Eukoenenia igrejinha (Palpigradi: Eukoeneniidae), a new cave-dwelling palpigrade from southeastern Brazil

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Abstract. *Eukoenenia igrejinha* sp. nov. is described on the basis of one male and one female from the cave Gruta da Igrejinha, state of Minas Gerais, southeastern Brazil. The new species exhibits morphological features unique among the South American species of *Eukoenenia* described to date, such as a single pair of paramedian thickened setae flanked by one slender seta on each side of opisthosomal sternites IV–VI and 9 pairs of setae on the first genital lobe of the female. Additionally, we provide the coxal formulae of the South American species *E. maquinensis, E. spelunca, E. improvisa* and *E. janetscheki*.

Keywords: taxonomy, morphology, Minas Gerais

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Several species of palpigrades have been described from Brazil in recent years, with the number of species increasing from two to fifteen since 2010 (Souza & Ferreira 2012a, b, 2016, 2018). Four of these species are distributed in a speleological unit known as the Quadrilátero Ferrífero -Conceição, located in Minas Gerais state, southeastern Brazil: the edaphic species Eukoenenia ferratilis Souza & Ferreira, 2011 and the troglobitic species E. navi Souza & Ferreira, 2018, E. eywa Souza & Ferreira, 2018 and E. neytiri Souza & Ferreira, 2018 (Souza & Ferreira 2011a, 2018). This region is considered one of the most important mineral provinces in Brazil, mainly due to its deposits of gold and iron ore, and it comprises a geologically complex area in which hundreds of caves have been recorded in different lithologies. Although most of these caves are inserted in ferruginous rocks (canga, iron ore, itabirite, compact hematite and laterites), there are also caves in carbonatic and siliciclastic rocks (Oliveira et al. 2011). During sampling carried out in the Gruta da Igrejinha, a cave inserted in dolomitic marble located in this important province, two specimens of Palpigradi belonging to a new species were found. Accordingly, the aim of this study is to describe Eukoenenia igrejinha sp. nov., a new palpigrade that exhibits unique morphological features when compared not only to species of this genus that occur in the same speleological unit, but to those in South America as a whole.

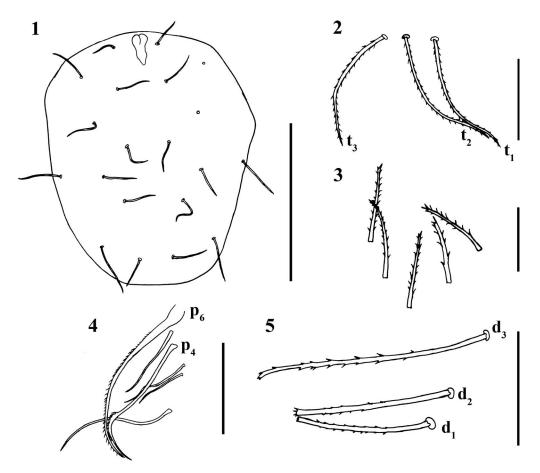
METHODS

The palpigrade specimens were collected with a fine brush in the inner portions of the cave Gruta da Igrejinha and stored in 70% ethanol. They are deposited in the Collection of Subterranean Invertebrates of Lavras (ISLA), Biology Department, Federal University of Lavras, Lavras, Minas Gerais, Brazil.

They were cleared in Nesbitt's solution, mounted individually on glass slides using Hoyer's medium (Krantz & Walter 2009), and studied under a Zeiss Axio Scope A1 microscope with phase contrast and DIC optics. An ocular micrometer was used for the measurements of selected body parts. In the description, measurements of the male are followed by measurements of the female in brackets, if available. Drawings were made with a camera lucida fitted to an Olympus BX40 phase contrast microscope.

