

## Two new troglobiotic palpigrades (Palpigradi: Eukoeneniidae) from Brazil

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### Abstract

Two new species of troglobiotic Brazilian palpigrades are described: *Eukoenenia jequitinhonha* sp. n., found in Lapa do Córrego do Vieira cave (Caraí, Minas Gerais) and *E. cavatica* sp. n., found in Cazanga cave (Arcos, Minas Gerais). The importance of documenting the occurrence of troglobiotic species, even if they are represented by only a single specimen, is discussed.

**Key words:** Caves, endemism, taxonomy, troglomorphism

### Introduction

Arachnids of the order Palpigradi are widely distributed in Brazilian caves. Although only four troglobiotic species have been formally described from Brazil (Souza & Ferreira 2010; 2011; 2012a; 2012b), many other troglomorphic species have been found in caves of differing lithologies (Souza & Ferreira 2010). A common characteristic of these species is their great rarity, based on the small number of individuals found and their restricted distributions. The Brazilian species *Eukoenenia spelunca* Souza & Ferreira, 2011 and *E. virgemdalapa* Souza & Ferreira, 2012 provide examples of this rarity, each being known only from the holotype. Furthermore, Brazilian troglobiotic species present extreme endemism and, in most cases, appear to be confined to a single cave. Elsewhere, however, recent collecting of specimens belonging to troglobiotic species originally described from only a few individuals have allowed the publication of complementary descriptions in some cases, such as those of the European species *Eukoenenia gasparoi* Condé, 1988 (Condé 1989; Christian *et al.* 2012) and *E. draco* (Peyerimhoff, 1906) (Mayoral & Barranco 2013).

This work describes two new troglobiotic species of Palpigradi from the state of Minas Gerais, Brazil: *Eukoenenia jequitinhonha* sp. n., based on a female found in a granite cave, and *E. cavatica* sp. n., based on a male collected in a limestone cave.

### Material and methods

The specimens were found under rocks, captured with a fine brush and placed in vials containing 70% ethanol. They were examined by clearing in Nesbitt's solution and mounting in Hoyer's medium on glass slides using standard procedures developed for mites (Krantz & Walter 2009). All measurements were taken using an ocular micrometer. Photos were taken with an AxioCam 105 Color camera on a Zeiss Axio Scope A1 microscope equipped with a phase contrast and DIC. Body length was measured from the apex of the propeltidium to the posterior margin of the opisthosoma.

The following abbreviations are employed, based on Barranco & Mayoral (2007): ti, tibia; bta1, basitarsus 1; bta2, basitarsus 2; bta3, basitarsus 3; bta4, basitarsus 4; ta1, tarsus 1; ta2, tarsus 2; ta3, tarsus 3; a, width of basitarsus IV at level of seta r; er, distance between base of basitarsus IV and insertion of seta r; grt, tergal seta length; gla, lateral seta length; r, stiff seta length; t/r, ratio between length of basitarsus IV and stiff seta length; t/er,

ratio between basitarsus IV length and distance to insertion of stiff seta; gla/grt, ratio between lengths of lateral and tergal setae; B/bta, ratio between lengths of prosomal shield and basitarsus IV; bta/ti, ratio between lengths of basitarsus IV and tibia IV. Setal nomenclature follows that of Condé (1955; 1990) and Christian & Christophoryová (2013).

The specimens are housed in the Coleção de Invertebrados Subterrâneos de Lavras, Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais (ISLA).

## Taxonomy

### Family Eukoeneniidae Petrunkevitch, 1955

#### Genus *Eukoenenia* Börner, 1901

##### *Eukoenenia jequitinhonha* sp. n.

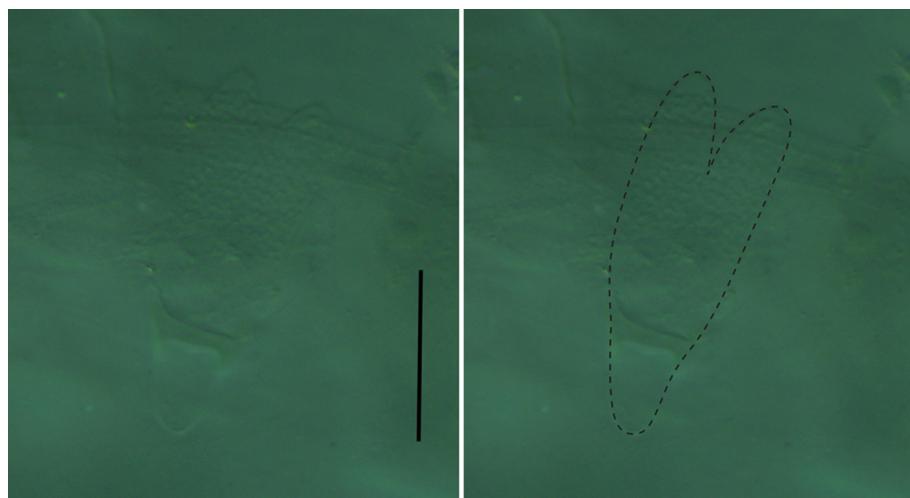
**Material examined.** Holotype: adult female (ISLA 4014) from Lapa do Córrego do Vieira cave, Caraí, Minas Gerais ( $17^{\circ}9'5.3''$  S/  $41^{\circ}30'15.5''$  W), Brazil, 10/VII/2004, leg. R.L. Ferreira.

**Etymology.** The specific name is a noun in apposition that refers to the Jequitinhonha river, in whose drainage basin the type locality is located. This river is one of the most important in the north of Minas Gerais state.

