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Contribution to the knowledge of *Siphonoperla* in Europe (Plecoptera: Chloroperlidae): *Siphonoperla korab* sp. n.

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Abstract

A new species of stonefly of the genus *Siphonoperla* from the Dinaric Mountains of Macedonia is described. The description of *S. korab* sp. n. is based on morphological and genetic characters. We also provide notes on habitat and distribution of this new taxon. Further notes on the biogeography of the genus are added.

Key words: Plecoptera, *Siphonoperla*, new species, penial structures, mtDNA, Macedonia

Introduction

Species of the genus *Siphonoperla* Zwick, 1967 are relatively small chloroperline stoneflies usually associated with cold spring habitats, but some species have been reported from lowlands. The systematics of the genus is much disputed (Weiss *et al*. 2011), and species are distinguished on morphological criteria. The genus *Siphonoperla* is distributed throughout Europe and occurs both in North Africa and in Central Asia. The genus currently includes thirteen described species (DeWalt *et al.* 2011): *S. hajastanica* (Zhiltzova), *S. libanica* Alouf, *S. lepineyi* (Navás), *S. baetica* (Aubert), *S. burmeisteri* (Pictet), *S. montana* (Pictet), *S. neglecta* (Rostock), *S. graeca* (Aubert), *S. taurica* (Pictet), *S. ottomoogi* Graf, *S. torrentium* (Pictet), *S. transsylvanica* (Kis) and *S. italica* (Aubert). In this study, we focus on a morphologically similar species group comprising *S. neglecta*, *S. graeca* and the new species *S. korab* sp. n.

Material and methods

Two specimens were collected using an aerial insect net and all material was stored originally in 70% ethanol but transferred to 95% ethanol for genetic analysis. The holotype and paratype are deposited in the collection of the Biologiezentrum Linz (LI-PLEC-2011/01), Austria. General distributions were compiled from data extracted from the following publications: Aubert (1953, 1956), Alouf (1992), Braasch & Joost (1976), Despax (1951), Fiałkowski & Kittel (2002), Fochetti (1995), Graf *et al.* (2008), Kimmins (1935), Kis (1963, 1974), Lillehammer (1988), Loskutova (2003), Murányi (2007), Sivec (1980), Soldán *et al.* (1998), Weiss *et al.* (2011), and Zwick (1971, 1981).

DNA extraction and Data analysis. Genomic DNA was extracted from three *Siphonoperla* species (*S. neglecta*, *S. graeca* and *S. korab* sp. n.) using DNeasy blood and tissue kits (Qiagen GmbH, Hilden, Germany). A PCR reaction was carried out to amplify a 650 bp region of the mitochondrial cytochrome oxidase I (*cox1*) gene

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using primers LCO-1490 and HCO-2198 (Folmer *et al.* 1994) in a 25µl reaction. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and sequenced in both directions using the PCR primers. Samples were analyzed on a 3500xL (Applied Biosystems) automated sequencer.

Forwards and reverse reads were assembled and edited using CodonCode Aligner v 3.5 (Codon Code Corporation, Dedham, USA) and contigs were compared to the NCBI nucleotide database using blastn queries (http://blast.ncbi.nlm.nih.gov). Phylogenetic analysis was conducted on three *Siphonoperla* species. We also included two other published *Siphonoperla* sp. *cox1* sequences (i.e. of *S. montana* and *S. ottomoogi*, GenBank Accession Nos. EU441195 and EU441202; Graf *et al.* 2008) as these showed >90% identity to our sequences in the blastn query and additionally *Perla* sp. as an outgroup (obtained from /ibol.org/). All sequences were aligned using ClustalW (Thompson *et al.* 1994) and a phylogenetic search was conducted using a maximum likelihood approach in PhyML v 3.0 (Guindon & Gascuel 2003) under a GTR model of evolution (as determined by Modeltest v 3.7, Posada & Crandall 1998) and bootstrapping of 1000 replications. Uncorrected pairwise *p*–distances among individuals were generated with Mega 4.0 (Tamura *et al.* 2007). All newly generated DNA sequences have been deposited in GenBank (Accessions JN084076-JN084078).

