

Original paper

First record of a representative of the order Palpigradi (Chelicerata: Arachnida) from Serbia

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Summary. This contribution refers to the first record of the genus *Eukoenenia* Börner, 1901 (Arachnida: Palpigradi) in Serbia. One adult male was discovered in Provalija Cave, a part of the Cerjanska Cave System, near Niš in eastern Serbia. Based on diagnostic morphological characters, habitat preferences and the distribution of similar taxa, we conclude that it belongs to the *Eukoenenia spelaea* species complex.

Keywords: Balkan Peninsula, Cerjanska Cave System, Eukoeneniidae, microwhip scorpions, troglobionts.

INTRODUCTION

Palpigradi, or microwhip scorpions, are tiny, unpigmented and eyeless arachnids that are often overlooked in their natural habitat. Species of this order inhabit the soils of tropical and subtropical regions of the world, but are also found in subterranean habitats (Condé 1996). In Europe, there are numerous records of palpigrades associated with caves (Mammola et al. 2021), with their northernmost reported distribution limit at 49° north in the Western Carpathians in Slovakia (Kováč et al. 2002). To date, Palpigradi have been found in most of Central and Southern Europe, but no findings have been reported from Serbia. During recent biospeleological exploration of caves in Serbia, an adult male was discovered in Provalija Cave, which is part of the Cerjanska Cave System near Niš. This is the first record of Palpigradi from Serbia.

MATERIAL AND METHODS

Site characteristics – cave description

The Cerjanska Cave System is one of the largest underground systems in Serbia; and is located in the northern part of the Kalafat Massif in the eastern part of the country, north of Niš (Fig. 1). Due to its exceptional geomorphological, hydrological and aesthetic value, the cave system has been protected as a natural monument since 1998, under the name ‘Cerjanska Cave’ by decree of the Government of the Republic of Serbia. The entire system developed in the limestone of Ljuti Vrh (Nešić 2023a). The first documented explorations of the cave date back to the early 1970s. The cave is richly decorated with speleothems, including stalactites, stalagmites, draperies, cave corals, and crystal flowers. In addition, helictites are especially abundant, and are one of the most re-