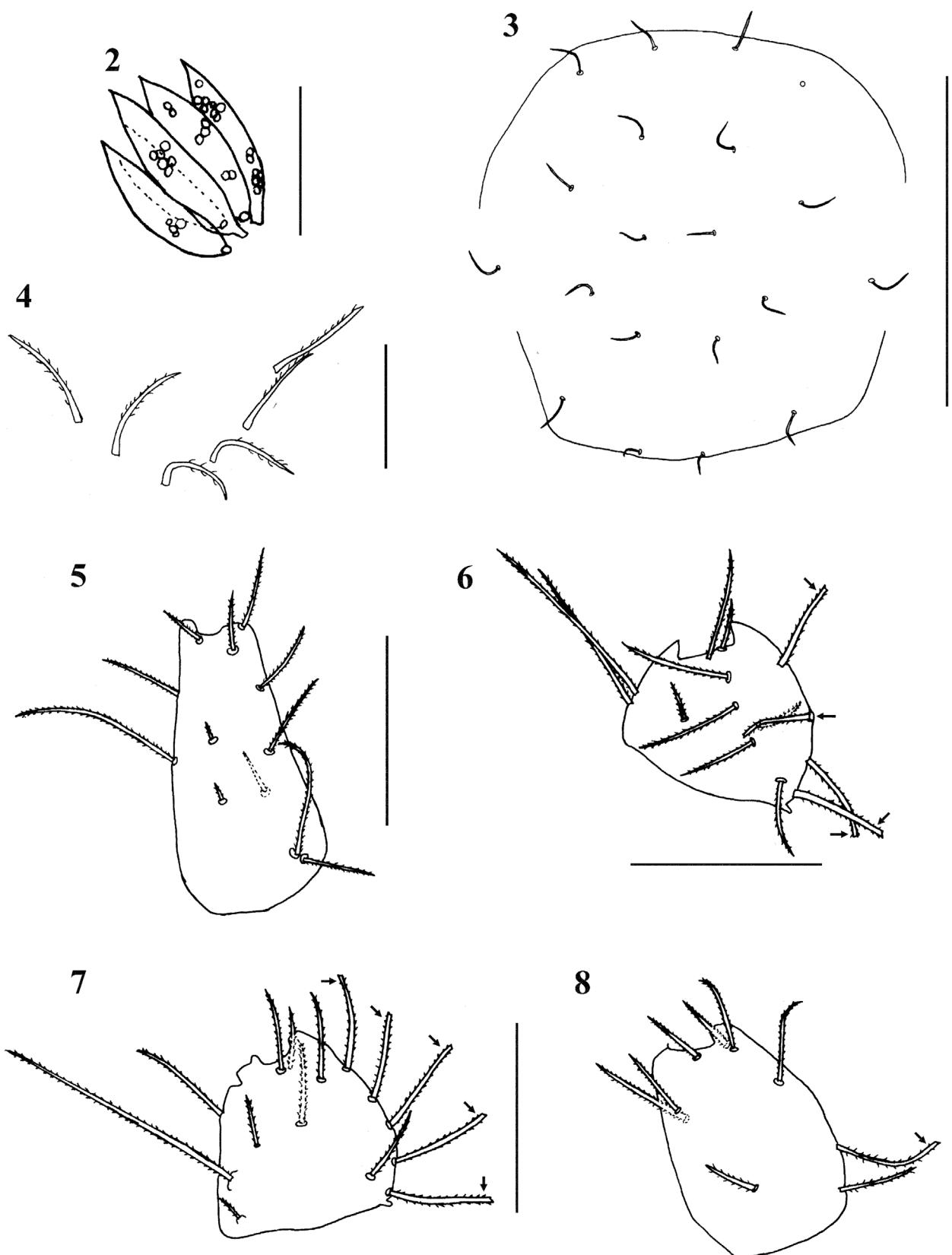
**Diagnosis.** This species differs from all others of the genus *Eukoenenia* by the following combination of characteristics: prosomal lateral organs with 5 pointed blades; metapeltidium with  $3 + 3$  setae ( $t_1, t_2, t_3$ ) with similar lengths; 6 deuto-tritosternal setae in U-shaped arrangement; chaetotaxy of coxae I–IV: 12 setae, 14 (4 thick and 10 normal setae), 14 (5 thick and 9 normal setae), 9 (1 thick and 8 normal setae); basitarsus IV with six setae; 2 + 2 thickened setae ( $a_1$  and  $a_2$ ) between a pair of normal slender setae ( $s$ ) on opisthosomal sternites IV–VI; opisthosomal tergites II–VI with 2 pairs of  $t$  setae ( $t_1$  and  $t_2$ ) between a pair of normal slender setae ( $s$ ). Values of indices: bta/ti 0.89; B/bta 1.8.

**Description of female.** Total body length (without flagellum): 1480  $\mu\text{m}$ .

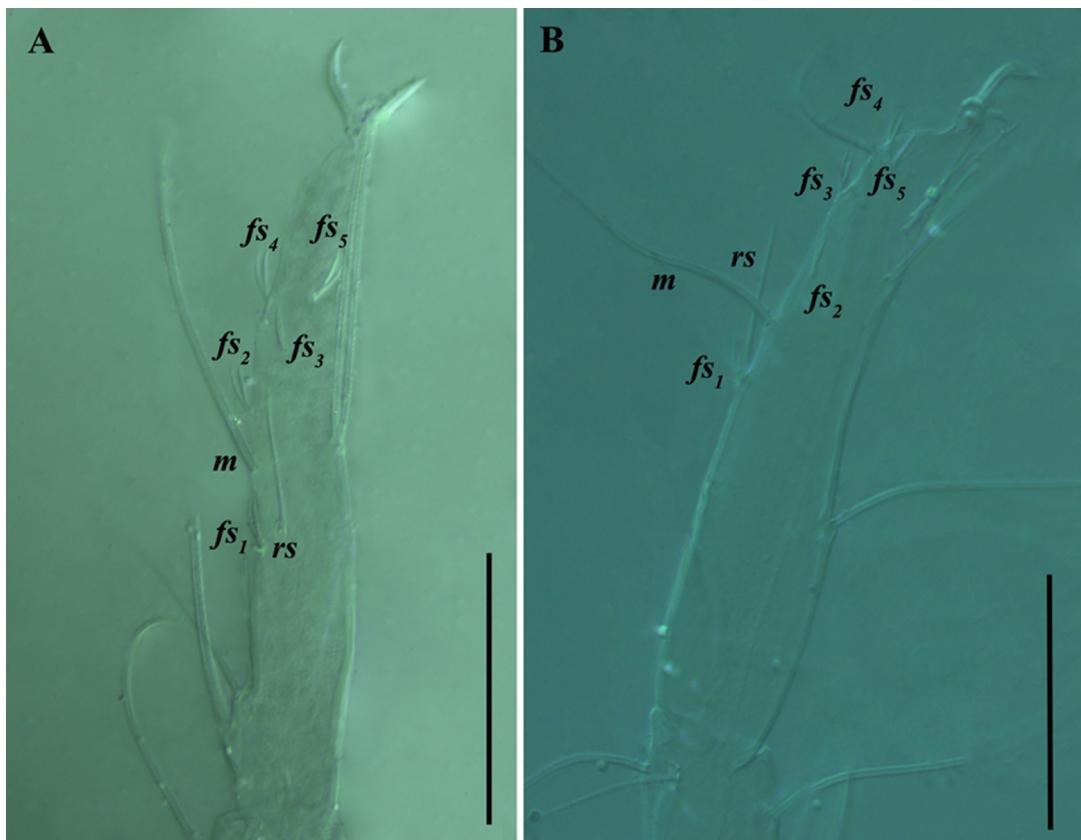
**Prosoma.** Frontal organ (43  $\mu\text{m}$  long) with two distally rounded branches, with irregular reticulation (Fig. 1). Lateral organ with five blades (longest 22  $\mu\text{m}$ ), reticulated and distally pointed (Fig. 2). Propeltidium 298  $\mu\text{m}$  long; with 10 + 10 short setae in five rows (Fig. 3). Metapeltidium with  $3 + 3$  setae ( $t_1, t_2, t_3$ ) of similar lengths (33  $\mu\text{m}$ , 33  $\mu\text{m}$ , 31  $\mu\text{m}$ ). Six deuto-tritosternal setae in U-shaped arrangement (Fig. 4). Hand of chelicera with 4 dorsal setae, 1 middle seta, 1 ventral seta and 1 seta inserted next to teeth of fixed finger; dorsal length of basal segment 200  $\mu\text{m}$ . Number of teeth on fingers not determined. Coxal chaetotaxy: coxa I with 12 setae, coxa II with 4 thick and 10 normal setae (the longest 103  $\mu\text{m}$ ), coxa III with 5 thick and 9 normal setae (the longest 128  $\mu\text{m}$ ) and coxa IV with 1 thick and 8 normal setae (Figs. 5–8).



