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Source: Arachnologische Mitteilungen: Arachnology Letters, 60(1) : 19-22

Published By: Arachnologische Gesellschaft e.V.

URL: <https://doi.org/10.30963/aramit6003>

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## ***Eukoenenia florenciae* (Arachnida: Palpigradi) from the Munich Botanical Garden – first record of microwhip scorpions in Germany**

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(both authors contributed equally to this study)



doi: 10.30963/aramit6003

**Abstract.** Seven female specimens of *Eukoenenia florenciae* (Rucker, 1903) were collected in greenhouses of the Munich Botanical Garden (Munich, Germany). Morphological determination is supported by sequencing and comparison of the 18S and 28S genes in BLAST. This is the first record of the arachnid order Palpigradi in Germany.

**Keywords:** distribution, Eukoeneriidae, Europe, greenhouses, SEM

**Zusammenfassung.** *Eukoenenia florenciae* (Arachnida: Palpigradi) aus dem Botanischen Garten München – Erstnachweis von Tasterläufern für Deutschland. Sieben weibliche Exemplare von *Eukoenenia florenciae* (Rucker, 1903) wurden in Gewächshäusern des Botanischen Gartens München gesammelt. Ergänzt wird die morphologische Bestimmung durch Sequenzierung und Vergleich der 18S- und 28S-Gene in BLAST. Dies ist der Erstnachweis der Arachniden-Ordnung Palpigradi für Deutschland.

Palpigrades are a poorly-known and obscure arachnid order (Harvey et al. 2006). They are regarded as the most enigmatic arachnids and as one of the rarest and smallest groups among all terrestrial arthropods (Giribet et al. 2014). First reported by Grassi & Calandruccio (1885) in the late 19<sup>th</sup> century, no photos of living specimens were published before the beginning of the 21<sup>st</sup> century (Kováč et al. 2002) and their extraordinary feeding habit was revealed even later (Srnč et al. 2013). The main features of these tiny, whitish, and eyeless soil and cave-inhabiting arachnids are the segmented opisthosoma, a flagellum with up to 15 bristle bearing articles, and the pedipalps, which are used for locomotion.

So far, 109 species from six genera and two families have been described – the Prokoeneriidae Condé, 1996 with two genera and seven known species, and the larger Eukoeneriidae Petrunkevitch, 1955 with four genera and 102 species (Giribet et al. 2014, Christian pers. comm. in 2020). The distribution of palpigrades ranges over parts of North America, South America, Africa, South Asia, Australia and Europe. All 38 species known from Europe belong to the family Eukoeneriidae and the genus *Eukoenenia* Börner, 1901 (Harvey 2013). From these, 34 species are restricted to Europe and are known from caves and shallow subterranean habitats. Other European species are *E. juberthiei* Condé, 1974, recorded from Greece and Lebanon (type locality) and *E. berlessei* (Silvestri, 1903) from Italy (type locality), France, Algeria and the Virgin Islands (USA) (Harvey 2013). Beside these, there are two subcosmopolitan species found in Europe: *E. mirabilis* (Grassi & Calandruccio, 1885) and *E. florenciae* (Rucker, 1903). Both species can be found in anthropogenic environments (e.g. in parks, botanical gardens or greenhouses). *Eukoenenia mirabilis* was described from Italy and is distributed in southern Europe, but up to now also recorded from Israel, northern Africa, Madagascar, Chile, South Africa, and Australia (Harvey et al. 2006). *Eukoenenia florenciae* is described from the USA, and also recorded from Colombia, Paraguay, Argentina, Nepal, Australia and the island of Tenerife (Christian &

Christophoryová 2013). Additionally, Condé (1981) referred all non-Mexican records from Bermuda, Réunion, Egypt, Morocco, Madagascar and Mauritius published as *E. hanseni* (Silvestri, 1913) to *E. florenciae*. Finally, the European records from France (Paris, as *Koeneria buxtoni* Berland, 1914) and Slovakia (Košice and Bratislava) were made in greenhouses (Christian & Christophoryová 2013). This study extends the distribution of *E. florenciae* to Germany and represents the first record of palpigrades from this country.

