Original paper

The bristle millipede family Lophoproctidae (Diplopoda, Polyxenida) in the Balkan Peninsula and surroundings

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Summary. In the present study, we examined a small sample of lophoproctids from the Balkan Peninsula and its surroundings. The millipede family Lophoproctidae is recorded from Serbia and Croatia for the first time, with *Lophoproctus coecus* Pocock, 1894 and *Lophoproctinus* (?) *inferus* (Silvestri, 1903) being involved, respectively. In addition, *L. coecus* is recorded for the first time from Bulgaria and the entire Balkan Peninsula. Finally, the genus *Lophoproctinus* Silvestri, 1948 is new to the Balkan fauna. Some remarks and the habitats of the new records are given, as well as taxonomic notes, variations in some specimens and a map with all records of the family in the Balkans and its surroundings.

Key words: Bulgaria, Croatia, Europe, Lophoproctinus, Lophoproctus, Penicillata, Serbia.

INTRODUCTION

The millipede family Lophoproctidae includes small, up to 4.2 mm long, relatively unpigmented and blind bristle millipedes characterized by a single posterior bundle and an elongate antennomere 8. The group comprises six genera, one of which is doubtful, and approximately 45 species (Short 2015a, b). Two of these five or six genera can be found in Europe, viz., *Lophoproctinus* Silvestri, 1948 and *Lophoproctus* Pocock, 1895 (Kime and Enghoff 2011). The genus *Lophoproctinus*, with five species, has a wider global distribution (Short 2015a; Recuero and Rodríguez-Flores 2023), with only one species, *L. inferus* (Silvestri, 1903), occurring in western Mediterranean Europe. On the other hand, the genus *Lophoproctus* is less widespread, with five species ranging from the western Mediterranean basin to Kyrgyzstan, Central Asia (Short 2015b, 2020). Of these, four species are known from Europe, viz., *L. coecus* Pocock, 1895, *L. jeanneli* (Brölemann, 1910), *L. lucidus* (Chalande, 1888), and the troglobiotic *L. pagesi* Condé, 1981 (Kime and Enghoff 2011; Short 2015b; Recuero and Rodríguez-Flores 2023).

The Balkan Peninsula, one of the refugial areas of Europe, and its surroundings seem to be *terra incognita* with regard to the diversity of lophoproctids. There are only a few records of Lophoproctidae from this area from the past: Tabacaru (1966) and Giurginca and Baba (2016) recorded *Lophoproctus lucidus* from several localities in Romania, while Stoev and Lapeva-Gjonova (2005) reported *Lophoproctus* sp. (with a footnote about the possibility of a new species) from ant nests in Bulgaria. The same Bulgarian record appeared later as *Lophoproctus* cf. *L. lucidus* (Bachvarova et al. 2017). However, Short (2015b) recently demonstrated that there was confu-

sion in the past over the identification of *L. lucidus* and *L.* coecus, which had been treated as synonyms until the work of Nguyen Duy-Jacquemin (1993). After examining new material and re-examining old samples, Short (2015b) has shown that L. coecus has a much wider distribution range, extending from the central Mediterranean to Kyrgyzstan, while L. lucidus is more restricted to the western Mediterranean (see Short 2015b: 214, fig. 5 and Recuero and Rodríguez-Flores 2023: 8, fig. 4). In this context, several earlier records of Lophoproctus lucidus from Romania by Tabacaru (1966), and of Lophoproctus sp. from Bulgaria by Stoev and Lapeva-Gjonova (2005) are rightly cited as nearly undetermined. However, Giurginca and Baba (2016) claimed that their discovery of Lophoproctus in Romania actually corresponded to L. lucidus. In any case, we will continue to consider those records as doubtful L. lucidus (Fig. 1, yellow circles) until clear evidence in the form of microphotographs or scanning electron micrographs becomes available.

Thanks to a small, newly collected sample of lophoproctids from the Balkan Peninsula and its surroundings, in the present study we confirm the presumed existence of the species *Lophoproctus coecus* in this area. Furthermore, the genus *Lophoproctinus* is found in the Balkan Peninsula for the first time.