The terminology of the setae follows Condé (1977, 1990), Christian & Christophoryová (2013) and Mayoral & Barranco (2017a): r, stiff seta; grt, tergal seta; gla, lateral seta; fs, forked seta: rs. rodlike seta: m. macroseta: p. proximal setae of basal segment of chelicera; d, distal setae of basal segment of chelicera; cs, curved seta; tb, trichobothria. All setae described here as "long fs" are furcate only near the tip, but a longitudinal groove divides the shaft into a barbed and a smooth strand, as described by Christian et al. (2012). Abbreviations follow Mayoral & Barranco (2013, 2017a), Christian & Christophoryová (2013) and Souza & Ferreira (2018): L, total length of body (from the anterior margin of the propeltidium to the tip of the opisthosoma, without flagellum); B, length of dorsal shield (propeltidium); P, pedipalpus; I-IV, legs I to IV; cx, coxa; tc, trochanter; fe, femur; pa, patella; 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 the basitarsus at level of seta r; dr, dgla and dgrt, distance between base of the basitarsus and insertion of seta r, gla and grt, respectively; B/IVbta, ratio between lengths of prosomal shield and basitarsus IV; IVbta/ti, ratio between lengths of basitarsus IV and tibia IV; Seg, dorsal length of the basal segment of the chelicera.

In the discussion section, we provide morphological information on the type specimen of *E. improvisa* Condé, 1979, from French Guiana, deposited at the Chelicerata Collection of the Natural History Museum of Geneva, and on an adult female of *E. cf. janetscheki* Condé, 1993 deposited at the Instituto Nacional de Pesquisas da Amazônia (INPA 0019, "Capoeira", close to Rio Tarumã-Mirim, 16 March 1990, Kempson Extractor 0–14 cm, J. Adis).



Figures 1–5.—*Eukoenenia igrejinha* sp. nov., male holotype: 1. Propeltidium and frontal organ (scale bar 150 μ m). 2. Setae of the metapeltidium (scale bar 50 μ m). 3. Setae of the deuto-tritosternum (scale bar 20 μ m). 4. Proximal series of setae ($p_1 - p_6$) on the basal segment of the chelicera (scale bar 40 μ m). 5. Distal series of setae ($d_1 - d_3$) on the basal segment of the chelicera (scale bar 40 μ m).

TAXONOMY

Family Eukoeneniidae Petrunkevitch, 1955 Genus Eukoenenia Börner, 1901

Koenenia Grassi & Calandruccio 1885:165 [junior primary homonym of *Koenenia* Beushausen 1884 (Mollusca: Bivalvia)].

Koenenia (Eukoenenia) Börner 1901:551.

Type species.—*Koenenia mirabilis* Grassi & Calandruccio 1885, by monotypy.

Eukoenenia igrejinha sp. nov.

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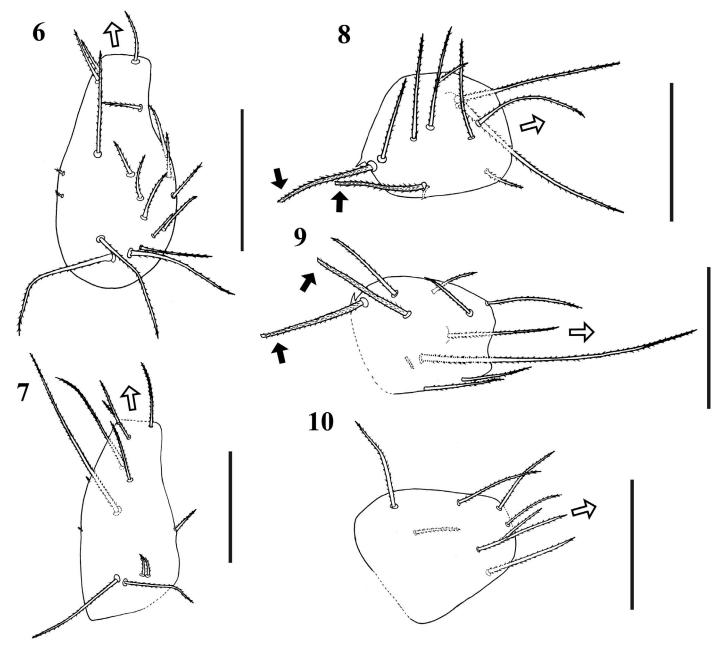
Material examined.—*Holotype male.* BRAZIL: *Minas Gerais*: Ouro Preto, Gruta da Igrejinha (20°27'1.44"S, 43°42'16.10"W), 2 August 2014, M.F.V.R. Souza (ISLA 13174).

