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New records for the Kosovo caddisfly fauna with the description of a new species, *Drusus dardanicus* sp. nov. (Trichoptera: Limnephilidae)

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Abstract

The Balkan Peninsula is one of the most important European hotspots of freshwater biodiversity. The region is, however, to a large extent insufficiently investigated. Here we present data on distribution of caddisflies in one particularly understudied area, the Republic of Kosovo. Our data include the first records of *Adicella altandroconia* Botosaneanu & Novak and *Halesus tessellatus* (Rambur) for the Kosovo caddisfly fauna, and a new locality for the recently described *Ecclisopteryx keroveci* Previši, Graf, & Vitecek. Further, we describe the new caddisfly species *Drusus dardanicus* sp. nov. from the Kopaonik Mountains. The new species belongs to the *D. discophorus* Species Group and differs morphologically from its most similar congeners (*D. discophorus* Radovanovi, *D. balcanicus* Kumanski, and *D. bureschi* Kumanski) mainly in exhibiting (1) subtriangular superior appendages; (2) a narrow, dorsal spinate area of tergite VIII; and (3) evenly rounded tips of intermediate appendages in caudal view. In phylogenetic analysis, *D. dardanicus* sp. nov. is well delineated and recovered as a sister taxon to *D. osogovicus* Kumanski, a species recorded from Bulgaria. The recent discovery of a new species and other rare or microendemic species presents important contributions to the knowledge on the rich freshwater biodiversity in Kosovo. These species face increasing anthropogenic pressure and threats to their conservation.

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Keywords

species description; Drusinae; freshwater biodiversity; Balkan Peninsula; conservation; taxonomy

Introduction

The Balkan Peninsula is recognized as one of the most important European freshwater biodiversity hotspots (Gottstein-Matošec *et al.* 2002, Kryštufek & Reed 2004) with high rates of endemism in Trichoptera (e.g., Kušini *et al.* 2014). Putatively, historic climate conditions and geological properties of the area induced enhanced speciation, resulting in high species richness and high proportions of cryptic diversity of aquatic biota (Bănescu 2004; Previšić *et al.* 2009, 2014a, 2014b; Zakšek *et al.* 2009; Weiss *et al.* 2014).

The limnephilid subfamily Drusinae Banks 1916 comprises eight genera, with the genus *Drusus* Stephens, containing the greatest number of species (e.g., Kumanski 1973; Sipahiler 1999, 2002; Malicky 2002, 2004). The majority of *Drusus* species are regional or micro-endemics inhabiting single mountain ranges in Europe, the Balkan Peninsula, and Asia Minor (Malicky 1979, 1983; de Moor & Ivanov 2008; Graf *et al.* 2008). There are currently 85 species and 6 subspecies placed within this genus, including 10 species described during recent years from the Balkan Peninsula, namely Albania, Bosnia and Herzegovina, Greece, and Montenegro (Malicky 2004, 2005; Kušini 2011; Oláh 2010, 2011; Oláh & Kovács 2013; Viteček *et al.* 2015). The new *Drusus* species described in this paper is the ninth species of this genus reported from the Republic of Kosovo (Ibrahimi *et al.* 2012a, 2012b, 2013). Despite some historical and recent efforts (e.g., Malicky 1986, 1999; Marinković - Gospodneti 1975, 1980; Oláh 2010; Oláh *et al.* 2013; Pongrácz 1923; Ibrahimi *et al.* 2012a, 2012b, 2013, 2014), Trichoptera diversity in the Republic of Kosovo remains insufficiently documented, and the country potentially sustains more (endemic) species.

In this contribution we present new distribution data on caddisflies for the Republic of Kosovo, and describe the new species *Drusus dardanicus* **sp. nov.**

Material and methods

Fieldwork and sampling

Caddisflies were collected from eight localities across Kosovo during 2009–2014, mainly from the Llap and Sitnica watersheds (Fig. 1).

