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A new amphibious species of the subterranean genus *Bureschia* Verhoeff, 1926 (Isopoda: Oniscidea: Trichoniscidae) from Serbia

Ivo KARAMAN¹ & Mladen HORVATOVIĆ^{2,*}

^{1,2}Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad,
Trg Dositeja Obradovića 2, SR – 21000 Novi Sad, Serbia.

^{1,2}Serbian Biospeleological Society, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia.

* Corresponding author: mladen.horvatovic@dbe.uns.ac.rs

¹ Email: ivo.karaman@dbe.uns.ac.rs

¹[urn:lsid:zoobank.org:author:7F67D4A8-5515-45DB-9425-D83D6A0243ED](https://zoobank.org/author:7F67D4A8-5515-45DB-9425-D83D6A0243ED)

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Abstract. *Bureschia serbica* sp. nov., is described from a cave in the Stara Planina Mt in Serbia as a second species in the genus. It is a troglobite amphibious species, predominantly aquatic like the nominal species of the genus, *Bureschia bulgarica*. The new species is adapted to amphibious life in running groundwater, unlike the nominal one that lives in subterranean lakes and ponds. Body structure changes, most noticeable the significant changes in the structure of the mouthparts, as a result of preadaptation in aquatic and amphibious Synocheta, are discussed.

Keywords. Taxonomy, troglobite, aquatic, preadaptation, mouthparts, Synocheta.

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Introduction

Amphibious and even aquatic ways of living in terrestrial isopods are widely present among Synocheta Legrand, 1946, two closely related families – Trichoniscidae Sars, 1899 and Styloniscidae Vandel, 1952. Trichoniscidae is a Holarctic, predominantly west Palearctic group, while Styloniscidae mainly comprises Gondwanan distribution. Synocheta is a group that has never sufficiently adapted to terrestrial conditions and has not conquered the terrestrial environment the way the most diversified oniscid group, Crinocheta Legrand, 1946, did. Synocheta representatives are among the oniscids most dependent on moisture. They are restricted to wet or very moist habitats as endogean forms (then often evolved in the direction of nanism) or epigeal-terricolous, often near springs and smaller watercourses. Where they live, most of them are often exposed to occasional submersion, which they, possessing the gill structures of endopodites of pleopods 3–5, easily survive. They are particularly numerous in karstic areas. In the karstification process, water is a crucial factor. It is, therefore, not surprising that they are by far the most dominant oniscid group in the subterranean fauna. Synocheta's diet also seems tied to

constantly humid places, so adapting to the subterranean way of life was not a large transition step for them. Some of them in the underground environment switched to the amphibious way of life. Such forms are widespread among subterranean Trichoniscidae representatives of the genera *Balearonethes* Dalens, 1977; *Bureschia* Verhoeff, 1926; *Mexiconiscus* Schultz, 1964; *Titanethes* Schiödte, 1849; some representatives of the genera *Alpioniscus* Racovitza, 1908; *Brackenridgia* Eigenmann & Ulrich, 1902; *Cyphonethes* Verhoeff, 1926; *Cyphoniscellus* Verhoeff, 1901; *Graeconiscus* Strouhal, 1940; *Scotoniscus* Racovitza, 1908 and *Trichoniscoides* Sars, 1899 (Dalens 1977; Schmalfuss 1979; Sket 1986; Karaman 2003; Taiti & Xue 2012; Taiti *et al.* 2018).

Some of the subterranean trichoniscids are truly aquatic – stygobionts such as *Typhlotricholigoides aquaticus* Rioja, 1953 from Mexico, *Cantabroniscus primitivus* Vandel, 1965 from Spain, three species of *Alpioniscus* from Sardinia, both species of *Macedonethes* Buturovic, 1955 from North Macedonia and *Brackenphiloscia vandeli* Ortiz, García Debras & Lalana, 1999 from Cuba. Some appear in completely unexpected environments for terrestrial isopods such as the stygobitic *Alpioniscus kuehni* (Schmalfuss, 2005), which from the coastal and inland caves reach the submarine caves of Sardinia (Taiti *et al.* 2018), tolerating partially salt water (Schmalfuss 2005). *Macedonethes stankoi* Karaman, 2003, from a fast-flowing underground stream, has evolved into an actual rhithral animal, unique among isopods (Karaman 2003).

Among the Styloniscidae, there are quite a lot of amphibious, aquatic and supposedly aquatic species, mainly described recently. According to current knowledge, they appear in two remote parts of the world: Thailand, Myanmar and southern China karstic regions and karstic regions of Brasil. *Thailandoniscus annae* Dalens, 1989 was the first described aquatic styloniscid. Described from the same region were the aquatic *Trogloniscus clarkei* Taiti & Xue, 2012 and *Trogloniscus trilobatus* Taiti & Xue, 2012 from China and the amphibious *Bamaoniscus lobatus* Taiti & Montesanto, 2020 from Myanmar (Dalens 1989; Taiti & Xue 2012; Taiti & Montesanto 2020). The aquatic genus *Chaimowiczia* Cardoso, Bastos-Pereira, Souza & Ferreira, 2021, with three species, was described from Brasil (Cardoso *et al.* 2021, 2022). From the same region the following are amphibious: *Spelunconiscus castroi* Campos-Filho, Araujo & Taiti, 2014; all ten species of *Xangoniscus* Campos-Filho, Araujo & Taiti, 2014 and the shelter-builder *Iuiuniscus iuiuensis* Souza, Ferreira & Senna, 2015 (Campos-Filho *et al.* 2014, 2016, 2022; Souza *et al.* 2015; Bastos-Pereira *et al.* 2017; Cardoso *et al.* 2020).

Bureschia bulgarica Verhoeff, 1926, from the monotypic genus, is one of the first described amphibious isopods. So far, it is known from three cave systems in the western part of the Stara Planina Mt in the Iskar River Basin in Bulgaria (Beron 2020). Specimens collected by our colleague Matija Petković in the westernmost part of the Stara Planina Mt in Serbia (Fig. 1) proved to be a new species of this genus and are described here as *Bureschia serbica* sp. nov.

