Distribution and morphology of the European Karst palpigrade *Eukoenenia gasparoi* (Arachnida: Palpigradi)

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**Abstract.** The first record of the palpigrade *Eukoenenia gasparoi* Condé 1988 in Croatia is reported. We review the distribution of this troglobiotic species endemic to the classical European Karst region, give an illustrated description of the adult stages and the juvenile female, and evaluate divergence among the populations. Morphological differences reflect individual-level rather than population-level variation.

**Keywords:** Cave, Croatia, endemism, first record, troglomorphy

Over 80% of the 28 palpigrade species recorded from Europe inhabit caves and related subterranean habitats (Harvey 2003; Christian 2009). *Eukoenenia gasparoi* Condé 1988 belongs to a group of seven troglobiotic species characterized by at least three blades in the lateral organ and seven (exceptionally six or five) setae on the basitarsus of the last pair of legs, while the remaining European species have only one blade or four basitarsal setae. The group includes Europe’s most distinctly modified cavernicolous palpigrades (Condé 1998) with consistently small distribution ranges: *E. brignolii* Condé 1979 from Apulia, Italy; *E. draco* (Peyerimhoff 1906) from Mallorca, with the subspecies *E. d. zariquieyi* (Condé 1951) from Catalonia, Spain; *E. grafitti* Condé & Heurtault 1994 from Sardinia, Italy; *E. hispanica* (Peyerimhoff 1908) from Aragon, Spain; *E. naxos* Condé 1989 from Irakleia, Greece; and *E. patrizii* (Condé 1956) from Sardinia, Italy. *Eukoenenia gasparoi* is currently the best documented member of this group and has been recorded from four localities. We report on habitat characteristics of a new location that widens the known distribution range of the species from the Classical European Karst landscapes in northeastern Italy and southwestern Slovenia toward the northwestern limits of Croatia. In order to detect possible intraspecific divergence, we supplement Condé’s (1988, 1990) data with a detailed morphological description of the Croatian specimens.

**METHODS**

We fixed the three specimens from the cave Radota jama in 70% EtOH, cleared them in 10% KOH and chloral phenol, and subsequently embedded them in a modification of the water soluble Swan medium (Rusek 1975). Next, we studied the slides under a Nikon E 600 microscope with a measuring eyepiece and phase contrast and DIC optics. Micrographs, taken with Nikon D 200 and Nikon 1 J1 cameras, were stacked and edited (contrast enhanced, motifs partly cut out) using Photoshop CS5.

Setae (in italics) are termed according to Condé (1988) except for the converse labeling of gla and grt (see footnote in Condé (1990:834). Abbreviations: B = length of propeltidium; ti = tibia; bta = basitarsus; ta = (telotarsus); a = maximum width of the basitarsal article (instead of the commonly used width at the insertion of seta r, because there the cross section of the basitarsus is not perfectly round: rotation of the article changes the apparent width); dg = r = shortest distance between the insertion of the seta and the base of the article; fs = forked seta(e). Indices (Table 1) are given as dividend/divisor.

The specimens are held in the collection of the Croatian Biospeleological Society, Zagreb.

**GENERAL DISTRIBUTION AND NEW RECORD OF E. GASPAROI**

The known localities of *E. gasparoi* (Fig. 1) are situated in the western part of the Classical European Karst, i.e., the Carso Triestino, the adjoining Low Littoral Karst, and its continuation in the karstic plateau of the Cićarja. Although the five caves are situated along a NW–SE line no longer than 45 km, the range of distribution extends over borderlands of Italy, Slovenia, and Croatia. *Eukoenenia gasparoi* is the only endemic palpigrade species of a region where two cavernicolous congeners, *E. spelaea* (Peyerimhoff 1902) and *E. austriaca* (Hansen 1926), have also been recorded (Zagmajster & Kováč 2006).