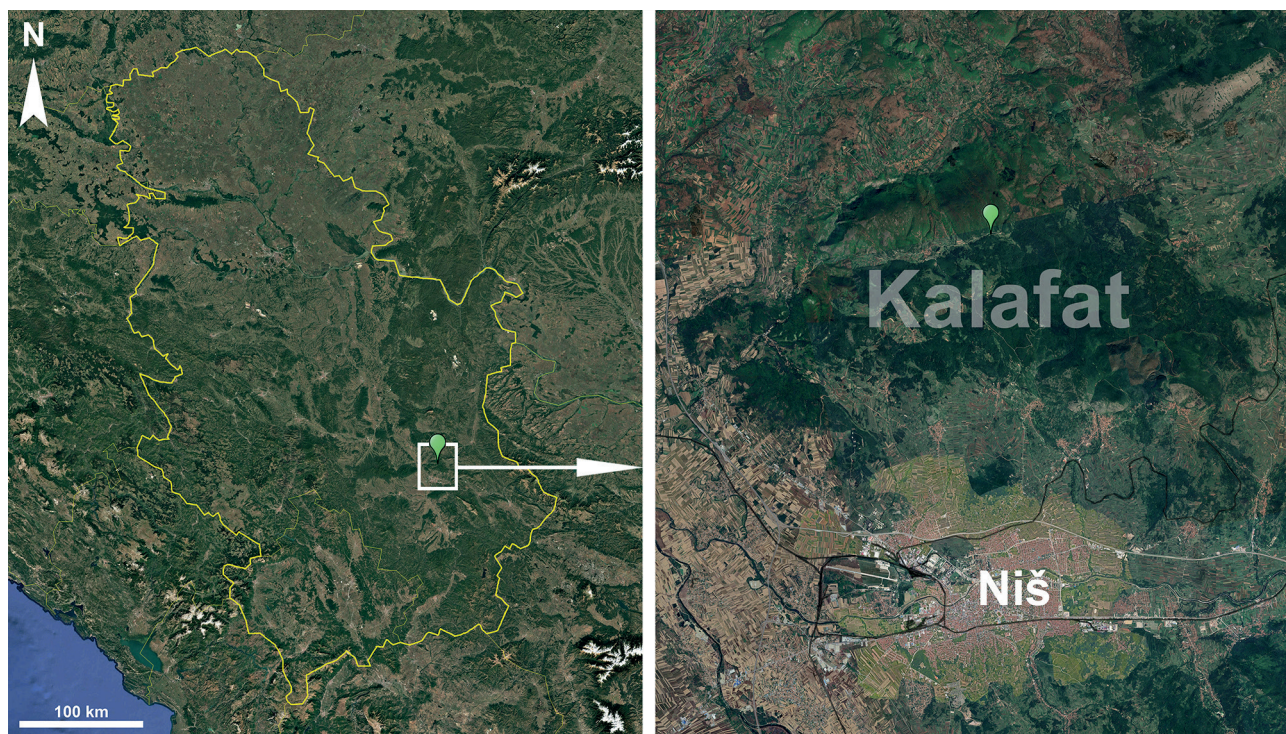


Fig. 1. Location of Provalija Cave.

markable features of Cerjanska Cave. According to the most recent data, Cerjanska Cave is the third longest cave system in Serbia, with 7,170 meters of explored passages. Four morphological and genetic units can be distinguished: Provalija Cave, Kravljanska Pit, the inflow passages, and Pećurina (Nešić 2023b; Nešić and Jović 2023).

Provalija Cave is the dominant component of the system. It represents a large vadose ponor cave, with a total length of 5,703 m, including 3,675 m of active river passage. It has a simple morphology and consists mainly of a river channel, which extends over two levels and has a length of 4,903 m, as well as several side passages with a combined length of 800 m (Nešić 2023b). The first biospeleological investigations of the Cerjanska Cave System were conducted at the end of 1999. Between 1999 and 2015, researchers from the Institute for Nature Conservation of Serbia, in collaboration with speleologists, surveyed several subterranean sites within the Kalafat Massif, including Provalija Cave. In 2017, biospeleological studies were conducted by members of the Serbian Biospeleological Society, and most recently, in 2024, by biospeleologists from the University of Belgrade – Faculty of Biology. According to Pavićević et al. (2023), 20 arthropod species have been recorded from Provalija Cave, the majority of which are troglophiles and troglonexes. However, four troglotic species are known from the cave: the harvestman *Paranemastoma bureschi* (Roewer, 1926); the millipedes *Dzibogosoma naissi* Makarov & Ćurčić, 2012 and *Balkanodes-*

minus serbicus Antić, Vagalinski, Stoev & Akkari, 2022; and the carabid beetle *Duvalius rtanjensis provalijae* Pavićević, Zatezalo & Popović, 2016 (Makarov et al. 2012; Antić et al. 2022; Pavićević et al. 2023).

Record specification

Serbia, Niš, Provalija Cave, about 1,000 m from the cave entrance, 1 adult male, on a very wet stone, 19.11.2024, leg. D. Antić.

One permanent slide has been deposited at the Institute of Zoology, University of Belgrade – Faculty of Biology, Belgrade, Serbia.

Permanent slide preparation and morphology study

Morphology of the specimen was studied using a Carl Zeis Axiolab 5 light microscope equipped with phase contrast. For this purpose, the specimen was boiled gently in an evaporation glass dish in 95% ethyl alcohol for approximately 1 minute to remove fat from the body. For clearing, the specimen was transferred to a 10% KOH aqueous solution in a concave glass dish for ca. 1 minute and then transferred to a dish containing chlorophenol until it was fully transparent. Finally, the specimen was mounted in Swann medium on a permanent slide and left to dry. After drying, the cover glass was ringed with Canada balsam to seal the mounting medium and prevent desiccation.

Map and photography

The distribution map was created in Google Earth Pro (version 7.3.6.10201) and Adobe Photoshop CS6. Photos of the living male *in situ* were taken with an Olympus Stylus Tough TG-6 digital camera. Images of morphological structures were recorded using a Leica DM2500 light microscope equipped with DIC (differential interference contrast). The final images were edited and combined using Adobe Photoshop CS6.

RESULTS

Taxonomy

Class Arachnida

Order Palpigradi

Family Eukoeneniidae

Genus *Eukoenenia* Börner, 1901

Diagnosis. Representatives of the genus exhibit the following diagnostic characters: opisthosomal sternites IV–VI without ventral sacs; segment IX is narrower than VIII, but wider than XI; and the opisthosomal sternites IV and VI each form a protuberance (Barranco and Harvey 2008).