Paratype. BRAZIL: *Minas Gerais*: 1 \Im , collected with holotype (ISLA 13173).

Diagnosis.—Frontal organ with two branches expanded and rounded at the distal end and without reticulation; four finely reticulated blades in the lateral organs; 5 setae on deutotritosternum; 10 pairs of setae on propeltidium; 3 pairs of setae on metapeltidium; cheliceral fingers with 9 teeth each; coxae II–IV with 2-2-0 thick setae; 6 setae (*grt*, *gla*, *r*, *esp* and 2 *esd*) on basitarsus of leg IV; opisthosomal tergites II–VI with two pairs of setae *t* between one setae *s* on each side; opisthosomal sternites IV–VI with one pair of setae *a* flanked by one seta *s* on each side; each half of the first lobe of male genitalia with 13 setae (2st + 9 + 2 fusules), second and third lobes with 4 setae on each half; first lobe of female genitalia with 9 pairs of setae.

Description.—Body length without flagellum: 935 (1110) μ m.

Prosoma. Frontal organ 30 (34) μ m long, the two branches are expanded and rounded at the distal end, reticulation absent (Fig. 1). Lateral organ with four pointed-lanceolate and finely reticulated blades (not shown because only the tips of the blades were visible). Propeltidium with 10+10 setae (Fig. 1). Setae t_1 , t_2 and t_3 of metapeltidium 77, 87 and 75 μ m long, respectively (Fig. 2). Labrum with 5+5 short setae (visible only in the paratype). Deuto-tritosternum with 5 setae in U-shaped arrangement (Fig. 3). Basal segment of chelicera 162 (230) μ m in dorsal length, with 6 proximal setae (p_6 the thickest and densely barbed; p_4 thicker than the remaining setae and densely barbed in its distal third) (Fig. 4), 3 distal setae smooth in their proximal third and with tiny projections in their distal part: d_3 longer (77 μ m) than d_1 (45 μ m) and d_2 (52 μ m) (Fig. 5); and 1 apical seta. Hand of chelicera with 7 setae: 4 dorsal



Figures 6–10.—*Eukoenenia igrejinha* sp. nov., male holotype: 6. Coxa of right pedipalp. 7. Coxa of right leg I. 8. Coxa of left leg II. 9. Coxa of left leg III. 10. Coxa of left leg IV. Filled arrows point to thickened setae, open arrows indicate the distal ends of the coxae. Scale bars 60 µm.

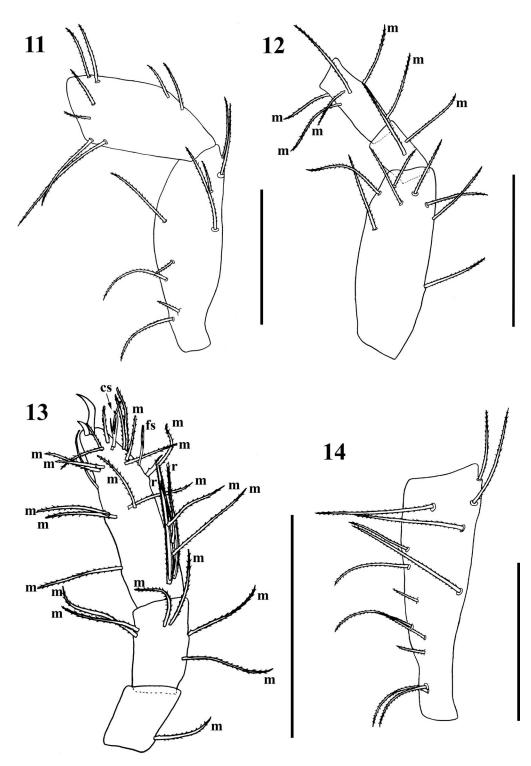
setae, 2 setae in its outer portion (1 close to articulation of movable finger and 1 on a tubercle close to the teeth of the fixed finger) and 1 seta inserted in its inner portion. Fingers with 9 teeth each.

