Coming out in a harsh environment: a new genus and species for a land flatworm (Platyhelminthes: Tricladida) occurring in a ferruginous cave from the Brazilian savanna

Ana Leal-Zanchet[1], Alessandro Damasceno Marques[1]

[1] Instituto de Pesquisas de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos, 93022-750 São Leopoldo, Rio Grande do Sul, Brazil

Corresponding author: *Ana Leal-Zanchet* (zanchet@unisinos)

Short title: New land planarian in Brazilian savanna

Abstract

A new genus and species of the Neotropical subfamily Geoplaninae, sampled within a cave in an area belonging to the Brazilian savanna (Cerrado phytophysiognomy), is described. The new genus shows rare features within Geoplaninae, namely sub-cylindrical body, poorly developed sub-epidermal musculature and a narrow creeping sole. Some features, such as a small body and a broad sensory margin in the anterior region of the body, as well as the fact that the holotype showed signs of recent copula, may indicate an adaptation to the subterranean environment, representing at least a troglophile. The holotype shows a secondary male copulatory organ located immediately behind the primary one, both communicating with the female atrium and

gonopore canal. Since the species seems to have low abundance and restricted distribution and its type-locality is affected by mining activities, major concern arises regarding its conservation.

Key words: Geoplaninae, land planarians, Neotropical region, subterranean fauna, taxonomy.

Introduction

In the Neotropical region, there have been addressed some faunal inventories on land triclads, especially in southern South America, most of them in areas of Atlantic Forest (Leal-Zanchet & Carbayo, 2000; Castro & Leal-Zanchet, 2005; Antunes, Marques & Leal-Zanchet, 2008; Leal-Zanchet & Baptista, 2009; Baptista, Oliveira & Leal-Zanchet, 2010; Amaral et al., 2014; Negrete, Colpo & Brusa, 2014). Some specific environments, however, are almost unexplored, such as the hypogean habitats. More than 11,000 caves have been documented in Brazil, representing about only 10% of the potential number of caves in the country (Auler et al., 2001).

Caves are relatively stable environments regarding thermal and moisture conditions (Barr, 1968; Rocha & Galvani, 2011; Pellegrini & Ferreira, 2012), thus favouring the occurrence of land planarians. However, their diversity in such environments is almost unknown. Their occurrence in subterranean habitats may be occasional, searching for a shelter, as was probably the case of a recently described species of *Pasipha* NAME (Leal-Zanchet & Marques, 2018). Some species may be adapted to the cave environment, completing their life cycle both in hypogean or epigean habitats (troglophiles) or inhabiting exclusively the cave (troglobites), using it for foraging and reproduction (Barr, 1968).

already published, right?
Autor desconhecido
Hoje, 16:00

~~The recently described flatworm species of~~ *Pasipha* NAME, AUTHORS was sampled in a ferruginous cave from an area belonging to the Brazilian savanna (Cerrado phytophysiognomy), on the eastern margin of the Serra do Espinhaço Plateau, in southeastern Brazil (Leal-Zanchet & Marques, 2018). In a different ferruginous cave from the same region, another fauna survey indicate~~d~~s the occurrence of a second flatworm species ~~in a different ferruginous cave~~. This flatworm shows features that allow its assignment to the Neotropical subfamily Geoplaninae, but it could not been assigned to any known genus. Thus, herein we provide ~~here~~ the description of a new genus and species of Geoplaninae to accommodate this flatworm.

It is already published, right?
Autor desconhecido
Hoje, 15:47

Material and Methods

A single flatworm was collected during the day by direct sampling in the entrance zone of a ferruginous cave (FSS-0081) in Conceição do Mato Dentro (18°56'48.1" S, 43°24'27.6" W), at an altitude of 810 m a.s.l., in the state of Minas Gerais, southeastern Brazil (Fig. 1). The specimen was fixed in 70% ethyl alcohol during field work. The preserved specimen was analysed regarding colour pattern, body shape and dimensions and then photographed under a stereomicroscope. Methods described by Rossi et al. (2015) were used for histological processing of the material and analysis of external and internal characters. The material was sectioned at intervals of 6 µm and stained with Goldner's Masson or Haematoxylin/Eosin methods.