Siphonoperla korab Graf, sp. n.

(Figs. 3a–b, 4c, 5)

Diagnosis. This species is defined in the male by the presence of a broad U-shaped, penial spine area, and in both sexes by a small rectangular and one spindle shaped sclerotisation of the pro- and mesosternite, respectively.

Description. General appearance genotypic, small, slender and yellowish; body length: male: 6.2 mm, with wings 8.0 mm; female: 8.0 mm, with wings 10.0 mm.

Head: pale, no dark markings between ocelli; maxillary and labial palps brownish; antennae pale proximally, from the 6th segment continuously brownish until the tip.

Pronotum: Completely pale with the exception of narrow brown marks medially, and on anterior and posterior margins. Additionally one delicate brownish median line is present.

Thorax: Meso- and metaterga with brownish W-shaped markings. Legs pale, tarsi and tarsal claw brownish. Pro- and mesosternite with a small rectangular and one spindle shaped sclerotisation (Fig. 4c).

Abdomen: Terga 2–7 with rectangular to triangular brownish patches. Tergum 1 with a broad brown stripe at the anterior margin covering nearly the entire segment. Sternites and cerci pale.

Male genitalia. Male epiproct slender (Fig. 5), inner margin smoothly convex not curved.

Basal area of everted penial sac lacking spinules. Penial spine area broad, U-shaped and prominent (length = 421.3 µm, width = 320.8 µm) (Fig. 3a). Marginal spines distinctly broader and longer than others. Two small additional spine areas ventrolaterally (in respect to the everted penis) of the base of the titillators. Titillators long (length = 1430.6 µm, Fig. 3b).

Female genitalia. Subgenital plate broad, covering half of the 8th segment. Slightly biconvex.

Eggs and larva. Unknown.

Material examined. Holotype male: Macedonia, Korab Mts., tributary of Žaba river, 41° 47’ 45.3’’N, 20° 36’ 18.3’’E, 1830m asl., 3.7.2010, leg. A. Previšić; Paratype: 1 female, same data.

Remarks. In the genus *Siphonoperla*, arrangements of spines of the penial armature are diagnostic (Weiss *et al*. 2011). Among those species with one median group of spines are *S. taurica*, *S. burmeisteri*, *S. graeca*, *S. neglecta* and *S. korab*. These species have additionally ventrolateral narrow fields of spines (Zwick 1971, Fig. 11f; Figs. 1c, 2b, 3b). Males of *S. neglecta*, *S. graeca* and *S. burmeisteri* are characterized by one median sclerotized isosceles triangle shaped armature (Fig. 2a), whereas *S. taurica* has one narrow sclerotised patch of spines (Kis 1974, Fig. 155e). In *S. burmeisteri* there is a prominent median stiffening of the apical portion of the penis sac (Zwick 1991, Kis 1974, fig. 154c). *Siphonoperla graeca* (Figs. 1a, c) has well sclerotized areas laterally at the base of the titillators. Within this morphologically similar group including *S. graeca*, *S. burmeisteri*, and *S. taurica*, the titillators are relatively short as compared to other members of the genus. *Siphonoperla korab* is apparently most similar to both *S. neglecta* and *S. graeca* but differs by the broad, larger U-shaped penial spine-field (Fig. 3a). In *S. neglecta*, the proportion between length and maximum width of the main spine-field is 1.496, in *S. graeca* it is 1.94, whereas in *S. korab* it is 1.31. Additionally, the relative dimensions of the spine field are diagnostic (maxi-

mum width: 239 µm; maximum length: 159 µm in *S. neglecta*; 209 µm and 107 µm in *S. graeca* and 421 µm and 320 µm respectively in *S. korab*). Titillator length of *S. korab* (1430.7 µm, Fig. 3b) is also similar to *S. neglecta* (1214.6 µm, Fig. 2b).

