa, southwest and southeast Oceania (Shelley & Golovatch 2011).

Regarding the restriction to the subterranean environment, there is no known species of the family that is restricted to these environments, although the spirostreptidan suborder Cambalidea has at least three troglobite species in the New World: *Cambala speobia* Chamberlin, 1953 and *Cambala reddeli inornatus* Causey, 1964 both from gypsite caves in Texas (USA) and *Mexicambala russelli* Causey, 1964 found in caves in Mexico (Causey 1964; Reddell 1994, White & Culver 2012). Depigmentation, reduced number of ocelli (*Cambala speobia* and *Mexicambala russeli* are anophthalmic) and increased body size have been considered as troglomorphic characters in these species (Causey 1964). Another specialization feature correlated with the subterranean environment is the elongation of appendages (including sensory) (Golovatch & Kime 2009), and in their respective descriptions only the elongation of the antennae and tarsal claws were cited in *Mexicambala russelli* (Causey 1964).

This work describes the species *Pseudonannolene spelaea* **n. sp**., found in ferruginous caves the state of Pará (Brazilian Amazon). The species stands out due to its troglomorphic characteristics, therefore being considered the first troglobite species of the family Pseudonnanolenidae.

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Material and methods

Collection and preservation:

Type specimens were collected during 2010 and 2011 and are deposited in the Zoology Collection, Seção de Invertebrados Subterrâneos (ISLA) at the Universidade Federal de Lavras (UFLA), Campus Universitário de Lavras, Minas Gerais, Brazil.

All specimens were captured with a fine brush and placed in vials containing 70% ethanol. Specimens were sought for visually throughout the floors and walls of the caves. Special attention was also paid to decaying vegetation (e.g. leaves and tree bark) and animal carcasses, though this material was only common near the cave entrances. Twenty-seven individuals were found (although all specimens were observed, few specimens were collected) in 9 caves in the municipality of Parauapebas, Pará State, Brazil. (GEM-1712, GEM-1735, GEM-1727, GEM-1714, GEM-1756, GEM-1770, GEM-1754, GEM-1774, GEM-1773). The caves are located in a large ferriferous deposit locally called Serra dos Carajás (Fig. 1).

Measurements and Drawings:

Measurements and drawings were made under a stereomicroscope (Stemi 2000 (ZEISS) and a *camera lucida* microscope (Leica MDLS). Dissections were made with fine entomological pins. Micrographic images were performed using the AxioCam ERc 5s program connected to the Primo Star microscope (ZEISS). The stereoscopic images were acquired using the Leica M205 A, with the program Leica Application Suite auto montage to combine the images. For the measurements of length and width of head, collum and anal segment, the maximum horizontal and vertical diameter was used in millimeters. For the length of legs, tarsal claws and antennae, the distance between two farther points in their extremities was used. The ratio between the lengths and widths of structures with midbody diameter was made using the midbody diameter as maximum measurement (100%).

Results

Order Spirostreptida Brandt, 1833

Suborder Cambalidea Cook, 1895

Family Pseudonannolenidae Silvestri, 1895

Epinannolenidae Chamberlin, 1922 (Shelley 2003: 10).

Genus *Pseudonannolene* Silvestri, 1895

Pseudonannolene spelaea Iniesta & Ferreira, new species
(Fig. 2–6)

Material examined. Holotype: 1 Male (fragmented) (ISLA 3797) from GEM – 1770 cave, Parauapebas/PA, Brazil, 21/X/2010, Oliveira, M.P. coll.
Paratypes: 1 Male (fragmented) (ISLA 3796) from GEM—1744 cave, Parauapebas/PA, Brazil, 20/IX/2010, Oliveira, M.P. coll.; 2 Female (ISLA 3794, ISLA 3795) from GEM—1712 cave, Parauapebas/PA, Brazil, 30/X/2010, Oliveira, M.P. coll.

Etymology. Name given in apposition as a reference to the Latin word *spelaea*, meaning “cave”.

Diagnosis. Distinct from all previously known species of the genus due to depigmented body and eyes (the eyes with few ocelli, between 10–15) and in the details of the gonopods.
Live specimens can be seen in Figure 6.

Description of Adults. Measurements: The measurements can be seen in Table 1.

Colour: translucent white.