The field work was conducted under a collection license granted by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA (permit number 02015.004286/2010-49).

Type-material is deposited in the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, state of São Paulo, Brazil (MZUSP). The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:A6418F77-34C3-413B-81E9-54296A74F2F6. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Abbreviations used in the figures:

cmc common muscle coat

cov common glandular ovovitelline duct

cs creeping sole

di dorsal insertion of pharynx

e eyes

fa female atrium

fc female canal

88**go** gonoduct

89**i** intestine

90**lu** pharyngeal lumen

91**med** main ejaculatory duct

92**mma** main male atrium

93**mo** mouth

94**mpv** main prostatic vesicle

95**n** nerve plate

96**o** ovary~~ies~~

97**oe** oesophagus

98**om** outer musculature of pharynx

99**ov** ovovitelline duct

100**pp** pharyngeal pouch

101**sbm** sub-intestinal transversal mesenchymal muscles

102**sd** sperm duct

103**sed** secondary ejaculatory duct

Not found in figures. Please add

Autor desconhecido
Hoje, 11:02

104**sg** shell glands

105**sm** sensory margin

106**sma** secondary male atrium

snm sub-neural transversal mesenchymal muscles

spm supra-intestinal transversal mesenchymal muscles

spv secondary prostatic vesicle

t testes

v vitellaria

ve ventral epidermis

vi ventral insertion of pharynx

vm ventral cutaneous musculature

Taxonomic description

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

***Difroehlichia* Leal-Zanchet & Marques, gen. nov.**

Type-species: *Difroehlichia elenae ~~Leal-Zanchet & Marques~~*, sp. nov. Monotypic.

Genus diagnosis: Geoplaninae with small and slender body, sub-cylindrical ~~in cross-section~~, with parallel margins; eyes mono- and trilobate, arranged along the body margins; sensory margin broad; sensory pits in an irregular row; creeping sole narrow, less than half of body width; sub-epidermal musculature poorly developed; sub-epidermal and mesenchymal

Either sub-cylindrical or elliptical in cross section
Autor desconhecido
Hoje, 10:44

Absent at the anterior tip of the body?
Autor desconhecido
Hoje, 10:45

musculatures without cephalic specializations; longitudinal mesenchymal muscles absent; pharynx cylindrical; prostatic vesicle intrabulbar; male atrium short with eversible penis; ascending portion of ovovitelline ducts lateral to female atrium, joining each other dorsally to female canal or atrium; female canal dorsally flexed; female atrium obliquely disposed; adenodactyls or musculo-secretory papillae absent.

Remarks

The occurrence of dorsal testes and sub-epidermal longitudinal muscles arranged in bundles ~~indicate that the new genus belongs~~conform to the subfamily Geoplaninae, which currently has 24 genera (Carbayo et al., 2013). The new genus, however, shows poorly developed sub-epidermal musculature and a narrow creeping sole, which are rare features within Geoplaninae (Ogren & Kawakatsu, 1990).

A morphological comparative analysis suggests that among Geoplaninae, only the genus *Xerapoa* has such a narrow creeping sole, being even~~r~~ narrower, with a width corresponding to one-third of body width (Froehlich, 1955; Ogren & Kawakatsu, 1990), whereas the creeping sole shows a width less than half of body width in *Difroehlichia*. In addition, *Xerapoa* shows sensory pits opening at the tip of small papillae, main nervous system two-chord shaped, eyes monolobate, ovaries close to the pharynx, ovovitelline ducts joining behind the female atrium and a horizontal female canal (Froehlich, 1955; Carbayo et al., 2013). Thus, the new genus can be easily differentiated from *Xerapoa* by having sensory pits opening through a broad sensory margin, a broad nervous plate, eyes mono- and trilobate, ovaries close to the anterior tip, ovovitelline ducts joining dorsally to female canal or atrium and female canal dorsally flexed.

Etymology

The new genus honours the researchers Dr. Claudio Froehlich and the late Dr. Eudóxia Froehlich, who developed an extensive and renowned study on land triclads. Gender: feminine.

Difroehlichia elenae ~~*Leal-Zanchet & Marques,*~~ sp. nov.