The locality of the first Croatian record, Radota jama (Figs. 2, 3), is an unbranched karst cave of 268 m length and 170 m depth, developed in dark brown miliolide limestone of the middle Paleocene. The entrance opens at 593 m a.s.l. on Mt. Žbevnica between the villages Rakitovec (Slovenia) and Brest (Croatia) on the Cićarja plateau in the northern part of...
the Istrian peninsula, a few hundred meters within the territory of Croatia. Bat guano is a major nutrient basis for the rich cavernicolous fauna that includes the snail *Zospeum spelaeum schmidti* (Frauenfeld 1854) (Slapnik & Ozimec 2004), the spider *Mesostalita nocturna* (Roewer 1931), the pseudo-scorpions *Chthonius spelaeophilus histrus* Beier 1931, *Trogl glochthonius doratodactylus* Helversen 1968 and *Neobisium reimoseri reimoseri* (Beier 1929), the woodlouse *Titanethes dahli* Verhoeff 1926 (Bedek et al. 2011), the centipede *Eupolybothrus obrovensis* (Verhoeff 1930), the springtails *Absolonia gigantea* (Absolon 1901) and *Troglopedetes pallidus* Absolon 1907 (det. M. Lukić), the lepidodrine beetle *Bathysciotes khevenhuelleri* (Miller 1852), and the pselaphine beetle *Machaerites kastavensis* Pavčeveč & Ozimec 2009. For the period between November 2010 and June 2011, permanently recording instruments yielded a mean air temperature of 9.1°C (8.4–9.5) and a mean relative humidity of 99.5% (97.1–100) in the cave.

**Table 1.**—*Eukoenenia gasparoi*, morphometric indices of all known specimens (leg I above, leg IV below the division; j = juvenile; see text for other abbreviations). New data are in bold, literature data are derived from Condé (1988, 1990). *1 = Grotta delle Perle, holotype; 2 = Grotta Azzurra di Samatorza; 3 = Vilenica jama; 4 = Radota jama; 5 = Grotta delle Perle; j1 = Caverna III del Monte Sedlen; j2 = Radota jama. * value for the opposite leg is 2.63.

|    | v1 | v2 | v3 | v4 | v5 | d1 | d2 | j1 | j2 | j1j2 |
|----|----|----|----|----|----|----|----|----|----|------|
| bta3/r | 1.00 | 1.08 | 1.09 | 0.98 | 1.03 |
| bta3/dr | 4.07 | 4.04 | 4.13 | 4.59 | 4.74 | 4.44 | 4.32 | 3.33 | 4.05 |
| bta3/ti | 0.38 | 0.37 | 0.36 | 0.37 | 0.36 | 0.36 | 0.35 |
| bta3/B | 0.24 | 0.24 | 0.24 | 0.20 | 0.22 |
| bta/a | 9.53 | 8.89 | 8.89 | 8.00 | 8.45 |
| bta/dr | 2.80 | 2.80 | 2.90 | 2.86 | 2.53 | 2.50 | 2.58 |
| bta/ti | 2.42 | 2.42 | 2.59 | 2.40 | 3.44 | 3.15* | 3.15 |
| bta/B | 0.81 | 0.81 | 0.79 | 0.77 | 0.78 | 0.82 |
| gla/grt | 0.64 | 0.66 | 0.65 | 0.68 | 0.77 | 0.69 | 0.71 |
| bta/B | 0.53 | 0.57 | 0.54 | 0.55 | 0.55 | 0.44 | 0.51 |

Family Eukoeneniidae Petrunkevitch 1955

Genus *Eukoenenia* Borner 1901

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

*Eukoenenia gasparoi* Condé 1988

*Eukoenenia gasparoi* Condé 1988:729–736, Figs. 4–8, table 3; Condé 1990:833–835, Figs. 1c, 2c; Condé 1998:917, 919; Zagmajster & Kováč 2006:25, Fig. 1.

**Material examined.**—One adult male (leg. S. Polak) and one juvenile female (leg. V. Zášek), collected from the underside of stones in the cave Radota jama on 27 November 2010 in the course of the Slovenian-Croatian Karst Underground Protection project (www.project-kup.org; Ozimec et al. 2011). One adult female (leg. R. Ozimec, 29 July 2001) from the same cave (Fig. 2).

**Diagnosis.**—A medium-sized, troglomorphic *Eukoenenia*. Adults with 3 blades in the lateral organ; 3 setal bases forming a wide V on the deuto-tritosternum; 10 +10 very short setae on the propeltidium; 1 +1 setae on the metapeltidium; 7 setae on the basitarsus of leg IV; tergites with only 1 +1 setae t, flanked by one seta s on each side; sternites III–VI with 4 +4 subequal setae; semiglobular bases of the fusules on the first genital valve of the...
male, along with 4+4 and 5+5 setae on the following valves; 2+5+4 setae on each half of the first genital valve of the female.