Coxal chaetotaxy. Pedipalp coxa with 19 setae (Fig. 6); coxa I with 13 setae (including 2 tiny microsetae) (Fig. 7); coxa II with 2 thick and 10 ordinary setae (including two macrosetae) (Fig. 8); coxa III with 2 thick and 9 ordinary setae (including 1 macroseta and 1 adjacent microseta) (Fig. 9) and coxa IV with 8 ordinary setae (thick seta absent) (Fig. 10).

Palp. tc with 9 setae; *fe* with 8 setae (Fig. 11); *ti* with 8 setae; *bta1* with 1 *m* and 1 normal seta; *bta2* with 1 normal seta and 5 *m* (Fig. 12); *ta1* with 1 *m*; *ta2* with 6 *m*; *ta3* with 1 long *fs*, 1 *cs*

with a conspicuous spine, 2 r, 12 m (one of the macrosetae with a conspicuous spine) and 8 normal setae (Fig. 13).

Leg I. tc with 13 normal setae, two of which are considerably smaller than the others (Fig. 14); fe with 9 normal setae; pa with 9 normal setae and 1 tb (Fig. 15); ti with 8 normal setae; bta1 with 1 normal setae, 1 m, 2 tb and 1 fs (inner branch shorter than the outer branch); bta2 with 3 m, 1 normal seta, 2 tb and 1 long fs (Fig. 16); bta3 with 1 r, 1 grt and 1 microseta; bta4 with 4 m, 1 normal seta, 1 tb and 1 long fs; ta1 with 2 r and 3 normal setae (one considerably smaller than the others); ta2 with 5 m, 1 tb and 1 long fs (Fig. 17); ta3 with 5 fs (branches with similar lengths) arranged as $fs_1/fs_2/fs_3/$



Figures 11–14.—*Eukoenenia igrejinha* sp. nov., male holotype: 11. Trochanter and femur of right palp. 12. Tibia – basitarsus 2 of right palp. 13. Tarsus 1 – tarsus 3 of right palp. 14. Trochanter of right leg I. Scale bars 80 µm.

 $f_{S_{4+5}}$, $rs (rs/f_{S_1} = 2.8)$, 2 r, 1 cs, 13 m and 5 normal setae (Fig. 18).

esp and are the most basal setae; gla level the seta r (seta r is slightly proximal to gla in the paratype) (Fig. 19).

IVbta. 5.9 (6) times longer than wide and with 6 setae (*grt*, *gla*, *r*, *esp* and 2 *esd*). Seta *r* 1.4 (1.6) times shorter than the tergal edge of segment and inserted in its proximal half (*dr*/IVbta=0.3); *esp*, *gla* and *grt* inserted in proximal half, *grt* level

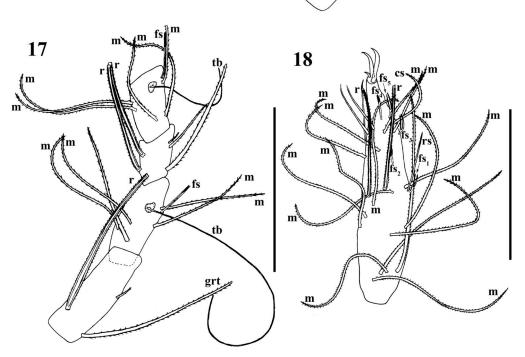
Opisthosoma. Tergites II–VI with 3 + 3 dorsal setae, two pairs of t setae (t_1, t_2) between a pair of slender setae (s). Sternite III with 2 + 2 setae. Sternites IV–VI each with 1 + 1 thickened setae (a_1) between a pair of slender setae (s) (inserted

m

tb

tb

15



16

Figures 15–18.—*Eukoenenia igrejinha* sp. nov., male holotype, right leg I: 15. Femur and patella. 16. Tibia – basitarsus 2. 17. Basitarsus 3 – Tarsus 2. 18. Tarsus 3. Scale bars 80 μm.