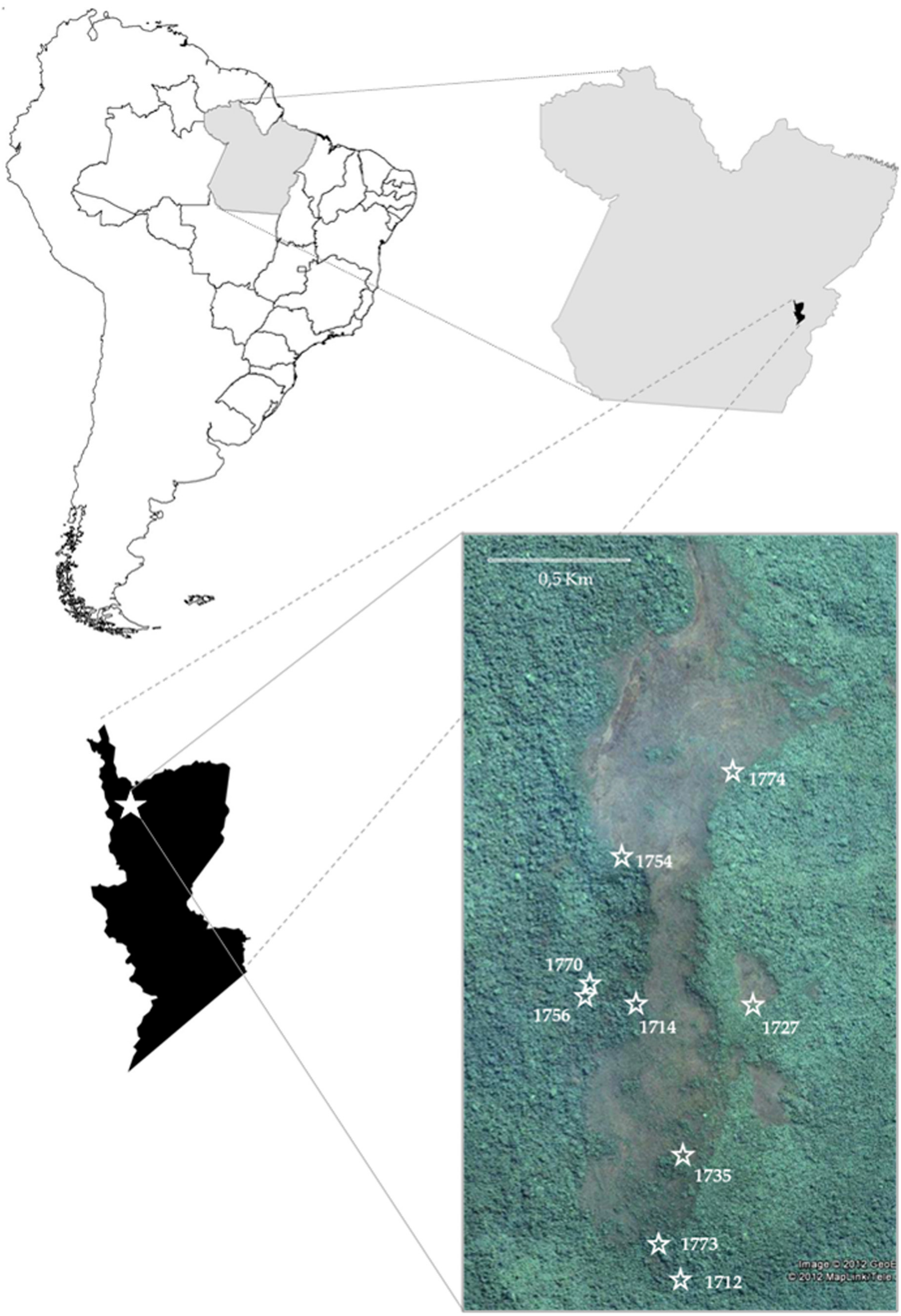

FIGURE 1. Distribution map of *Pseudonannolene spelaea* **n. sp.** Stars and numbers refer to caves.

FIGURE 2. *P. spelaea* **n. sp.**, paratype female (ISLA 3794) detail of anterior region.

TABLE 1. Measurements (in mm.) and ratios of selected body parts in relation to midbody vertical diameter (in parentheses) of *P. spelaea* **n. sp**. Abbreviations: Leng. = length; Diam; = midbody vertical diameter; A.S. = anal segment; L. = legs; T.C. = tarsal claw; Ant. = antennae.