Material and methods

Specimens were collected by forceps, from a stream, wet walls and the limestone cave floor. Specimens were preserved in the field in 70% ethanol and dissected in diluted glycerin. Dissections were done with micro-needles under a dissection microscope. All separated parts were placed in distilled water and then mounted on slides in Faure's mounting medium (40 g chloral hydrate, 10 g gum Arabic, 5 g glycerin, 5 g glacial acetic acid, 5 g glucose and distilled water). Microscopic photographs were done on a Zeiss Axio Imager A1. Drawings based on photographs were made in Adobe Illustrator CS6 on a Wacom Intuos 2 graphics tablet. The scanning electron microscopy (SEM) photographs were taken with a JEOL-JSM-64601v SEM microscope in a high vacuum. Dried samples mounted on stubs were prepared in a Baltec SCD005 sputter coater.

The acronyms used in the text are as follows:

SEM = Scanning electron microscope

ZZDBE = Zoological collection at the Department of Biology and Ecology, University of Novi Sad, Serbia

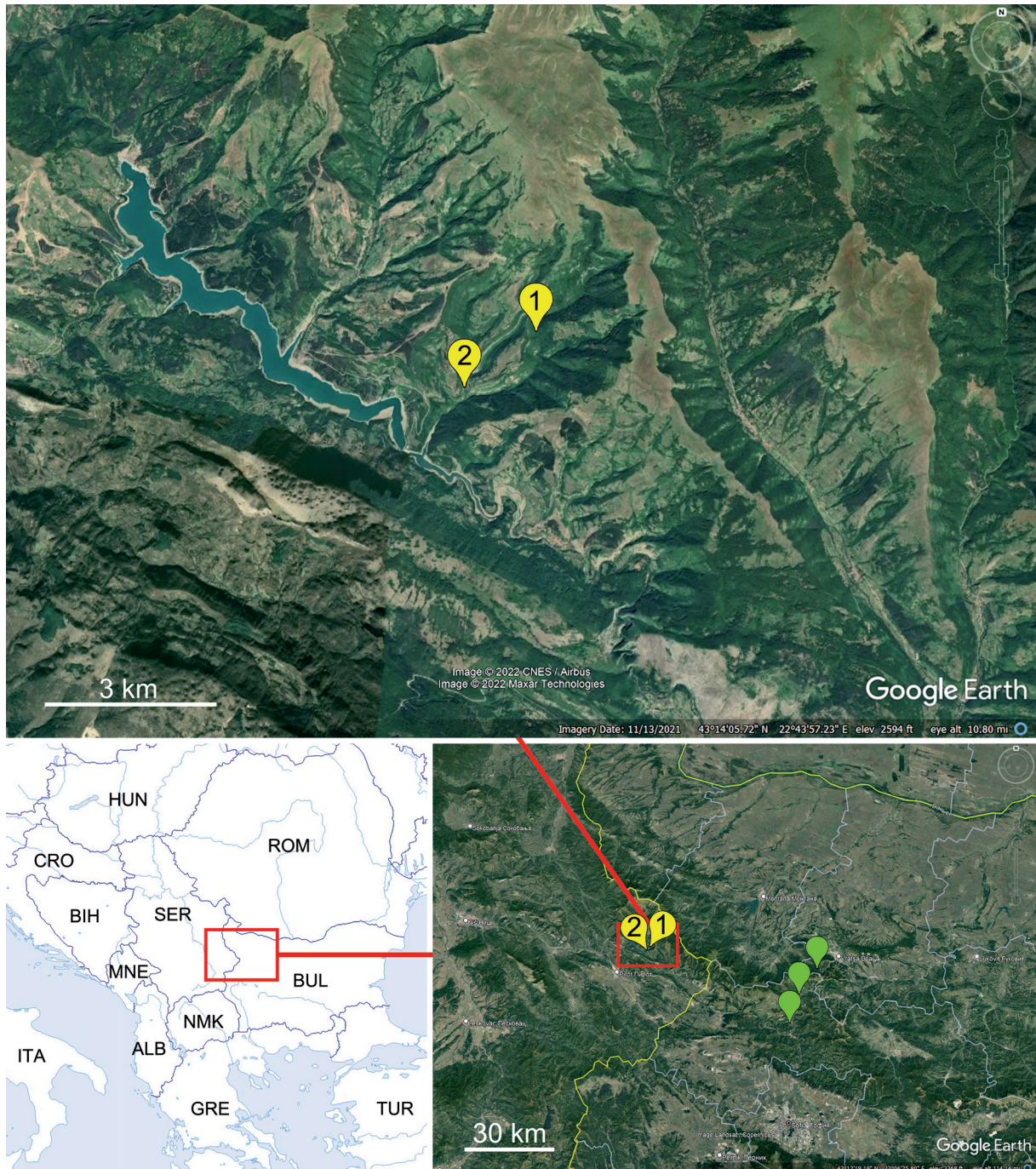


Fig. 1. Distribution of species of *Bureschia* Verhoeff, 1926. Yellow signs = *Bureschia serbica* sp. nov. (1- Suva dupka Cave, 2- small cave in Bela village, probable species site); green signs = *Bureschia bulgarica* Verhoeff, 1926 (Map from GoogleEarth Pro).

Results

Class Malacostraca Latreille, 1802
Order Isopoda Latreille, 1817
Suborder Oniscidea Latreille, 1802
Family Trichoniscidae Sars, 1899

Genus *Bureschia* Verhoeff, 1926

Type species

Bureschia bulgarica Verhoeff, 1926.

Bureschia serbica sp. nov.

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Figs 2–7

Diagnosis

A blind, medium-sized troglobite species with a narrow body (maximum length: ♂ 7 mm, ♀ 7.2 mm). Antennula with elongated articles and reduced number of aesthetascs (3). Antenna with flagellum of nine articles, longer than article 5 by a third of article length. Maxilliped endopodite wide with enormously widened, distally truncate endite. Male pleopod 1 endopodite basal segment with a solid outer projection at its tip and flagelliform terminal segment with thick base; exopodite distally with wide outwardly curved, quadrangular protruding tip. Male pleopod 2 endopodite with stout basal article, three times as long as wide; distal article three times as long as basal one, tapering to a whip-like tip, grooved in the apical third. Uropod basis enlarged posteriorly, as long as endopodite; exopodite and endopodite subequal in length, inserted at the same level.