caudal to the thickened setae); a pair of pores present between *a* setae on sternites IV–VI of the female. Setae *a* in the sternites IV–VI of male (52 μ m long) and female (60–62 μ m long) with similar shape, becoming gradually thinner from the base to the apex. Segments VII–XI with 8 setae each. The 2 dorsal setae (17 μ m long) on the intermediate ring of the flagellum are smaller than the 2 ventral setae (30 μ m long) (Figs 20–21). Pubescence of the opisthosomal segments IX–XI evenly short and dense. The flagellum of both specimens is missing.

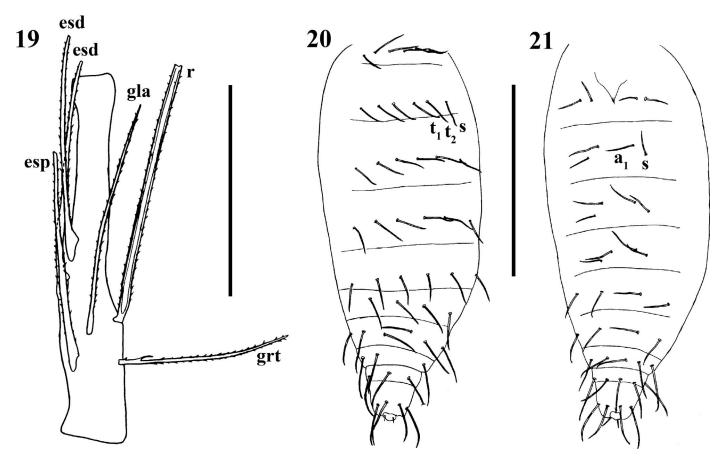
Male genitalia. 2 + 2 anterior (st_1, st_2) setae close to first lobe, 11+11 setae in the first lobe (including 2+2 fusules). Fusules close to each other, with a dilated conical base and a setiform end; internal canals not observed. Each half of second and third lobes subtriangular, with a simple and thin apex,

with 4 setae (w, x, y and z on the third lobe); orifices not observed (Fig. 22).

Female genitalia. First lobe with 9 + 9 setae presenting the following arrangement in rows: 2+2 sternal setae (st_1 , st_2), 2 + 2, 1 + 1, 1 + 1 and 3+3 distal setae (a_1 = 17µm, a_2 = 22µm, a_3 = 27µm); inner surface of the first lobe with a group of 3 orifices on each side and a medial pair of smaller orifices. Second lobe with 3 + 2 setae (it was not possible to observe the *x* seta on the right side) (x = 17 µm; *y* and *z* longer); presence of cuticular spines and a group of 4 orifices on each half (Figs. 23–24).

Measurements (in μ m) and ratios are given in Table 1.

Habitat.—The 930 meter long Gruta da Igrejinha has been systematically visited by speleological groups since the 1960s (Rosada et al. 2013). Being the biggest cave of the iron



Figures 19–21.—*Eukoenenia igrejinha* sp. nov.: 19. Left basitarsus IV of male holotype (scale bar 60 µm). 20, 21. Opisthosoma of female paratype, dorsal (20) and ventral (21) view (scale bar 400 µm).