Type material. Holotype MZUSP PL XXX: leg. *Carste Ciência e Ambiente*, 23 November 2016, Conceição do Mato Dentro (18°56'48.1" S, 43°24'27.6" W; altitude 810 m a.s.l.), state of Minas Gerais (MG), Brazil – anterior tip: transverse sections on 6 slides; anterior region at the level of the ovaries: sagittal sections on 5 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 5 slides; copulatory apparatus: sagittal sections on 6 slides; posterior region: sagittal sections on 4 slides.

Diagnosis

Fixed, *Difroehlichia elenae* is characterized by an almost homogeneous dark brown pigmentation over dorsal surface and body margins, pharynx cylindrical and short, prostatic vesicle tubular, unforked and vertically disposed with two portions: a proximal, narrow portion ~~narrower~~ and ventral, distal portion globose and dorsal, ejaculatory duct short, male atrium short and globose occupied by a large circular fold, male and female atria separated by a constriction, female atrium with folded walls and ample lumen, tapering to communicate with the gonopore canal, length of female atrium about 2/3 of male atrial length.

Unclear.

Autor desconhecido
Hoje, 10:58

both, main and secondary?

Autor desconhecido
Hoje, 11:06

Etymology

The new species pays homage to a friend, the late Dr. Elena Diehl, who contributed to the knowledge of the ecology of ants and termites in southern Brazil.

Type-locality: Conceição do Mato Dentro, state of Minas Gerais (MG), Brazil.

Description

External features

Aspect of living specimen unknown. Body elongate with parallel margins, sub-cylindrical ~~in cross section~~; anterior and posterior tips rounded (Fig. 2). ~~After fixation, length of~~ 13.5 mm long, maxima~~l~~um width of 1.5 mm, and maxima~~l~~um height of about 0.8 mm. Mouth at a distance from anterior tip of the body equivalent to 63% of body length and gonopore at 78% ~~of body length~~.

~~Fixed specimen with d~~Dorsal surface covered ~~by~~with fine, almost homogeneous dark brown pigmentation over light brown ground colour, which is apparent under stereomicroscope on the anterior tip and on the median dorsal region. Ventral surface grayish, ~~with~~ dark brown at the margins, ~~lateral parts bordered by the~~these bordered by the whitish sensorial margin in the anterior body half (Fig. 2).

Unclear. What is apparent? Do you mean “mottled pigment less densely distributed on the anterior tip and on the median dorsal region, so that this region becomes lighter” or something like this?
Autor desconhecido
Hoje, 11:13

Eyes, absent on the very anterior tip, arranged exclusively on the body margins, ~~absent on the very anterior tip~~ and almost imperceptible, even under the stereomicroscope due to the ~~highly~~ body pigment~~ed body~~. Close to the anterior tip, eyes monolobate ~~and~~, with pigment cups of

about 8 µm in diameter. ~~After that~~Behind, they become trilobate and with larger pigment cups (about 15–30 µm in diameter). The eyes become sparser towards the posterior tip.

Sensory organs, epidermis and body musculatures~~.~~

Anterior tip

Body flattened on anterior tip, becoming sub-cylindrical ~~in cross section~~ just after the tip. Epidermis low (3 µm) with few and sparsely distributed rhabditogen glands and few cyanophil glands with amorphous secretion. On the anterior tip, openings from erythrophil glands are absent.

The creeping sole occupies about 30% of body width, showing a tall, erythrophil epithelium with long and densely arranged cilia. Laterally to the creeping sole, the ventral epidermis is similar to the dorsal epidermis (Figs 3A–C).

The sensory margin is wide, occupying between 15% and 28% of body width on either side of body; it is ventromarginally arranged and shows low, ciliated epithelium (Figs 3A, C), receiving cyanophil glands with amorphous secretion. It contains one irregular row of sensory pits (15–30 µm ~~long~~deep), which occur as simple invaginations on either side of body in approximately the anterior third of body. ~~After~~Behind the tip, the sensory margin gradually becomes narrower and receives openings from erythrophil glands with finely granular secretion, usually 3-4 gland necks opening close to each other.