Etymology

The species is named after the country where it has been collected.

Material examined

Holotype

SERBIA • ♂; Stara Planina Mt, Bela, Suva dupka Cave; 28 Oct. 2017; M. Petković leg.; ZZDBE 1143/1.

Paratypes

SERBIA • 11 ♂♂, 7 ♀♀, 3 juvs; same collection data as for holotype; ZZDBE 1143/2.

Description

Holotype male

BODY. 6.8 mm long, colorless, smooth, outline as in Fig. 2A.

CEPHALON (Fig. 2A). With suprantennal line medially blunt; lateral lobes short and narrow, quadrangular, longer than wide.

PEREONITES (Fig. 2A). Posterior margin of pereonites 1–3 convex, 4–7 progressively concave.

PLEON (Fig. 2A). Slightly convex; pleonites 3–5 with slightly developed epimera.

PLEOTELSON (Fig. 2A). With a wide triangular distal part and rounded apex.

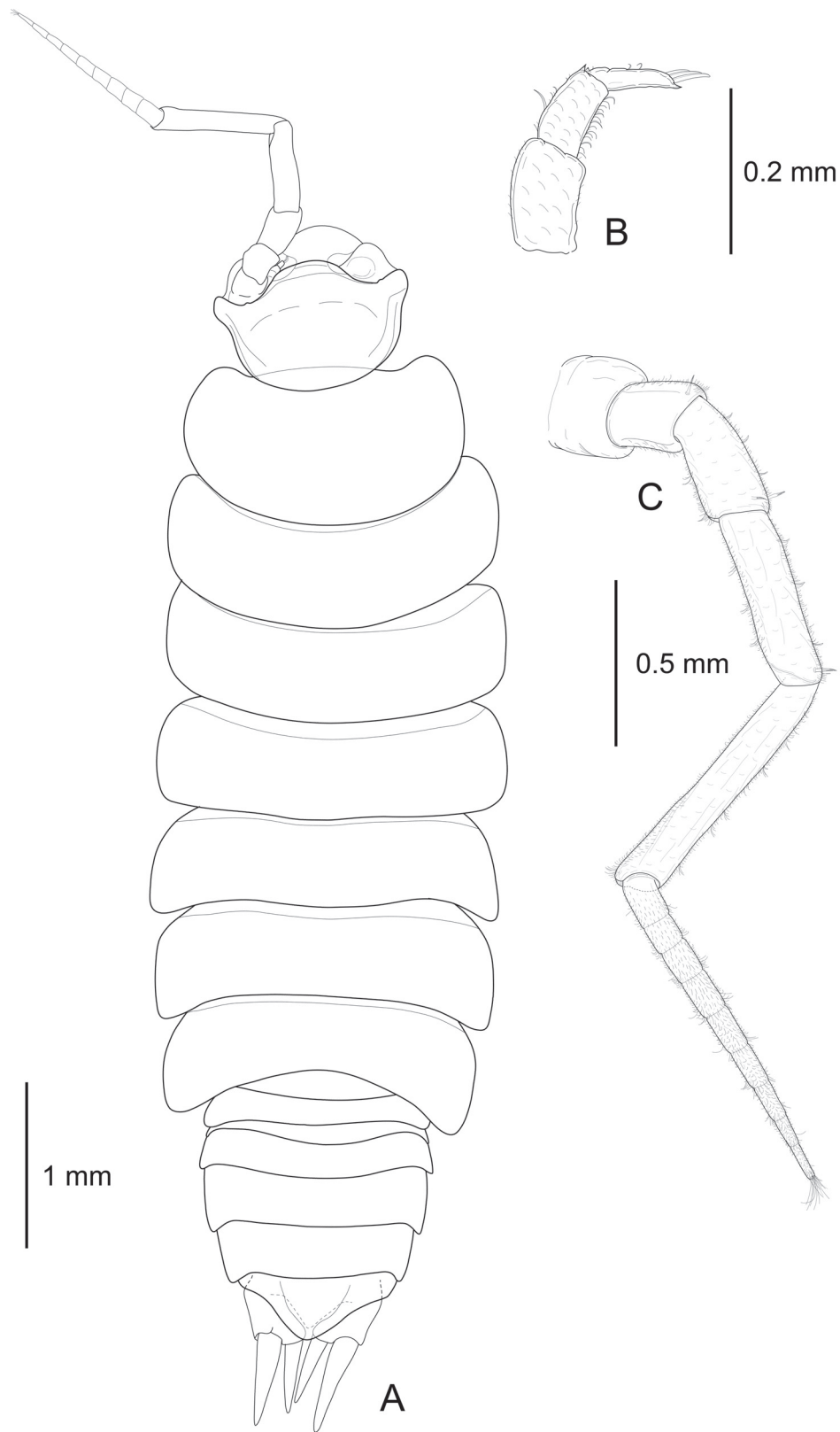


Fig. 2. *Bureschia serbica* sp. nov., paratype, ♂ (6.8 mm) from Suva dupka Cave (ZZDBE 1143/2). **A.** Adult specimen, dorsal view. **B.** Antennula. **C.** Antenna.

ANTENNULA (Fig. 2B). With elongated basal article; second article narrow, elongated, somewhat shorter than basal article; third article narrow, as long as second article, with three short aesthetascs and a spine on its tip.

ANTENNA (Fig. 2C). Moderately elongated; article 3 twice as long as broad; article 4 3.5 times as long as broad; article 5 shorter than flagellum; flagellum with 9 articles.

MANDIBLES. Right mandible (Fig. 3B) with two penicils; pars incisiva elongated, ending with two flat teeth; lacinia mobilis bottle-shaped distally with sparse small thorns; pars molaris elongated with one penicil. Left mandible (Fig. 3A) with three penicils; pars incisiva lateralis and pars incisiva medialis elongated with 3-4 short teeth on their tops; pars molaris oval in shape with one penicil.