		Body		Head		Collum					
	Body rings	Leng.	Diam.	Leng.	Wid.	Leng.	Wid.	A.S.	L.	T.C.	Ant.
Holotype	65	28	1.88	0.96 (0.51x)	1.12 (0.59x)	0.80 (0.43x)	1.40 (0.74x)	0.96 (0.51x)	1.12 (0.59)	0.12 (0.06x)	1.76 (0.94x)
Paratype ♂	60	26	1.74	0.88 (0.51x)	1.12 (0.64x)	0.96 (0.55x)	1.46 (0.84x)	0.84 (0.48x)	1.14 (0.64x)	0.10 (0.06x)	1.84 (1.06x)
Paratype ♀ 1	64	31	1.88	0.86 (0.46x)	1.20 (0.64x)	0.88 (0.47x)	1.40 (0.74x)	0.81 (0.43x)	1.04 (0.55x)	0.12 (0.06x)	1.94 (1.03x)
Paratype ♀ 2	62	25	1.88	0.80 (0.43x)	1.04 (0.55x)	0.80 (0.43x)	1.40 (0.74x)	1.07 (0.46x)	0.96 (0.51x)	0.12 (0.06x)	1.74 (0.93x)

Head (Fig. 2): Head glabrous and depigmented. Three small labral teeth (not modified), a row of 15 labral setae and above a row of 6 supralabral setae. Mandibles depigmented, glabrous and with 2 external teeth above, 4 internal teeth and 6 rows on pectinate lamellae. Eyes depigmented with 10 to 15 ocelli, very difficult to see.

Antennae depigmented and densely setose. Four terminal sensorial cones. First antennomere small, with setae exclusively positioned on the distal edge. Second and third antennomeres of similar sizes. Fourth antennomere a little longer than the fifth, and this possesses a wider, curved distal border. Sixth antennomere longer and wider than the fourth and fifth. Groups of basiconic sensilla measuring 15 µm, on the edge of the fifth and sixth antennomeres.

Gnathochilarium (Fig. 3): The structure of the gnathochilarium is similar to that observed in other species of the genus *Pseudonnanolene* with few differences in relation to the disposition of the setae. Basal gula (Gu) with setae. Rounded mentum (Me) with small setae on the distal portion. Stipes (St) with basally and distally rounded lateral borders. Promentum (Pme) with triangular aspect, divided in midline and with dispersed setae throughout the whole surface. Lamellae linguales (Ll) entirely separated by promentum.