The Llap River watershed is located in the northeastern part of Kosovo and originates from the Kopaonik Mountains on the state border between the Republic of Kosovo and the Republic of Serbia. The 76.5 km long Llap River is the largest tributary of the Sitnica River by length and catchment area. The Llap River catchment covers one-third (32.9%) of the Sitnica River catchment, and slightly more than one-quarter (26.7%) of the whole Kosovo basin catchment area (Fig. 1). The main spring branch of the Llap River is Murgullë Stream, fed by several smaller streamlets originating beneath Shatoricë Mountain in the central part of the Kopaonik Mountains. The specimens of *Drusus dardanicus* **sp. nov.** were collected from one of the streamlets 5 km above Bollosicë Village at 1335 m a.s.l. Several streamlets

were investigated up to 1700 m a.s.l., but *Drusus dardanicus* **sp. nov.** was found only at the above-mentioned locality. The spring area of this river is investigated for the first time regarding its caddisfly fauna.

We collected adult specimens of the new *Drusus* species with entomological nets and by handpicking from the riparian vegetation near the stream on 11.v.2014. Other caddisfly specimens reported in this paper were collected with entomological nets, handpicking, and nocturnal light trapping in the vicinity of the streams. Nocturnal light trapping follows the procedure of Malicky (2004), placing an ultraviolet light above a white pan of 60 cm diameter, filled 10 cm with water with a few drops of detergent added. All collected specimens were stored directly in 96% ethanol. All collected material (including the holotype) is deposited in the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina “Hasan Prishtina,” Prishtinë, Republic of Kosovo, except for 6 specimens of the new species: 3 paratypes deposited in the Croatian Natural History Museum in Zagreb (coll. Ku ini -Trichoptera), Croatia; 3 paratypes in the Biologiezentrum des Oberösterreichischen Landesmuseums, Linz, Austria.

Identification and taxonomical work

Specimens were identified using mainly publications by Malicky (2004), Kumanski (1985, 1988) and Previši *et al.* (2014b); systematic nomenclature follows Morse (2015).

Nomenclature of male terminalia of *Drusus dardanicus* **sp. nov.** follows Nielsen (1957, for *Limnephilus flavicornis* Fabricius) using the simplifying terms “superior appendages” for the lateral processes of segment X (cerci *sensu* Snodgrass 1935), and “intermediate appendages” for the sclerite and the anterior process of segment X (paraprocts *sensu* Snodgrass 1935). Comparative assessments of morphological features of *D. dardanicus* **sp. nov.** were based on the other specimens collected in Macedonia (*D. discophorus* Radovanovi 1942) and Bulgaria (*D. bureschi* Kumanski 1973 and *Drusus balcanicus* Kumanski 1973) or based on literature (Malicky 2004). Morphological features of genitalia of *D. dardanicus* **sp. nov.** were analysed from 9 male specimens. Illustrations were prepared following Thomson and Holzenthal (2010), in which pencil templates made with a camera lucida mounted on a compound microscope are digitized, edited, and inked in Adobe Illustrator (v. 16.0.4, Adobe Systems Inc.).

DNA extraction and PCR amplification

Specimens used for DNA analysis were cleared using either the Qiagen Blood and Tissue Kit for DNA-extraction according to the manufacturer’s recommendation and subsequent KOH-treatment (Böhm *et al.* 2011), or KOH-treatment (Malicky 2004). Whole genomic DNA was extracted from the abdomen or the thorax of adult or larvae specimens using the DNEasy Blood and Tissue Kit (Qiagen) according to the manufacturer’s protocol. Standard PCR procedures and primers were used (Table 1). PCR reactions were set up in 10 µl reactions. Unpurified PCR products were sequenced on an ABI 3177XL capillary sequencer at BiK-F using the PCR primers and two additional internal primers for D2 (D2UP-4 and D2DN-B, Zhou *et al.* 2007).

Sequences were edited in Geneious R6 (<http://www.geneious.com>, Kearse *et al.* 2012) and aligned using MAFFT v7 (Katoh & Standley 2013) as implemented in Geneious R6. Nucleotide substitution models for each partition were selected according to the Bayesian Information Criterion in the model test module of Mega v5.1 (Tamura *et al.* 2007) (Table 2). For phylogenetic analysis, the 28SnrDNA fragment was not partitioned. To examine species delineation and association of specimens from the Western Balkans, we inferred a phylogeny using all available sequences of the new species (Table 3). As outgroup taxa we used *Drusus discolor* Rambur 1842 (Limnephilidae: Drusinae) and *Anisogamus waringeri* Graf & Vitecek 2015 (in Graf *et al.* 2015) and *Melampophylax austriacus* Malicky 1990 (Limnephilidae: Stenophylacini) (Table 3).