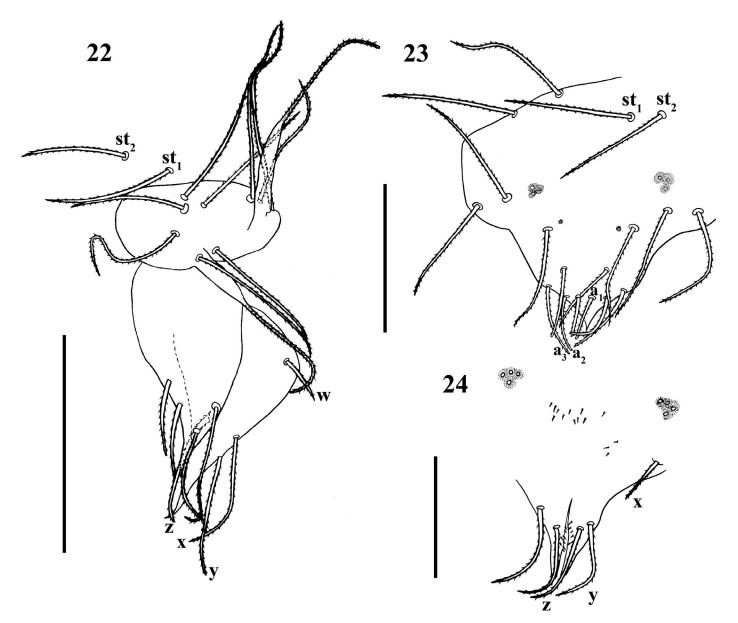
quadrangle region developed in dolomitic marble, Gruta da Igrejinha is currently protected within the "Parque Estadual Serra do Ouro Branco." In the past, however, the cave has been strongly affected by mining activities and has lost its original entrance owing to an explosion. One specimen of *E. igrejinha* was collected under a small rock in the last chamber of the cave, in an area far from the entrance and rich in organic matter. Invertebrates such as springtails were abundant there and may constitute potential prey. The second individual was also collected under a small rock at the end of an ascendant conduit laterally connected to this last chamber (Fig. 25). This location was poor in organic resources and invertebrates.

Etymology.—The specific epithet is a noun in apposition that refers to the name of the type locality. "Igrejinha" means "small church" in Portuguese.

DISCUSSION

Eukoenenia igrejinha sp. nov. can be readily distinguished from other species of *Eukoenenia* described to date by the chaetotaxy of the opisthosomal sternites IV–VI. Other species with only 1+1 paramedian *a* setae (*E. montagudi* Barranco & Mayoral, 2014; *E. deceptrix* Remy, 1959; *E. depilata* Remy, 1959; *E. remyi* Condé, 1974 and *E. christiani* Condé, 1988) have two or more slender setae on either side of these sternites (Remy 1959; Condé 1974, 1988; Barranco & Mayoral 2014), whereas *E. igrejinha* has a single seta.

Eukoenenia igrejinha differs from the other South American congeners in having nine pairs of setae on the first genital lobe of the female (a character state of only around a dozen of species worldwide: Remy 1959, 1960; Condé 1979a, 1993a; Montaño & Francke 2006; Mayoral & Barranco 2017a, 2017b); and by the presence of 2-2-0 thick setae on coxae II-IV (Condé 1979a, 1997; Souza & Ferreira 2010, 2011a, 2012a, b, 2016, 2018; Ferreira et al. 2011). The coxal formula is not stated in the original descriptions of E. grassii (Hansen, 1901), E. roquettei Mello-Leitão & Arlé, 1935, E. improvisa, E. janetscheki, E. maquinensis Souza & Ferreira, 2010 and E. spelunca Souza & Ferreira, 2011. Our study of the type material revealed 5-6-2 for E. maquinensis, 4-3-2 for E. spelunca (some coxal setae are broken off in the type, so that the number of thick setae could only be inferred from the shape of the insertions) and 3-3-? for E. improvisa (the chaetotaxy of coxa IV was not clearly visible in the type specimen). We observed 3-3-1 thick coxal setae for a female of E. cf. janetscheki from "Capoeira" near Rio Tarumã Mirim (a place from which specimens for the complementary description came) (Condé 1997). Thus, the coxal formula remains totally unknown only for E. roquettei and E. grassii. The descriptions of these species are deficient from a modern perspective and the type material is inaccessible, since the holotype of both species have been lost (Condé 1986). The



Figures 22–24.—*Eukoenenia igrejinha* sp. nov.: 22. Genital lobes of male holotype, left side (scale bar 80 μm). 23. First genital lobe of female paratype (scale bar 60 μm). 24. Second genital lobe of female paratype (scale bar 40 μm).

non-reticulated frontal organ is also distinctive, when considering the species for which this feature is described or illustrated (Ferreira et al. 2011; Souza & Ferreira 2011a, 2012a, b, 2016, 2018) and when compared to the type specimens of *E. maquinensis* and *E. spelunca*.