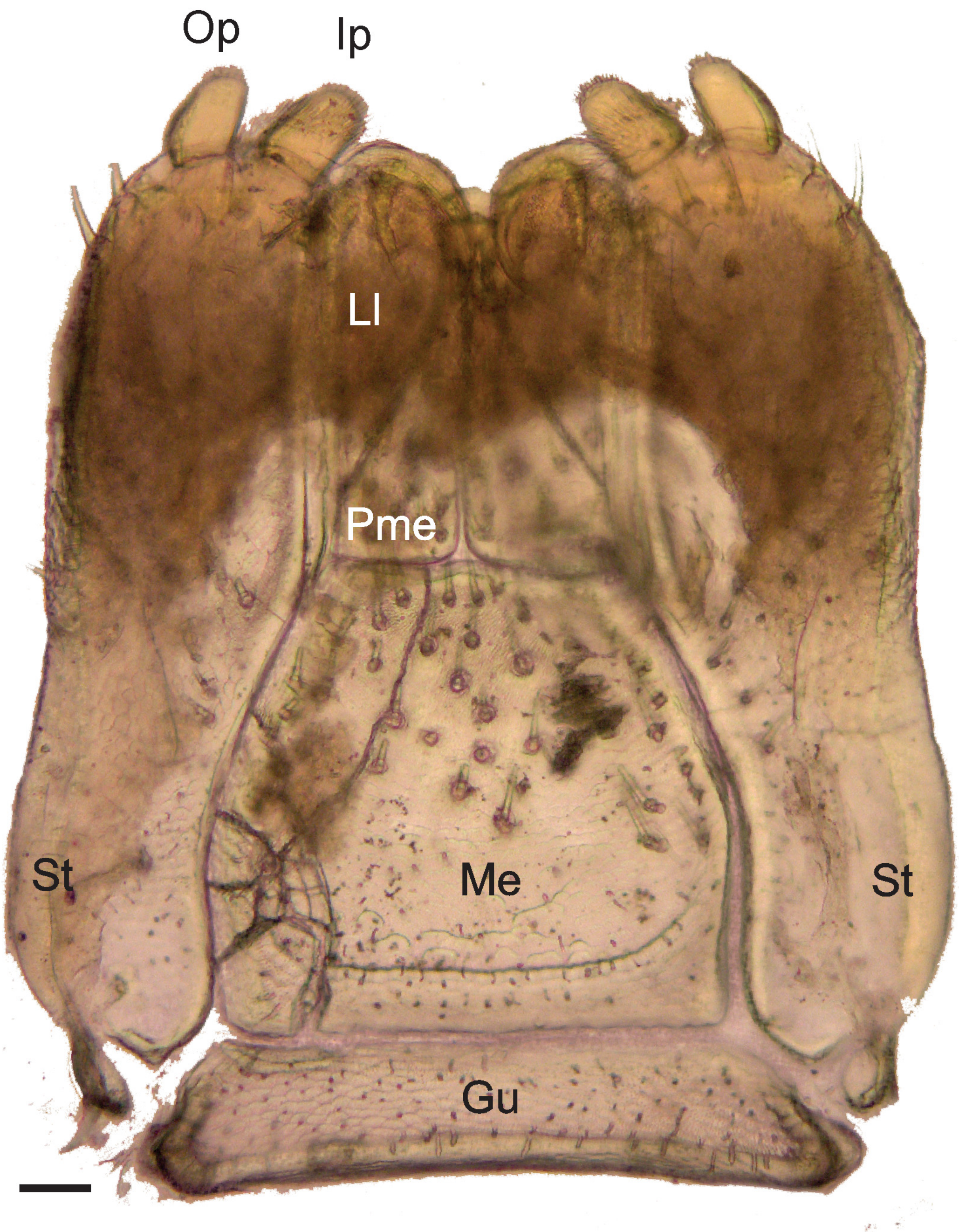

FIGURE 3. *P. spelaea* **n. sp.**, holotype male (ISLA 3797), micrographic images. Detail of *Gnatochilarium*. **Abbreviations:** Gu = gula; Me = mentum; St = stipes; Pme = pro- mentum; Ll = laminae linguales; Op = outer palp; Ip = inner palp. **Scale bars:** 10 µm.

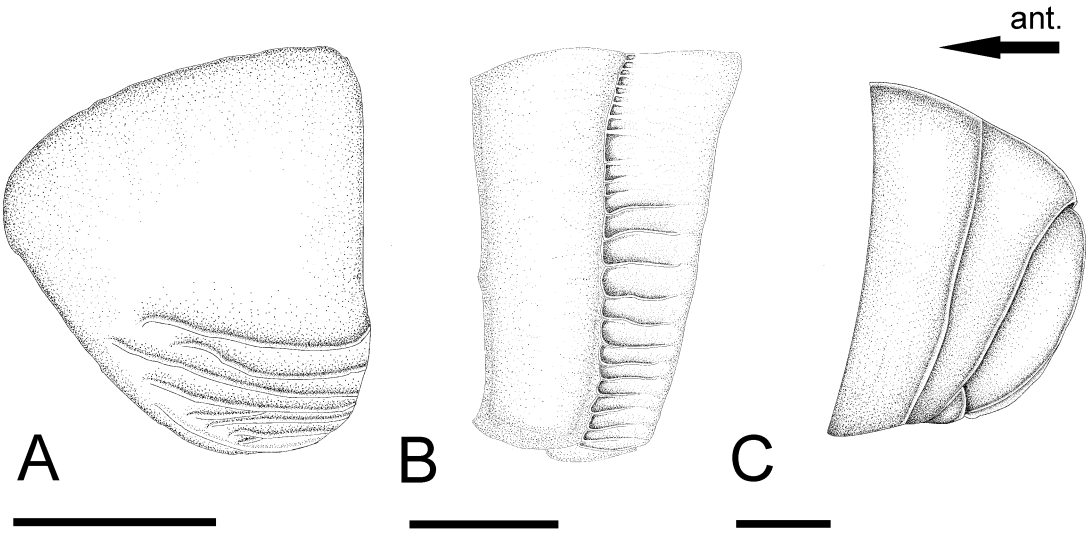

FIGURE 4. *P. spelaea* **n. sp.**, paratype female (ISLA 3794), Schematic drawings. **A:** *Collum*; **B:** Anterior segment; **C:** Anal segment. **Scale bars:** A = 500 µm; B = 500 µm; C = 500 µm.