Bayesian inference of the concatenated dataset (mtCOI5-P + mtCOI3-P + CADH + WG + 28SnrDNA) was performed in MrBayes 3.2 (Ronquist *et al.* 2012), implementing the respective substitution models. Six parallel runs with two chains each were carried out (50x10⁶ generations, sampling every 10,000th generation). Stationary distribution of runs in the same optimal tree space was assumed if the average standard deviation of split frequencies reached values below 0.01. Additionally, MrBayes parameter files were examined in Tracer v1.6 (Rambaut *et al.* 2014) to assess if runs had reached a stationary phase and converged on model parameters. A majority clade credibility tree was estimated based on trees sampled by MrBayes after discarding the first 1,250 trees of each run as burn-in.

Results

During this investigation a total of 17 caddisfly taxa were collected belonging to 14 genera (Table 4). Two species, *Adicella altandroconia* Botosaneanu & Novak 1965 and *Halesus tessellatus* (Rambur 1842), were newly recorded in the Republic of Kosovo. Further, the new micro-endemic species *Drusus dardanicus* **sp. nov.** is described.

Rhyacophilidae

Rhyacophila loxias Schmid 1970

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 23.vii.2014, leg. Halil Ibrahimi (3 males, 4 females); same data except 24.vii.2014, light trap, leg. Halil Ibrahimi, Fitesa Asllani Ibrahimi, Irsa Ibrahimi and Ildir Ibrahimi (6 males). Podujevë Municipality, Brecë Village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 11.v.2014, leg. Halil Ibrahimi (1 male).

Rhyacophila polonica McLachlan 1879

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 23.vii.2014, leg. Halil Ibrahimi (3 males); same data except 24.vii.2014, light trap, leg. Halil Ibrahimi, Fitesa Asllani Ibrahimi, Irsa Ibrahimi and Ildir Ibrahimi (1 male). Prishtinë Municipality, Keqekollë Village, middle section of the stream, 715 m a.s.l., 42.740626°N, 21.336689°E, 14.v.2014, leg. Halil Ibrahimi (12 males); same data except 17.v.2014, leg. Halil Ibrahimi (3 males, 1 female). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from the Mitrovicë side, side spring of

Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 21.v.2014, leg. Halil Ibrahimi (11 females, 5 males).

Philopotamidae

Philopotamus montanus (Donovan 1813)

KOSOVO: Podujevë Municipality, Shatoricë Mountain, stream above Bollosicë Village, 1330 m a.s.l., 43.118169°N, 20.99330°E, 11.v.2014, leg. Halil Ibrahimi (12 males, 2 females); same data except 12.v.2014, light trap, leg. Halil Ibrahimi (3 males).

Wormaldia occipitalis (F.J. Pictet 1834)

KOSOVO: Podujevë Municipality, Brecë Village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 11.v.2014, leg. Halil Ibrahimi (2 males). Prishtinë Municipality, Keqekollë Village, middle section of the stream, 715 m a.s.l., 42.740626°N, 21.336689°E, 14.v.2014, leg. Halil Ibrahimi (2 males).

Hydropsychidae

Hydropsyche sp

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 23.vii.2014, leg. Halil Ibrahimi (8 females); same data except 29.vii.2014, leg. Halil Ibrahimi (3 females). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 21.v.2014, leg. Halil Ibrahimi (2 females). Podujevë Municipality, Shatoricë Mountain, stream above Bollosicë Village, 1330 m a.s.l., 43.118169°N, 20.99330°E, 11.v.2014, leg. Halil Ibrahimi (2 females).

Uenoidae

Thremma anomalum McLachlan 1876

KOSOVO: Podujevë Municipality, Shatoricë Mountain, stream above Bollosicë Village, 1330 m a.s.l., 43.118169°N, 20.99330°E, 11.v.2014, leg. Halil Ibrahimi (1 male).

Goeridae

Silo graellsii A. E. Pictet 1865

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 3.vi.2014, leg. Halil Ibrahimi (1 male). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 21.vi.2014, leg. Halil Ibrahimi (1 male).