MAXILLA 1. Outer endite (Fig. 4A) with 10 elongated teeth, outer 4 stronger than others, 2 inner slightly shorter than others; three elongated pectinate seta distally; inner endite (Fig. 4B) with three penicils, terminal one with a broad base, densely setose, almost as long as proximal one.

MAXILLA 2 (Figs 4C, 5). Outer lobe significantly reduced, with 3 rod-like setae on its tip and a row of sparse tiny elongated setae on outer margin; terminally, both sides of inner lobe with brush-like surfaces – dense rows of curled setae (Fig. 5), more elongated on its terminal and outer edge.

MAXILLIPED (Fig. 4D). With strongly widened endite. Endite distally truncate, with large and wide penicil near medial margin and a row of long setae on its distal margin. Latero-distal margin slightly protruding, distally truncate with a row of setae terminally; outer margin protruding in its basal half; palp on medial edge with four pronounced lobi with setae.

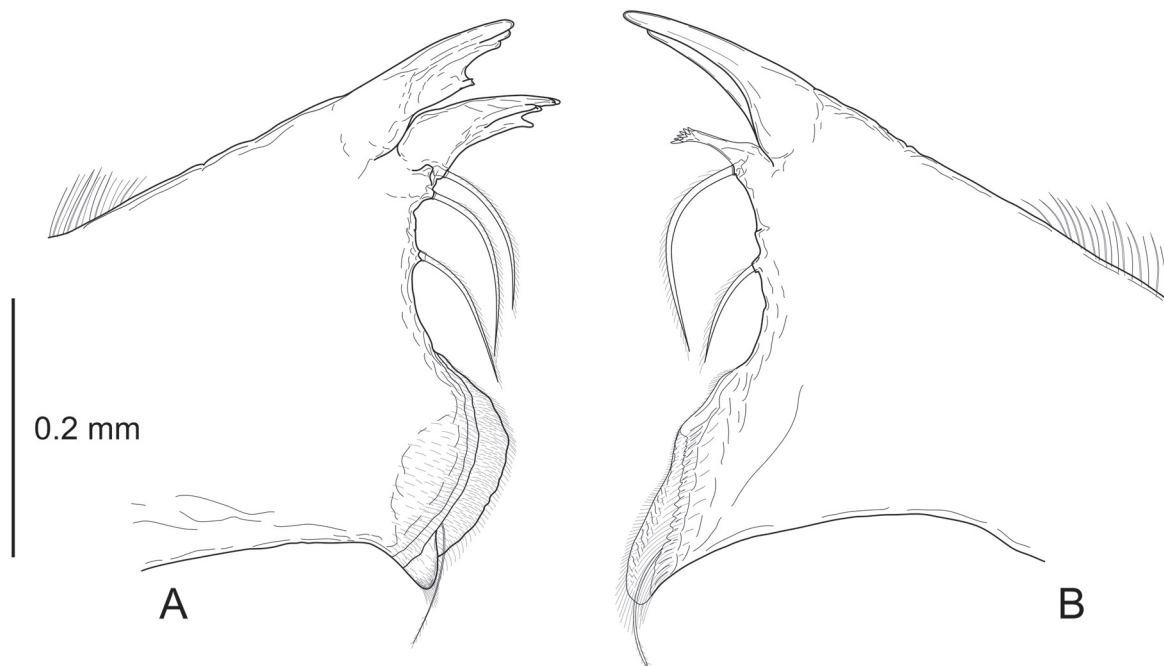


Fig. 3. *Bureschia serbica* sp. nov., paratype, ♀ (7.2 mm) from Suva dupka Cave (ZZDBE 1143/2). **A.** Left mandible. **B.** Right mandible.

PEREPODS. Pereopods 1–2 similar in shape; 3–7 progressively longer, similar in shape. Pereopod 1 (Fig. 6A) merus longer than carpus; ventral surfaces of carpus, merus and propodus covered with small pointed scales. On pereopod 2, such scales are smaller than on pereopod 1, sparsely covering ventral surface of propodus only. Other legs without such pointed scales. Pereopod 7 (Fig. 6B) merus shorter than carpus, propodus longer than carpus. Water conducting structures on pereopods 6 and 7 missing.

