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***Pseudonannolene lundi* n. sp., a new troglobitic millipede from a Brazilian limestone cave (Spirostreptida: Pseudonannolenidae)**

LUIZ FELIPE MORETTI INIESTA^{1, 2} & RODRIGO LOPES FERREIRA¹

¹*Centro de Estudos em Biologia Subterrânea, Setor de Zoologia Geral, Departamento de Biologia, Universidade Federal de Lavras, Minas Gerais, Brazil*

²*Corresponding author. E-mail: drops@dbi.ufla.br*

Abstract

Pseudonannolene lundi n. sp., is described from Lapa Sem Fim Cave, a limestone cave from Luislândia municipality, Minas Gerais State, Brazil. The species is the eighth troglobitic millipede and the third of the genus *Pseudonannolene* described from Brazilian caves. *Pseudonannolene lundi* shows pronounced depigmentation and decrease of body size, as found in other troglobitic species belonging to the genus. The gonopod has a robust and evident internal branch, and a solenomere slightly trianguliform.

Key words: *Pseudonannolene*, Cave, Conservation, Neotropics, troglobitic

Introduction

In Brazil, the troglobitic fauna is of great importance to cave conservation. According to the decree 6.640/2008, Brazilian caves containing at least one endemic troglobitic species are considered as of maximum relevance, thus cannot be destroyed. Unfortunately, other caves that are not considered of maximum relevance can be severely impacted. Currently, seven troglobitic millipedes species are known from Brazil (Schubart 1946a, 1946b, 1957; Golovatch & Wytwer 2004; Iniesta *et al.* 2012; Iniesta & Ferreira 2013a, 2013b). Among these, two species belong to the genus *Pseudonannolene* Silvestri, 1895: *P. spelaea* Iniesta & Ferreira 2013 from iron ore caves of Para state (Iniesta & Ferreira 2013a), and *P. ambuatinga* Iniesta & Ferreira 2013 from limestone caves of Minas Gerais state (Iniesta & Ferreira 2013b). For these species, the strong depigmentation, a reduction of the number of ocelli and a relative decrease in body size in comparison to non-troglobitic species are recognized as troglomorphic traits (Iniesta & Ferreira 2013a). In this paper, we describe a new, third troglobitic species of *Pseudonannolene*, from a Brazilian limestone cave in Minas Gerais state.

Material and methods

Collection and preservation: Type specimens were collected during 2014 and are deposited in the Zoology Collection, Seção de Invertebrados Subterrâneos (ISLA) at the Universidade Federal de Lavras, Campus Universitário de Lavras, Minas Gerais, Brazil. All specimens were collected by hand and fixed in vials containing 70% ethanol.

Photography and scanning electron microscopy (SEM): Dissections were made with fine entomological pins. The images were obtained using the AxioCam 506 color connected to a stereoscope Axio Zoom.V16 (ZEISS). 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%).

Systematic

Order Spirostreptida Brandt, 1833

Suborder Cambalidea Cook, 1895

Family Pseudonannolenidae Silvestri, 1895

Subfamily Pseudonannoleninae Silvestri, 1895

Genus *Pseudonannolene* Silvestri, 1895

Pseudonannolene Silvestri, 1895. *Annali del Museo Civico di Storia Naturale di Genova*, 34: 775. Type species: *Pseudonannolene typica* Silvestri, 1895, by monotypy. Online publication: Sierwald, P. (ed.). 2006. *Nomenclator Generum Diplopodorum*, version 2.

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(Fig. 1–3)

Material examined: Holotype: 1 ♂ (ISLA 8684) from Lapa Sem Fim cave (UTM 23K – 539.855 – 8.214.614), Luislândia/MG, Brazil, 17/IV/2014. Collected by R. L. Ferreira.

Paratypes: 2 ♂ (ISLA 8685, 8686) and 3 ♀ (ISLA 8687, 8688, 8689) from Lapa Sem Fim cave (UTM 23K – 539.855 – 8.214.614), Luislândia/MG, Brazil, 17/IV/2014. Collected by R. L. Ferreira.