Limnephilidae

Drusus botosaneanui Kumanski 1968

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 3.ix.2014, leg. Halil Ibrahimi (1 male). Podujevë Municipality, Breçë village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 4.ix.2014, leg. Halil Ibrahimi (3 males). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 3.ix.2014, leg. Halil Ibrahimi (2 males).

Drusus dardanicus sp.nov. Ibrahimi, Ku ini & Vitecek

(Figs. 2A–E, 3A–C)

Type material—Holotype (1 male) and paratypes (2 males): KOSOVO: Podujevë Municipality, Shatoricë Mountain, stream above Bollosicë Village, 1330 m a.s.l., 43.118169°N, 20.99330°E, 11.v.2014, leg. Halil Ibrahimi. Holotype deposited in the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina “Hasan Prishtina,” Prishtinë, Republic of Kosovo. Paratypes (3 males): Same collection and locality data, deposited in the Croatian Natural History Museum, Zagreb (coll. Ku ini -Trichoptera), Croatia. Paratypes (3 males): Same collection and locality data, deposited in the Biologiezentrum des Oberösterreichischen Landesmuseums, Linz, Austria; specimen identifiers for 2 males: fDsp4301M, fDsp4302M.

Diagnosis—Males of the new species are most similar to *Drusus discophorus*, *D. bureschi*, and *D. balcanicus* but differ in exhibiting (1) superior appendages short, triangular in lateral view; (2) dorsally bulging spinate area of tergite VIII narrow and embracing a small median indentation in dorsal view; (3) tips of intermediate appendages evenly rounded in caudal view, arising from a small base in dorsal view; (4) parameres each with 2 strong caudal spines and a subterminal dorsal protrusion bearing several smaller spines. *Drusus discophorus* males have elongate suboval superior appendages in lateral view; a broad, flat spinate area of tergite VIII embracing a wide, less sclerotized median indentation; medially constricted tips of intermediate appendages in caudal view, arising from a broad base in dorsal view; parameres each with a single, median dorsad spine and several medial, recumbent small spines. *Drusus bureschi* males have elongate subtriangular superior appendages in lateral view; a broad, long, flat spinate area of tergite VIII embracing a small median indentation; tips of intermediate appendages with a median dorsad protrusion in caudal view, arising from a broad, long base in dorsal view; parameres each with a single, median dorsal protrusion bearing several small spines. *Drusus balcanicus* males have subquadratic superior appendages in lateral view; a broad, oval, flat spinate area of tergite VIII embracing a small median indentation; slender, short tips of intermediate appendages in caudal view, intermediate appendages with two lateral, medial, proximal protrusions in dorsal view; parameres each with a single, median dorsal protrusion bearing several linearly oriented small spines.

Description—General appearance: Habitus dark, sclerites and tergites dark brown; cephalic and thoracic setal areas pale; cephalic, thoracic and abdominal setation blonde, abdominal setation concentrated on dorsal protuberances on abdominal segments VI & VII; legs brown to fawn, proximally darker; haustellum and intersegmental membranous integument pale, whitish; wings light brown, translucent, with blonde setae. Male maxillary palps each 3-segmented. Each male forewing 11–12 mm long. Spur formula 1,3,3 in males.

Male genitalia—Tergite VIII dark brown, in dorsal view distinctly incised anteriorly, with lighter areas around fused alveoli; setation concentrated near anterolateral borders of spinate area; transverse width of spinate area about $1/3^{\text{rd}}$ of tergite VIII width, spinate area appearing as two dorsally bulging, subtriangular posterolateral lobes medially connected by band of spines, embracing small medial indentation. Abdominal segment IX ventrally as wide as dorsally in caudal view; in lateral view medially with distinct, rounded caudal protrusion, lacking ventral protrusion. Superior appendages in lateral view short, subtriangular, dorsally straight, ventrocaudal edge longest. Intermediate appendages in lateral view medially bulging caudad, tips rounded, directed dorsad, rough; in dorsal view tips approximately parallel-sided, arising anteromedially, extending laterad with bar-shaped, laterally rounded, rough tip; in caudal view approximately trapezoidal, tips rounded. Inferior appendages (gonopods sensu Snodgrass 1935) in lateral view robust, proximally slightly constricted, distal part subovate, curved dorsad; in dorsal, ventral and caudal views with small median protrusion; in caudal view inferior appendages subovate; setal alveoli fused, creating rugged, less-sclerotized ventral area. Parameres simple, each with 2 distinct apical thorn-like spines and dorsal protrusion at their bases bearing several smaller spines.