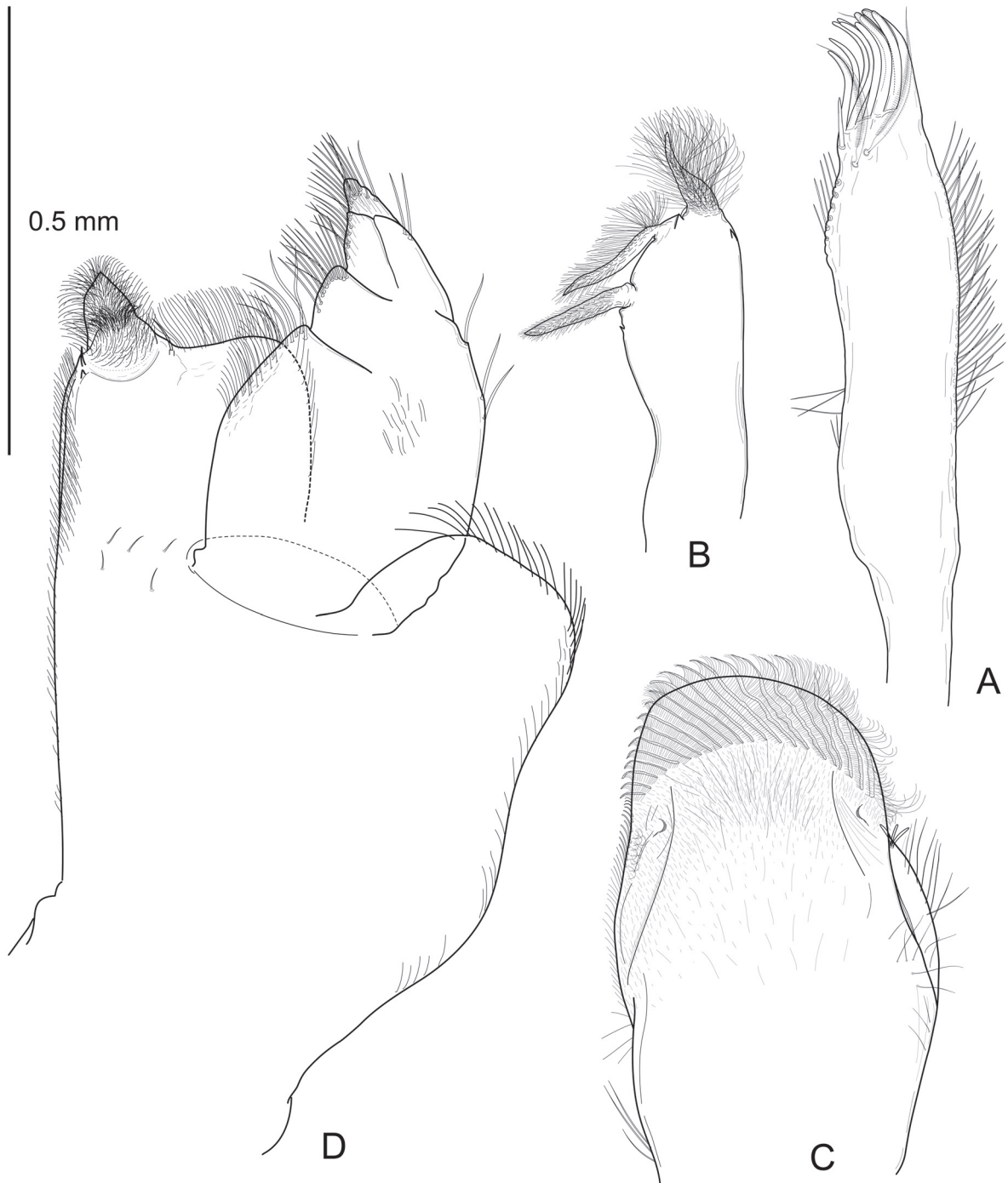


Fig. 4. *Bureschia serbica* sp. nov., paratype, ♂ (6.8 mm) from Suva dupka Cave (ZZDBE 1143/2). **A.** Maxilla 1 outer endite. **B.** Maxilla 1 inner endite. **C.** Maxilla 2. **D.** Maxilliped.

Elongated dactylar seta (usually developed in Trichoniscidae) does not exceed top of unguis on all pereopods.

GENITAL PAPILLA (Fig. 7A). Conical, elongated in apical part.

PLEOPOD 1 (Fig. 7B). Endopodite basal segment skittle-shaped with a solid outer projection at its tip and a long flagelliform terminal segment with a thick base; outer projection as long as wide. Exopodite wide, with a fringe of short setae on medial margin; distally with wide, outwardly curved, quadrangular protruding tip.

PLEOPOD 2 (Fig. 7C). Endopodite biarticulated; basal article stout, three times as long as wide; distal article elongated, three times as long as basal one, tapering to a whip-like tip, grooved in apical third; Exopodite wide, oval, with slightly bent distal margin and with a fringe of short setae on its outer margin.

PLEOPODS 3–5 (Fig. 6C–E). Vascularized endopodites, triangular in shape, with an enlarged basal part. Exopodites 3 and 4 tetragonal, exopodite 5 triangular; with a fringe of setae on their distal, medial and outer margins and sparse setae covering their outer surfaces.

UROPOD (Fig. 2A). Basis dilated posteriorly; exopodite and endopodite subequal in length, conical, inserted at same level and with a tuft of several short setae at apex.

Females

Differ from males only in the structure of pleopods 1 and 2.

Remarks

The new species is also amphibious, but it differs partially from the nominal *B. bulgarica* in its way of life. The new species has adapted to amphibious life in running groundwater as opposed to the nominal one in subterranean lakes and ponds. It is readily distinguishable from the nominal one by the structure of the antennula, antenna, mouthparts, pereopods and male pleopods. We compared specimens of the new species with specimens of *B. bulgarica* from Lakatnik and with Verhoeff's original description. The antennula third article of *B. serbica* sp. nov. is shorter than the basal article and has three short aesthetascs, vs longer with 6 aesthetascs in the nominal species. Antenna flagellum is shorter in the new species. Pars incisiva of both mandibles are more elongated in the new species than the in nominal one.

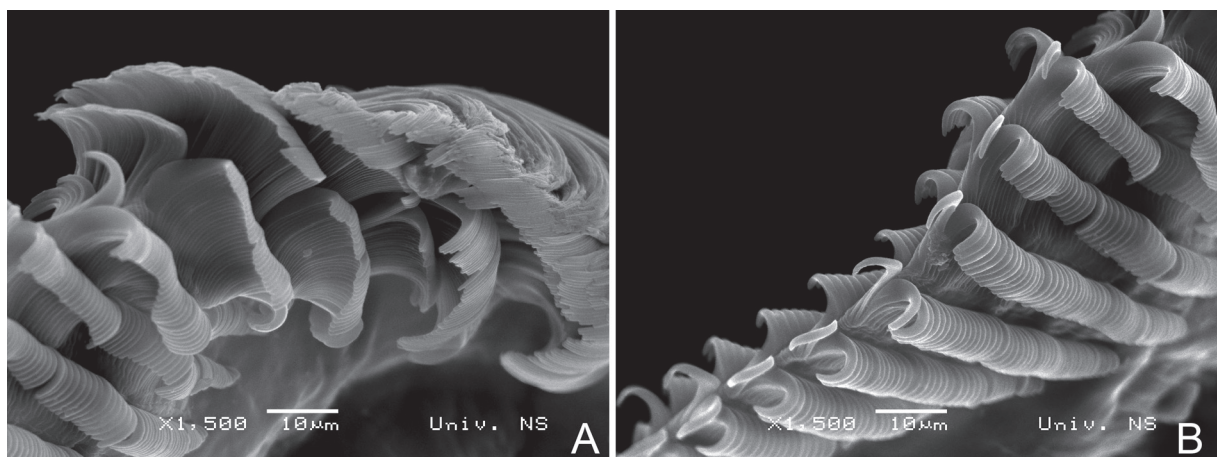


Fig. 5. *Bureschia serbica* sp. nov., paratype, ♂ (6.8 mm) from Suva dupka Cave (ZZDBE 1143/2), maxilla 2 (SEM). **A.** Medial edge. **B.** Terminal edge.