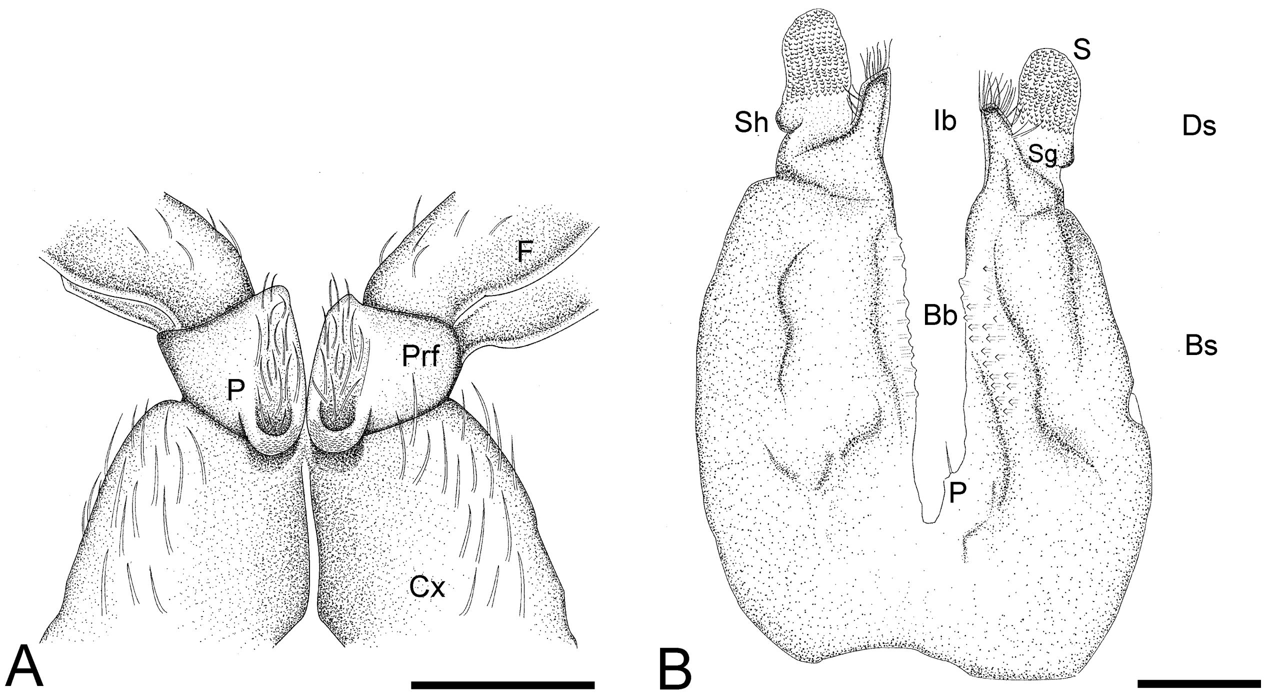

FIGURE 5. *P. spelaea* **n. sp.**, holotype male (ISLA 3797), stereoscope images. **A:** Schematic drawing of the first male leg pair; **B:** Schematic drawing of the gonopod (oral view). **Abbreviations:** Prf = pre-femur; P = process; F = femur; Cx = coxa; Bs = basal section; Ds = distal section; Sh = shoulder; Bb = basiconic bristles; P = process; Ib = internal branch; S = solenomere; Sg = seminal groove. **Scale bars:** 200 µm.

Trunk: Body with 60 to 65 rings. Collum (apodous ring) glabrous and depigmented (Fig. 4A). Transverse lateral striae present. Terga with prozonite and metazonite distinct only by a transverse suture. Metazonite with striae in lateral region, being more closely spaced in the anterior region due to the smaller width of trunk (Fig. 4B) (sutures variable among individuals). Opening of the repugnatorial glands starting from the fifth ring. Limbus smooth. Pre-anal ring depigmented (Fig. 4C) with dorsal projection covering part of the anal valve; this with few setae on the opening.