Eukoenenia spelaea species complex

Christian et al. (2014) defined the *Eukoenenia spelaea* complex based on the following characters: lateral organ with 3 or more blades; coxae of legs I–IV with 0–4–4–1 thick setae; leg I ta3 with 4 forked setae; leg IV bta with 4 setae; metapeltidium with 3 setae; tergite II with 2+2, tergites III–VI usually with 3+3 setae t, median seta absent; sternites IV–VI with 3+3 or more setae a; female with 3+3 apical setae on the first genital lobe and 3 setae on the second genital lobes; male with 3 setae on the second and 4 on the third genital lobes; opisthosoma with a distinct narrowing between segments VIII and IX.

Morphological analysis

The male from Provalija Cave shows: chelicera with 8 teeth (Fig. 2A); lateral organ on each side of the prosoma with 3 blades (Fig. 2B); deutotritosternum with 5 setae arranged in the shape of the letter V; setal pattern of opisthosomal sternites IV–VI as a1, a2, a3, s1, s2; sternal pores present; segment XI of opisthosoma with 11 setae; pattern of forked setae on tarsal segment 3 on leg I (leg I ta3): 1+1+2 forked setae, seta *rs* in lateral position (Fig. 2C); setal pattern of male genital plate: each half of 1st lobe with 2 sternal setae,

