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Two new species of *Pseudonannolene* Silvestri, 1895 from Brazilian limestone caves (Spirostreptida: Pseudonannolenidae): synotopy of a troglophilic and a troglobiotic species

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Abstract

Pseudonannolene ambuatinga **n. sp.** and *Pseudonannolene saguassu* **n. sp.** are described from individuals collected in limestone caves in the municipality of Pains, Minas Gerais, Brazil, which comprises a scenario of great conflict between speleological preservation and limestone extraction. The two species differ in their gonopod morphology and in size and color. *Pseudonannolene ambuatinga* **n. sp**. is regarded as the second troglobite species known for the family. This is the first report for Brazil of syntopy in two congeneric species in the same cave.

Key words: Diplopoda, *Pseudonannolene*, Cave, Brazil, Conservation, Neotropics, Troglobiotic

Introduction

The genus *Pseudonannolene* Silvestri, 1895 is often found in Brazilian caves of different lithologies (Mauriès 1974; Fontanetti 1996a; Trajano *et al.* 2000; Ferreira 2004; Trajano & Bichuette 2010; Souza-Silva *et al.* 2011; Iniesta & Ferreira 2013) and is thus considered as typically troglophile, establishing populations in both the external and subterranean environment (Culver & White 2005). Currently in Brazil, among the twenty known species, nine have been described from individuals found in caves, but only one is considered a troglobiont (Iniesta & Ferreira 2013). In Brazil, studies on the genus began at the end of the nineteenth century with descriptions of epigean species (Brölemann 1909). The first species described from Brazil, *Pseudonannolene longicornis* Porat, found in 1888 in the state of São Paulo, was initially described as *Alloporus longicornis* (Spirostreptidae), and later relocated in the genus *Pseudonannolene* (Brölemann 1909). The first species directly described in this genus was *Pseudonannolene alegrensis* Silvestri 1897 (state of Rio Grande do Sul) described by the Italian Filippo Silvestri (Brölemann 1909). Subsequently, several other species have been described from different regions (Silvestri 1902; Brölemann 1909; Mauriès 1987) making this genus the most diverse in the family in Brazil.

The first troglophilic species described was *Pseudonannolene strinatii* Mauries, 1974, found in the Gruta das Areias in the state of São Paulo (Mauriès 1974). Subsequently, others have been described (Mauriès 1987; Fontanetti 1996b), the most recent being *Pseudonannolene tocaiensis* Fontanetti, 1996 (Fontanetti 1996a), found in the Toca Cave (state of São Paulo). According to Trajano & Bichuette (2010), individuals found in this environment are usually associated to organic debris of animal origin, such as bat guano deposits (especially from hematophagous species). However, such authors apparently based their findings on few observations on the genus, reaching a generalized conclusion about an alleged preference for certain organic resources. These organisms can feed in a huge variety of organic debris inside caves, not only on bat guano. Furthermore, in most cases, organisms of this genus are found walking on the floor or on the walls of caves, and it is not rare to observe specimens buried under the cave sediments. In addition to taxonomic work referring to the genus, other works were published in Brazil on the reproductive histology, physiology and cytogenetics of some species (Penteado & Hebling-Beraldo 1991; Fontanetti 1991; Fontanetti 2000; Freitas & Fontanetti 2003; Campos & Fontanetti 2004).

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Although relatively little is known about the biology of species of this genus, the presence of more than one species in the same cave in Brazil has never been registered. From this perspective, the objective of the present work is to describe two new species of the genus found in caves, *Pseudonannolene saguassu* **n. sp**. and *Pseudonannolene ambuatinga* **n. sp**., the last considered a troglobiont. In at least one of the studied caves (Gruta do Éden) the co-existence of the two species was verified, which constitutes the first case of syntopic species of this genus in Brazil, although this may be "accidental" syntopic (see discussion). Nevertheless, the region where the species were found is subject to considerable anthropogenic impact (Zampaulo 2010), especially the destruction of caves due to mining activities.

Material and methods

Collection and preservation: Type specimens were collected during 2009 and 2012 and are deposited in the Zoology Collection, Seção de Invertebrados Subterrâneos at the Universidade Federal de Lavras (UFLA), Campus Universitário de Lavras, Minas Gerais, Brazil. All specimens were captured with a brush and placed in vials containing 70% ethanol, according to Ferreira (2004). The collections were made in seven limestone caves in the municipality of Pains, Minas Gerais state.

Photography and scanning electron microscopy (SEM): 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 obtained 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 observation on a LEO EVO 40 XVP scanning electron microscope (Leo Electron Microscopy), samples were mounted on aluminum support stubs, placed on a film of aluminum foil with carbon tape and sputter-coated with gold using a Baltec SCD 050. For the measurements of body length, length of legs, tarsal claws and antennae, the distance between two farthest points on their extremities was used. For the diameter, the maximum vertical diameter was used. The ratio between the lengths 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 ambuatinga Iniesta & Ferreira, 2013

(Figs. 2–6)

Material examined: Holotype: 1 Male (ISLA 2267) from Gruta Loca d'água de baixo, Pains/MG, Brazil, 28/I/2009.

Paratypes: 1 Male (fragmented, ISLA 2272) from Gruta Loca d'água de baixo, Pains/MG, Brazil, 28/I/2009; 2 Males (fragmented, ISLA 2273, ISLA 2275) from Gruta do Éden, Pains/MG, Brazil, 15/III/2012; 1 Female (ISLA 2274) from Gruta do Éden, Pains/MG, Brazil, 15/III/2012; 4 Female (fragmented, ISLA 2268, ISLA 2269, ISLA 2270, ISLA 2271) from Gruta Loca d'água de baixo, Pains/MG, Brazil, 28/I/2009; 4 females (ISLA 2276, ISLA 2277, ISLA 2278, ISLA 2279) from Gruta do Éden, Pains/MG, Brazil, 15/III/2012.