**FIGURE 1.** *Eukoenenia jequitinhonha* sp. n. Frontal organ, dorsal view. Scale bar 20  $\mu\text{m}$ .



**FIGURES 2–8.** *Eukoenenia jequitinhonha* sp. n. 2, lateral organ, dorsal view; 3, propeltidial chaetotaxy; 4, deuto-tritosternal setae; 5, coxa I; 6, coxa II; 7, coxa III; 8, coxa IV. Arrows in Figs 6–8 indicate thick setae. Scale bars: 20 µm (Fig. 2), 40 µm (Fig. 4), 100 µm (Figs 5–8), 250 µm (Fig. 3).



**FIGURE 9.** Tarsus 3 of leg 1 of the new species showing the modified setae (*rs* = rod seta, *fs* = forked seta, *m* = macroseta). A, *Eukoenenia jequitinhonha* sp. n.; B, *Eukoenenia cavatica* sp. n. Scale bars: 50  $\mu$ m.

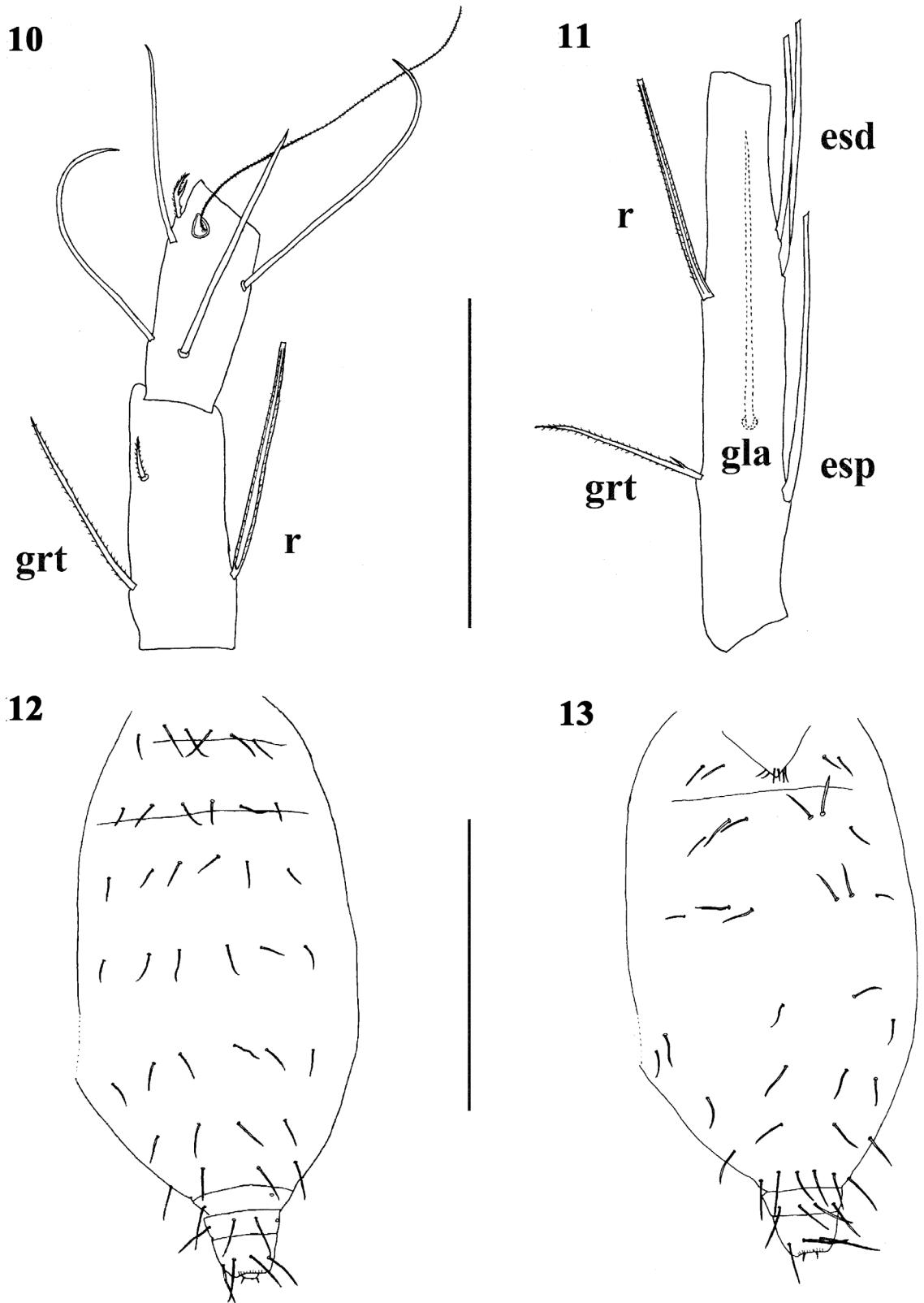
Pedipalp: ta3 with 2 forked setae. Length of pedipalp articles ( $\mu$ m): ti 152.5; bta1 67.5; bta2 75; ta1 37.5; ta2 50; ta3 75.