Cutaneous musculature thicker ~~in comparison~~ to that in pre-pharyngeal region, especially considering its relation to the body height, gradually diminishing in thickness towards anterior tip. Mesenchymal musculature slightly thicker in cephalic region than in pre-pharyngeal region,

From this point on, the text is not telegraphic any more. I would adopt only one style.
Autor desconhecido
Hoje, 11:33

It seems to be wrong (after Fig. 3A). Do you mean “ventral body surface flat in anterior tip”? I just would delete “flattened on anterior tip, becoming”.
Autor desconhecido
Hoje, 11:30

Interesting; in most cases, sensory margin is not ciliated.
Autor desconhecido
Hoje, 15:34

Absent at the anterior tip of the body?
Autor desconhecido
Hoje, 15:38

Which layers?
Autor desconhecido
Hoje, 11:43

with an additional transverse layer located below the nerve plate (Figs 3A–C); thickness gradually diminishes towards anterior tip.

How it is named?

Autor desconhecido
Hoje, 11:43

Pre-pharyngeal region

Creeping sole (Figs 3D–F) with erythrophil, tall epithelium (twice taller than the rest of the epidermis), showing an irregular height and densely arranged, long cilia~~.—I;~~ its width corresponds to between 40% and 50% of body width. Three gland types discharge through dorsal epidermis and body margins, as well as in the lateral portions of the ventral surface of pre-pharyngeal region: rhabditogen cells with xanthophil secretion with relatively short rhammites (about 10 µm long), cyanophil glands with amorphous secretion and sparse erythrophil glands with finely granular secretion. Sparser rhabditogen cells with smaller rhabdites open through creeping sole. Glandular margin absent (Fig. 3E).

Or rhammites?

Autor desconhecido
Hoje, 11:46

Cutaneous musculature with usual three layers (circular, oblique and longitudinal layers), longitudinal layer thin and with small bundles (Figs 3E–F). Mc:h 3%. Ventral and dorsal musculatures with similar thickness (about 8–12 µm) at sagittal plane ~~in pre-pharyngeal region~~, similar to the epidermal height, excepting on creeping sole; almost twice thicker on body margins (about 18 µm).

What is this?

Autor desconhecido
Hoje, 11:40

did not understand. Rewrite.

Autor desconhecido
Hoje, 11:48

Mesenchymal musculature (Figs 3D–F) poorly developed, mainly composed of two layers: (1) supra-intestinal transverse (about 2–3 fibres thick) and sub-intestinal transverse (about 3–4 fibres thick). In addition, there are oblique and dorso-ventral fibres.

Poorly developed mesenchymal layers are usually best seen on horizontal sections (you do not have). This might be the case. Thus, I would not stress that there are only two and would discuss it

Autor desconhecido
Hoje, 11:52

Pharynx

Pharynx cylindrical and short, nearly 4% of body length, occupying almost all length of pharyngeal pouch (Fig. 4A). Pharyngeal dorsal insertion slightly posteriorly shifted, but still located in anterior third of pharyngeal pouch. Mouth in median third of pharyngeal pouch. Oesophagus short (Fig. 4A); oesophagus: pharynx ratio 12%.

Pharynx and pharyngeal lumen lined with ciliated, cuboidal epithelium with insunk nuclei. Outer pharyngeal musculature comprised of a thin subepithelial layer of longitudinal muscles, followed by a thicker layer of circular fibres. Inner pharyngeal musculature comprises a thick subepithelial layer of circular fibres, followed by a thinner layer of longitudinal fibres. Outer and inner musculatures have a maximum thickness of about 15 µm, gradually becoming thinner towards pharyngeal tip. Oesophagus lined with ciliated, cuboidal to columnar epithelium, with a few insunk nuclei, and ~~covered~~underlain by ~~with~~ a thin muscle layer (about 10 µm) comprised of circular fibres interposed with longitudinal fibres.