Female, pupa and larva unknown.

Etymology—The species epithet was formed by masculinizing the historic name of a region in the Balkan Peninsula inhabited by the Dardanii tribe, ‘Dardania,’ whose language is supposed to be one of the Proto-Albanian dialects. Roman historians noted the people, and documented a mine (‘*metalla Dardanica*’) and corresponding town (‘*municipium Dardanicum*’), which was partly excavated, in the vicinity of the type locality.

Results of phylogenetic species delimitation—In a B/MCMCMC based on partial sequence data from five loci, monophyly of all species, including the putative new species, was highly supported (Fig. 4). Relationships among species in the most derived clade are not resolved. However, *Drusus dardanicus* sp. nov. is recovered as the highly supported sister to *D. osogovicus* Kumanski 1980. The clade ((*D. osogovicus* + *D. dardanicus* sp. nov.) + *D. bureschi* + *D. balcanicus* + *D. discophoroides* Kumanski 1979) is sister to *D. muranyorum* Oláh 2010, and (((*D. osogovicus* + *D. dardanicus* sp. nov.) + *D. bureschi* + *D. balcanicus* + *D. discophoroides*) + *D. muranyorum*) is recovered as sister to *D. popovi* Kumanski 1980, with *D. discophorus* basal to this clade.

Ecology, systematics and distribution—Larval stages of Drusinae are typical inhabitants of crenal to epirhithral sections of streams (Graf *et al.* 2008). Adults of the new species were collected in the vicinity of a typical Drusinae larval habitat—a first order stream of 2–2.5 m width, surrounded by dense riparian vegetation. Substrate was dominated

by meso- to macrolithal, with some larger boulders covered with water moss, and abundant organic matter (in the form of cut trees, branches and twigs, and leaves). Male morphology and position of the species in the phylogenetic analysis suggest a grazing feeding ecology of the larvae (Pauls *et al.* 2008). The species *D. dardanicus* **sp. nov.** belongs to the *D. discophorus* Species Group, as corroborated by both comparative morphology and molecular genetic analysis.

Based on the currently available regional collection data, the new species putatively is a micro-endemic of the Western Balkans, restricted to the watershed of the Llap River.

***Chaetopteryx bosniaca* Marinkovi -Gospodneti 1959**

KOSOVO: Podujevë Municipality, Shatoricë Mountain, stream above Bollosicë Village, 1330 m a.s.l., 43.118169°N, 20.99330°E, 11.xi.2014, leg. Halil Ibrahimi (2 males, 3 females). Podujevë Municipality, Brecë Village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 11.xi.2014, leg. Halil Ibrahimi (3 males). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 12.xi.2014, leg. Halil Ibrahimi (3 males, 4 females).

***Ecclisopteryx keroveci* Previši , Graf & Vitecek 2014b**

This species of *Ecclisopteryx* was mentioned previously as *E. cf. dalecarlica* Kolenati 1848 by Ibrahimi *et al.* (2014).

KOSOVO: Deçan Municipality, Lloqan Mountain, above Lloqan Village, middle section of Lloqan River, 1333 m a.s.l., 42.5518°N, 20.1624°E, 13.vi.2014, leg. Halil Ibrahimi and Agim Gashi (2 males, 5 females).

***Halesus digitatus* (Schrank 1781)**

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 3.ix.2014, leg. Halil Ibrahimi (1 male).

***Halesus tessellatus* (Rambur 1842)**

KOSOVO: Podujevë Municipality, Zakut Village, middle section of Llap River, 673 m a.s.l., 42.985264°N, 21.150753°E, 26.x.2014, leg. Nexhip Sejdiu (5 males, 1 female); same data except 5.xi.2014, leg. Nexhip Sejdiu (9 males).

***Potamophylax pallidus* (Klapálek 1899)**

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 3.ix.2014, leg. Halil Ibrahimi (1 male). Podujevë Municipality, Brecë Village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 4.ix.2014, leg. Halil Ibrahimi (2 males). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 3.ix.2014, leg. Halil Ibrahimi (4 males).