Leg I: with 7 trichobothria in usual arrangement and 9 forked setae (*fs*): 1 on ta2, bta4, bta2 and bta1 and 5 on ta3 (arranged as *fs*<sub>1</sub>/*fs*<sub>2</sub>/*fs*<sub>3</sub>/*fs*<sub>4+5</sub>): *fs*<sub>1</sub> is inserted in proximal half of the segment and slightly proximal to rod seta (*rs*) (*fs*<sub>1</sub>/*rs* = 0.29); macroseta (*m*) inserted halfway between *fs*<sub>1</sub> and *fs*<sub>2</sub> (Fig. 9A). Bta3 2.8 times longer than wide, with 3 setae (grt 55  $\mu$ m; r 75  $\mu$ m). Seta *r* slightly shorter than segment (77.5/75  $\mu$ m, t/r = 0.72), inserted in proximal third and surpassing distal margin of segment (72.5/17.5  $\mu$ m, s/er= 4.1) (Fig. 10). Measurements of leg I ( $\mu$ m): ti 165; bta1+2 142.5; bta3 77.5; s 72.5; a 27.5; grt 55; r 75; er 17.5; bta4 65; ta1 35; ta2 40; ta3 137.5.

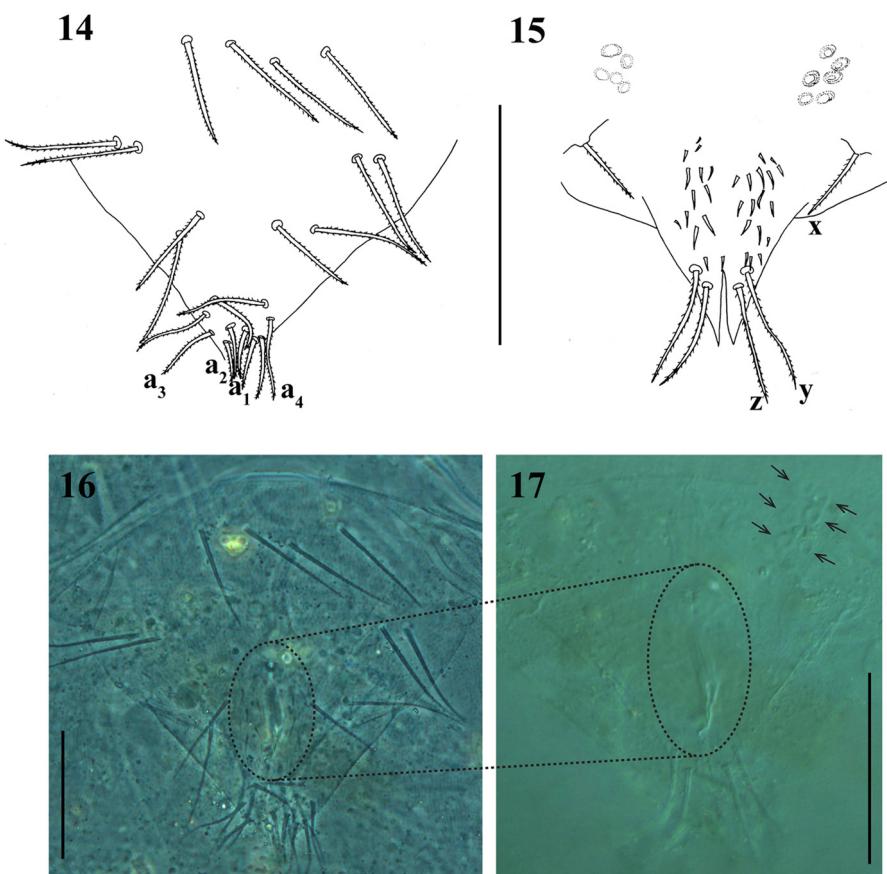
Leg IV: basitarsus long, 7.3 times longer than wide, with 6 setae (grt, gla, r, esp and 2 esd). Stiff seta *r* 2.4 times shorter than the tergal edge of segment (165/67.5  $\mu$ m, t/r = 2.4) and inserted in its distal half (165/95  $\mu$ m, t/er= 1.7); esp, gla and grt inserted in proximal half, grt level with esp, gla more distal (Fig. 11). Measurements of leg IV ( $\mu$ m): ti 185; bta 165; ta1 65; ta2 87.5; s 157.5; a 22.5; grt 52.5; gla 87.5; r 67.5; er 95. Values of indices: bta/ti 0.89; gla/grt 1.6; B/bta 298/165 1.8.

*Opisthosoma.* Tergites II–VI with 3 + 3 dorsal setae, two pairs of *t* setae (*t*<sub>1</sub>, *t*<sub>2</sub>) between a pair of slender setae (*s*) (Fig. 12). Sternite III with 2 + 2 setae. Sternites IV–VI each with 2 + 2 thickened and conical (width decreasing slightly from base to apex) setae in middle (*a*<sub>1</sub> and *a*<sub>2</sub>, 52.5–55  $\mu$ m), between a pair of slender setae (*s*, 42.5–45  $\mu$ m); a pair of pores present between *a*<sub>1</sub> setae on sternites IV–VI (Fig. 13). Segments VII–X with 8 setae each and segment XI with 7 setae.

Female genitalia: Chaetotactic formula 7+4/3. First lobe with 11 + 11 setae in 5 transverse rows: 2+2 sternal setae (*st*<sub>1</sub>, *st*<sub>2</sub>) followed by 2 + 2, 2 + 2, 2 + 2, 1 + 1 and 4+4 distal setae, of which *a*<sub>1</sub>, *a*<sub>2</sub>, *a*<sub>3</sub> and *a*<sub>4</sub> measure 19, 21, 27 and 34  $\mu$ m, respectively (Fig. 14). Second lobe with 3 + 3 setae (*x*, *y*, *z*), measuring 24, 41 and 38  $\mu$ m, respectively (Fig. 15); cuticular spines present (Figs 15–16); 6 glandular orifices on one side and 5 on the other (Figs. 15 and 17). Shape of spermatheca as shown in Figs 16 and 17.