MATERIAL AND METHODS

For preliminary examination and identification, specimens were mounted in glycerine as temporary microscope preparations and observed with a Carl Zeiss Axioscope 40 microscope at the University of Belgrade - Faculty of Biology, Institute of Zoology, Belgrade, Serbia (IZB). A detailed analysis of the individuals was carried out at Deakin University, Victoria, Australia, using light microscopy and scanning electron microscopy. For light microscopy, specimens were mounted on slides in Hoyer's medium, dried at 60 °C and examined with an Olympus CX 41 compound microscope. Scanning electron micrographs were obtained of selected whole specimens that were dehydrated in a graded series of ethanol, at 80%, 90% and 100%, and then air-dried. Specimens were then mounted on stubs using adhesive tabs, sputter-coated with gold and examined with a Philips XL20 scanning electron microscope.

Photographs of specimens *in situ* were taken with an Olympus Stylus Tough TG-6 and Canon EOS 400D digital cameras. The distribution map was created using Google Earth Pro (version 7.3.6.9345) and Adobe Photoshop CS6. The final images and plates were processed with Adobe Photoshop CS6.

The material is deposited in the Institute of Zoology, University of Belgrade – Faculty of Biology (IZB).

We also checked the citizen science project iNaturalist

for additional records (https://www.inaturalist.org/; accessed on October 31, 2023).

RESULTS

The analysis of 15 Lophoproctidae specimens from four localities revealed the occurrence of *Lophoproctus coecus* in the territory of the Balkan Peninsula and its surroundings, in Serbia and Bulgaria, while two specimens from a cave in Croatia were identified as *Lophoproctinus* (?) *inferus*. The family Lophoproctidae is recorded from Serbia and Croatia for the first time, with *Lophoproctus coecus* and *Lophoproctinus* (?) *inferus* being involved, respectively. *Lophoproctus coecus* is recorded from Bulgaria for the first time, while the genus *Lophoproctinus* was recorded from the territory of the entire Balkan Peninsula for the first time as well. Note that three additional records of Lophoproctidae in the Balkan Peninsula are based only on pictures.

Taxonomy

Class Diplopoda de Blainville in Gervais, 1844 Order Polyxenida Lucas, 1840 Family Lophoproctidae Silvestri, 1897

Genus Lophoproctinus Silvestri, 1898

Type species. *Lophoproctus inferus* Silvestri, 1903 (by original designation).

Diagnosis. Distinguished from other genera of Lophoproctidae by a combination of the presence of three thick sensilla basiconica on antennomere VI and more than four linguiform processes along the entireanterior margin of the labrum.

Lophoproctinus (?) inferus (Silvestri, 1903)

Material examined. CROATIA ● 2 ♂♂; Pelješac, Ponikve, Ston, Bijelo jezero Cave; 29 March 2019; leg. A. Kirin; (IZB).

Remarks. This is the first record of the family Lophoproctidae in Croatia. At the same time, this is the first record of the genus *Lophoproctinus* in the Balkan Peninsula (Fig. 1, pink triangle).

Habitat. So far only known from one cave in the Balkans.

Taxonomic note. The original description of *Lophoproctinus inferus* (Silvestri, 1903) (see Silvestri 1903) was incomplete with no detail of important diagnostic characters including size, antennal sensilla, leg chaetotaxy, telotarsus and labrum. Fortunately, Silvestri (1948) and Condé (1951, 1953) provided a limited further description of the species,

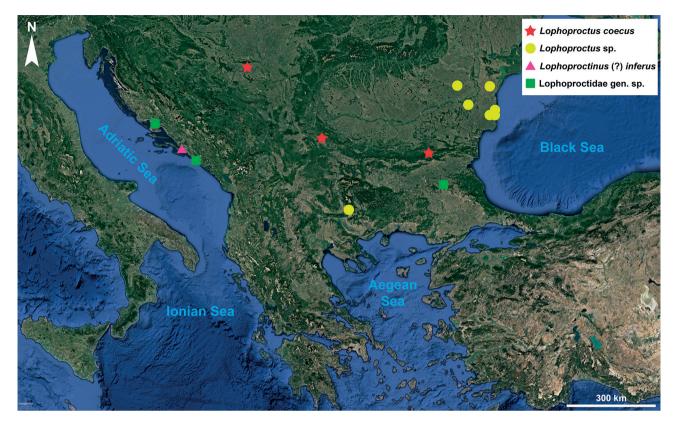


Fig. 1. Finding localities of the family Lophoproctidae on the Balkan Peninsula and its surroundings. (yellow circles = literature records; red stars, green squares and pink triangle = new records.)

on the basis of which the Croatian specimens have been tentatively identified as *L. inferus*. However new collections need to be made from the type locality and a more complete description given to confirm the identification, as well as to clarify the status of the subspecies *L. inferus maurus* Marquet & Condé, 1950 (see Marquet and Condé 1950) as a separate species.