Do you mean that not all nuclei are insunk?
Autor desconhecido
Hoje, 11:56

Reproductive organs

Testes in one irregular row on either side of body, located between intestinal branches (Fig. 3D). Testes occurring~~begin posteriorly to ovaries,~~ about 4 mm from behind anterior tip (30% of body length), i.e., posterior to ovaries, and ~~extend~~ back to slightly posterior to pharynx. Sperm ducts lateral to ovovitelline ducts, forming spermiducal vesicles posteriorly to pharynx. Laterally to penis bulb, sperm ducts ascend, enter the muscle coat and open terminally into the proximal portion of the prostatic vesicle. Intrabulbar prostatic vesicle tubular, unforked and vertically disposed (Figs 5, 6B). This vesicle shows two portions: proximal portion narrow~~er~~ and ventral; distal portion globose and dorsal (Figs 5, 6A–B). Ejaculatory duct short and narrow, arising from posterior region of prostatic vesicle and opening into the proximal portion of male

atrium (Fig. 6C), dorsally displaced. Male atrium short and globose with irregular contour and a large circular fold (Figs 5, 6A). Distal region of male atrium communicates with female atrium and gonopore canal through a constriction (Figs 5, 6A). Dorsolaterally to the gonoduct, a secondary male copulatory organ occurs (Figs 5, 6A, D–E), being displaced to the right and communicating with the distal portion of the female atrium and the gonoduct. It contains a smaller circular fold than that of the primary male atrium, receiving entally the opening of a short ejaculatory duct and a tubular, horizontally disposed prostatic vesicle (Figs 6D–E). A single, incompletely developed sperm duct opens into the most proximal portion of this prostatic vesicle.

Confusing. Figure legends and Figures account for main and secondary structures (prostatic vesicles, ejaculatory ducts, and male atria). Since there is no reference to them (secondary male atrium excepted) in the text, comprehension of copulatory organs is heavily hindered. Please rewrite.
Autor desconhecido
Hoje, 12:12

No mention to the "eversible penis"?!
Autor desconhecido
Hoje, 15:30

Epithelial lining of prostatic vesicle ciliated and columnar, with variable~~irregular~~ height, receiving finely granular erythrophil secretion, probably mixed (erythrophil core and a chromophobic peripheral part). In addition, distal portion of prostatic vesicle receives coarse granular erythrophil secretion. Muscularis of prostatic vesicle thin (about 6–8 µm thick), constituted of interwoven longitudinal, circular and oblique fibres (Fig. 6C). Ejaculatory duct lined with cuboidal to columnar epithelium, with irregular height and showing sparse cilia. Few glands with amorphous, cyanophil secretion open into this duct. Muscle coat of ejaculatory duct thin (about 3 µm), comprised of circular and longitudinal fibres. Male atrium lined with non-ciliated epithelium, showing microvilli and receiving finely granular, erythrophil or mixed secretion from glands with subepithelial cell bodies. Dorsal wall and proximal region of male atrium, including the circular fold, lined by columnar epithelium, the cells of which are united to each other by their basal halves, whereas their apical cell halves are free, giving a corrugated appearance to the epithelium (Fig. 6C). Ventral wall and distal region of male atrium lined with

cuboidal to flat epithelium. Muscularis of male atrium comprised of circular fibres followed by longitudinal fibres, thicker proximally (15–20 µm) than distally (3–5 µm).

Vitelline follicles, situated between intestinal branches, well-developed (Figs 3D–F, 4B). Ovaries globose, somewhat pear-shaped, measuring about 0.15 mm in both longitudinal and transversal axes. They are situated dorsally to ventral nerve plate (Fig. 4B), about 3 mm from anterior tip (22% of body length). Ovovitelline ducts emerge from lateral walls of ovaries, dorsally displaced (Fig. 4B), and run posteriorly immediately above ventral nerve plate. Distal sections of ovovitelline ducts run postero-medially, lateral to female atrium and unite dorsally to female canal, forming a short common glandular ovovitelline duct (Figs 5, 7A). Proximal portion of female atrium with an antero-dorsally directed female canal. Female atrium ~~almost~~roughly ovoid, obliquely inclined, with folded walls and ample lumen, tapering to communicate with the gonopore canal (Figs 5, 6A, 7A). Length of female atrium about 2/3 of male atrial length.