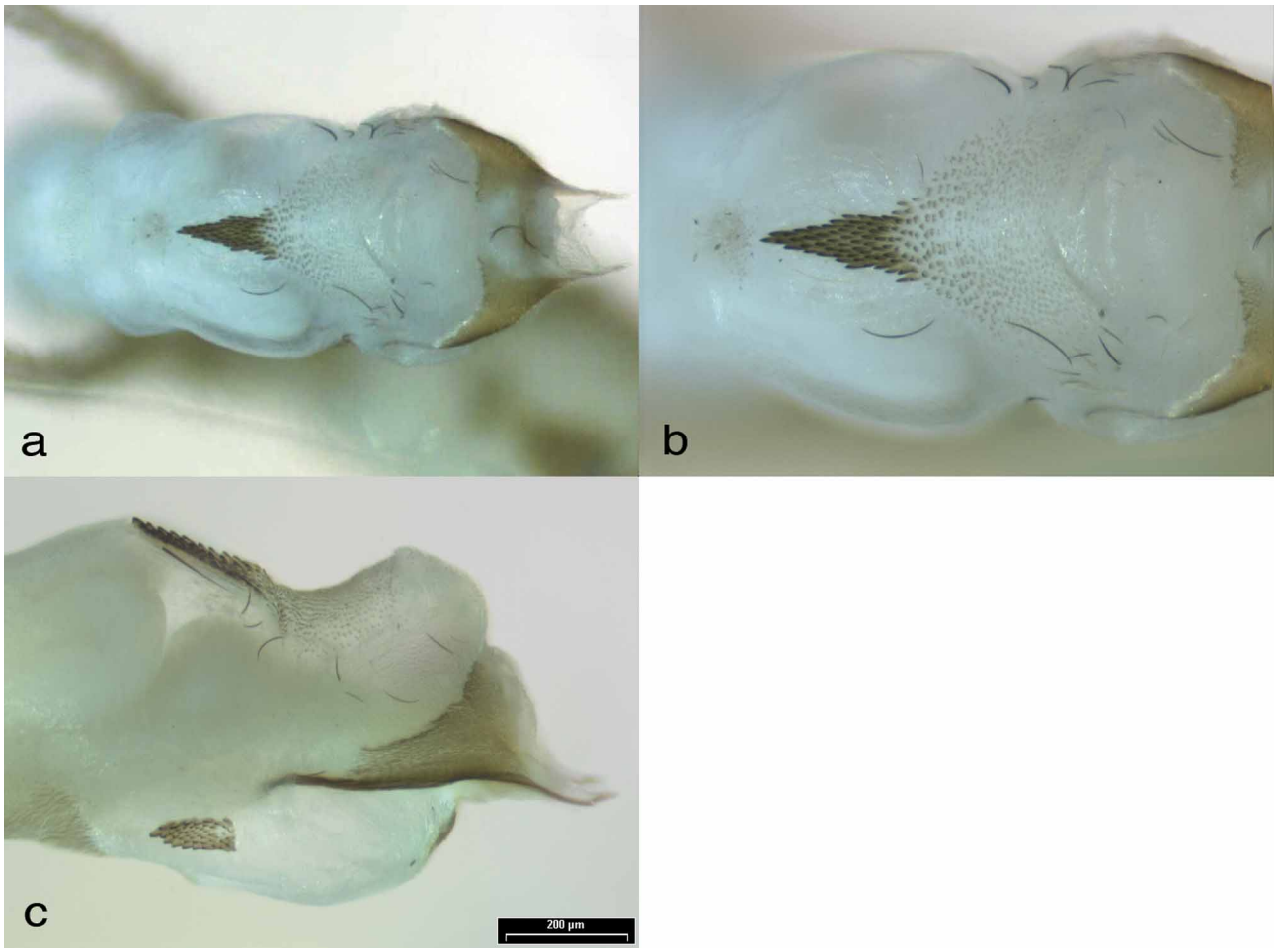

FIGURE 1. *Siphonoperla graeca*: penial armatures a), dorsal view; b) dorsal view; close up; c) right lateral view.