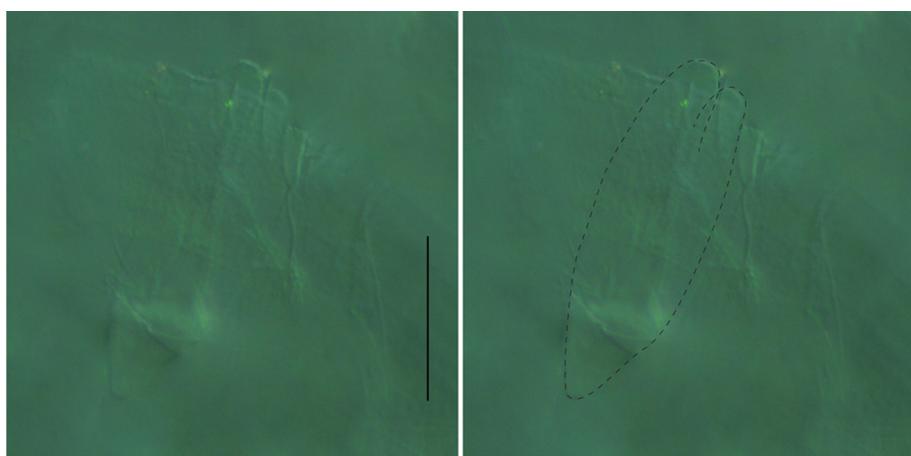
**FIGURES 10–13.** *Eukoenenia jequitinhonha* sp. n. 10, basitarsi 3–4 of leg I; 11, basitarsus IV; 12, opisthosoma, dorsal view; 13, opisthosoma, ventral view. Scale bars: 100 µm (Figs 10–11), 500 µm (Figs 12–13).



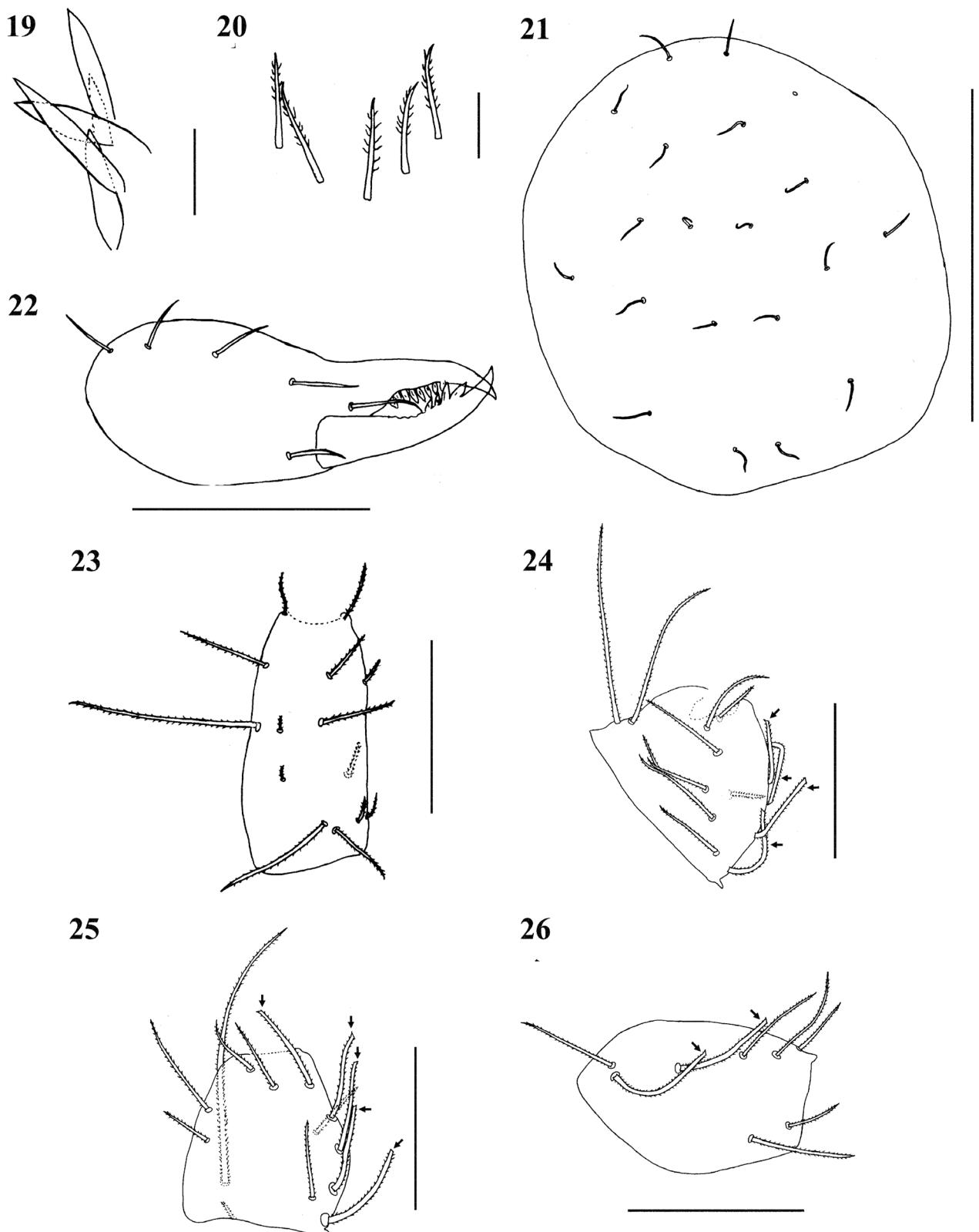
**FIGURES 14–17.** *Eukoenenia jequitinhonha* sp. n. 14, first lobe of female genitalia; 15, second lobe of female genitalia; 16, first and second genital lobes overlapped, showing the setae of the first lobe and the cuticular spines and the sclerotized parts of the spermatheca on the second lobe (delimited by the elliptical dashed lines); 17, second genital lobe, with the spermatheca indicated by the elliptical dashed line and the glandular pores indicated by the arrows. Scale bars: 50 µm (Figs 16–17), 60 µm (Fig. 15), 100 µm (Fig. 14).