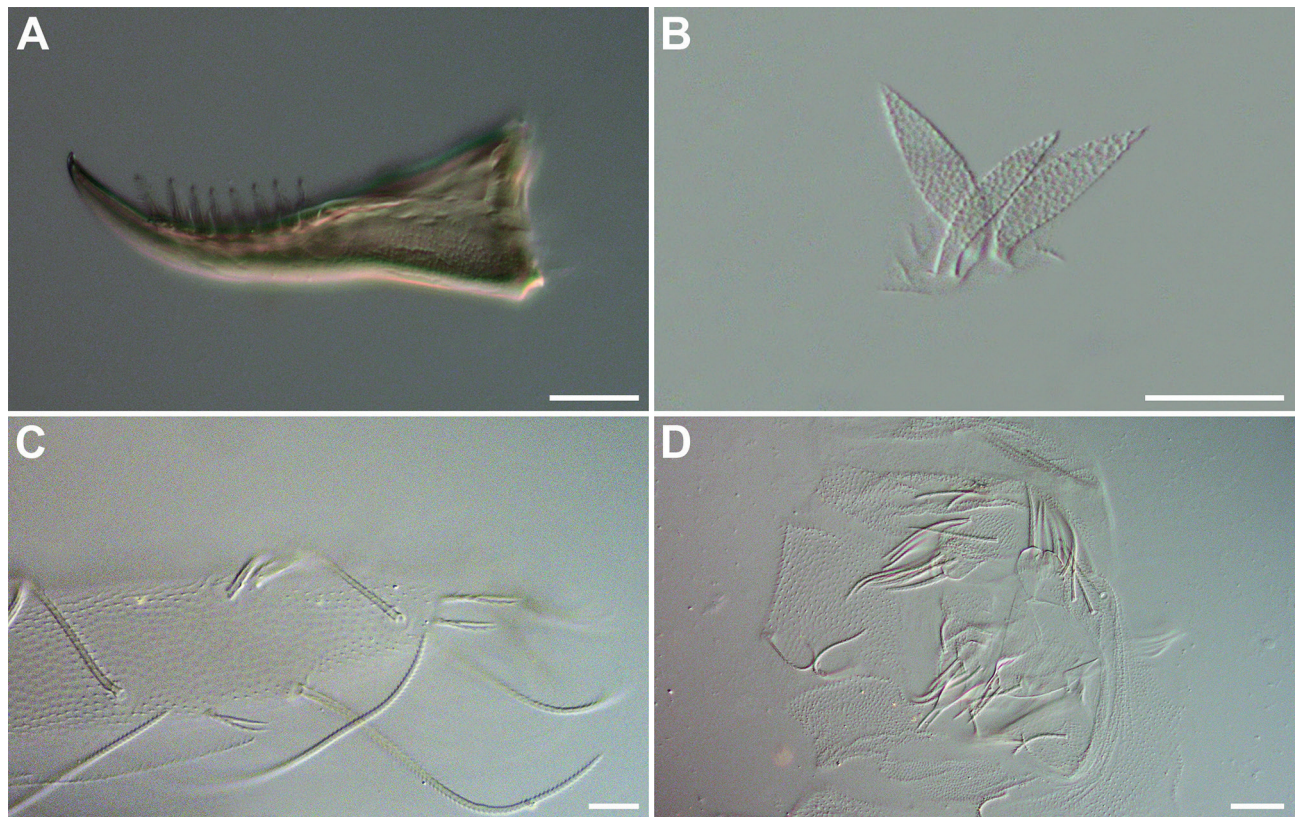


Fig. 2. Morphological characters observed in the male of *Eukoenenia* cf. *spelaea* (Peyerimhoff, 1902) from Provalija Cave. **A**, terminal segment of chelicera (finger); **B**, lateral organ; **C**, pattern of forked setae on tarsal segment 3 of leg I; **D**, male genital plate. (Scale bars: 0.01 mm).

9 setae on lobe extension (3 lateral, 6 submedian) and 2 apical fusules, setal pattern of 2nd and 3rd folds unclear (Fig. 2D); flagellum with 7 segments.

The flagellum is a morphological structure with some taxonomic significance. Due to its fragility, it sometimes breaks off partially in living animals and is often completely lost in preserved specimens. Both apply to the animal identified from Provalija Cave. Before it was captured, it had a broken flagellum with 7 segments (Fig. 3), instead of the 12–15 segments found in intact specimens of the *E. spelaea* complex.

DISCUSSION

Because this was the first record of a palpi-grade in Serbia, efforts were made to identify the specimen at the species level. Initial inspection of the freshly mounted animal revealed that the genital plate belonged to an adult male. A setal pattern of a1, a2, a3, s1, s2 on the opisthosomal sternites IV–VI strongly indicated affiliation with the *E. spelaea* complex. The presence of 3 or more setae 'a' per hemisternite is one of the (albeit not exclusive) features of this complex (Christian et al. 2014). Only *E. austriaca* (Hansen, 1926), which appears to be related to *E. spelaea*, has 2 setae 'a', like many other *Eukoenenia* species.

During the examination, the sample was damaged

under the cover glass, rendering some morphological details unrecognizable. However, what we were able to discern was compatible with an assignment to the *E. spelaea* complex: the pattern of the forked setae on tarsal segment 3 of leg I, the number of blades in the lateral organ, and the number and arrangement of setae on the deutotritosternum. Only the number of setae on opisthosoma XI was slightly higher than in most populations of the *E. spelaea* complex (11 vs. 9–10). Until further diagnostic characteristics can be examined in other palpi-grades from Provalija Cave, we refer to this population as *Eukoenenia* cf. *spelaea* (Peyerimhoff, 1902).

The known distribution range of *Eukoenenia spelaea* with its four subspecies extends from France, west of the Rhône, across the Alpine arc to the western Carpathians and the northern Dinarides. The other species of the *E. spelaea* complex (*E. austriaca*, *E. bonadonai* Condé, 1979, *E. lanai* Christian in Christian et al. 2014, *E. roscia* Christian in Christian et al. 2014, *E. strinatii* Condé, 1977) inhabit much smaller areas and are distributed within the large range of *E. spelaea*, with the exception of the South Carpathian *E. margaretae* Orghidan, Georgesco & Sârbu, 1982 and *E. condei* Orghidan, Georgesco & Sârbu, 1982. The record of *E. cf. spelaea* in Provalija Cave extends the distribution range of the entire species complex to the south.

The species in the *E. spelaea* complex are only moderately troglomorphic: morphological adaptations to cave



Fig. 3. Habitus of the collected male of *Eukoenenia* cf. *spelaea* (Peyerimhoff, 1902) from Provalija Cave. Photos: D. Antić.

life, such as elongated extremities, an elongated flagellum and a higher number of blades in the lateral organ are not very pronounced. Like the vast majority of European palpigrades, they are troglobiont, and restricted to subterranean habitats. The fact that two of them (*E. spelaea* and *E. austriaca*) have been found in Austria, not only in caves but also near the surface in loose sediments (Christian 2004) does not contradict this: such habitats are considered 'shallow subterranean habitats' sensu Culver and Pipan (2014). The species identified in Provalija Cave can also be considered to be troglobiont.

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