The maxilla 1 inner endite is with a longer terminal penicil in *B. serbica*. The maxilliped endopodite latero-distal margin is somewhat narrower than in the nominal species. Pereopod articles of the new species are stubbier compared with the same in *B. bulgarica*. Those differences are mostly connected with the different ways of life. The male pleopod 1 exopodite is stubbier than in *B. bulgarica*, with a wider outer projection at its tip. The male pleopod 2 endopodite basal article is short, one-third the

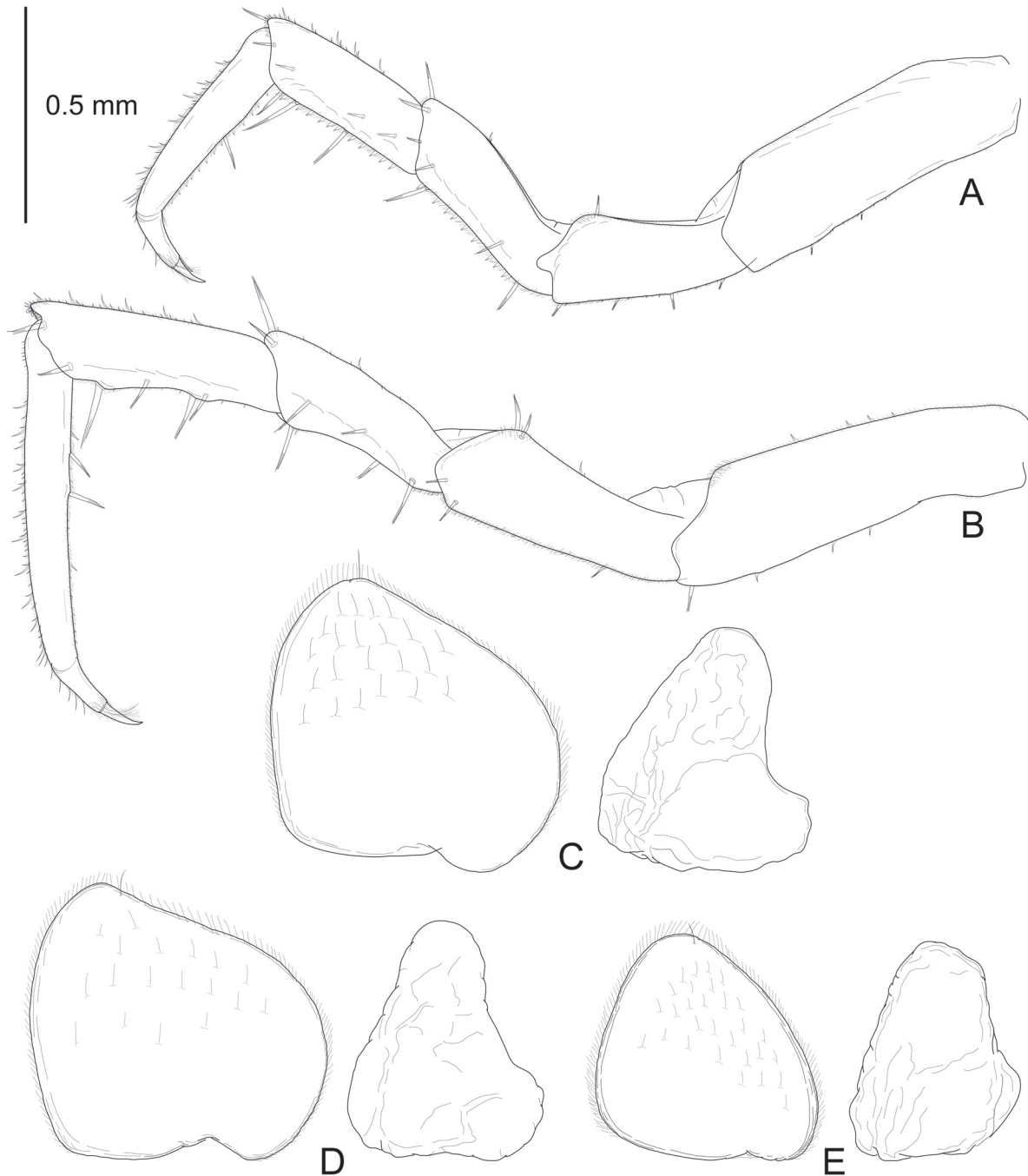


Fig. 6. *Bureschia serbica* sp. nov., holotype, ♂, from Suva dupka Cave (ZZDBE 1143/1). **A–B.** Pereopods. **C–E.** Pleopods, exopodite and endopodite. **A.** Pereopod 1. **B.** Pereopod 7. **C.** Pleopod 3. **D.** Pleopod 4. **E.** Pleopod 5.