#### *Eukoenenia cavatica* sp. n.

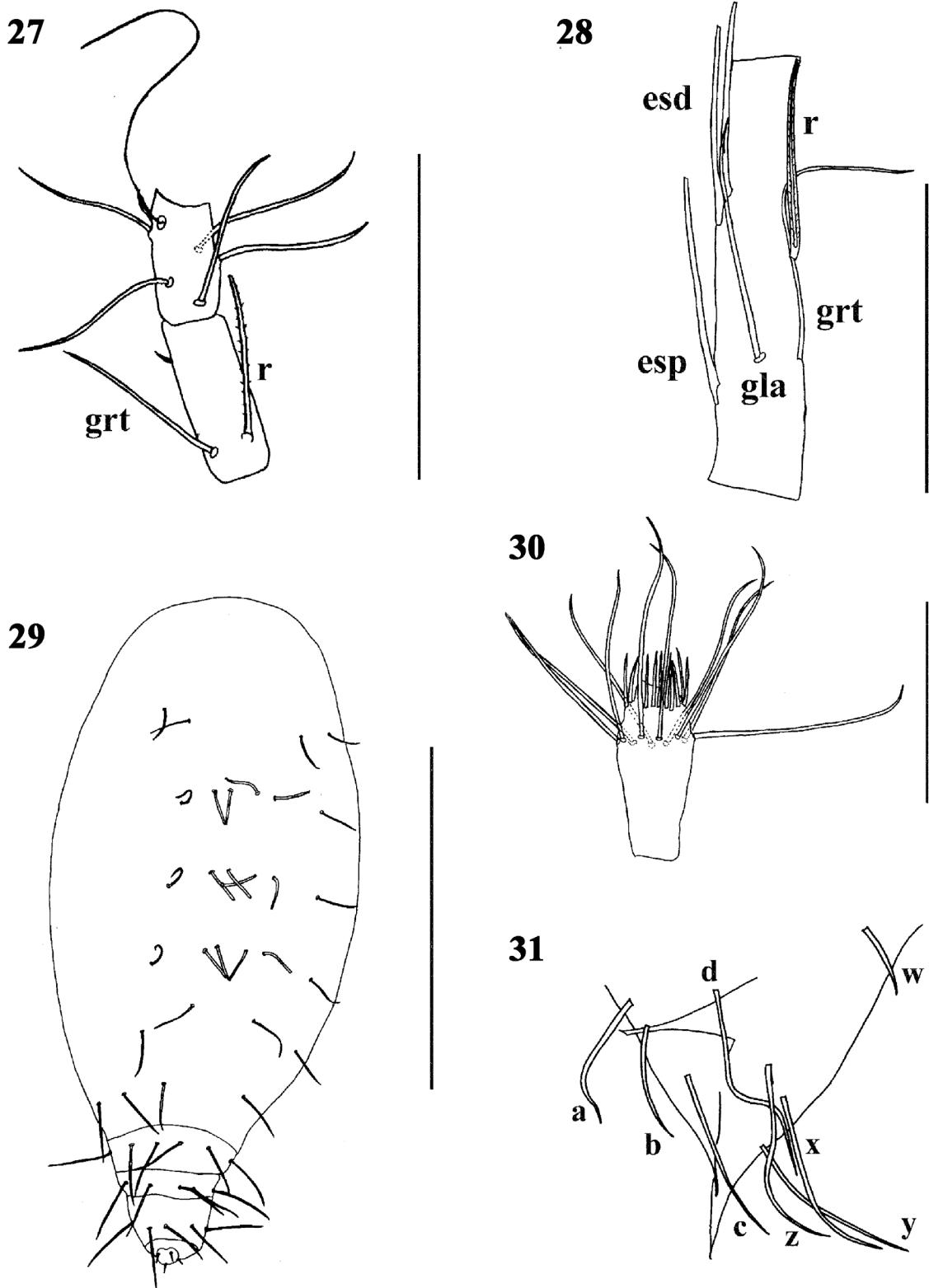
**Material examined.** Holotype: adult male (ISLA 4135) from Gruta Cazanga cave, Arcos, Minas Gerais ( $20^{\circ}16'58.9''$  S/  $45^{\circ}35'46.5''$  W), Brazil, 30/I/2009, leg. R. L. Ferreira.



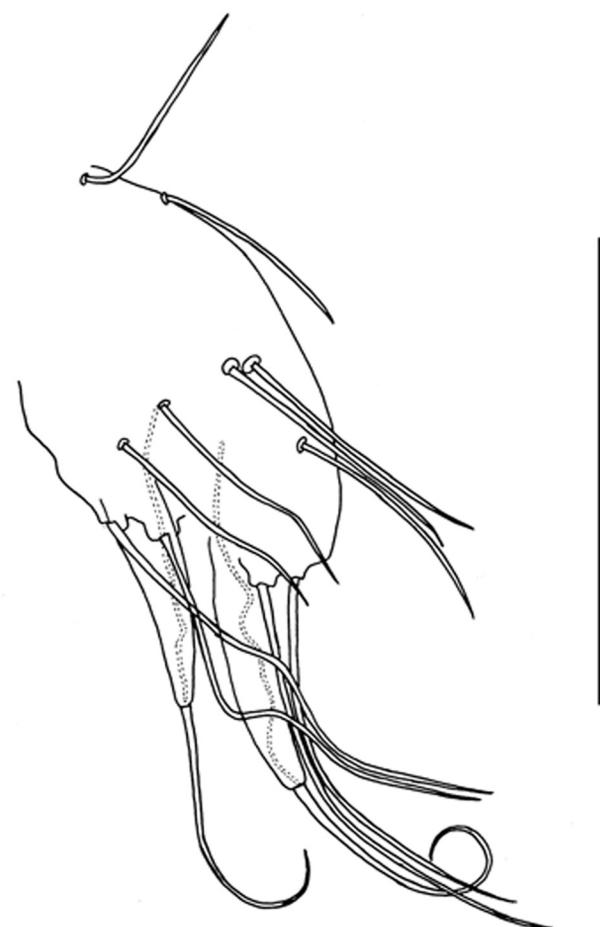
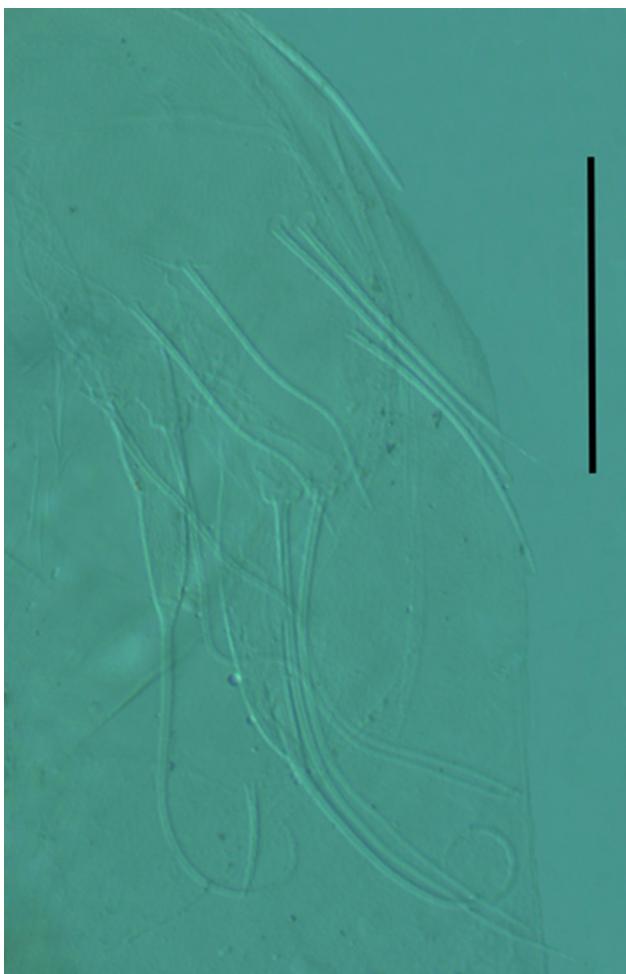
**FIGURE 18.** *Eukoenenia cavatica* sp. n. Frontal organ, dorsal view. Scale bar: 20 µm.