Genus Lophoproctus Pocock, 1894

Type species. *Lophoproctus coecus* Pocock, 1894 (by original designation).

Diagnosis. Distinguished from other genera of Lophoproctidae by a combination of the presence of four thick sensilla basiconica on antennomere VI and more than four linguiform processes along the entire anterior margin of the labrum (Fig. 2).

Lophoproctus coecus Pocock, 1894 Figs 2, 3A–E

Material examined. SERBIA • 1 \bigcirc , 1 subadult \circlearrowleft , 1 exuvia, 2 damaged adults; Stara Planina Mountain, Pirot, village of Temska, no name cave I (smaller), lat 43.26588°,

lon 22.55247°, in the litter layer inside the cave near the entrance; 10 July 2012; leg. M. Šćiban & D. Antić; IZB. • 1 adult; same locality as previous; 3 June 2021; leg. M. Šević, D. Stojanović & D. Antić; IZB. • 1 \bigcirc ; Vojvodina, Ruma, Kudoš neighbourhood, lat 45.010333°, lon 19.804451°, wet part of DA's backyard, under a brick; 11 July 2022; leg. D. Antić; IZB. BULGARIA • 2 $\bigcirc \bigcirc$; Oblast Sliven, Stara Reka, village of Kipilovo, near an old wooden bridge, lat 42.8862°, lon 26.2456°, roadside, *Carpinus, Fagus, Corylus, Acer* etc, under one stone; 9 June 2022; leg. D. Antić & B. Vagalinski; IZB.

Remarks. This is the first record of the family Lophoproctidae in Serbia. *Lophoproctus coecus* is new to the Bulgarian fauna and this is the first recorded find on the Balkan Peninsula (Fig. 1, red stars). It is of interest to note that Fig. 3A shows *L. coecus* with caudal bristles in a splayed defensive stance, in contrast to undisturbed lophoproctids with caudal bristles tightly bundled together (Fig. 3B–E). Most of the detachable caudal bristles are tipped with hooks which serve to entangle potential predators. The shed bristles are then renewed at the next molting. Penicillata employ this defensive strategy, lacking the chemical defences found in most Chilognatha groups (Eisner et al. 1996).

Habitat. This species is currently known from two localities in Serbia. One locality is in the suburbs of the town

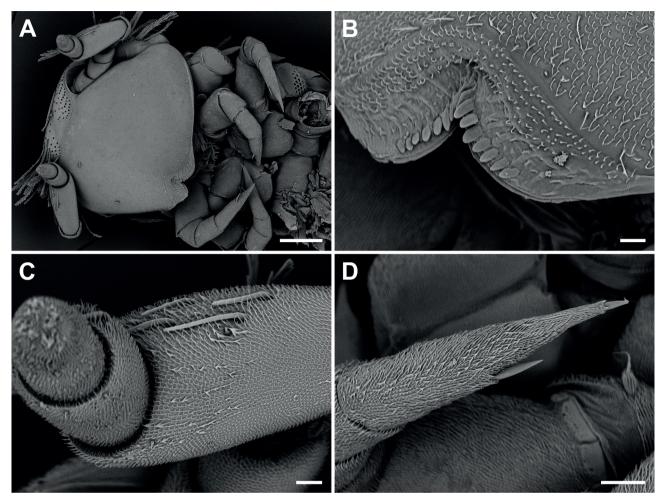


Fig. 2. *Lophoproctus coecus* Pocock, 1894 from Bulgaria. **A**, head and anterior part of body, anterior and ventral views respectively; **B**, labrum with linguiform processes, anterior view; **C**, left antennomeres VI–VIII with characteristic arrangement of four thick sensilla basiconica on antennomere VI; **D**, tarsus 2 and telotarsus. (Scale bars: A = 0.1 mm; B, C = 0.01 mm; D = 0.02 mm.)

of Ruma in the north of the country. The find comes from a garden located in the backyard of the lead author of the present study (DA). Although various soil arthropods have been collected or re-observed in this garden for several years, a single specimen of *L. coecus* was found only recently. The second site is a small cave by the river Temštica, near the village of Temska on Stara Planina in the south-eastern part of Serbia. It seems that the population is established in this cave, as specimens were found there twice in the same leaf layer in the entrance area of the cave almost 10 years apart. The only confirmed find of this species in Bulgaria comes from a natural habitat in the south-eastern part of the country, near the village of Kipilovo. All individuals were found under a stone in a bush next to a forest path located at the entrance of the forest.