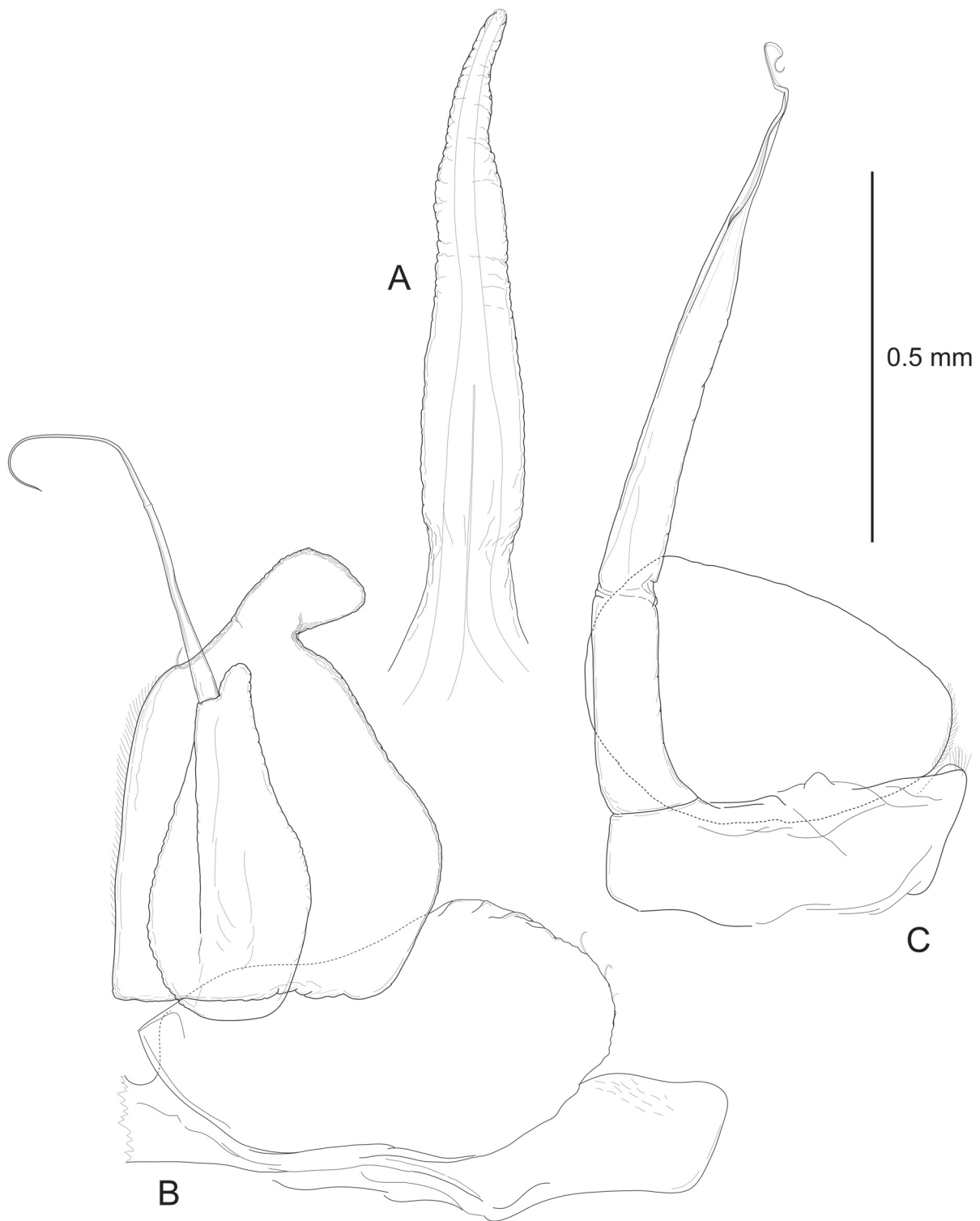


Fig. 7. *Bureschia serbica* sp. nov., holotype, ♂, from Suva dupka Cave (ZZDBE 1143/1). **A.** Genital papilla. **B.** Pleopod 1. **C.** Pleopod 2.

length of the terminal one, compared to the same in *B. bulgarica* where it is half of the length of the terminal one. The new species is also smaller than the nominal one.

In Suva dupka cave, we watched a specimen that entered a small shallow stream that descended the rock slope at about 35°, moving against a strong water current and raising a wave of water in front of it. We assume that we observed the specimen in its regular activities (feeding) and not that it entered the stream disturbed by our presence.

Another finding place of this species (noted by Matija Petković) is probably a small cave in Bela village with a stream flowing out of it. Specimens were noted in subterranean running water in the cave. Unfortunately, we have had no opportunity to examine specimens from that population for this study, but the closeness to the type locality and the same way of life suggest their conspecificity.

Discussion

Among amphibious trichoniscids, we recognize two categories: predominantly terrestrial and predominantly aquatic species. This distinction is based mainly on experience, which may not be entirely precise due to limitations regarding the period of the year, the time that researchers spend in caves, and the fact that encounters with these animals are rare. Predominantly terrestrial amphibious species, such as *Titanethes albus* (C. Koch, 1841), were occasionally found in water, mostly individually, where they could survive for a long time if necessary (Sket 1986). They do not depend on water for their diet, although they obviously feed in the water (pers. obs.). Most amphibious species belong to this category, including *Cyphonethes herzegowinensis* (Verhoeff, 1900) and some species of the genera *Alpioniscus*, *Graeconiscus*, *Brackenridgia*, *Trichoniscoides*, *Scotoniscus*, etc. Predominantly aquatic amphibious species are primarily found in water and often in groups. Adaptation to a predominantly aquatic amphibious lifestyle is closely linked to a change in diet in subterranean habitats. However, it remains unclear whether they feed on bacteria biofilms there or by water filtration or if they combine all available feeding options in water, which seem to be more numerous than terrestrial ones. Answering these questions will require more extensive studies that are not easy to accomplish. However, it is clear, that this is a preadaptation caused by the need for energy sources in an environment that is generally not rich in them. This transition is relatively easy to achieve in Synocheta due to their adaptive respiratory system. Preadaptation to the aquatic way of life is manifested by significant changes in the structure of the mouthparts. Some changes are peculiar (in aquatic *Typhlotricholigoides aquaticus*), but in most, they are of the same type, similar among phylogenetically very distant species. The tendency is noticeable. Generally, in all aquatic and predominantly aquatic amphibious species the maxillipeds are strongly widened distally (*T. aquaticus*, *C. primitivus*, *Macedonethes skopjensis* Buturović, 1955 and *M. stankoi* among Trichoniscidae and all amphibian and aquatic Styloniscidae), or with widened maxilliped endite which takes a quadrangular shape (*Bureschia*, *Alpioniscus* (*Illyrionethes*) *stochi* Taiti & Argano, 2018, *A. (Illyrionethes) sideralis* Taiti & Argano, 2018 and *A. (Illyrionethes) kuehni*). Except for *T. aquaticus* and *C. primitivus*, in all aquatic and predominantly aquatic amphibious species the maxilliped palp medial edge has more or less pronounced setose lobes. There is also a noticeable tendency to reduce the outer lobe of the maxilla 2. Antennulae in aquatic and predominantly aquatic amphibious species often have an elongated narrow third article and a reduced number of aesthetascs.

The structure of the respiratory pleopods is missing in many descriptions, so it is challenging to create a general picture; however, it is to be expected that their size (Dalens 1989), as well as the presence of an increased number of setae, plumose setae or other setae of delicate forms, indicate an aquatic lifestyle (Karaman 2003; Taiti & Montesanto 2020).