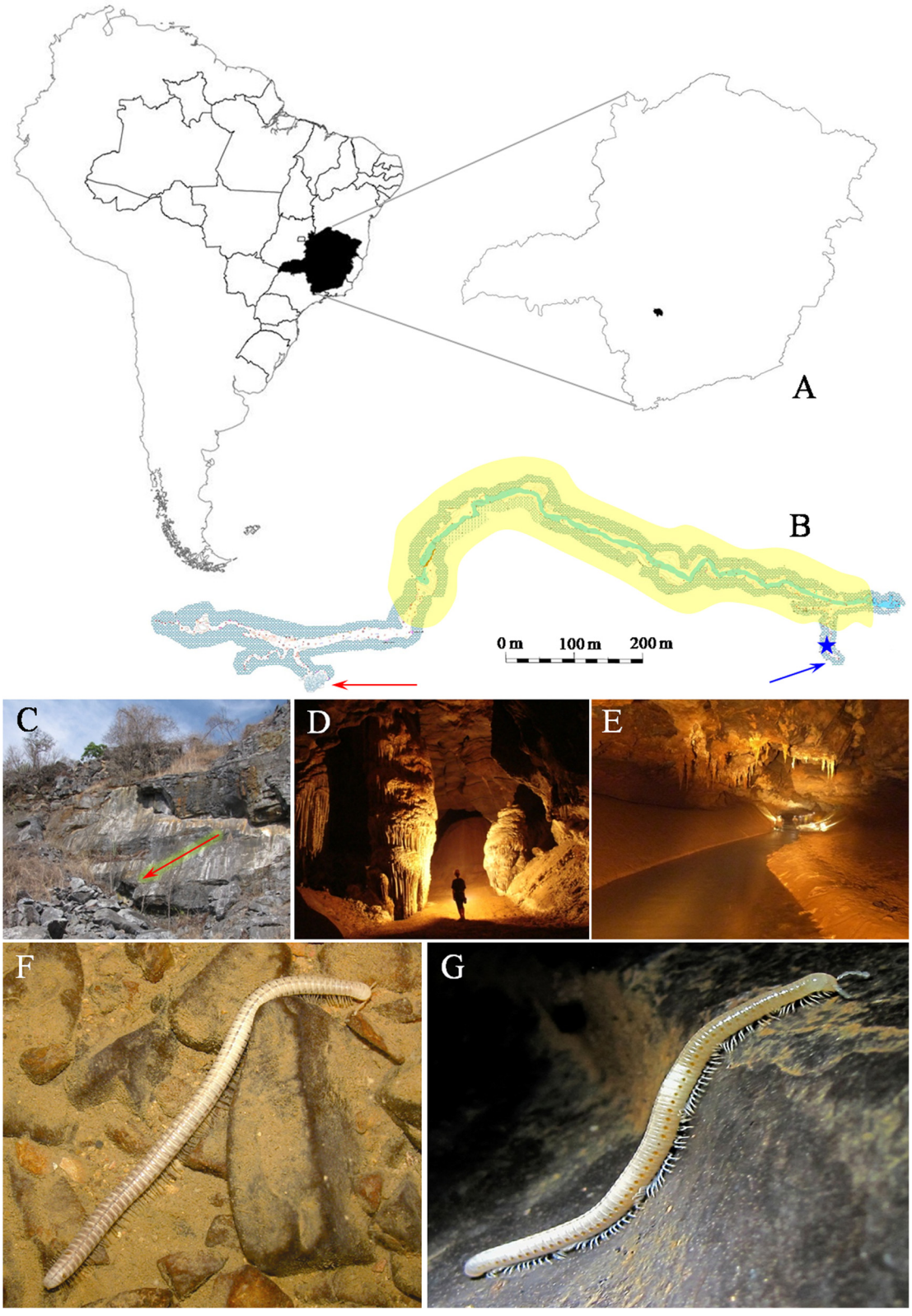

FIGURE 1. Distribution map of *P. ambuatinga* **n. sp.** and *P. saguassu* **n. sp.** A) Map of Brazil and Minas Gerais state, indicating the location of the Pains municipality; B) Map of the Gruta do Éden. The arrows show the cave entrances (blue arrow—natural entrance; red arrow—artificial entrance, opened by mining activities). The blue star indicates the area where *P. saguassu* was found and the yellow area shows the population distribution of *P. ambuatinga* inside the cave; C) Artificial entrance of the Gruta do Éden (red arrow); D) Upper conduits of the cave; E) Stream conduit, in which the population of *P. ambuatinga* lives; F) *P. ambuatinga* **n. sp.** found walking under the water; G) *P. ambuatinga* **n. sp.** on a rock.

FIGURE 2. *P. ambuatinga* **n. sp.**, paratype female (ISLA 2269), stereoscope images. Detail of anterior region;