Etimology. The specific epithet honors the Danish naturalist Peter Wilhelm Lund, considered the founder of speleology as a science in Brazil. Peter Lund has worked for decades in the caves of Minas Gerais state, and made remarkable contributions to Brazilian paleontology. In addition, we intend to extend the honor to a caving group of the same name (Espeleo Grupo Peter Lund) for their contributions to our knowledge regarding the caves in the north of Minas Gerais state, as well as the support given to us during our collections in the area.

Comparative diagnosis. *P. lundi* is similar to the species *P. ambuatinga* and *P. spelaea* in relation to depigmentation and reduction of body size, which have evolved as a result of restriction in the subterranean environment (Iniesta & Ferreira 2013a, b). The gonopod of *P. lundi* resembles *P. strinatii* (Areias Cave, São Paulo), mainly in the structure of the distal section. The internal brach is more robust and apparent. The solenomere is slightly trianguliform, varying in the format as in *P. ambuatinga* and *P. saguassu* (more trianguliform) in relation to *P. strinatii*, with a lateral/diagonal tip.

Description of adults. *Measurements:* Length from 54 up to 61 mm; maximum midbody diameter between 2.4 and 2.8 mm; body rings ranging between 62 to 68; length of antennae ranging from 2.6 to 2.9 mm (relation to diameter ranging 1.03 to 1.08); length of legs 2.10 to 2.3 mm (relation to diameter ranging 0.82 to 0.87); length of tarsal claw 0.12 to 0.16 mm (relation to diameter ranging 0.05 to 0.06).

General characteristics: Body whitish (Fig. 2). Trunk with metazona distinguished of prozona for a transversal suture and a weak difference in tonality. Tergites and collum depigmented. Lateral region of rings with transversal striae. Anal ring, valve and hypoproct depigmented. Head glabrous and depigmented. Labrum with a row of 15 labral setae and 6 supralabral setae. Mandibles depigmented with 2 external teeth evident, 4 internal teeth and 8 pectinate lamellae. Eyes depigmented with 27 to 33 ocelli; Antennae depigmented and densely setose. First antennomere smaller than others. Groups of basiconic sensilla on the edge of the fifth and sixth antennomeres (difficult to see). Four sensory cones on apical of sixth antennomere.

Male characteristics: The first male pair of legs with elongated coxae (**Cx**), two times longer than wide; pre-femoral process elongated and larger than pre-femur (**Pf**) (Fig. 3C). Process (**P**) densely setose from the basis up to distal portion (Fig. 3D). Gonopod elongated and well-sclerotized (Fig. 3A, B). Coxae reduced (**Cx**); basal section (**Bs**) with length larger than width. Basiconic sensilla (**B**) arranged in rows along the internal edge of **Bs**. A short shoulder (not visualized in SEM). Distal section (**Ds**) with length similar to half of **Bs**. Internal branch (**Ib**) robust and enlarged; similar to a shield of solenomere (**S**). Some bristles of **Ib** exceeding the apex of **S**. **S** slightly trianguliform; a long spine (**Sp**) on apex and lateral tip rounded; evident squamous surface on external side.