Variations. Variation in the number of sensilla basiconica was observed in the Serbian specimens, with some specimens displaying the typical pattern of sensilla on anten-

nomere VI but an extra sensillum (most commonly adjacent to the most proximal sensillum) on the other antenna. None of the Bulgarian specimens showed this variation; however, one subadult specimen that was about to moult presented with 2 sets of sensilla as well as an extra spine on Tarsus 2. It is interesting to note that the moulting lophoproctids were also brown in colour (Fig. 3A, B), in contrast to the remaining off-white specimens (Fig. 3C–E).

Records of Lophoproctidae on the Balkan Peninsula based on pictures only

So far, there are only two records of Lophoproctidae from the Balkan Peninsula in the Citizen Science project iNaturalist. One record comes from Croatia, near Omiš (https://www.inaturalist.org/observations/70672522), while the second record is from Bulgaria, Yambol district (https:// www.inaturalist.org/observations/151280947). The Bulgarian record is provided with some details. Two specimens were found between the villages of Razdel and Malko Kirilovo, lat 42.048032°, lon 26.649010°, on 14 March 2023 under a small stone in a small meadow between wooded areas (Vassil Vassilev pers. comm.). In addition to these two iNaturalist entries, we also received an image (Fig. 3F) of Lophoproctidae taken by Roman Ozimec in the coastal Šipun Cave, near Cavtat in Croatia.

Since we have not examined this material, we have left the identification at only the level of family (Fig. 1, green squares). Considering the general distribution, it is very likely that the Bulgarian find belongs to the genus *Lophoproctus*, and it is most probably *L. coecus*. Both Croatian finds are on the Adriatic coast and not so far from the site of *Lophoproc-tinus* (?) *inferus*.

DISCUSSION

The results of a re-examination of European species of *Lophoproctus* indicate that *L. coecus* is widespread throughout Europe, with its distribution extending into Central Asia (Short 2015b). However, the study noted a large gap in our knowledge of *Lophoproctus* in the Balkans and Turkey,

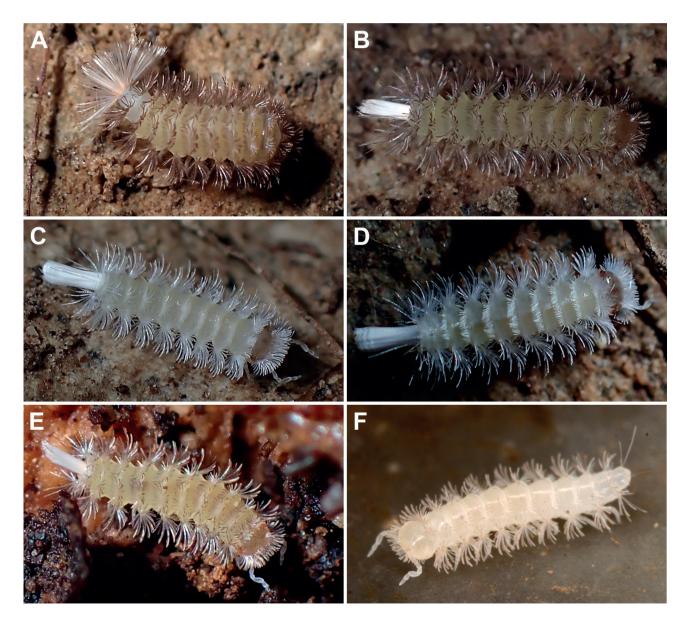


Fig. 3. Lophoproctids from the Balkan Peninsula *in situ*. **A**–**E**, *Lophoproctus coecus* Pocock, 1894; **F**, Lophoproctidae gen. sp. **A–D**, specimens from near Kipilovo, Bulgaria, **E**, specimen from no name cave I, near Temska, Serbia; **F**, specimen from the Šipun cave, Croatia. (Photographs: **A–E**, D. Antić, Olympus Stylus Tough TG-6; **F**, R. Ozimec, Canon EOS 400D.)

and it was predicted that *L. coecus* should occur in these regions. The present study confirms the presence of *L. coecus* in the Balkans and supports the prediction that the species of *Lophoproctus* previously collected in Bulgaria is *L. coecus*.

The presence of *Lophoproctinus* (?) *inferus* in Croatia was unexpected, and indicates that its collection may represent the western edge of its distribution, as this species has not been collected to date in the Caucasus region where many *L. coecus* were found.

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