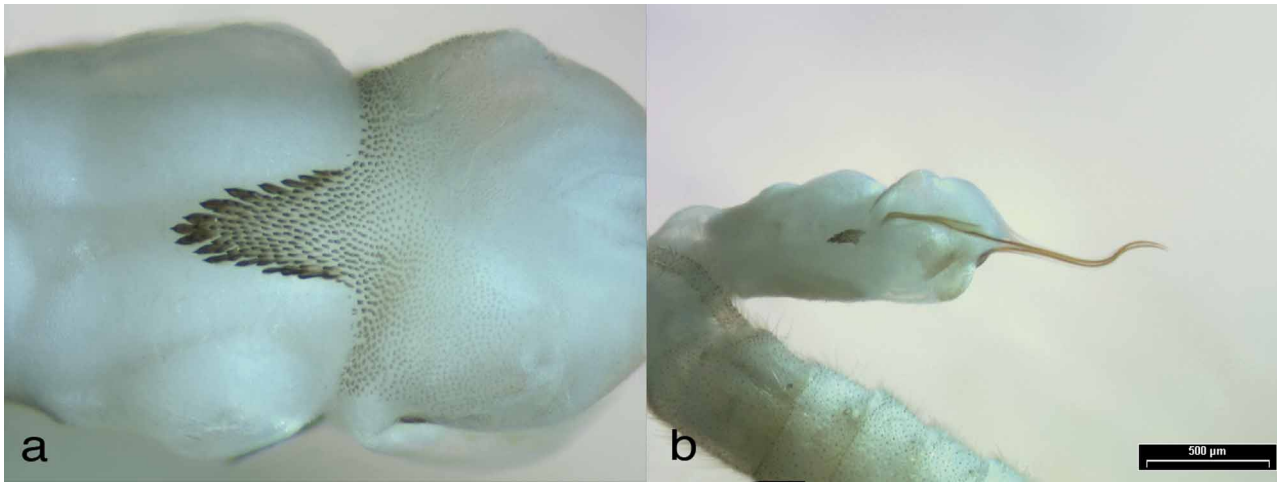

FIGURE 2. *Siphonoperla neglecta*: penial armatures a), dorsal view; b) right lateral view.