**FIGURES 19–26.** *Eukoenenia cavatica* sp. n. 19, lateral organ, dorsal view; 20, deuto-tritosternal setae; 21, propeltidial chaetotaxy; 22, chelicera; 23, coxa I; 24, coxa II; 25, coxa III; 26, coxa IV Arrows in Figs 24–26 indicate thick setae. Scale bars: 20 µm (Figs 19–20), 80 µm (Figs 24–25), 100 µm (Figs 23, 26), 150 µm (Fig. 22), 250 µm (Fig. 21).



**FIGURES 27–31.** *Eukoenenia cavatica* sp. n. 27, basitarsi 3–4 of leg I; 28, basitarsus IV; 29, opisthosoma, ventral view; 30, first flagellar segment; 31, second and third lobes of male genitalia. Scale bars: 40 µm (Fig. 31), 100 µm (Fig. 28), 150 µm (Figs 27, 30), 500 µm (Fig. 29).



**FIGURE 32.** *Eukoenenia cavatica* sp. n. First lobe of male genitalia. Scale bars: 50 µm (photograph) and 100 µm (drawing).

**Etymology.** The specific name is a Latin word meaning “living in a cave” and refers the troglobiotic nature of the species.

**Diagnosis.** This species differs from all others of the genus *Eukoenenia* by the following combination of characteristics: 5 pointed blades in prosomal lateral organ; metapeltidium with 3 + 3 setae ( $t_1$ ,  $t_2$ ,  $t_3$ ),  $t_1$  and  $t_3$  of similar length and  $t_2$  longest; 5 deuto-tritosternal setae in U-shaped arrangement; chaetotaxy of coxae I-IV: 14 setae, 13 (4 thick and 9 normal setae), 13 (5 thick and 8 normal setae), 8 (2 thick and 6 normal setae); basitarsus IV with 6 setae; 2 + 2 thickened setae ( $a_1$  and  $a_2$ ) between a pair of normal slender setae ( $s$ ) on opisthosomal sternites IV-VI; opisthosomal tergites II–VI with 2 pairs of  $t$  setae ( $t_1$  and  $t_2$ ) between a pair of normal slender setae ( $s$ ); male genitalia with 13 + 13 setae on the first lobe (including 2 + 2 fusules), 4 + 4 on the second and 4 + 4 on the third. Values of indices: bta/ti 0.86; B/bta 2.3.

**Description of male.** Total body length: 1410 µm (without flagellum).

**Prosoma.** Frontal organ (45 µm long) with two branches distally rounded, with irregular reticulation and inserted on a conical base (Fig. 18). Lateral organ with 5 long blades (longest 52 µm), reticulated and distally pointed (Fig. 19). Five deuto-tritosternal setae in U-shaped arrangement (Fig. 20). Propeltidium 325 µm long; with 10 + 10 short setae in 5 rows (Fig. 21). Metapeltidium with 3 + 3 setae ( $t_1$ ,  $t_2$ ,  $t_3$ ),  $t_1$  and  $t_3$  of similar length (57.5 µm, 55 µm),  $t_2$  longest (100 µm). Hand of chelicera with 5 dorsal setae, 1 ventral seta and 1 seta inserted near teeth of fixed finger; both fingers with nine teeth (Fig. 22); dorsal length of basal segment 200 µm. Coxal chaetotaxy: coxa I with 14 setae, coxa II with 4 thick and 9 normal setae (the longest 100 µm), coxa III with 5 thick and 8 normal setae (the longest 122 µm) and coxa IV with 2 thick and 6 normal setae (Figs. 23–26).

Pedipalp: ta3 with 2 forked setae. Length of pedipalp articles (µm): ti 150; bta1 55; bta2 67.5; ta1 37.5; ta2 50; ta3 75.



**FIGURES 33–35.** *Eukoenenia cavatica* sp. n. 33, Limestone outcrop in which Cazanga cave is located (note that original vegetation only remains near the outcrop); 34 and 35, live specimen.

Leg I: with 7 trichobothria in usual arrangement and 9 forked setae ( $fs$ ): 1 on ta2, bta4, bta2 and bta1 and 5 on ta3 (arranged as  $fs_1/fs_2/fs_3/fs_{4+5}$ ):  $fs_1$  inserted in distal half of segment, at the same level as rod seta ( $rs$ ) ( $fs_1/rs = 0.28$ ); macroseta ( $m$ ) inserted halfway between  $fs_1$  and  $fs_2$  (Fig. 9B). Bta3 2.5 times longer than wide, with 3 setae ( $grt$  77.5  $\mu m$ ;  $r$  72.5  $\mu m$ ). Seta  $r$  similar in length to the segment (75/72.5  $\mu m$ ,  $t/r = 1.03$ ), inserted in proximal third and surpassing distal margin of segment (70/20  $\mu m$ ,  $s/er = 3.5$ ) (Fig. 27). Measurements of leg I ( $\mu m$ ): ti 160; bta1+2 115; bta3 75; s 70; a 30;  $grt$  77.5;  $r$  72.5; er 20; bta4 62.5; ta1 40; ta2 42.5; ta3 145.

*Leg IV.* Basitarsus long, 5.5 times longer than wide, with 6 setae (*grt*, *gla*, *r*, *esp* and 2 *esd*). Stiff seta *r* 2.1 times shorter than the tergal edge (137.5/65 µm, t/r = 2.1) and inserted approximately in middle of segment (137.5/71 µm, t/er= 1.9); *esp*, *gla* and *grt* inserted in proximal half, *grt* level with *gla*, *esp* more proximal (Fig. 28). Measurements of leg IV (µm): ti 160; bta 142; ta1 54; ta2 80; s 130; a 25; *grt* 94; *gla* 75; *r* 65; er 71. Values of indices: bta/ti 0.86; *gla*/*grt* 0.80; B/bta 325/142 2.3.