Lepidostomatidae

Lepidostoma basale (Kolenati 1848)

KOSOVO: Podujevë Municipality, Brecë Village, side spring of Llap River, 838 m a.s.l., 43.071470°N, 21.077644°E, 11.v.2014, leg. Halil Ibrahimi (2 males). Mitrovicë Municipality, Bajgorë area, entrance into Kaçandoll Village from Mitrovicë side, side spring of Kaçandoll River by the main road, 1262 m a.s.l., 42.979°N, 21.0509°E, 19.v.2014, leg. Halil Ibrahimi (1 male).

Leptoceridae

Adicella altandroconia Botosaneanu & Novak 1965

KOSOVO: Prishtinë Municipality, Mramor Village, lower section of the stream about 1 km before discharge into Batllavë Lake, 667 m a.s.l., 42.635211°N, 21.275649°E, 30.vi.2009, leg. Halil Ibrahimi (1 male).

Sericostomatidae

Oecismus monedula (Hagen 1859)

KOSOVO: Mitrovicë Municipality, Mazhiq Village, middle section of the stream, 850 m a.s.l., 42.8688°N, 20.8271°E, 3.vi.2014, leg. Halil Ibrahimi (1 male).

Discussion

Systematic position and distribution of *Drusus dardanicus* sp. nov

The combination of three gene fragments (mtCOI3-P, 16SnrDNA, WG) was previously demonstrated to resolve phylogenetic relationships of Drusinae (Pauls *et al.* 2008). Here, we use a set of five gene fragments to discriminate species using phylogenetic methods in a Bayesian framework. Bayesian phylogenetic inference based on the combination of five gene fragments (mtCOI5-P, mtCOI3-P, CADH, WG, 28SnrDNA) suggests monophyly of the putative *Drusus* species, and a highly supported sister taxon relationship of (*D. dardanicus* sp. nov. + *D. osogovicus*). Morphology and molecular sequence data indicate a position of the new taxon among the *D. discophorus* Species Group. According to Kumanski (1988) and Oláh (2010) this group comprises nine taxa: *Drusus discophorus* (including *D. discophorus pallidus* Kumanski 1989 and *D. discophorus rhodopaeus* Kumanski 1989), *D. bureschi*, *D. balcanicus*, *D. discophoroides*, *D. popovi*, *D. osogovicus*, and *D. muranyorum*.

The nominate species (*D. discophorus*) of the Species Group was described from the Jabllanica Mountains in Macedonia (Radovanovi 1942), the other taxa of this group are restricted to Osogovo (*D. osogovicus*), Stara Planina (*D. balcanicus*, *D. bureschi*, *D. popovi*), Vitosha (*D. balcanicus*, *D. discophorus pallidus*), Belasica (*D. discophoroides*), Rhodopes (*D. discophorus rhodopaeus*), Rila (*D. discophorus pallidus*), and Pirin (*D. discophorus pallidus*) Mountains in Bulgaria (Kumanski 1988), while the newest described species of this group, *Drusus muranyorum*, is currently known only from the type locality in Sapka Mountains in Greece (Oláh 2010) (Fig. 5). Interestingly, *Drusus discophorus* has also been reported from Bjeshkët e Nemuna Mountains in Kosovo and Montenegro (Marinkovi -

Gospodneti 1980); however, these latter records cannot be confirmed as the material is lost. Considering the highly disjunct and limited distribution of Drusinae species in the Balkan Peninsula (Previši *et al.* 2009, 2014a), it is possible that records of *D. discophorus* from Kosovo and Montenegro are misidentifications of the then-undescribed *Drusus krusniki* Malicky which is a frequent inhabitant of Bjeshkët e Nemuna Mountains in Albania, Kosovo, and Montenegro (Ibrahimi *et al.* 2012b; Oláh 2010). *Drusus dardanicus* **sp. nov.** is the tenth known species of this group. Considering the limited range of other *Drusus* species and the presented collection data, the new taxon is expected to be a local endemic of Shatorica Mountain and surrounding areas in the Kopaonik range of mountains.

New records for the caddisfly fauna of Kosovo

Adicella altandroconia and *Halesus tessellatus* found during this investigation are first records of these species for the Kosovo caddisfly fauna. *Halesus tessellatus* is a widespread species that occurs all over Europe. However, the collection of the species in the Republic of Kosovo represents the first record for ecoregion 6 (after Illies 1978; Graf *et al.* 2008).