Ovovitelline ducts a~~nds well as~~ common ovovitelline duct lined with cuboidal to columnar, ciliated epithelium and ~~covered with~~underlain by intermingled circular and longitudinal muscle fibres (6–10 µm thick). Shell glands ~~with~~discharge their fine~~ly granular,~~ erythrophil, ovoid ~~secretion with ovoid~~ granules ~~empty~~ into the common ovovitelline duct and into the posterior sections of the ovovitelline ducts (Figs 5, 7A). Female canal lined with columnar, ciliated epithelium, receiving abundant strongly cyanophil, amorphous secretion from cell bodies located posterior to the copulatory organs. Female atrium lined with columnar to pseudostratified, ciliated epithelium (generally 10–30 µm), with stratified appearance in some places (70 µm), thus irregular in height. Some ciliated lacunae occur in this epithelium (Fig. 7A). Glands of female atrium of two types: with cyanophil, amorphous and with finely granular,

This description is lacking clear statement of the organs described: main and/or secondary prostatic vesicles, ejaculatoy ducts, male atria?
Autor desconhecido
Hoje, 14:41

What do refer these measueres to, circular, longitudinal, both?
Autor desconhecido
Hoje, 14:12

Do you mean dorso-lateral?
Autor desconhecido
Hoje, 14:15

In the figure legend, add which ovary was represented (either that on the left or on the right side)
Autor desconhecido
Hoje, 10:57

Grey is difficult to see. Instead of grey color for representing organs out of main plane, please use a different color, such as blue, red, green.
Autor desconhecido
Hoje, 11:00

Do you mean “in regions where it reaches up to 70 µm”
Autor desconhecido
Hoje, 14:24

erythrophil secretions. Musculature of female canal and atrium poorly developed (10–15 µm thick), composed of intermingled longitudinal and circular fibres (Figs 5, 6D).

Common muscle coat (Figs 5, 6A, C–E), consisting of longitudinal, oblique and circular fibres, poorly developed (15–50 µm thick), thicker around ental portion of male atrium, forming penis bulb. Male and female atria with continuous muscle coat. Gonoduct large, almost straight at the sagittal plane (Figs 5, 6A). Lining epithelium of gonoduct columnar, ciliated, receiving openings of two types of glands, one producing a finely granular erythrophil and the other an amorphous, slightly cyanophil secretion. Muscularis of gonopore canal (10–15 µm) comprised of subepithelial circular fibres and subjacent longitudinal fibres.

Remarks

The holotype is relatively well preserved, despite its direct fixation in 70% ethanol during field work (Fig. 2). This specimen shows parts of an arthropod in its intestine. Female atrium contains sperm and erythrophil, coarse granular secretion in its lumen, some sperm mixed with a slightly stained, apocrine secretion, indicating recent copula. This apocrine secretion also occurs in the gonoduct.

Ecology and distribution

The type-locality of *Difroehlichia elenae* belongs to the Brazilian savanna (Fig. 8), in an area characterized by rocky outcrops with lateritic cover. The cave surroundings show an open vegetation composed of herbs, shrubs and trees, associated with ferruginous rocks, which is characteristic of rupestrian complexes (Rapini et al., 2008, Oliveira et al., 2018). The type-locality is located close to that of a recently described species of *Pasipha*, in an area planned for

mining activities (Leal-Zanchet & Marques, 2018). The sampling place is a low ferruginous cave (maximal height of 1.8 m), showing 6.3 m of horizontal projection and an area of 9 m^2. It is composed of banded iron rocks (Fig. 8B) covered by a crushed lateritic cap with some quartzite fragments. The cave is located at the basis of a 4 m high vertical slope (Fig. 8A). The sampling site ~~wa~~is in the entrance zone, which corresponds to 60% of the cave area. Only two samplings were carried out in the area in June (dry season) and November 2016 (wet season), the single specimen of *Difroehlichia elenae* being collected in the latter. Other invertebrates, such as spiders (Ctenidae and Sicariidae) and insects (*Zelurus*, *Eidmanacris* and larvae of Lampyridae), also occurred in the cave.

Discussion

The new species described herein shows a heavily pigmented body and eyes along almost the entire body length, as presented by epigean species. However, it shows a small body and a broad sensory margin in the anterior half of the body. Such characteristics may indicate an adaptation to the subterranean environment. Adaptive features, such as hypertrophy of sensory organs and reduction of body pigmentation, as well as reduction or absence of eyes, are usually found in troglobites (Barr, 1968).