### **Material and methods**

Between 23. Oct. and 28. Nov. 2019 seven female specimens of *Eukoenenia florenciae* were collected in the following greenhouses of the Munich Botanical Garden (GERMANY, Bavaria, Upper Bavaria, Munich City, Nymphenburg): house 1 (orchid house) and house 2 (tropical economic plants house, Fig. 1), 48.1635°N, 11.5018°E (WGS84), 516 m a.s.l., 20–21°C, 70–90% humidity. Five specimens were collected during daytime by hand under dark, flat stepping stones, and the other two specimens through sieving and subsequent Winkler extraction. The substrate for sieving was taken from the soil under and nearby the stepping stones. Winkler extraction ran for ten days. All material was fixed in pure 80–96% ethanol. Photo series were taken with a NIKON V1 camera mounted on a LEICA Z16 APO stereo microscope. Up to 15 photos were combined to a single composite image with a greater field of depth using Helicon Focus 5.3 (HeliconSoft). For SEM preparation, an adult specimen was dehydrated in a graded acetone series, dried chemically (HMDS – hexamethyldisilazane), and coated with gold using a BIO-RAD Sputter Coater. SEM pictures were made with a LEO 1430VP electron microscope at 10–20 kV. Three whole specimens (two adults and one juvenile) were taken for DNA extraction, amplification and sequencing of COI, 18S and 28S genes, which were carried out by AIM (Advanced Identification Methods GmbH, Munich). 18S and 28S sequences were uploaded in BLAST and compared with previously published sequences of palpigrades (Zhang et al. 2000). The other four specimens (two adults and two juveniles) are deposited in the Bavarian State Collection of Zoology, Arthropoda varia section under the registration numbers ZSMA20190394–0397. DNA sequences of the 18S and 28S genes of the three studied specimens are available from GenBank under the accession numbers MT827869–

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Academic editor: Theo Blick

submitted 15.1.2020, accepted 4.9.2020, online 25.9.2020



**Fig. 1:** Collecting site of *Eukoenenia florenciae* in the tropical economic plants house (house 2) of the Munich Botanical Garden

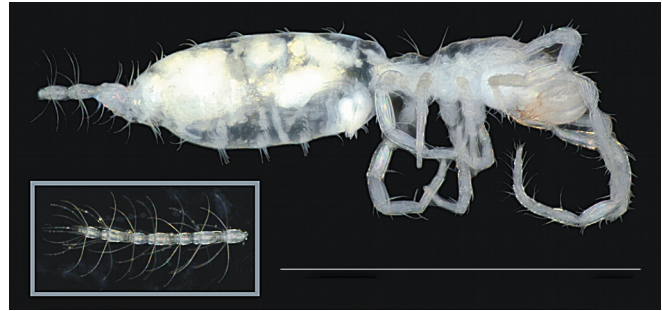
7871 (18S) and MT827873–75 (28S). Terminology of setae follows Christian & Christophoryová (2013).

## Results

Despite our best efforts, e.g. use of an aspirator or a small paintbrush, it was not possible for us to collect any complete specimen bearing the flagellum. The Winkler extraction of two specimens led to complete loss of the flagellum. Flagella of the hand-collected specimens were in one specimen completely lost, in one specimen nearly half lost, and in two specimens complete but broken off (Fig. 2). Our morphological determination as *Eukoenenia florenciae* was successful, especially by analyzing the following morphological features, which correspond to the original description by Rucker (1903) and to Christian & Christophoryová (2013):

1. The presence and positions of the single forked seta and the rod seta on the distal part of the pedipalp's last tarsal article (Fig. 3a).
2. The presence, positions, and count (4–4–2) of the thick setae on coxae II–IV (Fig. 3b).
3. The presence and positions of the five forked setae (1 + 2 + 2 from proximal to distal), the rod seta, the macroseta, and the curved seta on the distal part of the first leg's last tarsal article (Fig. 3c–f).
4. The chaetotaxy of the sternites IV–VI with 4 + 4 setae (shown for sternites IV+V in Fig. 3h).
5. The lateral organ with three blades.
6. The deuto-tritosternum with five setae.
7. Propeltidium with 10 + 10 setae and metapeltidium with 2 + 2 setae. Furthermore, the examination of the genital lobes (Fig. 3g) shows that all seven collected specimens are females. From these there are four adult and three juvenile specimens. Total body lengths (without flagellum) range between 850 µm and 900 µm in the juveniles, and between 1100 µm and 1350 µm in the adults.