Description. — Male: Appearance as in Fig. 4. Body length (without the broken-off flagellum) 1560 μm. B = 440 μm. Pubescence mostly short and dense, somewhat longer and more sparse on the limbs, on opisthosomal segments IX–XI, and on labrum and deuto-tritosternum.

Frontal organ (crushed, hence overall length not determined) with a basal piece as long as the two broadly lanceolate branches, the latter abruptly narrowing to the rounded tip. The three elements of the lateral organ (37 μm) are relatively thick and evenly reticulated (Fig. 5). Propeltidium with 10+10 short setae (maximum 12 μm). Chaetotaxy of the metapeltidium reduced to 1+1 setae t2 (76 μm). Labrum with the usual cuticular pattern and 5+5 setae (maximum 14 μm). Deuto-tritosternum with 3 subequal setae (maximum 57 μm) forming a widely obtuse angle (Fig. 6). Basal article of the chelicera with a proximal group of 6 setae (seta 4 and 6 thickened), in the distal half with 3 aligned setae (the last one longest), and 1 apical seta. Hand of the chelicera with 1 ventral and 6 dorsal setae. Fingers of the chelicera with 8 teeth.

Pedipalp in the proximal half of ta3 with a rodlike seta (23 μm) and 3 forked setae (fs) successively arranged in the distal half of the article (as in Fig. 7). The stiff smooth branches of the fs are about the same length (15–17 μm), the flexible barbed branch of the distal seta is moderately longer, that of the proximal seta considerably longer (48 μm). The proximal fs has, like other setae on the distal articles of the limbs, a denticle near its base. Length of pedipalp articles (μm): ti = 258, bta1 = 81, bta2 = 128, ta1 = 60, ta2 = 78, ta3 = 79. Leg I with 7 trichobothria at the usual positions and altogether 10 fs. The 6 fs of ta3 are arranged as in Fig. 8: 1 long + 2 short fs near the tip, followed by 1+1 and 1 short fs in equal distances towards the base of the article. The remaining 4 fs are inserted near the distal ends of ta2, bta4, bta2, and bta1; they are all long. Short fs (15–18 μm) are split almost down to the base; long fs (30–54 μm) are only furcate near the tip, but a longitudinal groove divides the shaft into a barbed and a smooth strand. The bta3 of leg I (Fig. 9) is over four times longer than wide and bears 1 short distal and 2 long setae: grt is a little longer than r, both are inserted within the proximal third of the article and surpass the base of bta4. On
bta4, the trichobothrium, the f/s, and 2 of the 5 ordinary setae are placed in the distal half. Measured values of leg I (μm): ti = 301, bta1+2 = 247, bta3 = 108, a = 26, grt = 104, dgrt = 20, r = 99, dr = 25, bta4 = 91, ta1 = 43, ta2 = 47, ta3 = 193. Coxa of legs II and III each with 3 thickened setae (they are more cylindrical, apparently softer, and have an inclined barbule at the tip); rest of coxal chaetotaxy hardly readable. Leg IV with a very long and slender bta (nearly nine times longer than wide, Fig. 10), which bears 7 setae: grt, 2 esp, gla, r and 2 esd. Seta r is shortest, grt longest; all but gla carry a minute, oblique barbule at the tip. Measured values of leg IV (μm): ti = 310, bta = 240, a = 27, grt = 127, dgrt = 50, esp = 109/96, desp = 43/59, gla = 90, dglα = 67, r = 84, dr = 100, esd = 87/89, desd = 152/158, ta1 = 84, ta2 = 96. Indices of leg I: bta3/a = 4.15, bta3/r = 1.09, bta3/dr = 4.32, bta3/ti = 0.36, bta3/B = 0.24. Indices of leg IV: bta/a = 8.89, bta/r = 2.86, bta/dr = 2.40, bta/ti = 0.77, bta/B = 0.55.