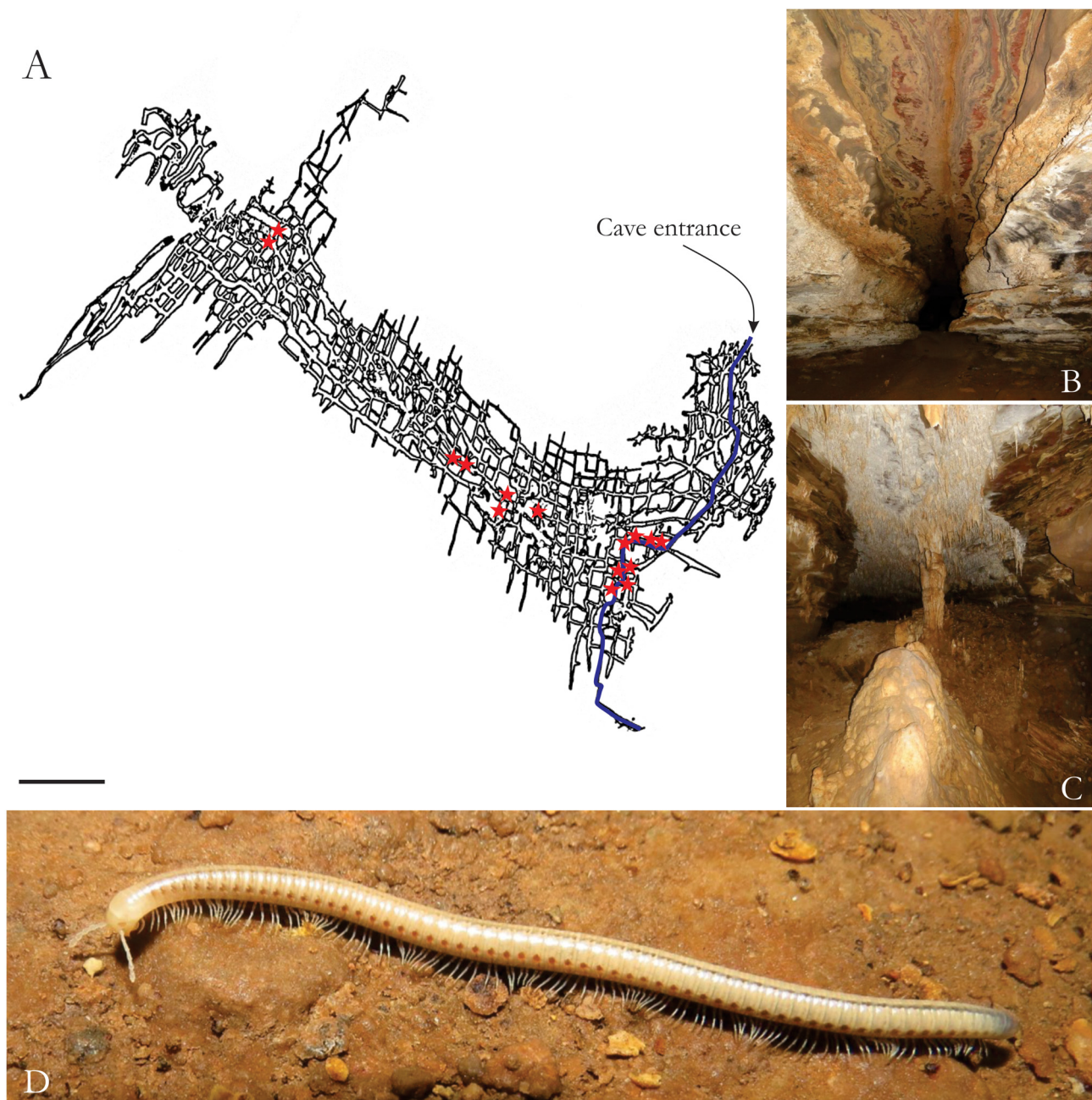


FIGURE 1. Distribution map of *Pseudonannolene lundi* n. sp. A) The map of Lapa Sem Fim cave, indicating with red star the specific location where the species was found and with blue line the subterranean river (credits for the cave map: Grupo Bambui de Pesquisas Espeleológicas). Scale bar corresponding to 60 meters; B) A dry conduit of the cave; C) An inner moist chamber; D) *Pseudonannolene lundi* n. sp., living specimen.