Amplification and sequencing of the COI gene, which is commonly used for DNA barcoding, failed in all three specimens used for DNA extraction. However, sequencing of the 18S and 28S gene was successful in all three specimens. Our morphological species determination is supported by the comparison of the 18S and 28S sequences of our specimens



**Fig. 2:** *Eukoenenia florenciae*, adult female (ZSMA20190395), broken flagellum in frame, scale bar: 1 mm

with sequences of palpigrades in BLAST. For 18S, there is an accordance of 99.28–99.83% with the two existing *E. florenciae* sequences. The next closest matches are *E. spelaea* (Peyerimhoff, 1902) (95.43–95.81%) and *E. strinatii* Condé, 1977 (95.06–95.64%). For 28S, there is an accordance of 99.84–100% with the two existing *E. florenciae* sequences and the next closest matches are again *E. spelaea* (94.79–95.10%) and *E. strinatii* (94.63–94.93%). However, sequences of palpigrades are available in public databases only for a limited number of species.

## Discussion

To date, there have been no records of palpigrades from Germany. However, several species are known from caves in the Alps in Austria, Italy and France. Some Austrian localities are even near the southern border of Germany (Blick & Christian 2002). Hence, one could expect palpigrades in caves in the German Alps too. Our study represents the first record of palpigrades in Germany. However, it is not from a natural habitat, but from an anthropogenic environment – a greenhouse of the Munich Botanical Garden.

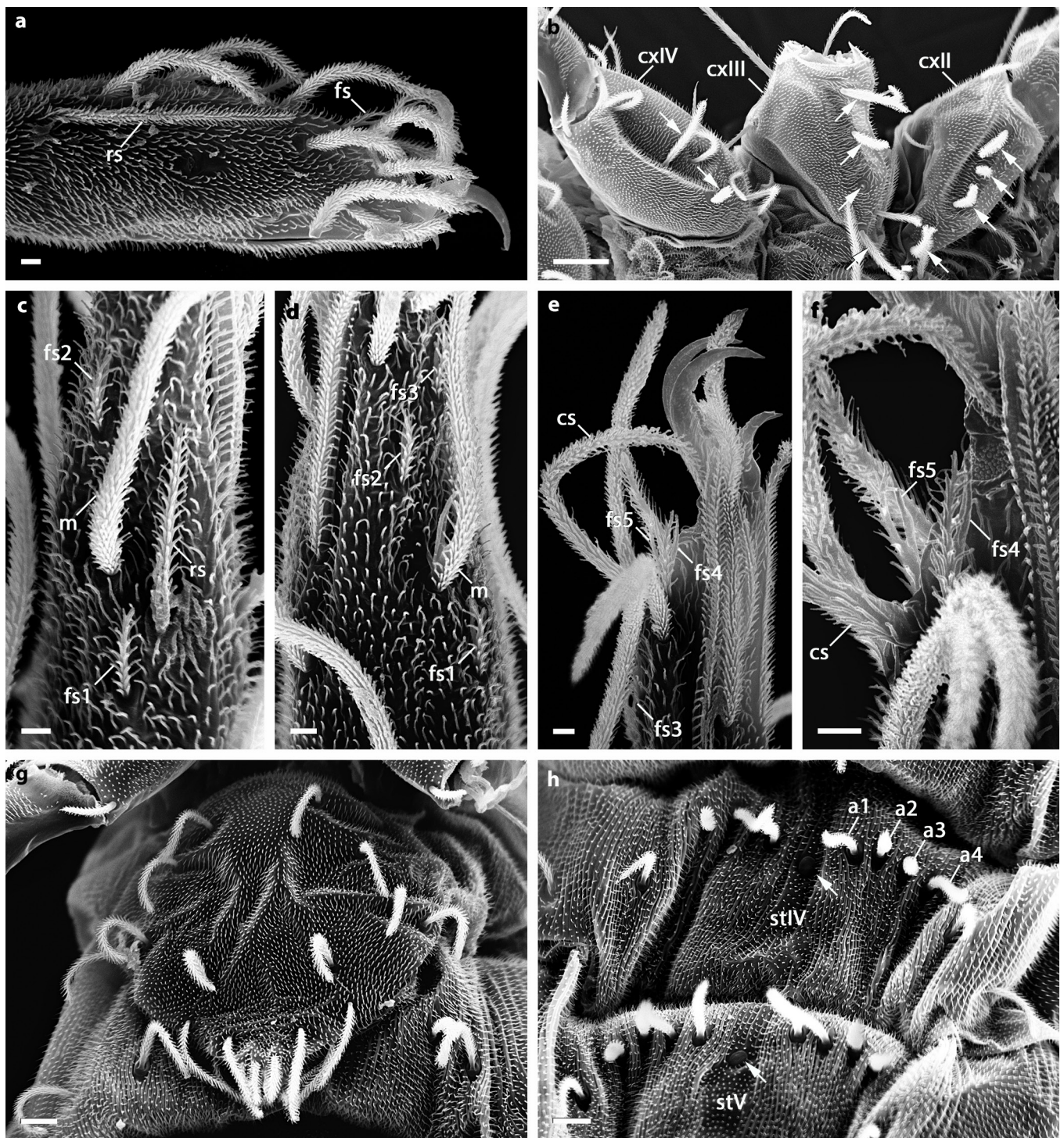
Rucker (1903) reported in her species description, that out of a series of 182 (!) specimens there was only a single one completely intact, including the flagellum. Christian & Christophoryová (2013) reported that there was not a single flagellum preserved out of four specimens from a Bratislava greenhouse, and Šestáková et al. (2017) could only collect one damaged specimen in a Košice greenhouse. Hence, it seems to be a general problem in collecting this species, or probably palpigrades in general.