Concerning the similarities with the South American *Eukoenenia*, the new species shares the chaetotaxy of opisthosomal tergites II–VI with all the troglobitic *Eukoenenia* from Brazil described to date and with the edaphic species *E. improvisa* and *E. janetscheki*. The lack of one *esp* seta on basitarsus IV is also a feature displayed by the species previously mentioned, except for *E. janetscheki* (in the case of *E. virgemdalapa* Souza & Ferreira, 2012, the *gla* setae is also absent). The propeltidium with ten pairs of setae is present in most species, with exception of *E. maquinensis, E. sagarana*

Souza & Ferreira, 2012, *E. navi, E. eywa* and *E. neytiri.* The chaetotaxy of the three lobes of the male genitalia resembles that of *E. cavatica* Souza & Ferreira, 2016, *E. spelunca, E. navi, E. eywa* and *E. neytiri* (Condé 1979a, 1993b, 1997; Souza & Ferreira 2010, 2011a, b, 2012a, b, 2016, 2018; Ferreira et al. 2011). The presence of 4 blades on the lateral organs is shared with the edaphic species *E. janetscheki* (Condé 1993b, 1997). *Eukoenenia improvisa* is known from a single specimen that has an asymmetric condition for this character (four blades on the left side and three on the right side), which precludes determination of the usual number of blades forming the lateral organs of this species (Condé 1979a).

The chaetotaxy of the palp and leg I (which are features that have only been recently described by taxonomists) of the new species differs from that of the Brazilian species *E. navi*, *E.*

L	935 (1110)	Ibta3	52 (60)	IVpa	100 (117)
В	232 (260)	Ibta3 – dr	12 (12)	IVti	117 (137)
Seg.	162 (230)	Ibta3 – r	72 (75)	IVbta	100 (120)
Pcx	95 (120)	Ibta3 – a	17 (22)	IVbta – a	17 (20)
Ptc	115 (135)	Ibta3 – grt	75 (80)	IVbta – dr	32 (37)
Pfe	92 (117)	Ibta3 – dgrt	5 (10)	IVbta – r	70 (75)
Pti	97 (117)	Ibta3/a	3.06 (2.73)	IVbta – gla	62 (67)
Pbta1	35 (45)	r/Ibta3	1.38 (1.25)	IV – grt	47 (56)
Pbta2	47 (57)	dr/Ibta3	0.23 (0.2)	IV – dgrt	20 (30)
Pta1	30 (35)	grt/Ibta3	1.44 (1.33)	IV – dgla	30 (50)
Pta2	32 (40)	dgrt/Ibta3	0.1 (0.17)	IVbta/a	5.88 (6)
Pta3	67 (75)	Ibta4	45 (52.5)	IVbta/r	1.43 (1.6)
Icx	110 (130)	Ita1	32 (40)	dr/IVbta	0.32 (0.31)
Itc	130	Ita2	32 (37)	IVta1	50 (60)
Ife	85 (105)	Ita3	115 (125)	IVta2	62 (70)
Ipa	100 (122)	IVex	75 (87)	IVbta/ti	0.85 (0.88)
Iti	97 (120)	IVtc	85 (82)	B/IVbta	2.32 (2.17)
Ibta1+2	67 (85)	IVfe	85 (112)		

Table 1.—Measurements (in µm) and ratios of the male holotype and the female paratype (in parentheses, if available) of *Eukoenenia igrejinha* sp. nov.