Adaptive changes of all mentioned structures are obvious if we compare closely related species that belong to the terrestrial categories with those that are aquatic and predominantly aquatic, amphibious species.

This is the case with species of the genus *Alpioniscus* in Sardinia (three terrestrial vs three aquatic), three terrestrial species of *Trogloniscus* compared with two aquatic or the aquatic *Thailandoniscus annae* with the terrestrial *Thailandoniscus brehieri* Taiti & Montesanto, 2020 and *Thailandoniscus whitteni* Taiti & Montesanto, 2020 (see Dalens 1989; Schultz 1995; Taiti & Xue 2012; Taiti *et al.* 2018; Taiti & Montesanto 2020). *Thailandoniscus brehieri* shows specific characteristics (antennulae and setation of pleopod 3–5 exopods) that indicate a possible amphibious way of life, which the authors (Taiti & Montesanto 2020) pointed out.

Otherwise, very conservative characteristics such as mouthparts in the changed conditions show great adaptability. However, they are not the only ones. Some further changes in adaptation to different physical conditions in the aquatic environment are also visible in the antenna structure of some genera. The antenna in those species that live in less turbulent waters is elongated, while those that live in running waters as aquatic or amphibious have shortened antennae, especially their flagella (absolute or relative to the fifth article). That is evident by comparing species of the genus *Bureschia*, and *Alpioniscus kuehni* with *A. stochi* Taiti & Argano, 2018 and *A. sideralis* Taiti & Argano, 2018 (see Taiti *et al.* 2018).

A species that has undergone significant changes in adaptation and even acquired an entirely new organ (structure) is the rhithral *Macedonethes stankoi*. Based on some features that result from adaptation, a great expert on Oniscidea, Dr Ionel Tabacaru (2021), has singled out this species into a separate genus which he named *Karamanoniscus*, in honor of the first author of the present study.

Unfortunately, we cannot agree with this change because it reflects the different physical conditions of the environment for which the two stygobite species, *M. skopjensis* (nominal taxon) and *M. stankoi*, have adapted, and not a reflection of their phylogenetic distance. Both species are stygobites of a broad mountain massif, Jakupica Mt, located at its two ends. Several troglobite taxa of the genus *Alpioniscus* s. str. close to *Alpioniscus boldorii* Arcangeli, 1952 are also present on the slopes of the same mountain area and neighboring terrain (Buturović 1954, 1955b; unpubl. data).

The characteristic that Tabacaru (2021) stated as one that denies the kinship of *M. skopjensis* and *M. stankoi* is the difference in the number of penicils of the right mandible (one vs two). That characteristic can vary among related species, which was presented in Karaman (2003), and among species of the genus *Alpioniscus* (*Illyrionethes*) from Sardinia (Taiti *et al.* 2018). Tabacaru also overlooked the fact that the presence of reduced penicils (well developed in *M. skopjensis*) on both pars molaris was mentioned in the description of *M. stankoi*. The complete reduction of these penicils in some species of the genus *Alpioniscus* (*Illyrionethes*) from Sardinia (Taiti *et al.* 2018) suggests that this is probably the result of adaptation to an aquatic lifestyle. As for the structure of male pleopods in three species of *Macedonethes*, they are basically of the *Alpioniscus* s. str. type, the group of species close to *A. boldorii*. The strong propodite at the base of the strong pleopod 2 endopodite, as well as the structure of the male pleopod 2 of *M. castillonensis* (Cruz & Dalens, 1989), does not correspond to species of the genus *Spelaeonethes* Verhoeff, 1932 (where the species was originally placed) but rather to species of the subgenus *Alpioniscus* s. str. The characteristics of the maxillipeds and uropods of *M. castillonensis* show similarities with those of *M. skopjensis* and *M. stankoi* (Buturović 1955a; Cruz & Dalens 1989; Karaman 2003). Whether this is the result of convergence (then *M. castillonensis* is an aquatic or predominantly aquatic, amphibious species) or whether it reflects phyletic closeness remains an open question. More significant differences in some details of the structure of the male pleopods result from preadaptation to the aquatic way of life of *M. skopjensis* and *M. stankoi*. The unusual structure of the pleopod 1 exopodite and pleopod 2 endopodite of *M. stankoi*, as well as entirely new structures (organs) on the sternite of the 5th male and female pereonites, are the result of adaptation to the conditions of running water, providing the possibility of copulating under those conditions. The close relation of *M. skopjensis* and *M. stankoi* is indicated by the almost identical structure of the maxilliped. We do not exclude the possibility that in

some further revision, these three species will appear as close to the species of the subgenus *Alpioniscus* s. str. Some important details of the structure of *M. skopjensis* are unknown, and the unknown whereabouts of the holotype is a serious handicap in further consideration of the relationship between these species. Although we know almost nothing about the biology of *Macedonethes skopjensis*, it is most likely not a crenophilic species (Taiti & Xue 2012). If it were, it would have been collected in larger numbers, and not just one specimen. Stanko Karaman, who collected it as a hydrobiologist, searched the sources for the stygobionts he studied (amphipods and non-oniscidean isopods), which the groundwater occasionally brought to the surface. It is probable that the *M. skopjensis* specimen also arrived from that environment. Next to the river Treska below the Sveta Petka village is a strong spring called Vrelo, which must be the spring in question. The course of the river Treska through that part of the gorge was submerged by the accumulation in 1938. The first author was at that source about 10 years ago, when the accumulation was emptied for a while due to the construction of a dam upstream, but he could not find specimens of the species. We hope that speleologists who occasionally organize diving into this spring will find and collect new specimens of this species. So far, unfortunately, that has not happened.

There is a well-founded fear that this species may be lost forever.

One type of amphibious way of life is manifested in some species belonging to the category of hygropetric (cave wall flowstones with water seeps) cave inhabitants (Sket 2004) with a characteristic pereopod structure and slightly modified mouthparts. *Cetinjella monasterii* Karaman & Horvatović, 2018 and some still undescribed Balkan styloniscid species belong to this category.

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