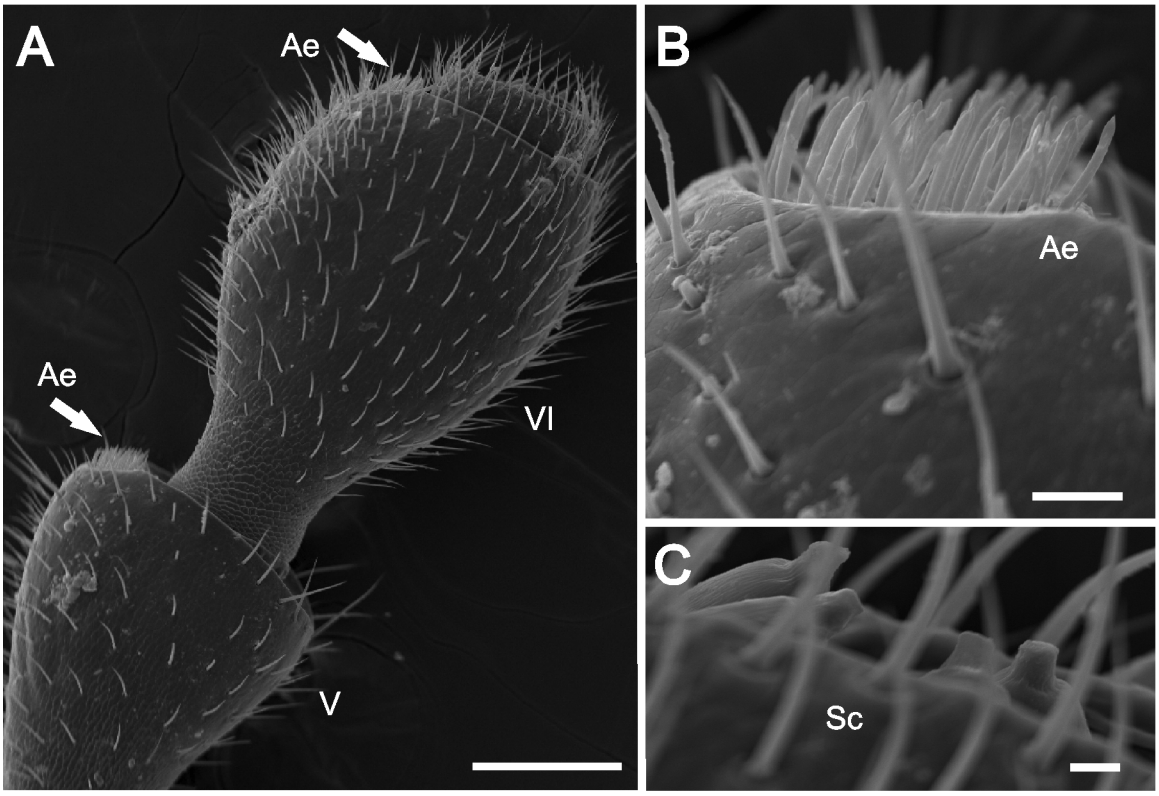

FIGURE 3. *P. ambuatinga* **n. sp.**, paratype male (ISLA 2272), SEM, antenna view. A) 5° and 6° antennomeres; B) Fifth antennomere with detail of aesthetascs; C) Last antennomere with detail of sensory cones. **Abbreviations:** Ae = aesthetascs; Sc = Sensory cones. **Scales bars:** A = 200 µm; B = 20 µm; C = 10 µm.

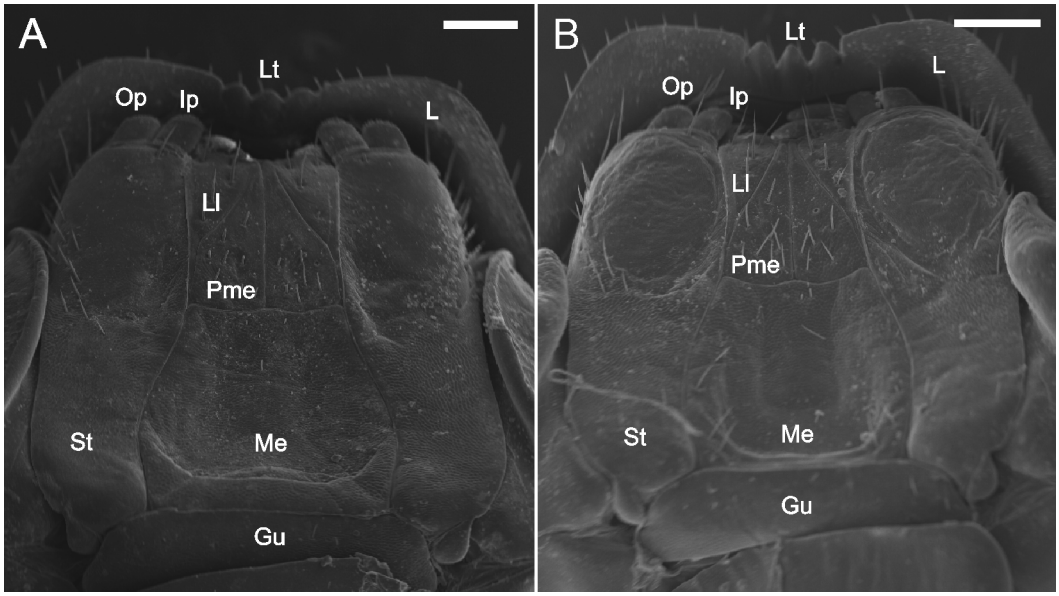

FIGURE 4. *P. ambuatinga* **n. sp.**, paratype female (ISLA 2268) and paratype male (ISLA 2272), SEM, *gnatochilarium* view. A) Female; B) Male. **Abbreviations:** Gu = gula; St = stipes; Me = mentum; Pme = pro-mentum; Ll = laminae linguaies; L = labrum; Lt = labrum teeth; Op = outer palpus; Ip = inner palpus. **Scales bars:** 200 μm.

Etimology. *Ambuatinga* is formed by a combination of words: "*Ambua*," means "millipede" and "*tinga"* means "white", both words coming from the Tupi-Grarani (Brazilian Indian languages). Therefore, *ambuatinga* means "white millipede." It is to be treated as a noun in apposition.

Diagnosis. Body and eyes depigmented. Eyes with 27-33 ocelli. Basal section of gonopod 0.75 times longer than width and distal section 0.5 times longer than width. Solenomere rhomboid, spine directed laterally.

Measurements: Length from 40 up to 44 mm; maximum midbody diameter between 2.40 to 3.36 mm; body rings ranging between 61 to 66; length of antennae ranging from 2.5 to 2.89 mm (relation to diameter ranging 0.86 to 1.04); length of legs 1.60 to 2.11 mm (relation to diameter ranging 0.67 to 0.63); length of tarsal claw 0.1 to 0.14 mm (relation to diameter of 0.04).

Color: Whitish.

Description of Adults. *Head* (Fig. 2): Head glabrous and depigmented. Three small labral teeth, a row of 15 labral setae and above a row of 6 supralabral setae. Mandibles depigmented, glabrous and with 2 external teeth, 4 internal teeth and 10 pectinate lamellae (difficult to see). Eyes depigmented with 27 to 33 ocelli, arranged in 4-5 rows. Antennae depigmented and densely setose. First antennomere small, with setae exclusively positioned on the distal edge. Second and third antennomeres of similar sizes. Fourth and fifth antennomeres shorter than third (Fig. 3A), the width in the fifth being longer than the fourth. Sixth antennomere longer and wider than the fourth and fifth and with four terminal sensory cones (Fig. 5C). Groups of basiconic sensilla on the edge of the fifth and sixth antennomeres (Fig. 5B).

Gnathochilarium (Fig. 4A; B): *Gula* (**Gu**) with short setae. *Mentum* (**Me**) rounded with few setae visible. Males with a concavity deeper in **Me** than the female and *Stipes* (**St**) more rounded in distal region. **St** with basally and distally rounded lateral borders. *Promentum* (**Pme**) divided into two triangular parts separated by a midline suture. *Laminae linguaies* (**Ll**) triangular, entirely separated by **Pme**.

Trunk: Tergites and collum depigmented. Lateral region of rings with transverse striae present (striae variable in individuals). Anal shield and anal valve slightly pigmented.

First male pair of legs (Fig. 5A): The first leg pair is modified and densely setose. Sternum (**St**) small and rounded. Coxae (**Cx**) larger than those of remaining legs and densely setose. Prefemur (**Prf**) with thin oral process parallel (**P**) to the coxa (Fig. 5B). Postfemur (**Psf**) and tibia (**Tb**) reduced (Fig. 5C). Femur (**F**) longer and wider than other podomeres. Terminal claw (**Tc**) not modified (Fig. 5D).