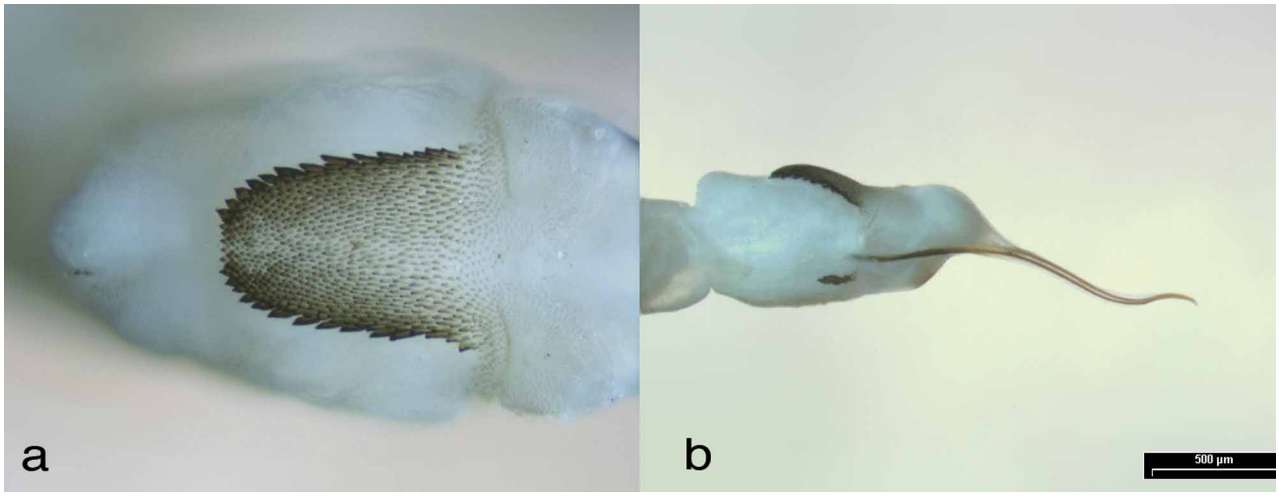

FIGURE 3. *Siphonoperla korab* sp. n.: penial armatures a), dorsal view; b) right lateral view.

Siphonoperla graeca resembles *S. burmeisteri* in its penial spine area (Murányi 2007, fig. 86), and has very short titillators (Figs. 1a, c). The length from the base to the tip is 588 µm, and overlaps the penis by 272.8 µm only. In *S. korab,* the excess length is 991.9 µm, and in *S. neglecta* 775.2 µm, respectively.

In contrast to *S. neglecta* and *S. korab* the median spine area of *S. burmeisteri* (Kis 1974, fig. 154c) and *S. graeca* bears spines in size and degree of sclerotisation similar to those at the margins (Fig. 1b), whereas both *S. neglecta* and *S. korab* have much larger and darker marginal spines (Figs. 2a, 3a). Both *S. neglecta* and *S. graeca* have prominent dark sclerotisations on thoracic sternites (Figs. 4a, b), but *S. korab* exhibits notable modifications (Fig. 4c). In dorsal view, the epiproct of *S. korab* (Fig. 5) is similar to that of *S. neglecta* and not that broad at the base as in *S. graeca* and *S. burmeisteri*. Its inner margin lacks a sharp curve as in *S. montana*.

Intraspecific variation of penial armatures seems to be low in the genus and apparent hybrids are unknown. Although we analysed only one male and one female of *S. korab* the genetic and morphological differences to other compared species are distinct.

Genetics. PCR amplification and sequencing of *coxI* was successful for the three *Siphonoperla* species. Overall species sequences were clearly similar to previously published data (Graf *et al.* 2008) based on a blastn search. Combining our sequences with blastn query results produced an aligned matrix of 5 taxa and 442 characters. In the maximum likelihood *coxI* gene tree (ln L = -1685.47326; Fig. 7) *S. korab* sp. n. sequence was phylogenetically more similar to other *Siphonoperla* species of the *neglecta*-group (*S. graeca*, *S. neglecta*) with divergence ranging from 3.8–5.0%, and genetically more distant to *S. ottomoogi* and *S. montana,* with 10.2% and 9.7% of divergence between sequences.

Zoogeographical notes. The genus *Siphonoperla* exhibits as most Plecoptera, a general increase in diversification from northern to southern latitudes of Europe. Only a few species (*S. torrentium* and *S. burmeisteri*) are distributed in Northern Europe, with *S. burmeisteri* extending far to the east (St. Petersburg and the Pechora Basin), and southwards to the Czech Republic (Bojková 2009) and Bulgaria (one disputable single record only) but did not disperse into Western Europe. In contrast the Mediterranean peninsulas are inhabited by several species such as *S. baetica* (Spain), *S. graeca* (Bosnia-Herzegovina, Montenegro, Macedonia, Greece, Albania), *S. libanica* (Anatolia, Lebanon), *S. transsylvanica* (Romania, Bulgaria), and *S. italica* (Italy). The generally eastern-distributed *S. neglecta* is widespread (from the Balkans westwards to the margins of the Austrian Alps to the mountainous ranges of Slovakia, the Czech Republic, but also western Germany). A similar distribution is apparent for *S. taurica* with an eastward extension into the Crimean Peninsula. *Siphonoperla montana* has a more restricted distribution occurring in the Beskids Mountains, the Šumava Mountains in the Czech Republic, the Alps, and the Apennine and the Dinaric Mountains. A smaller area is inhabited by the microendemic *S. ottomoogi* (few records in the Eastern Alps, Austria) and the subspecies *S. torrentium manevali* (Massiv Central, France). *S. hajastanica* (Armenia), *S. libanica* (Anatolia, Lebanon) and *S. lepineyi* (Northern Africa) do not occur in Europe. Although the status of several taxa (especially of the *S. torrentium* group) is much-disputed (Weiss *et al.* 2011), the high degree