The total body lengths of our specimens are similar to the specimens of Christian & Christophoryová (2013) from Bratislava and Tenerife, Condé (1981) from Paris, and Condé (1951) from Egypt, and like these, once more, only about one half the length of the Texan specimens in the original description. Maybe Rucker's (1903) measurements included the flagellum. She only used the term “size”. This could be an explanation for the discrepancy.

Besides the successful sequencing of the 18S and 28S genes, we failed to amplify the COI gene in this study. This seems to be a general problem in palpigrades. Giribet et al. (2014) had similar problems. In their elaborate phylogenetic analysis, the COI gene was amplified successfully for only about one third of the specimens.

*Eukoenenia florenciae* is perhaps the most widespread palpigrade species in the world and parthenogenesis probably facilitates its dispersal. However, it is still unknown if it is obligate or facultative, no males were found so far. Most re-





**Fig. 3:** *Eukoenenia florenciae*, adult female (ZSMA20190394). **a.** left pedipalp: distal part of last tarsal article, scale bar: 2  $\mu$ m; **b.** right coxae II–IV, arrows show thick setae, scale bar: 20  $\mu$ m; **c–f.** left leg I: distal part of last tarsal article, scale bars: 2  $\mu$ m; **c–d.** positions of forked setae 1–2, rod seta and macrosetae; **e.** positions of forked setae 3–5 and curved seta; **f.** details of forked setae 4 and 5; **g.** female genitalia, scale bar: 10  $\mu$ m; **h.** chaetotaxy of sternites IV and V, arrows show unpaired gland openings, scale bar: 10  $\mu$ m; a1–a4: setae of sternites, cs: curved seta, cxII–IV: coxae II–IV, fs: forked seta of pedipalp, fs1–fs5: forked setae 1–5 of leg, m: macroseta, rs: rod seta, stIV–V: sternites IV–V. Setal nomenclature after Christian & Christophoryová (2013)

cords of this species – including the locus typicus – are from anthropogenic environments, and so its native range is as uncertain as the possible existence of males. Only the record from Nepal (Siwalik region, southern Nepal) is from an undisturbed forest environment (Condé 1979: sub *E. hanseni*, 1997), from there only females were collected as well. Maybe the species originates from this region.

In any case, today *E. florenciae* has a subcosmopolitan distribution. Its introduction to the greenhouses of the Munich

Botanical Garden can be reconstructed to a certain extent. So far, *E. florenciae* is known from three other greenhouses in Paris, Bratislava and Košice (Condé 1981, Christian & Christophoryová 2013, Šestáková 2017). Like other institutions, the Munich Botanical Garden has a lively exchange of plants – including the soil as habitat for the palpigrades – with other botanical gardens. Accordingly, there was also an exchange with Košice (Cactaceae) and Paris (open air plants) in the past (E. Bayer pers. comm.). Furthermore, there is a perma-

nent in-house rotation of plants and a frequent exchange of the soil. Possibly, in this way *E. florencae* was introduced to the orchid house and the tropical economic plants house in Munich. This species is probably even more widespread in greenhouses of other botanical gardens.

### Acknowledgements

We thank Ehrentraud Bayer, vice director of the Munich Botanical Garden for kind permission to collect palpigrade specimens, as well as the technical staff of the greenhouses, especially Bert Klein and Harald Frank, for friendly help and support. Furthermore, we want to thank Erhard Christian for providing important information and the reviewers Jana Christophoryová, Lubomír Kováč and Erhard Christian, as well as the editors Theo Blick, Petr Dolejš and Konrad Wiśniewski for constructive contribution to the manuscript.

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