The opisthosoma shows a sudden narrowing between segments VIII and IX. Tergites II–VI with one seta t (probably t3) and one seta s. Tergal setae s are considerably shorter than t, more slender and lack a typical basal ring (Fig. 13). Sternite III with st2 and st3. Sternites III–VI each with a pair of sternal

Figures 7,8.—Eukoenenia gasparoi, female from Radota jama, forked setae on terminal limb segments: 7. Pedipalp, ta3, with the base of the big forked seta enlarged to show the denticle ; 8. Leg I, distal half of ta3, with one long (arrow) and five short forked setae. Scale = 20 μm.

Figures 9–12.—Eukoenenia gasparoi from Radota jama: 9. Male, leg I, basitarsus 3 and 4, the arrow points to the forked seta next to the trichobothrium of basitarsus 4; 10. Male, leg IV, basitarsus; 11. Female, leg IV, basitarsus; 12. Juvenile female, leg IV, basitarsus. Scale = 100 μm.
pores and the setae $a_1$, $a_2$, $s_1$ and $s_2$ (Fig. 14), in the terminology of Condé (1988). The four setae of each hemisternum are of similar length, identical structure, and inserted at equal distances, so that they might as well be interpreted as $a_{1-4}$. On sternite VI, the setae $a_1$ are 66 μm long and 77 μm apart. Segments VII–XI with 7, 9, 8, 8, and 8 (maximum 101 μm) setae. The flagellum is lost.

Shape of the first genital lobe (Fig. 15) uncertain due to folding. It probably conforms to Fig. 8 in Condé (1988), just as the setation does. 2+2 sternal setae ($st_1$, $st_2$) are followed, on the lobe in the proper sense, by 11+11 phaneres including 2+2 fusules. Each fusule is inserted on top of a glabrous half-sphere through which the effenter duct shines (Fig. 16). The two flaps of the second lobe each with 4, those of the third lobe each with 5 setae (Fig. 15).

**Female:** Complementary observations (characters conform with those of the male partly omitted). Body length (without the broken-off flagellum) 1590 μm. B = 450 μm. Blades of the lateral organ maximum 35 μm. Zeta $i_2$ on the metapeltidium 89 μm. Deuto-protosternal setae maximum 58 μm. Pedipalp on ta3 with a rodlike seta and 3 $fs$ (Fig. 7) arranged and shaped just as in the male. Length of pedipalp articles (μm): $t_i = 252$, bta1 = 97, bta2 = 129, ta1 = 59, ta2 = 79, ta3 = 84. Coxa of the pedipalp with 19 setae, coxae of legs I–IV with 15 (0 thickened) / 14 (3 thickened; Fig. 17) / 12 (3 thickened) / 8 (0 thickened) setae. Leg I with trichobothria and $fs$ as in the male (Fig. 8), shape and chaetotaxy of bta3 and bta4 the same. Measured values of leg I (μm): $t_i = 301$, bta1+2 = 247, bta3 = 109, $a = 27$, gtr = 108, $dgtr = 18$, $r = 101$, $dr = 23$, bta4 = 98, ta1 = 48, ta2 = 53, ta3 = 199. The proportions of the basutarsus of leg IV are almost the same as in the male, but the insertions of the setae (particularly $gla$ and $r$) differ (Fig. 11). Measured values of leg IV (μm): $ti = 309$, bta = 249, $a = 28$, $gtr = 126$, $dgtr = 59$, $esp = 106/107$, $desp = 65/74$, $gla = 97$, $dgla = 76$, $r = 86$, $dr = 96$, $esd = 91/95$, $desd = 172/174$, $ta1 = 82$, $ta2 = 96$. Indices of leg I: bta3/a = 4.04, bta3/r = 1.08, bta3/dr = 4.74, bta3/ti = 0.36, bta3/B = 0.24. Indices of leg IV: bta/a = 8.89, bta/r = 2.90, bta/dr = 2.59, bta/ti = 0.81, bta/B = 0.55. Setation of the opisthosomal segments II–VI as in the male. On sternite VI the setae $a_1$ are 73 μm long and 64 μm apart. Segments VII–XI with 7, 9, 8, 8, and 8 (maximum 120 μm) setae.