In addition, the holotype of *D. elenae* shows signs of recent copula, which may indicate that this flatworm is using the cave environment for reproduction, representing at least a troglophile. Land flatworms usually show low dispersion ability and may have strict ecological requirements that limit their occurrence to specific habitats, some species being affected by the conservation state of the habitat (Carbayo, Leal-Zanchet & Vieira, 2002; Baptista, Oliveira &

Leal-Zanchet, 2010; Amaral et al., 2014; Negrete, Colpo & Brusa, 2014). In other faunal inventories and ecological surveys, many species of land planarians were represented by unique or few specimens (Castro & Leal-Zanchet, 2005; Antunes, Marques & Leal-Zanchet, 2008; Leal-Zanchet & Baptista, 2009; Baptista, Oliveira & Leal-Zanchet, 2010; Amaral et al., 2014; Negrete, Colpo & Brusa, 2014). Similarly, only a single specimen of *D. elenae* was sampled in the faunal survey in ferruginous caves in the eastern margin of the Serra do Espinhaço Plateau. Hence, it is difficult to draw firm conclusions concerning the adaptation of this species to the cave environment.

The holotype of *D. elenae* shows a secondary male copulatory organ immediately behind the primary one. It probably is in an initial stage of development, since it receives the opening of a single, poorly developed sperm duct. Its occurrence may be either an anomaly or a functional adaptation, constituting a populational feature or even a specific or generic characteristic, the latter occurring, for example, in some earwigs (Kamimura, 2006). The finding of other representatives of the species is also necessary to shed light on this question.

The area, where the cave is located, has been intensively sampled, but the species was collected exclusively in its type-locality (unpublished data). Thus, the species seems to have a restricted distribution and, considering the mining impacts in its type-locality, a major concern for its conservation is raised.

Conclusions

The new genus described herein shows rare features within Geoplaninae, such as sub-cylindrical body, poorly developed sub-epidermal musculature and a narrow creeping sole. The holotype of

the new species has a small body and a broad sensory margin in the anterior region of the body, and showed signs of recent copula. Such features may indicate an adaptation to the subterranean environment, but since the specimen shows a heavily pigmented body and eyes along almost the entire body length, it should be interpreted as at least a troglophile. The holotype shows a secondary male copulatory organ located immediately behind the primary one, both communicating with the female atrium and gonopore canal. Considering that the type-locality is affected by mining activities, and the species seems to have a low abundance and restricted distribution, major concern arises regarding its conservation.

Why do you say “at least”? This statement is found along the text. What is beyond a troglophile?
Autor desconhecido
Hoje, 14:38

Acknowledgements

The Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Nr. 306853/2015-9), Anglo American Brasil and Carste Ciência e Ambiente are thanked for financial support to this study. We also acknowledge Carste Ciência e Ambiente for samplings and information about the type locality, as well as for the map and photos of the sampling place. We thank G. Iturralde for the photo in Fig. 2A and the laboratory technician L. Guterres for her help in section preparation. M.Sc. E. Benya is acknowledged for an English review of the text.

References

Amaral SV, Hack IR, Iturralde GG, Leal-Zanchet AM. 2014. Land flatworms (Platyhelminthes: Tricladida) in remnants of deciduous forest in the northeast region of southern Brazil. *Biota Neotropica* 14:1–6. http://dx.doi.org/10.1590/S1676-06020140045