of diversification and speciation at the Southern Peninsulas like the Balkans remains obvious. *S. korab* is a cold spring-species of higher elevations (Fig. 6) probably restricted to the Korab Mountain Range.

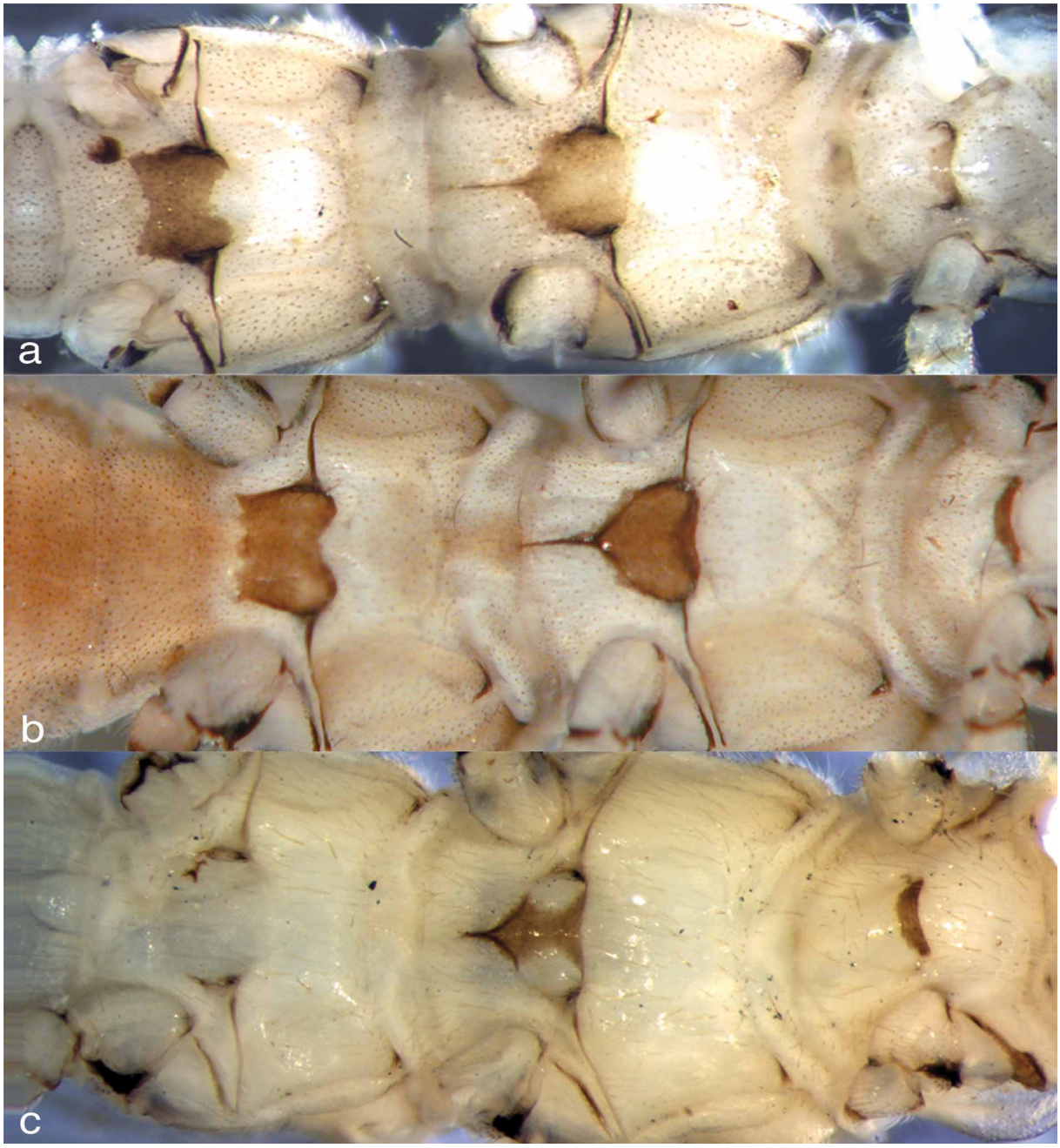

FIGURE 4. Thoracal sternites, a): *S. graeca*; b): *S. neglecta*, c): *S. korab* sp. n.

Similar scattered distributions of microendemic species of the