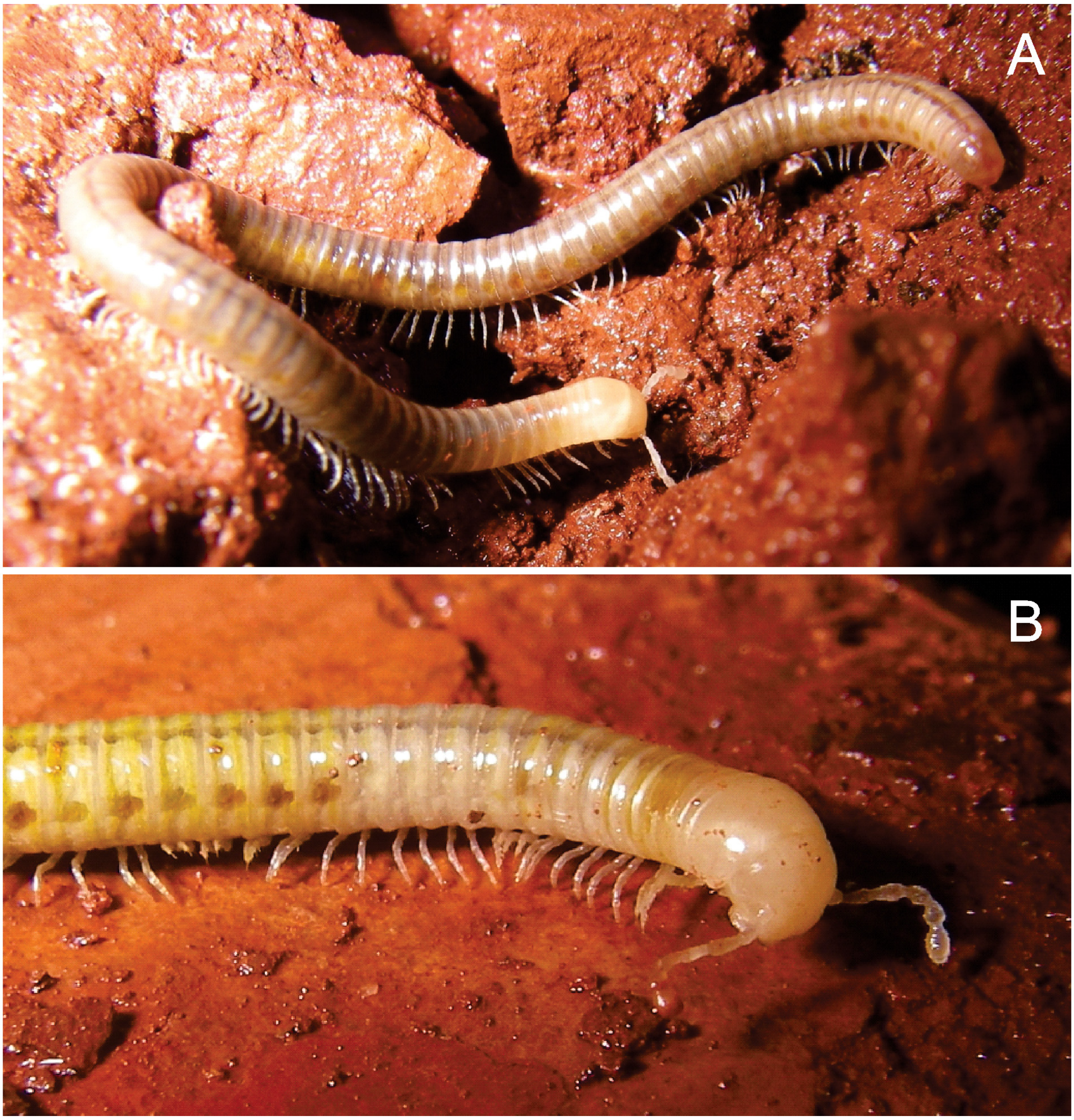

FIGURE 6. *Pseudonannolene spelaea* **n. sp.**, live specimens.

First Male Leg Pair (Fig. 5A): The first leg pair is modified. Sternum reduced and not extending between the coxae (Cx). The coxae larger in comparison with the remaining legs. Pre-femur (Prf) with oral process parallel (P) to the coxae. Sensory bristles in the mesal region of process and small lobes on the surface of the anterior margin. The length of femur (F) is about 2 times longer than width. Post-femur (Psf) and tibia (Tb) with width similar to length. Tarsus with width 1.5 times shorter than length. Terminal claw not modified.

Gonopod (Fig. 5B): Structure is typical of the genus, composed of a reduced coxal part separated from telopodite by a rather vague lateral sulcus; telopodite terminating in two well-developed processes, one a seminal branch (solenomere, S) with a micro-squamate region, the other an internal branch (Ib), the latter densely setose. Gonopods relatively short and stout, about 2 times longer than wide, with a glabrous basal section (Bs) and a process (P) next to the sternal region, P supporting a seta. Basal section beset with papillae (Bb) extending onto a membranous area between coxal part and distal portion. The latter section (Ds) is 1.75 times longer than basal width and about 2.5 times shorter than Bs together with coxal part. Ds divided into a complex S and a digitiform Ib, both separated by a concave region (Sg, seminal groove?). S flattened and rounded, with a micro-squamate anteromedian face. Smooth region basal to S with a lateral lobe like a small shoulder (Sh). Ib somewhat shorter than S, with setae, presumably sensory ones, both at apex and lateral margin.

Discussion

The species *Pseudonannolene spelaea* **n. sp.** differs substantially from other species of the genus by a combination of characteristics, many of which are due to isolation in, and adaption to, the subterranean environment. The number of ocelli observed in *P. spelaea* (10–15 ocelli) is quite different to those of epigean species, which can vary from 20 (in *P. rocana*, *P. pusilla*, anf *P. parvula*) to 40 ocelli (in *P. segmentata*, see discussion below, concerning troglomorphic traits in the species). The body length (in males) is also quite distinct when comparing *P. spealea* (26–28 mm) to other species of the genus. This length can vary from 30 mm (in *P. rocana*, *P. augularis* and *P. microzoporus*) up to 72 mm (in *P. segmentata*). Despite this differences in body sizes, in most species there are few differences in number of body rings, typically around 60–65 rings. The exceptions are *P. rugosetta* (51 rings) and *P. microzoporus* (47 rings), and *P. paulista* (74 rings) (Silvestri 1902; Mauriès 1987).