eywa and E. neytiri by the number of setae on tibia, basitarsus 1 and tarsus 1 of the palp, and on tibia and basitarsus 2 of leg I. The total number of setae on tarsus 1 of leg I is the same (five) in these four species, however, in the new species two normal setae are replaced by two r setae (Souza & Ferreira 2018). In addition, the morphology of the forked setae on tarsus 3 of palp and on basitarsus 4 and tarsus 2 of leg I of the new species is different from the other three species, being

similar to the long forked setae found in the European species *E. gasparoi* Condé, 1988 (Christian et al. 2012).

The value of the ratio B/btaIV (2.17–2.32) of *E. igrejinha* is closer to that in troglobitic species (average value = 1.69) than that of edaphic species (average value = 3.51) (Mayoral 2015). Nonetheless, the value of the ratio IVbta/ti (0.85–0.88) is similar to that in other Brazilian edaphic species (average values of 0.87, 0.84 and 0.90 for *E. ferratilis, E. potiguar* and

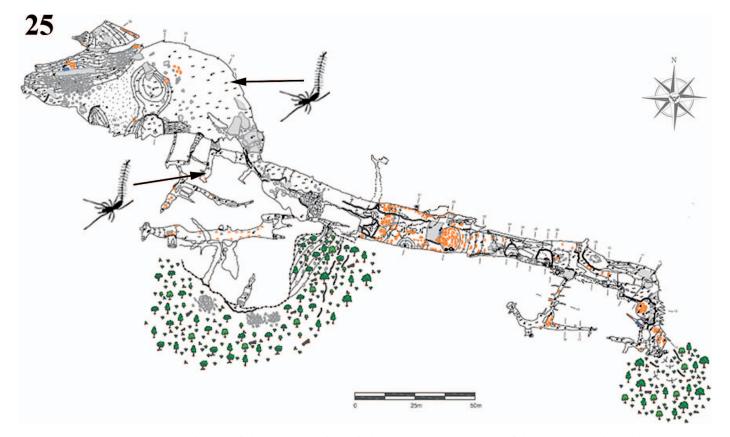


Figure 25.—Map (ground floor) of the cave Gruta da Igrejinha with the sampling spots of the two palpigrades marked.

E. janetscheki, respectively) (Condé 1993b; Ferreira et al. 2011; Souza & Ferreira 2011a). On the other hand, the presence of four blades on the lateral organs is a feature shared with only about 8% (E. janetscheki and E. pauli Condé, 1979) of the edaphic Eukoenenia described to date, since the remaining species (with exception of *E. depilata* Remy, 1959) have three or fewer blades (Remy 1959; Condé 1979b, 1993b). It is important to highlight that an increase in the number of blades on the lateral organs is considered to be an important troglomorphic trait for Palpigradi (Mayoral 2015). Thus, this character is not very common in soil-dwelling palpigrades and also occurs in some troglobitic species, including E. strinatii Condé, 1977 and E. margaretae Orghidan et al., 1982 (Condé 1977; Orghidan et al. 1982). Being so, this species has an intermediate morphology, although it shares more characteristics with troglomorphic than edaphomorphic species. Several caves have been sampled in different parts of the Iron Quadrangle region during environmental impact studies but E. igrejinha has only been found in the type locality so far. Accordingly, it is possible that this species represents a troglobite. However, a deeper investigation of cave and soil invertebrates in the surrounding area is needed to clarify its distribution and degree of dependence on the subterranean environment.

Rosada et al. (2013) granted the highest relevance to Gruta da Igrejinha due to its geological and historical uniqueness. According to the Brazilian decree on the use of natural subterranean cavities, this cave must not be destroyed or irreversibly damaged. However, the management plan that is supposed to limit human impact upon the Gruta da Igrejinha has never been implemented. Visitors can access all sections since there are no delimited trails in the cave, and speleologists and students frequently use Gruta da Igrejinha for training purposes. As a consequence, trampling may become a threat to this and other species.

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