Gonopod (Fig. 6A)*:* Coxae (**Cx**) reduced, glabrous and joined with basal section of telopodite. The basal section (**Bs**) of telopodite with surface more membranous than **Cx**. Shoulder absent. Length is about 0.75 times longer than width. **Bs** beset with basiconic sensilla (**B**) extending toward the internal margin of section (Fig. 6D).

Distal section (**Ds**) (Fig. 6B) sturdier and with length about 0.5 times longer than width. **Ds** divided into solenomere (**S**) (Fig. 6C) and an internal branch (**Ib**), separated by a seminal groove (**Sg**). **Ib** (coxosternal branch) like a shield of solenomere, densely setose. **S** rhomboid, with two different regions of squamous surface, a lateral surface more rounded and another spiniform in anteromedian region. Presence of a spine (**Sp**) in apex directed laterally.

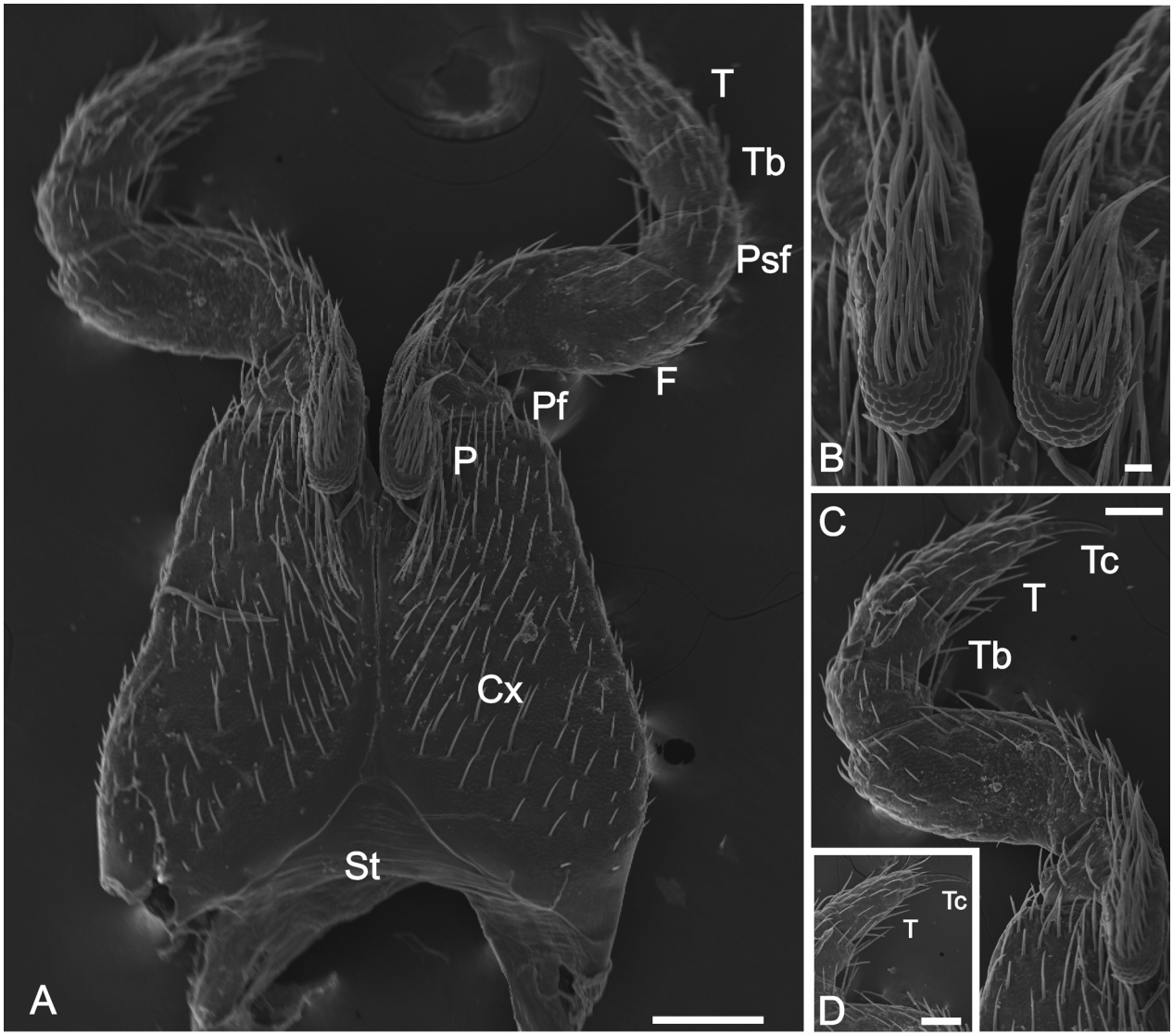

FIGURE 5. *P. ambuatinga* **n. sp.**, paratype male (ISLA 2272), SEM of the first pair of legs. A) First pair of legs; B) Detail of process on the pre-femur; C) Detail of the left leg; D) Detail of tarsus and claw. **Abbreviations:** St = sternum; Cx = coxae; P = process; Pf = pre-femur; F = femur; Psf = post-femur; Tb = tibia; T = tarsus; Tc = tarsal claw. **Scale bars:** A = 200 µm; B = 20 µm; C = 100 µm; D = 100 µm.

Remarks. Individuals of *P.ambuatinga* were observed in seven caves in the Pains region (Minas Gerais, Brazil), totaling 327 individuals, although few were collected for the study. In all caves, the organisms were always found in aphotic areas, far from entrances, also showing a clear preference for extremely humid locations. The largest populations were found in caves with underground rivers, such as the Gruta do Éden (Fig. 1B, C, D, E) and the Loca d´água de baixo. Hundreds of individuals were observed in these caves. The organisms showed clear preference for plant remains that accumulated on the banks of rivers and for underground deposits of bat guano (especially that of the bat *Desmodus rotundus*, a hematophagous species). Furthermore, we observed completely submerged organisms, walking along the bottom of streams (Fig. 1F), in the Gruta do Éden and Loca d´água de baixo. Some individuals were collected alive and reared in the laboratory. The terrarium was modified to contain two regions, one photic and the other aphotic (covered with aluminum foil and black plastic). Under these

conditions, individuals exhibited clear preference for the aphotic area of the terrarium as expected, with some specimens burying themselves in sediments and remaining there.