genus *Drusus* (Trichoptera) among other aquatic organisms of Dinaric Mountains have been documented and recently discussed as allopatric speciation events due to Pleistocene fragmentations (e.g. Previšić *et al.* 2009, Oláh 2010, Kučinić *et al.* 2011).

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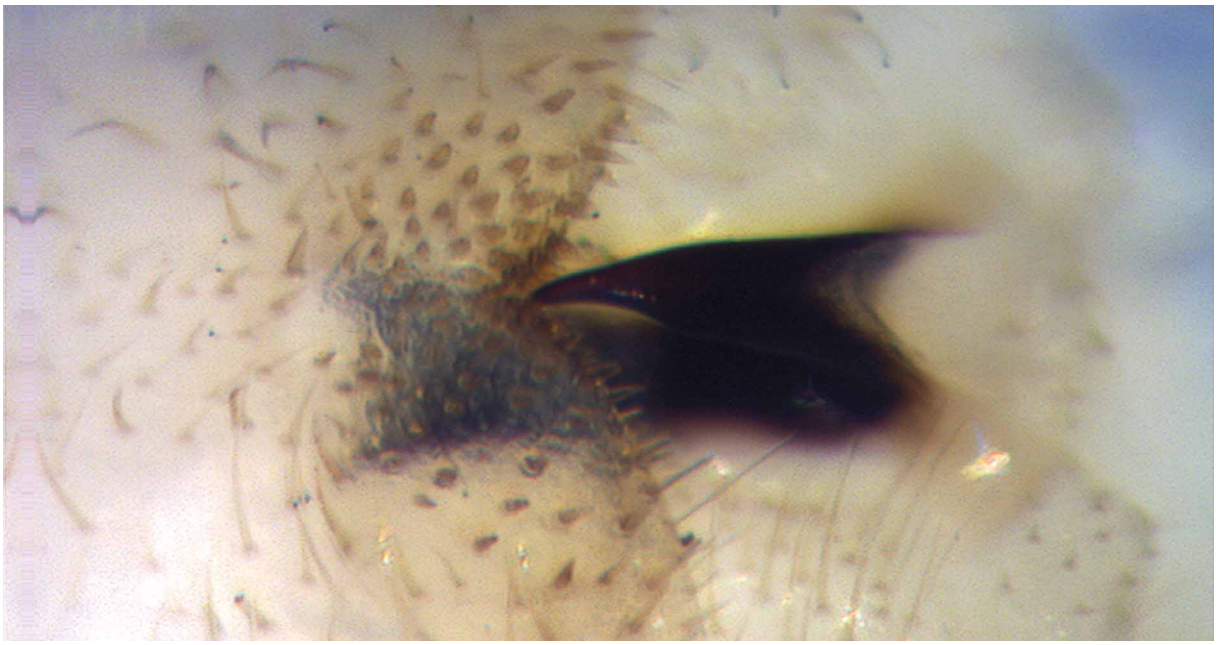

FIGURE 5. *Siphonoperla korab* **sp. n.**: epiproct.