Comparing *P. spelaea* with recent descriptions of other species of the genus from Brazil (especially troglophilic species from Brazilian caves), the depigmentation (of the body and eyes) and the reduction of the ocelli number is evident. The antennae of the new troglobitic species, when compared to those of *P. chaimowiczi* (Fontanetti 1996a) and *P. strinatii* (Mauriès 1974) possess two main differences. The first includes the complete depigmentation in *P. spalaeus* compared to the strongly pigmented antennae of the troglophilic species. The second difference includes the length and thickening of the groups of basiconic sensilla present on the fifth and sixth antennomeres. In *P. chaimowiczi*, *P. strinatii* and *P. anapophysis* (Fontanetti 1996a) the basiconic sensillas are noticeably shorter than those observed in *P. spelaea* (that measure about 15 µm). The exact sensory function of the sensillae is not known, but they may comprise a strong indication of adaptation by the species to subterranean life. Unfortunately, these sensillae could not be compared to those present on other species of the genus, because these structures have not been detailed in their descriptions. The comparisons conducted here were based on observations carried out on specimens of *P. chaimowiczi* and *P. strinatii* present in the ISLA collection.

Mauriès (1974) mentioned, for *P. strinatii*, a certain degree of depigmentation for the body (brownish) as well as for the eyes, as a trend toward a reduction in the number of ocelli. This author attributed these characteristics to the evolution of the species in the subterranean environment. However, although Mauriès (1974) has associated such features to development in caves, this does not seem to be the case. These characteristics are apparently just a result of maintaining an ancestral condition. The eyes of organisms of this species have numbers of ocelli (20–25) similar to those observed for epigean species, besides the depigmentation not being evident. In comparison with epigean species, the reduction in the number of ocelli in *P. spelaea* is evident (only 10–15 ocelli). *Pseudonnanolene segmentata* (Silvestri 1902) found in Paraguay, possesses 40 ocelli, and another Brazilian species, *Pseudonannolene longicornis* Porat, 1889 has 31 to 43 ocelli (Mauriès 1987). Therefore, the number of ocelli of described epigean species of the genus varies from 20 to 43, distributed among 3–5 rows.

According to Fontanetti (2002), another important taxonomic trait of the group is the prefemoral process in the first pair of legs of the males. Concerning this process, *P. spelaea* is similar to *P. tocaienses* (Fontanetti 1996b), *P. mesai* (Fontanetti 2000), *P. chaimowiczi* (Fontanetti 1996a), *P. strinatii* (Mauriès 1974) in presenting processes parallel to the coxa. Such a characteristic differs from *P. anapophysis* (Fontanetti 1996a), which does not present such a process.

According to Shear (1969) and Golovatch & Kime (2009) the troglobitic species of a genus are often larger than the epigean species. However, depending on the availability of food (classic characteristic of oligotrophy in the caves) size reduction possesses a compensatory function for the lack of resources in the environment (Enghoff 1992). As such, some species of troglobitic arthropods can escape the tendency toward increased body size, running the opposite to the expected, smaller than usual (Souza & Ferreira 2011). Accordingly, *P. spelaea* is 27 mm long on average, while *P. anapophysis* (Fontanetti 1996a), *P. chaimowiczi* (Fontanetti 1996a) and *P. imbirensis* (Fontanetti 1996a) are 90 mm, 80–130 mm and 60 mm long, respectively. As in other cave dwelling Arthropoda, some factors may influence the decrease in body size, such as escaping predation, rapid development in order to reproduce and ability to move around in small spaces (Culver *et al.* 1995; Culver *et a*l. 2010).

The known New World troglobite species for the order, *Cambala speobia*, *Cambala reddeli inornatus*, *Mexicambala russelli* and *Pseudonannolene spelaea* **n. sp.** differ from epigean by two main features: the a depigmentation and reduction (or absence) of ocelli. In *Mexicambala russelli* there is also elongation of the antennae and tarsal claws. These features can represent adaptations of species in this environment, mainly due to absence of light. Thus, the elongation of structures (sensory or not, like the antennae and tarsal claw) is an of positive survival value. Besides, these traits also can be attributed to other adaptations, like soil-dwelling. However, adaptation in soil has still not been evidenced in the genus.

Descriptions of new species normally include more precise comparisons of that new species with others of the same genus, suggesting possible affinities. In the present case, however, little is known about Neotropical *Pseudonannolene*. Moreover, the extreme modification of *P. spelaea* as a result of evolution in the subterranean environment makes it difficult to associate this species with any other known species of the genus.