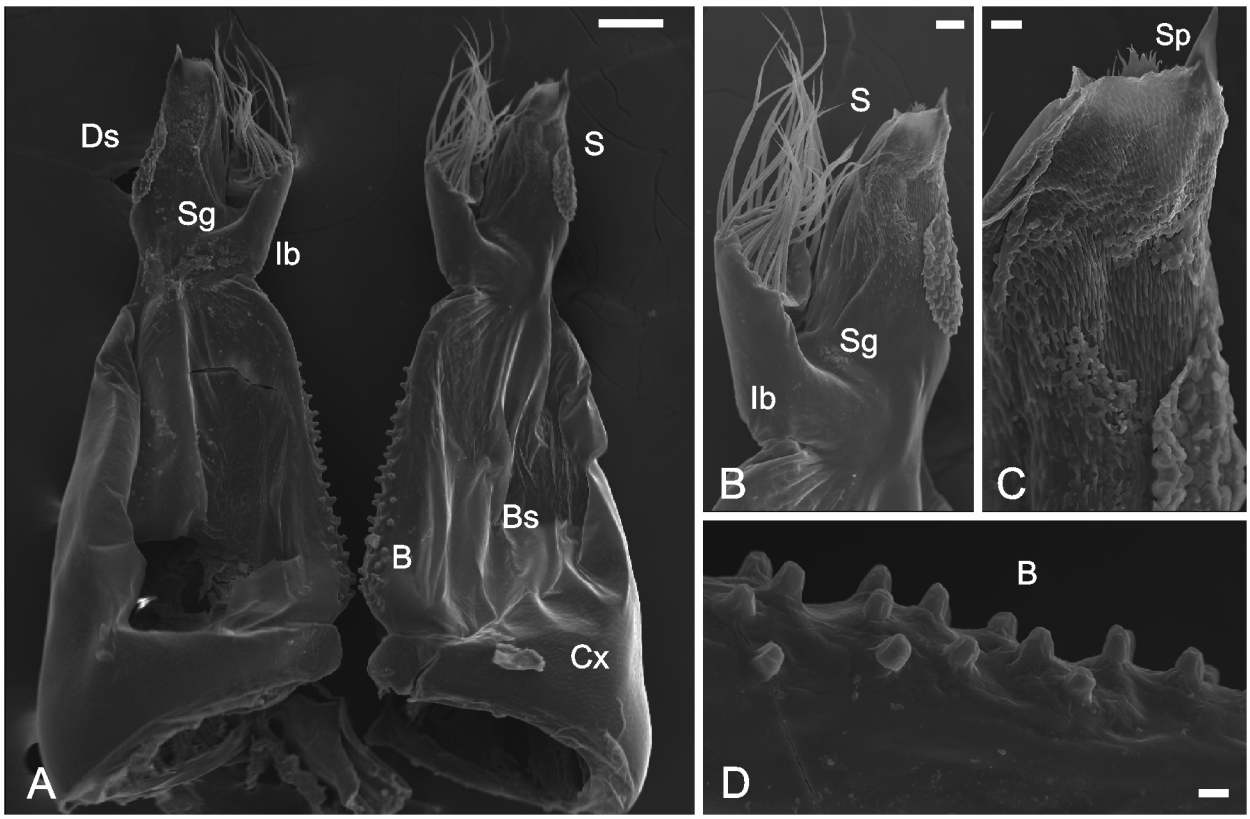

FIGURE 6. *P. ambuatinga* **n. sp.**, paratype male (ISLA 2272), SEM of the gonopod. A) Gonopod; B) Detail of distal segment of the telopodite; C) Detail of the solenomere; D) Basiconics bristles. **Abbreviations:** Cx = coxae; B = basiconic bistles; Bs = basal segment; Ds = distal segment; Sg = seminal groove; Ib = internal branch; S = solenomere; Sp = spine. **Scale bars:** A = 100 µm; B = 20 µm; C = 10 µm; D = 10 µm.

***Pseudonannolene saguassu* Iniesta & Ferreira, 2013.**
(Figs. 7–10)

Material examined: Holotype: 1 Male (Fragmented, ISLA 2266), from Gruta do Éden, Pains/MG, Brazil, 15/III/2012.

Etimology. *Saguassu (saguaçú)* comes from the Tupi-Grarani origin (Brazilian Indian languages), meaning "those with big eyes". This name obviously refers to the numerous ocelli observed in this species. It is to be treated as a noun in apposition.

Diagnosis. Body with 69 rings and brownish coloration. About 45 Ocelli, pigmented. 30 labral setae and mandible with 11 pectinate lamellae. Gonopod with rhomboid solonomere and a central spine. Basal section 0.75 times longer than wide and distal section about 0.5 times longer than wide.

Measurements: Length of 65.16 mm; maximum midbody diameter of 3.68 mm; 69 body rings; length of antennae of 3.67 mm (relation to diameter 0.99); length of legs 2.60 mm (relation to diameter 0.71); length of tarsal claw 0.2 mm (relation to diameter 0.05).

Color: Brownish.

Description of adults. *Head* (Fig. 7): Head glabrous and pigmented. Three small labral teeth, a row of 30 labral setae and above a row of 6 supra labral setae (one apparently missing). Mandibles pigmented, glabrous and with 2 external teeth above, 5 internal teeth and 11 pectinate lamellae. Eyes pigmented with 45 ocelli, distributed into 5–6 rows. Antennae pigmented and densely setose. First antennomere small, second and third antennomeres of similar sizes, fourth and fifth antennomeres shorter than third. Sixth antennomere longer and wider than the fourth and fifth. Groups of basiconic sensilla on the edge of the fifth and sixth antennomeres. Four terminal sensory cones.

FIGURE 7. *P. saguassu* **n. sp.**, holotype male (ISLA 2266), stereoscope images. Detail of anterior region;

Gnathochilarium (Fig. 8A; B): *Gula* (**Gu**) with short setae. *Mentum* (**Me**) rounded and with base wider than the latter. *Stipes* (**St**) with base shorter than distal region. *Promentum* (**Pme**) with two trianguliform parts and *Laminae linguales* (**Ll**) entirely separated by **Pme**.

Trunk: Body with 69 rings. Tergites and collum pigmented (homogeneously brownish). Lateral transverse striae present (striae variables into individuals). Anal shield and anal valve pigmented like the trunk.