*Opisthosoma.* Tergites II–VI with 3 + 3 dorsal setae, 2 pairs of *t* setae (*t<sub>1</sub>*, *t<sub>2</sub>*) between a pair of slender setae (*s*). Sternite III with 2 + 2 setae. Sternites IV–VI each with 2 + 2 thickened and cylindrical setae (basal and apical parts of similar width) (*a<sub>1</sub>* and *a<sub>2</sub>*; 49–53 µm) in middle between a pair of slender setae (*s*; 56–59 µm) (Fig. 29). Segments VII–XI with 8 setae each. Only the first flagellar segment remains. It has a row of ten long setae in its distal half and a row of spike-like setae at the apex (Fig. 30).

*Male genitalia.* Chaetotactic formula 2+9+2f/4/4. First lobe with 13+13 setae, (including 2+2 fusules). Fusules close to each other, with a dilated conical base (*f<sub>1</sub>* 48 µm; *f<sub>2</sub>* 55 µm), a setiform end and internal canals (represented by dotted lines in Fig. 32). Second lobe with a simple, sharp apex (without bifurcation), with 4 + 4 setae (*a*, *b*, *c*, *d*). Third lobe also subtriangular, well developed, with 4 + 4 setae (*w*, *x*, *y*, *z*), with a bifurcate tip (Fig. 31).

## Discussion

The two new species described here have many characteristics in common, but they can be distinguished mainly by the number of setae on coxae, the relative positions of the setae *r*, *gla*, *grt* and *esp* on basitarsus IV, and by the relative length of the *t<sub>2</sub>* setae on the metapeltidium (similar to *t<sub>1</sub>* and *t<sub>3</sub>* in *E. jequitinhonha* sp. n and longer than *t<sub>1</sub>* and *t<sub>3</sub>* in *E. cavatica* sp. n). Furthermore, they present differences on the lengths of the setae and segments, with *E. jequitinhonha* sp. n. (bta/ti 0.89; B/bta 1.8) presenting more elongated members than *E. cavatica* sp. n. (bta/ti 0.86; B/bta 2.3). Their holotypes differ in number of setae on deuto-tritosternum, but the difference is minimal (5 versus 6) and fits on the range of intraspecific variation known for other species (e.g. species from *Eukoenenia spelaea* complex: Christian et al. 2014; *Eukoenenia draco*: Mayoral & Barranco 2013). The difference in the shape of the thick setae on opisthosomal sternites IV–VI (conical on *E. jequitinhonha* sp. n. and cylindrical on *E. cavatica* sp. n.) is also a not reliable feature to distinguish the new species, since it is known to show sexual dimorphism in others species (e.g. *Eukoenenia sagarana* Souza & Ferreira 2012 and *E. maroccana* Barranco & Mayoral 2007).

Unfortunately, the genitalia could not be compared because *E. jequitinhonha* sp. n. is represented by an adult female and *E. cavatica* sp. n. by an adult male. The number of setae on basitarsus IV is the same as that in the other Brazilian troglobiotic species (except for *E. virgemdalapa*, which presents only five setae). The number of setae of the abdominal tergites and sternites IV–VI is similar to that of *E. spelunca* and *E. virgemdalapa*.

An increase in the number of elements in the lateral organs is a general tendency in troglobiotic species (Condé 1998), which provide other examples of lateral organs formed of five elements. *E. jequitinhonha* sp. n. also presents elongation of the appendages, as reflected by the bta IV/ti ratio (0.89), which is closer to the average value of troglobiotic species (0.95) than to that of endogeous species (0.79) (Condé 1996). In addition, the value of the propeltidium/bta IV ratio (1.8) is similar to that of troglobiotic species, in which it is below 2 (Conde 1998). *E. cavatica* sp. n. has a ratio bta/ti (0.86) intermediate between those of troglomorphic and endogomrophic species. Although the flagellum of the holotype was lost during collection, it was quite long, approximately 1.7 times longer than the body, as can be seen in figures 34–35. This value is similar to or higher than those found in the troglobiotic species *Eukoenenia patrizii* (Condé, 1956) (1.75), *E. condei* Orghidan, Georgesco & Sarbu, 1982 (1.4), *E. austriaca* (Hansen, 1926) (1.34) and *E. gasparoi* (1.3) (Condé 1998). Moreover, a large number of samples had been taken from epigeous habitats around the caves in this region and no specimen of this species was found. Only the endogeous sub-cosmopolitan *E. florenciae* was found outside and inside the other caves (about 300) sampled in the region of Arcos and Pains. This provides strong evidence that *E. cavatica* is endemic to Cazanga cave.

The cave in which *E. jequitinhonha* sp. n. was found (Lapa do Córrego do Vieira cave) has a granite lithology, being a typical talus cave. Such caves are formed by the removal of soil and sediments between large granite blocks shaped by spheroidal weathering, resulting in a cavity that is often broadly associated with other endogeous habitats. Although there are few studies on the fauna associated with granitic caves, there is evidence that the number of troglobiotic species tends to be lower than in other lithologies, except in the case of talus caves (Souza-Silva et al. 2011). Unfortunately, this cave is located in a region severely degraded by human activities, including

the presence of extensive pasture and monoculture areas, as well as a highway built a few dozen meters from the cave. It is noteworthy that *E. jequitinhonha* sp. n. is the first troglobiotic palpigrade species to be found in a granite cave, since all other troglobiotic species described to date occur in caves formed by carbonate rocks. The apparent rarity of this new species, associated with its status as the only troglobite species of Palpigradi associated with a granite cave, underlines the need to preserve this cave.

The only described Brazilian species of *Eukoenenia* that does not occur in carbonate caves is *Eukoenenia ferratilis* Souza & Ferreira 2011, which is widely distributed in iron ore caves in Minas Gerais state. However, it is not a troglobiotic species, since it has also found outside caves (unpublished data).

*E. cavatica* sp. n. was found in a limestone cave located in a region seriously threatened by mining and agricultural activities (Fig. 33). Furthermore, the cave itself is severely altered, especially due to unregulated tourist visits. Although the cave has been visited on three occasions by the authors of this work, only a single specimen was found, which indicates its rarity (and eventual vulnerability).

Harvey *et al.* (2011) emphasized that is virtually impossible to sample and know the whole biota. Therefore, the identification of the most vulnerable elements of the fauna, such as troglobiotic species, is of utmost importance for conservation efforts. According to these authors, is vital to preserve endemic taxa that suffer from degradation and loss of habitat. Thus, the formal description of the new species treated here, even from a single specimen, is of the utmost importance for the preservation of these caves and their surroundings, since Brazilian law requires the preservation of caves with rare troglobiotic taxa.

## Acknowledgements

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