The family Pseudonannolenidae, described in 1895 by Silvestri, is defined largely by the characteristics of gnathochilarium, the separation of the promentum the main identification feature, and only one pair of gonopods (Silvestri 1985; Pocock 1910; Adis 2002). This feature allows its differentiation from other families, including Cambalidae, to which belong the only troglobitic species of the order known so far from North and South America. According to the original description, the definition of the genus *Pseudonannolene* is given by the presence of multi-serial eyes, sub-clavate antenna (six antennomeres) in which the second, third and fourth antennomeres have greater lengths, but these characters may be inadequate in the present day to diagnose the genus. The gonopod morphology is also another genus-differential feature. It is noteworthy that the presence of basiconic bristles on the gonopod is represented as a serrated structure by some authors (Silvestri 1895). This feature was also erroneously observed in other descriptive works (Fontanetti 2000) in which the bristles were identified as dentiform processes. Finally, *Pseudonannolene spelaea* **n. sp.** can be unequivocally placed in Pseudonannolenidae due to its gnathochilarium (promentum) traits and also placed in *Pseudonannolene* due to the gonopod morphology. Genera considered near *Pseudonannolene* are *Typhlonannolene* (*Typhlonannolene adaptus* Chamberlin, 1923, the only species described of the genus, the description being made only from females) found in British Guiana (South America), *Epinannolene* (Mauriès 1987) for which species are known in Central America (Loomis 1968) and in the Brazilian Amazon (Mauriès 1987; Adis 2002) and *Cambalomma* Loomis, 1941, found in Central America. *Typhlonannolene* presents mandibles with 9 to 10 pectinate lamellae, the repugnatorial pores present in the fifth segment, besides the only species described for the genus being also anophthalmic (although not found in caves) and the gnathochilarium being similar to that of *Epinannolene* (Chamberlin 1923). It is noteworthy that the validity of the genus remains uncertain due to the absence of males described. *Epinannolene* has mandibles with about seven lamellae, the fourth segment apodal and the repugnatorial pore present in the sixth (or fifth) segment (Brölemann 1903). In the description, the author pointed out the great similarity of the genus to *Pseudonannolene*, and differences include the absence of middle division in the *promentum* and the presence of 10 lamellae in *Pseudonannolene* (Brölemann 1903; Mauriès 1987). Other characteristics which also differentiate them are podous rings from which begin the repugnatorial pores and the gonopod morphology. In *Epinannolene* there is a tendency to have an elongated solenomere, that has a bifid morphology. Furthermore, the gonopod in representatives of this genus, is separated into two portions, distal and basal (Mauriès 1987). In relation to *Cambalomma* Loomis, 1941 the similarity is due to the presence of basiconic sensilla on the fifth and sixth antennomere, repugnatorial pores also in the fifth segment (Loomis 1941) and the division of the promentum (Florez & Hoffman 1995). Other genera such as *Physiostreptus* Silvestri, 1903, *Holopodostreptus* Carl, 1913, *Choctella* Chamberlin, 1918, *Phallorthus* Chamberlin, 1952 are also considered near *Pseudonannolene* due to the reduction of posterior gonopod (Florez & Hoffman 1995).

Over time, the family Pseudonannolenidae underwent major modifications in relation to its taxonomic position. It is emphasized that the taxonomic status of some groups within the order Spirostreptida (like Pseudonannolenidae) remains unclear (Hoffman & Florez 1995; Korsós & Johns 2009). Shelley (2003) brought important considerations about these problems of taxonomic order involving this group.

Habitat, ecological considerations and threats: The caves where the specimens were collected are located in an iron ore outcrop regionally known as “Morro II”. Most caves occurring there are associated to the superficial ferruginous breccia (canga formation). Several caves are known in the area, and the species was found in nine caves. These caves are small, with sizes varying from 29 meters (GEM 1754) to 204 meters (GEM 1727). However, all of those caves are connected to a huge network of small channels (canaliculi), typically found in the canga formation, which considerably enhances the habitats for the subterranean fauna in iron ore caves (Souza-

Silva *et al.* 2011). In all cases, the individuals were found in the inner portion of the caves, which have very distinct conditions from those observed near the entrances. Those areas are usually aphotic and very moist.

The Brazilian cave fauna has been systematically studied only in the last few years. New species are currently being discovered and more than 500 troglobitic invertebrate species were found in different areas of Brazil during the past five years (Ferreira, unpublished data). However, this singular fauna is endangered, especially due to anthropogenic activities which are threatening their habitats. Until 2008, all caves in Brazil were protected by law. Unfortunately, the national legislation was altered and caves all over the country can now be exploited. To ensure the complete conservation of a cave in Brazil, it is necessary, from a biological point of view, to prove the occurrence of at least one endemic troglobitic species. Therefore the description of *P. spelaea*, besides contributing to the knowledge of this genus in the Neotropics, ensures the protection of the caves which the species inhabits.

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