First male pair of legs (Fig. 9A): The first leg pair is modified and densely setose. Coxae (**Cx**) with basis longer than distal region. Distal region with a shoulder. Sternum not visible. Prefemur (**Prf**) with thin oral process parallel (**P**) to the coxa, more rounded in distal (Fig. 9B). Post-femur (**Psf**) and tíbia (**Tb**) reduced. Femur (**F**) elongated. Terminal claw (**Tc**) not modified.

Gonopod (Fig. 10A; B): Gonopod short and stout. Coxae (**Cx**) little visible and glabrous. The basal section (**Bs**) of telopodite glabrous, 0.75 times longer than wide. Shoulder absent. Basiconic sensilla (**B**) extending along the membranous margin from basal section. Distal section (**Ds**) about 0.5 times longer than width and with two well-developed processes, the solenomere (**S**) and an internal (seminal) branch (**Ib**). **S** rhomboid and with a squamous surface coating the structure. Spine (**Sp**) present in center of the apex. **Ib** (coxosternal branch?) digitiform and supporting setae at distal region that exceeds the length of **S**. Short seminal groove (**Sg**) between **S** and **Ib**.

Remarks. Morphological analysis was performed by stereoscopic observations and light microscopy in a single male specimen. The specimen was found in an area between the twilight and aphotic zones of Gruta do Éden, near the vertical entrance (Fig. 1B). In the same cave, there is a huge population of *P. ambuatinga*, which is distributed especially in the main conduit of the cave, however, cut by a stream. Accordingly, this cave represents the first case of syntopic species of *Pseudonannolene* occurring in Brazilian caves. However, it is very likely that *P. saguassu* actually represents an "accidental" species, since only a single specimen was found during many visits paid to the cave. Since the external (epigean) environment was not properly sampled, it is plausible to assume that the main habitat of this species is the external semi-deciduous forest which covers the area. Therefore, it is not possible to consider the species as "rare" based on the fact that only a single specimen was collected.

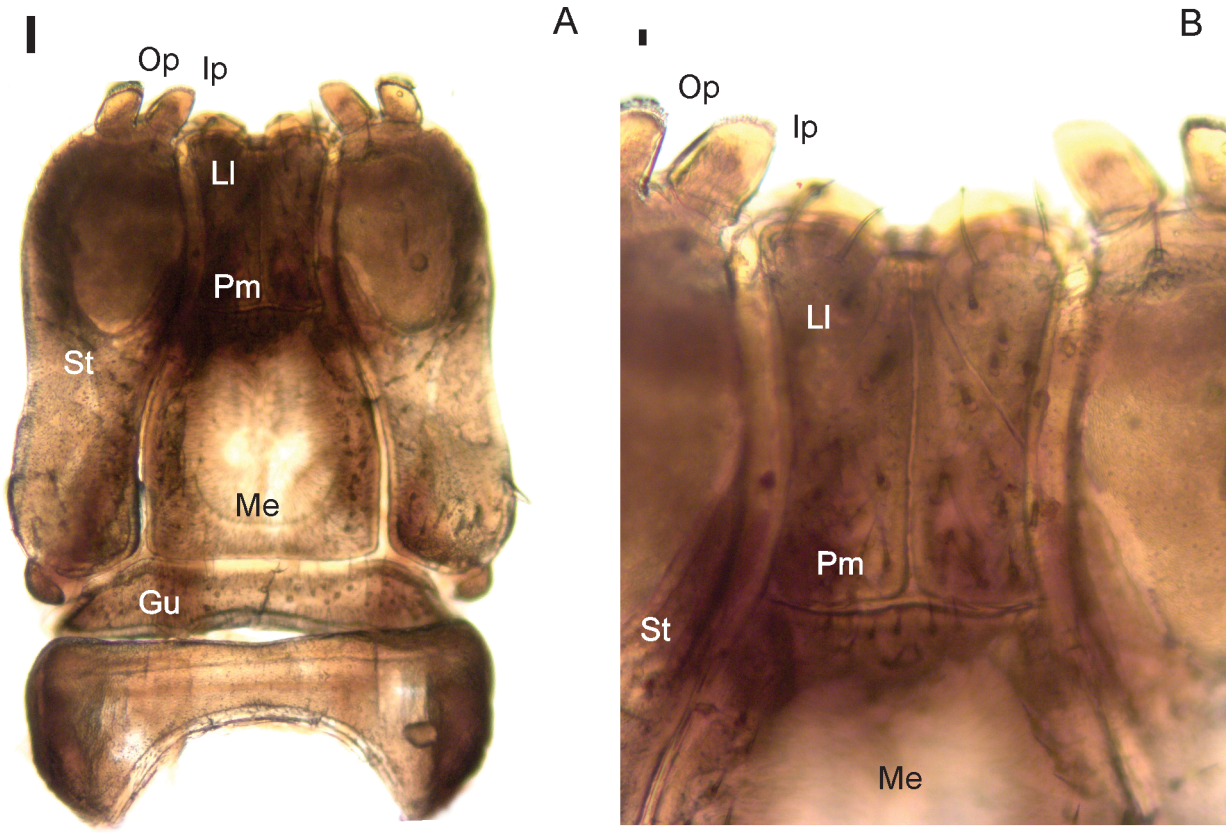

FIGURE 8. *P. saguassu* **n. sp.**, holotype male (ISLA 2266), micrographic images. A) gnatochilarium; B) Detail of the gnatochilarium. **Abbreviations:** Gu = gula; St = stipes; Me = mentum; Pme = pro-mentum; Ll = Laminae linguaies; Op = outer palpus; Ip = inner palpus. **Scales bars:** A = 100 µm; B = 20 µm.