FIGURE 6. Habitat of *Siphonoperla korab* **sp. n.** (Photo by I. Stanković)

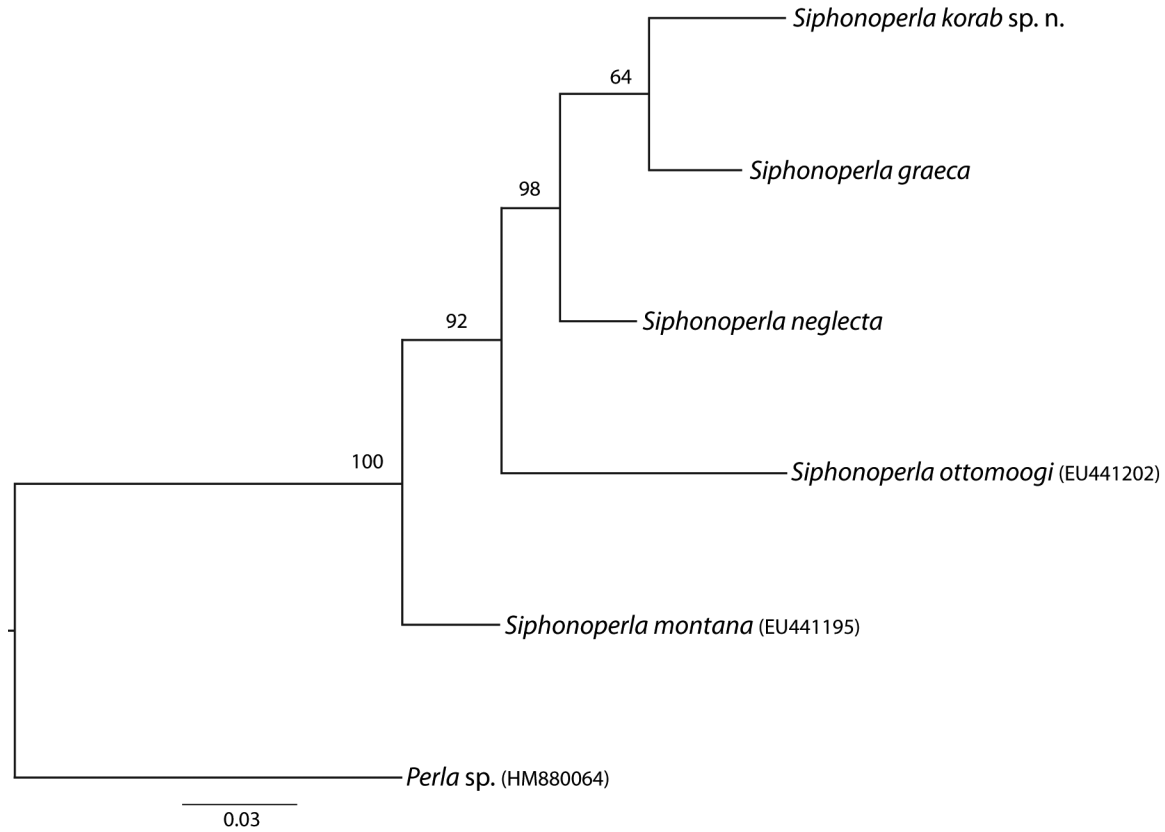

FIGURE 7. Maximum likelihood phylogenetic tree of *coxI* using a GTR model of evolution, including newly sequenced *S. korab* **sp. n.**, *S. neglecta* and *S. graeca*, and highly ranked sequences according to blastn queries (with GenBank alphanumeric accession codes). Numbers on the branches are values of bootstrap support from maximum likelihood analysis.

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