First genital lobe with 22 setae, each half has 2 basal + 5 + 4 apical. The basal setae are longest (maximum 75 μm), the apical seta $a_4$, inserted somewhat above $a_{1-3}$, is 35 μm long (Fig. 18). The two flaps of the second genital lobe each with 3 setae.

**Juvenile female:** Body length (without the broken-off flagellum) 1440 μm. B = 365 μm. Frontal organ (39 μm) shaped as in the adult. Lateral organ (Fig. 19) with two blades (31 μm). Labrum with 4+4 setae. Deuto-tritosternum (Fig. 20) with 1 seta (47 μm). Chelicera with complete setation, fingers with 7 teeth.

Length of pedipalp articles (μm): $ti = 193$, bta1 = 68, bta2 = 105, ta1 = 53, ta2 = 69, ta3 = 71. Propeltidium with 11+11 short setae (i.e., with one pair more than the adult 5). Metapeltidium with 1+1 setae (74 μm). Legs with trichobothria, $fs$, and thickened coxal setae as in the adult. Measured values of leg I (μm): $t_i = 230$, bta1+2 = 171, bta3 = 81, $a = 24$, gtr = 81, $dgtr = 11$, $r = 79$, $dr = 20$, bta4 = 76, ta1 = 42, ta2 = 42, ta3 = 170. Basutarsus of leg IV (Fig. 12) with incomplete setation ($gla$ and 1 $esp$ missing). Measured values of leg IV (μm): $ti = 227$, bta = 186, $a = 22$, gtr = 89, $dgtr = 35$, $esp = 82$, $desp = 26$, $r = 72$, $dr = 59$, $esd = 77/74$, $desd = 111/110$, ta1 = 74, ta2 = 86. Indices of leg I: bta3/a = 3.38, bta3/r = 1.03, bta3/dr = 4.05, bta3/ti = 0.35, bta3/B = 0.22. Indices of leg IV: bta/a = 8.45, bta/r = 2.58, bta/dr = 3.15, bta/ti = 0.82, bta/B = 0.51.

Primordia of genital valves are developed on opisthosomal segments II and III (Fig. 21). On the unpaired, but mediodistally cleft anterior valve of the juvenile ♀ 4+4 long and 2+1 minute subapical setae; the longest, most anterior pair (51 μm) probably represents 1+1 $st_1$. The posterior valve is composed of two lobes, each with 1 short seta. Two long setae at either side of the posterior valve are the sternal setae of segment III, $st_2$ and $st_3$.

Chaetotaxy of tergites II–VI as in the adult. Sternites IV–VI with $a_1$, $a_2$, and $s_1$; setae $s_2$ are missing (Fig. 22). On sternite VI, the setae $a_1$ are 61 μm long and 79 μm apart. All gland orifices are visible. Segment VII with 7, XI with 8 setae (chaetotaxy of VIII–X unclear).

**Variability.**—Table 1 compiles all available indices of the presently known specimens. The values lie closely together, suggesting that there has been virtually no morphological divergence among the Italian, Slovenian, and Croatian populations of *E. gasparoi*.

**DISCUSSION**

Cavernicolous palpigrades differ in morphological characteristics thought to be influenced by the subterranean environment; some species appear more troglomorphic, while others appear less so (sensu Christiansen 1962). Although
Eukoenenia gasparoi has a moderately long flagellum of 1.30 times the body length (Conde 1990) and a common number of blades in the lateral organ, the elongated ambulatory and sensory limbs immediately suggest troglomorphy. This impression is supported by indicator indices such as the basitarsus IV proportion. Conde (1998) reports length/width values between 3.22 and 10.22 for cave-dwelling palpigrades, with E. gasparoi (9.53, holotype) ranking among the most troglomorphic species. In specimens from Radota jama, this ratio is 8.89 (♂ and ♀) and 8.45 (juv. ♀). According to Conde (1996), the length ratio basitarsus IV/propeltidium separates soil from cave palpigrades, averaging 0.28 in the former and 0.59 in the latter. Again the Croatian specimens turn out to be troglomorphic: 0.55 (♂ and ♀) and 0.51 (juv. ♀). The two indices suggest increasing troglomorphism from the juvenile to the adult stage, and so does the length of the foreleg. It is about 1.30 the body length in the adult specimens from Radota jama, but just 1.07 in the juvenile female.