394Antunes MC, Marques DIL, Leal-Zanchet AM. 2008. Composição das comunidades de planárias
395 terrestres (Platyhelminthes, Tricladida, Terricola) em duas áreas de floresta estacional
396 semidecidual do sul do Brasil. *Neotropical Biology and Conservation* 31:34–38.
397Auler A, Rubbioli E, Brandi R. 2001. *As grandes cavernas do Brasil*. Belo Horizonte: Grupo
398 Bambuí de Pesquisas Espeleológicas.
399Baptista VA, Oliveira SM, Leal-Zanchet AM. 2010. Inventário de planárias terrestres
400 (Platyhelminthes, Tricladida) em remanescente de Floresta Estacional Decidual do Sul do
401 Brasil. *Biota Neotropica* 10:247–252.
402Barr TC. 1968. Cave ecology and the evolution of troglobites. In: Dobzhansky T, Hecht MK,
403 Steere WC, Eds. *Evolutionary biology*. Boston: Springer, 35–102.
404 https://doi.org/10.1007/978-1-4684-8094-8_2
405Carbayo F, Álvarez-Presas M, Olivares CT, Marques FPL, Froehlich EM, Riutort M. 2013.
406 Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification:
407 proposal of taxonomic actions. *Zoologica Scripta* 42:508–528.
408 https://doi.org/10.1111/zsc.12019
409Castro RA, Leal-Zanchet AM. 2005. Composição de comunidades de planárias terrestres
410 (Platyhelminthes) em áreas de floresta estacional decidual e de campo na região central do
411 Rio Grande do Sul, Brasil. *Acta Biologica Leopoldensia* 27:147–150.
412Froehlich, CG. 1955. Sobre a morfologia e taxonomia das Geoplanidae. *Boletim da Faculdade*
413 *de Filosofia, Ciências e Letras da Universidade de São Paulo*, Série Zoologia, 19*:*195–279.
414Kamimura Y. 2006. Right-Handed penises of the earwig *Labidura riparia* (Insecta, Dermaptera,
415 Labiduridae): evolutionary relationships between structural and behavioral asymmetries.
416 *Journal of Morphology* 267:1381–1389. https://doi.org/10.1002/jmor.10484

Leal-Zanchet AM, Baptista VA. 2009. Planárias terrestres (Platyhelminthes, Tricladida) em remanescentes de Floresta com Araucária. In: Fonseca CR, Souza AF, Leal-Zanchet AM, Dutra T, Backes A, Ganade G, Eds. *Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável*. Ribeirão Preto: Holos, 199–207.

Leal-Zanchet AM, Carbayo F. 2000. Fauna de planárias terrestres (Platyhelminthes, Tricladida, Terricola) da Floresta Nacional de São Francisco de Paula, RS, Brasil: uma análise preliminar. *Acta Biologica Leopoldensia* 22:19–25.

Leal-Zanchet A, Marques A. 2018. Searching for shelter in a ferruginous cave? A new species of *Pasipha* (Platyhelminthes, Tricladida) from a plateau in the Brazilian savanna. *Zookeys* 776:13–25. doi: 10.3897/zookeys.776.26308

Negrete L, Colpo KD, Brusa F. 2014. Land Planarian Assemblages in Protected Areas of the Interior Atlantic Forest: Implications for Conservation. *PLoS ONE* 9:e90513. doi:10.1371/journal.pone.0090513

Ogren RE, Kawakatsu M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola). Part I: Geoplaninae. *Bulletin of the Fuji Women's College, Ser. 2* 28:79–166.

Oliveira PA, Pereira IM, Messias MCTB, Oliveira MLR, Pinheiro AC, Machado ELM, Oliveira JLA. 2018. Phytosociology of the herbaceous-subshrub layer of a rupestrian complex in Serra do Espinhaço, Brazil. *Acta Botanica Brasilica* 32:141–149. https://doi.org/10.1590/0102-33062017abb0225

Pellegrini T, Ferreira R. 2012. Metodologias diferenciadas aumentam a eficiência de inventários faunísticos em cavernas. *Arquivos do Museu de História Natural e Jardim Botânico* 21:111–121.

Rapini AA, Ribeiro PL, Lamberti S, Pirani JR. 2008. A flora dos campos rupestres quartzíticos da Cadeia do Espinhaço. *Megadiversidade* 4:16–24.

Rocha BN, Galvani E. 2011. Microclima de ambientes cavernícolas: estudo de caso da Gruta da Santa, Parque Estadual de Intervales, SP. *Revista Brasileira de Climatologia* 9:21–34. http://dx.doi.org/10.5380/abclima.v9i0.27508

Rossi I, Amaral SV, Ribeiro GG, Cauduro GP, Fick I, Valiati VH, Leal-Zanchet AM. 2015. Two new Geoplaninae species (Platyhelminthes: Continenticola) from Southern Brazil based on an integrative taxonomic approach. *Journal of Natural History* 50:1–29. https://doi.org/10.1080/00222933.2015.1084057