Notes. *P. lundi* n. sp. comprises the third troglomorphic species of the genus described from Brazilian caves. This species presents some specific troglomorphic traits (morphological adaptation to the subterranean environment), such as pronounced depigmentation and a reduction of body size. Regarding these adaptations, some aspects deserve comments. Regarding the lack of pigmentation, it is worth noting that is undoubtedly a result of restriction in an aphotic environment, although some troglomorphic species belonging to the genus do not necessarily have a notable depigmentation (*personal observation*). In addition to the depigmentation, the reduction of body size can be an indicator of the restrictions in the subterranean realm. Indeed, the three troglomorphic species from the genus do not exceed 44 mm in length (maximum of 66 body rings) (Iniesta & Ferreira 2013a, b), while some Brazilian non-troglomorphic species, in average, have lengths around 61.5 mm (Iniesta & Ferreira 2013a, b, Iniesta & Ferreira 2014). According to Culver et al. (1995, 2010), the reduction of body size in some cave-dwelling arthropod is known, in

which the size depends of the specific habitat in caves, or its interactions with other groups of arthropods (e.g. predation).



FIGURE 2. *Pseudonannolene lundi* n. sp., stereoscope image. Detail of anterior region of a female.

Regarding to the decrease on the number of ocelli, it did not prove to be necessarily a differential feature for the troglobitic species. Both troglobitic species, *P. lundi* and *P. ambuatinga*, have numbers of ocelli similar to other non-troglobitic Brazilian species (Mauriés 1974, 1987; Fontanetti 1996a, b; Iniesta & Ferreira 2013a, b; Iniesta & Ferreira 2014). The exception is *P. spelaea*, which has a maximum of 15 ocelli (Iniesta & Ferreira 2013a). The number of ocelli is quite variable between non-troglobitic species, varying from 20 to almost 50 (Iniesta & Ferrera 2013b; Iniesta & Ferrera 2014). Accordingly, it is quite difficult to assume the presence of a reduced number of ocelli in any species, since for all cases the closest relative species is unknown, given the lack of a phylogenetic analysis for the genus. So, if the ancestor of *P. lundi* had around 30 ocelli, we could consider that a reduction did not occur. However, if this species descends from an epigean species with originally around 50 ocelli, the reduction would be obvious. Unfortunately, lack of knowledge regarding the phylogenetic relationships among the known species prevents us from actually assessing whether there was (or not) a reduction in the number of ocelli in troglobitic species from this genus.

According to Iniesta & Ferreira (2013a), another possible troglomorphic trait in the genus is the elongation of the sensilla basiconica on the antennae. In the species *P. spelaea* the elongation is remarkable in comparison to other non-troglobitic species from Brazil. However, to confirm the modification of this trait, some future work will be required. For *P. lundi*, the sensilla are larger than in other species, but no comparative measurement was made.

Ecological remarks. For many troglobitic myriapods, as centipedes, the knowledge regarding the environment in which the species live certainly contributes to establishing if the species is actually restricted to the subterranean environment (Ázara & Ferreira 2014). This is also true for *Pseudonannolene*, since there are many species (mostly non-troglobitic) living in caves. Accordingly, the species' habitat should be characterized, since in some cases, the population can only be found in specific inner chambers of a cave.