Figures 15, 16.—Eukoenenia gasparoi, male from Radota jama: 15. Total aspect of the genital valves; black rings indicate setal bases of the second valve, white rings those of the third valve. Scale = 50 μm; 16. First genital valve. Arrows point at the hemispheric bases of the fusules. Scale = 20 μm.
The length of basitarsus IV in relation to the tibia has also been considered informative for the degree of cave adaptation. Condé (1990) established bta/ti values of 0.70–1.00 for most of the cavernicolous palpigrades and 1.10 for the extremely troglomorphic E. naxos. Souza & Ferreira (2010) determined a bta/ti value of 1.07 for E. maquinensis from Brazil, a troglobiotic species with an excessively long flagellum. In E. gasparoi, however, the basitarsus of leg IV is clearly shorter than the tibia (bta/ti between and 0.77 and 0.82), suggesting moderate troglomorphy. The apparent inconsistency between bta/B and bta/ti leads us to conclude that any index, taken individually, may be an unreliable indicator of palpigrade troglomorphy. A long basitarsus may be caused either by an elongation of this very structure, or of the entire leg, or both. Furthermore, the comparison among related species shows that morphological changes in response to subterranean life do not necessarily evolve at the same pace, if they evolve in parallel at all. Many troglobiotic animals, cave fishes included, display a mosaic of more or less troglomorphic traits (Romero 2011). In E. naxos, for instance, all relevant characters indicate “most advanced subterranean evolution”, except the number of blades in the lateral organ (Condé 1990).

The vast majority of the 27 native European plus one introduced palpigrade species occur in caves (Christian 2009), but only six resemble E. gasparoi in regard to btaIV chaetotaxy. None of them has more than two setae t on the tergites III–VI, and two species (E. naxos, E. patrizii) share with E. gasparoi the reduction to a single seta t. Number, distribution, and shape of the forked setae on the distal articles of pedipalp and leg I separate E. gasparoi from the species of the mirabilis, spelaea, and subangusta complexes, but it remains unclear whether this is a shared character state of all the seven species with an elevated number of setae on btaIV. These definitely troglobiotic palpigrades, supposed descendants from an ancient tropical fauna (Condé 1998), are probably endemic to closely confined areas. Some have been recorded solely from the type locality, but considering the mostly accidental records of palpigrades in caves, one need not necessarily presume spot endemism. Eukoenenia gasparoi surpasses related species in the density of records, so that the area of distribution is taking shape. The record reported here expands the known range from the Classical European Karst in the hinterland of Trieste (Italy) and Divača (Slovenia) some 30 km to the southeast, by a small distance into Croatian territory. Two other, closely related palpigrade species, E. spelaea and E. austriaca, partly overlap the range of E. gasparoi, but have much wider distributions. Eukoenenia gasparoi, however, is endemic to the area traditionally called “the Karst”; it is the Karst palpigrade par excellence. Intensified biospeleological research might uncover occurrences of E. gasparoi in other caves of the Čišarija and the heartland of Istria, but the species is obviously missing in the well-investigated Postojna and Kočevoj regions. We do not know of any current threat to E. gasparoi, but the small area of distribution makes the species appear vulnerable. The maintainance of the populations lies in the responsibility of the three countries.

The specimens from Radota jama consolidate the key characteristics of E. gasparoi and make adults easily determined. Inspection of the deuto-tritosternum alone would be sufficient for identification, as the reduced chaetotaxy (three setal bases forming a wide V in adult specimens) is unique among European palpigrades. Consideration of additional
characters (see Diagnosis) resolves all doubt about species affiliation.

*Eukoenenia gasparoi* appears to be morphologically uniform. Although existing descriptions (Condé 1988, 1990) give no information on the propeltidial chaetotaxy of juvenile specimens, we assume that the additional pair of setae on the propeltidium of the juvenile female from Radota jama is due to individual variation. The same applies to the unstable insertion height of certain setae of basitarsus IV (compare Figs. 10 and 11). As even the left and the right basitarsus of one individual can differ (e.g., asterisk in Table 1), we follow Condé (1988, 1990) in regarding this variation as taxonomically irrelevant.

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