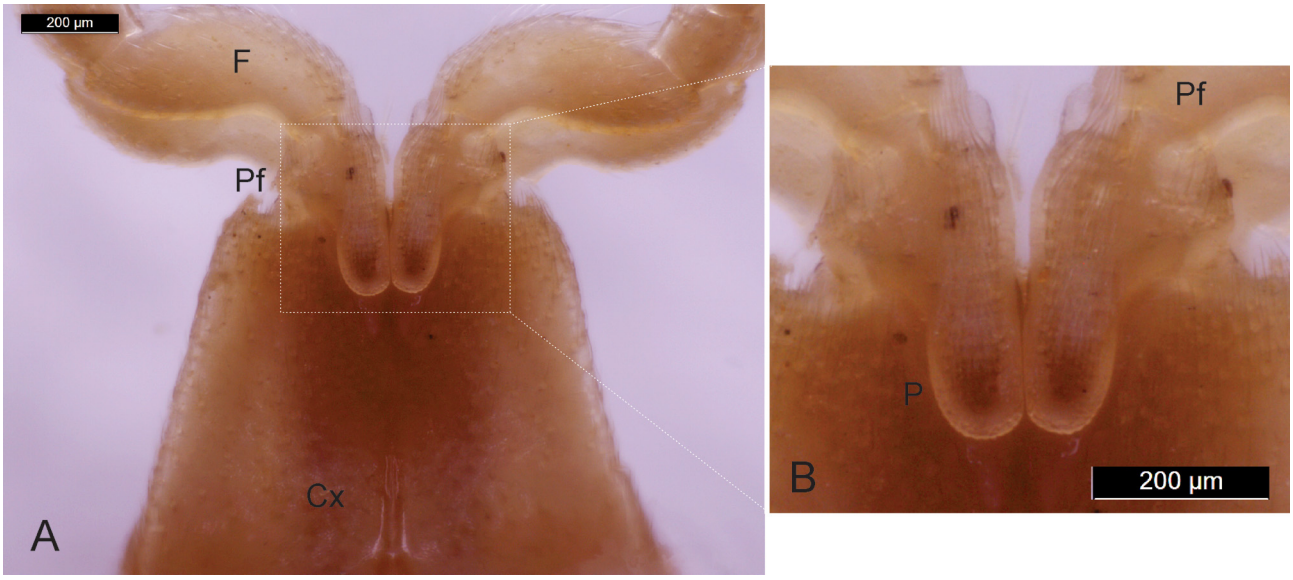

FIGURE 9. *P. saguassu* **n. sp.**, holotype male (ISLA 2266), stereoscope images. A) First pair of legs; B) Details of the oral process. **Abbreviations:** Cx = coxae; P = process; Pf = pre-femur; F = femur.

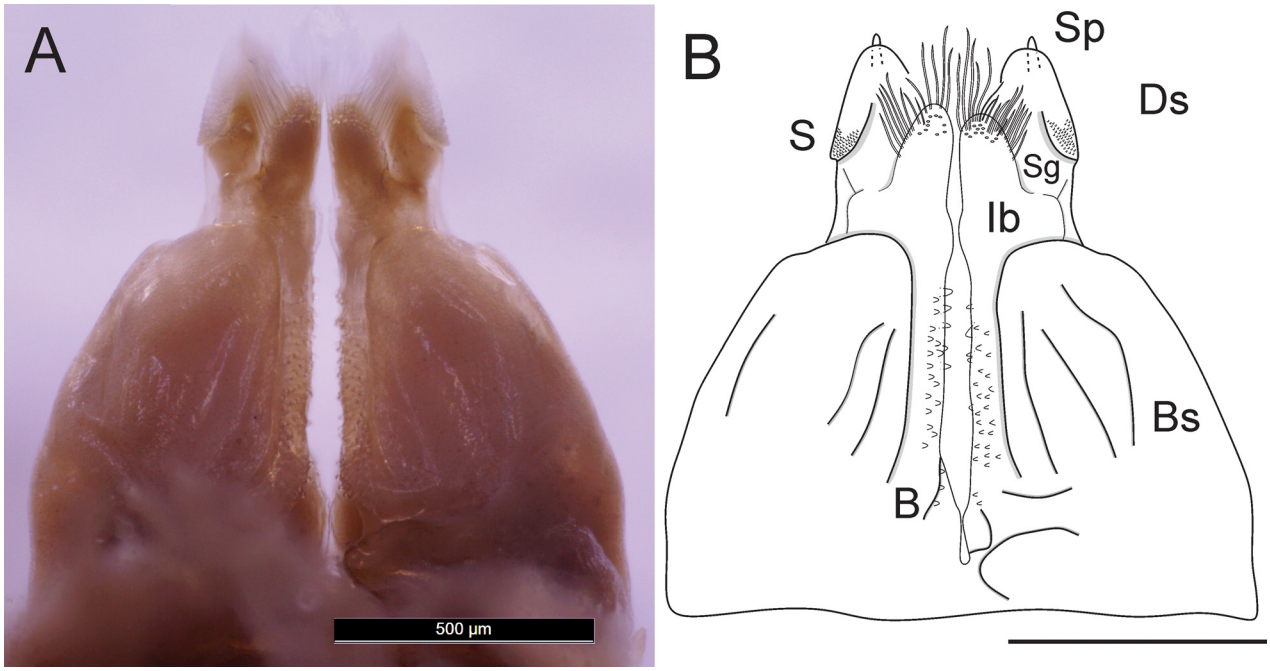

FIGURE 10. *P. saguassu* **n. sp.**, holotype male (ISLA 2266), stereoscope images and schematic drawings. A) Gonopod; B) Schematic drawing of the gonopod. **Abbreviations:** Bs = basal segment; Ds = distal segment; B = basiconic bistles; Ib = internal branch; S = solenomere; Sp = spine; Sg = seminal groove. **Scale bars:** 500 µm.

Discussion

Affinities. *Pseudonannolene* differs from closely related genera, such as *Epinannolene* and *Typhlonannolene* (the latter, of uncertain taxonomic position) in the longitudinal separation in the promentum and the general structure of the gonopod. Although the gonopod description is essential for effective species diagnosis, this character was little (or insufficiently) detailed in old descriptions, and in some cases comparison with other species becomes difficult. According to Fontanetti (2002) a character of importance in differentiating species of the genus is the oral process of the pre-femur of the first pair of legs of males, which displays differentiation in form and may even be absent, as in *P. anapophysis* (Fontanetti 1996b; Fontanetti 2002). Considering the characteristics that differentiate *Pseudonannolene* species, *P. saguassu* **n. sp**. and *P.ambuatinga* **n. sp** clearly differ from other species of the genus.

The gonopods of the two species show similarities in the ratios between the length and width of the telopodite basal and distal section. The rhomboidal appearance of the solenomere is also similar in the two species and *P. anapophysis* (Fontanetti 1996b), although the position of the apophysis at the apex is not the same (not illustrated in *P. anapophysis*) and by presenting a difference in solenomere proportions. On the internal branch, the digiform structure in *P. saguassu* is similar to *P. anapophysis* (Fontanetti 1996b) and *P. strinatii* (Mauriès 1974), while *P. ambuatinga* has similarity to *P. tocaiensis* (Fontanetti 1996a), *P. chaimowiczi* and *P. imbirensis* (Fontanetti 1996b) in the branch having a shield shape compared to the solenomere. However, it is noteworthy that *P. saguassu* and *P. ambuatinga* feature gonopodal structures more similar, in general, to each other compared to those of other Brazilian species.