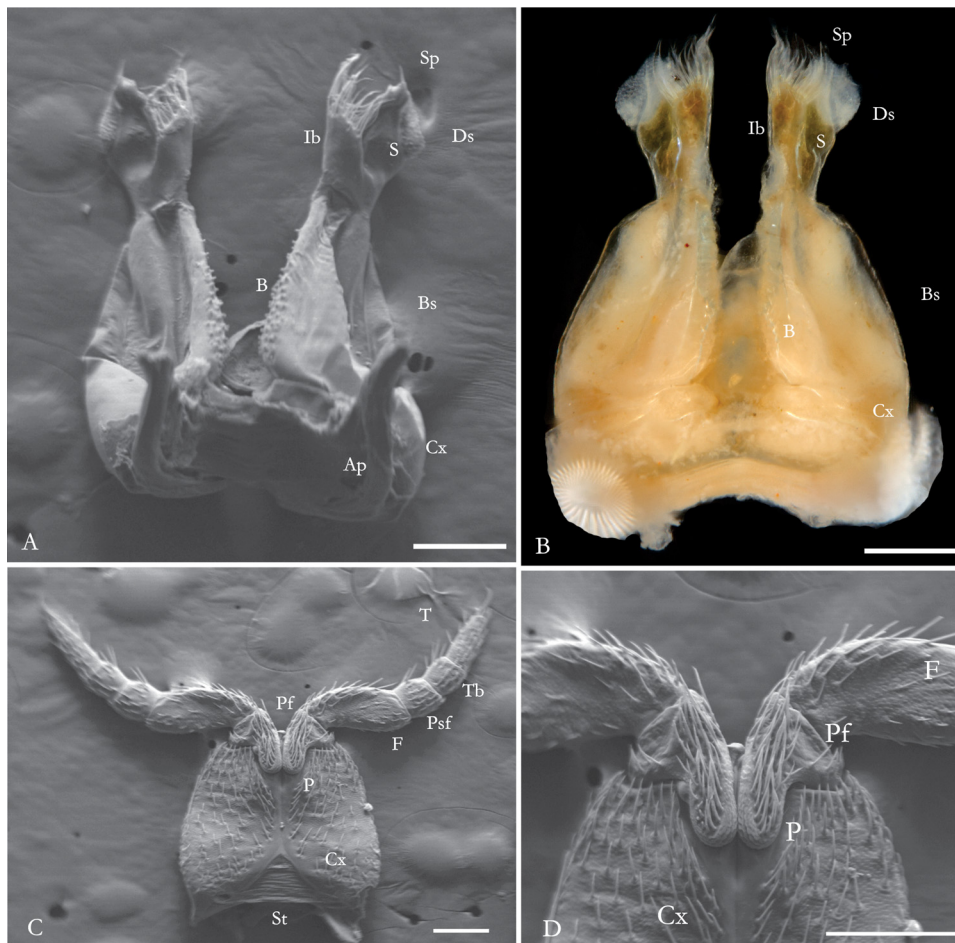


FIGURE 3. *Pseudonannolene lundi* n. sp., SEM image. A) Gonopod (200 μ m); B) Gonopod (0.2 mm); C) First male pair of legs (200 μ m); D) Detail of pre-femoral process (200 μ m). **Abbreviations:** Ib = internal branch; Sp = spine; S = solenomere; Ds = distal section; B = basiconic sensilla; Bs = basal section; Ap: apodeme; Cx = coxae; T = tarsus; Tb = tibia; Psf = post-femur; F = femur; Pf = pre-femur; P = process; St = sternum. **Obs:** The shoulder and the lateral tip of S in figure A were modified in the drying process for the SEM.

The Lapa Sem Fim cave comprises the largest cave in Minas Gerais state, with around 15 km of explored galleries. The cave morphology is quite unusual, with an intricate system of conduits. There are only two known entrances (Figure 1A), each one located in the extremities of the only drainage (which is intermittent) existent in the cave. Most of the galleries are extremely dry (Figure 1B), being located in an upper level in relation to the drainage conduit. In a few areas, there are some moist chambers, in which the humidity comes from percolating water. Such chambers are usually full of speleothems. Specimens of *P. lundi* were observed only in moist areas inside the cave, though in very distinct regions (Figure 1C). No specimen was observed close to entrances. The biggest aggregation was observed in the middle part of the drainage conduit, although specimens were usually located in upper areas. In the inner portions of the cave, specimens were only found on those moist chambers, full of speleothems. Specimens seem to feed on distinct organic debris, as vegetal debris (mainly near the drainage conduit) or bat guano (all over the cave), though those resources were very rare, especially in areas far from the drainage. At least 5 other caves were sampled in the vicinity, but specimens of *P. lundi* were only observed in Lapa Sem Fim cave, suggesting that the species might be endemic to that cave.

Although Brazilian caves are under risk due to the new legislation, the population of *P. lundi* seems to be relatively safe. The cave is rarely visited by locals and is well preserved. Furthermore, the large size of the cave, allied to its atypical morphology, are attributes that certainly enhance the relevance of the cave.

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