In relation to oral processes in the first pair of legs of males, both species have a form similar to that found in *P. tocaiensis*, *P. mesai*, *P. chaimowiczi*, *P. leucocephalus*, *P. strinatii*, *P. ophiulus* and *P. halophila* (Fontanetti 2002).

Regarding somatic characters in *P. ambuatinga,* 6 supra-labral setae and 15 labral setae were observed, as in *P. spelaea* (Iniesta & Ferrera 2013) while in *P. saguassu* 6 supra-labral and 30 labral setae were observed. On the number of body rings there were no significant differences, *P. ambuatinga* has 61-66 rings while *P. saguassu* has 69, the average in South American species being 60 rings (Silvestri 1902; Mauriès 1974; Mauriès 1987). The body size also showed no large differences in comparisons with other species, although *P. saguassu* (65 mm in length and 3.7 mm in maximum vertical diameter) is larger than *P. ambuatinga* at 40 mm in length and 2.8 mm vertical diameter. Among the species of the genus, the body length ranges from 30 mm, as in *P. rocana* and *P. augularis* to 72 mm in *P. segmentata*. In comparing the species pigmentation, the difference becomes remarkable only in

relation to *P. ambuatinga* due to its depigmentation (see discussion below). *P. saguassu* of brownish tint has a clearly different shade from other species of the genus, which present a blackish color and differences between the prozonite (lighter region) and metazonite (Mauriès 1974; Fontanetti 1996a; Fontanetti 1996b). The whitish coloration observed in *P. ambuatinga* is discussed below.

Troglomorphic traits. Characters such as the number of ocelli, body size and pigmentation are variable among species of the genus, but may also have been modified after restriction (or isolation) in the subterranean environment. In addition, another important character that suggests troglomorphy (though little discussed in the descriptions of the genus), includes groups of basiconic sensilla found on the distal edge of the fifth and sixth antennomeres. In the troglobiont species *P. spelaea* (Iniesta & Ferreira 2013) the sensillae are elongaged when compared to non-troglobiotic species (inferring their possible enhanced sensory function). However, these structures can present as modified only in very troglomorphic species, since in *P. ambuatinga* such bristles are not more elongated than those present in *P. saguassu* and other troglophile species such as *P. strinatii* and *P. chaimowiczi* (Mauriès 1974; Fontanetti 1996b).

P. ambuatinga presents pronounced depigmentation when compared to other epigean species. However, this characteristic was found to be variable (some specimens found in the Gruta do Éden had faint pigmentation). In addition, individuals reared in the laboratory gradually became pigmented, indicating that these organisms are not actually albino. Such a condition, associated with the non-elongation of the antenna sensillae (as observed in *P. spelaea*) may possibly indicate a recent isolation in the subterranean environment, so that many traits still do not show as regressed (such as eyes and pigmentation) or hypertrophied (such as antenna sensilla).

According to Shear (1969) and Golovatch & Kime (2009) troglobiotic species can often show a trend of increasing body size. In this case, the species *Pseudonannolene ambuatinga* presents a smaller size when compared to *P. saguassu* and to other non-troglobite species., This feature may result from the evolution of the species for locomotion in small spaces, or for its rapid development, since the number of body rings does not greatly vary in relation to other species. This decrease of the body size of troglobiotic species has been observed in *P. spelaea* (Ferreira & Iniesta 2013) and in other groups (Culver *et al.* 1995; Culver *et al.*, 2010; Souza & Ferreira 2011).

Regarding the number of ocelli, *P. ambuatinga* has 27 to 33, while *P. saguassu* has about 45. Among the described species, there is a variation from 20 to 40 ocelli, with the exception of the troglobite species *P. spelaea* with 10 to 15 ocelli (Iniesta & Ferrera 2013). Although *P. ambuatinga* does not have an apparent reduction in the number of ocelli compared to other species of the genus, this species has a markedly lower number of ocelli than that observed in the *P. saguassu* species. In summary, *P. ambuatinga* is probably a troglobite species. However, given the high degree of ignorance of the epigean fauna of the region (and even the country), it would be advisable to carry out external collections for the actual diagnosis of the troglobiosis (in relation to its restriction to the subterranean environment or not) to be confirmed.

Conservation issues. According to Zampaulo (2010) the region where *Pseudonannolene ambuatinga* **n. sp.** and *Pseudonannolene saguassu* **n. sp.** were found is currently a location of major social and environmental importance. Since the mid-1960s the region has suffered from intense investment in mining activities (sometimes illegal) for the production of cement, lime and soil correctives. Such activities have caused irreversible alterations in the landscape of the area resulting in a scenario of great conflict between (bio) speleological preservation and limestone extraction. The region has also experienced strong impacts on its natural vegetation, thus leading to habitat loss (Cavalcanti *et al.* 2012) and alterations in drainage near the caves (Zampaulo 2010), directly influencing the allocation of resources into the caves, which are primarily dependent on the surrounding forests for the generation of organic matter. The extraction of limestone also leads to alterations in the caves, ranging from partial suppression of conduits (as noted in the Gruta do Éden, where *P. saguassu* and the largest population of *P. ambuatinga* were found) to the total destruction of caves due to detonation of explosive (Zampaulo 2010).

The caves located in this karst system make up one of the priority areas for conservation of invertebrates in the state of Minas Gerais, due to the richness of endemic and/or threatened species, thus constituting an area of extreme biological importance (Zampaulo 2010). Therefore, because of the threat status of that system, species description becomes a strong ally for preservation of the area. In Brazil, the current legislation (Decree nº 6.640/ 2008) allows that caves may be destroyed except those which are defined as being of maximum relevance. The presence of a troglobiotic species raises the cave to maximum relevance status, as long as the rarity of that species is verified. Thus, the description of *P. ambuatinga* as troglobiont likely increases the relevance of the caverns to which it is linked, causing such caves to become legally preserved.

Zampaulo (2010) defined the region as one of the richest in troglobiotic species in Brazil. Seventy-nine troglobitic species were found in caves in the area, only one of which has been described to date, which shows the great importance of the description of new species to ensure the area's preservation.

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