Adicella altandroconia is a rare local endemic of the Balkans, known from the Southern Carpathians, Bulgaria, and a single locality in Greece (Kumanski 1985; Graf *et al.* 2008).

A re-examination (Previši *et al.* 2014b) of the material published as *Ecclisopteryx* cf. *dalecarlica* (Ibrahimi *et al.* 2014) from Kosovo has shown that the *Ecclisopteryx* species from Lumbardhi i Pejës River in Kosovo represents a population of the newly described *Ecclisopteryx keroveci*. During this investigation this species was collected at a new locality, the Lloqan River. The record of *Thremma anomalum* is the first for the Black Sea watershed in Kosovo. Previously it was known only from numerous localities in the Adriatic Sea watershed in Kosovo (Ibrahimi *et al.* 2014).

Balkan springs, streams and rivers—harbours of unknown aquatic biodiversity in peril

The description of a new micro-endemic *Drusus* species in the Hellenic Western Balkans augments the number of endemic Drusinae species (14 of 21 species; Graf *et al.* 2008; Graf & Schmidt-Kloiber 2011; Previši *et al.* 2014b). Drusinae and other highland caddisflies inhabit isolated coldwater springs and stream habitats, which, in combination with low dispersal capacity and geological processes (such as karstification; Previši *et al.* 2009, 2014a), putatively enhanced speciation and fostered the high rates of endemism in Western Balkan caddisflies. A total of 38 Drusinae species are now reported from the Western Balkans (Graf *et al.* 2008, Oláh 2010, 2011; Oláh & Kovács 2013; Previši *et al.* 2014b; Vitecek *et al.* 2015; this study), and the description of *Drusus dardanicus* **sp. nov.** accentuates the importance of the region as a hot-spot of freshwater diversity.

The collection of *A. altandronica*, *H. tessellatus*, *T. anomalum* and other species contributes to the knowledge of caddisfly biodiversity in the Republic of Kosovo. Further, these data promote the significance of faunistic studies in Europe, particularly in emerging economies that will be subject to profound socio-economic changes and thus human resource exploitation.

Vulnerability of endemic freshwater biota to global change and (anthropogenic) habitat degradation is particularly high (Hering *et al.* 2009; Bálint *et al.* 2011; Conti *et al.* 2014). Construction of hydropower plants was demonstrated to be one of the major threats to freshwater biodiversity in emerging economies such as the Republic of Kosovo (Zarfl *et al.* 2014). The main drivers of habitat degradation in the Balkans are construction of small hydropower plants and associated damming of small streamlets (Freyhof 2012, Schwarz 2012), such as the stream at the type locality of *D. dardanicus* **sp. nov.**. Thus, the description of the new species and the additions to the faunal records of the Republic of Kosovo in combination with recent taxonomic efforts in the Balkans (e.g., Oláh 2010, 2011; Oláh & Kovács 2013; Ku ini *et al.* 2013; Previši *et al.* 2014b; Vitecek *et al.* 2015, this study) demonstrate the necessity of further comprehensive investigations on an underestimated portion of the biodiversity in Southern Europe. Also, the discovery of *D. dardanicus* **sp. nov.** portends the extinction of known and unknown biodiversity in the Western Balkans under common energy policy paradigms.

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Figure 1.
The watersheds of the Llap and Sitnica rivers with indicated main tributaries: magenta color, Sitnica River watershed; blue color, Llap River watershed.

Figure 2.

Male genitalia of *Drusus dardanicus* sp. nov. 2A, right lateral view; 2B, right paramere in right lateral view; 2C, ventral view, 2D, caudal view, 2E, dorsal view. Scale bar 1 mm.

Drawn by Vitecek.

Figure 3.
Male genitalia of *Drusus dardanicus* sp. nov.. 3A, right lateral view; 3B, caudal view; 3C, dorsal view.

Figure 4.

Results of phylogenetic inference. B/MCMCMC species tree analysis for nine *Drusus* species (24 terminal taxa) based on 3444-bp-long sequence from 5 loci (mtCOI5-P + mtCOI3-P + 16SmtDNA + CADH + 28S). Bold branches indicate highly supported (pp 0.95) clades.

Figure 5.
Distributions of *Drusus discophorus*-Group taxa. The species inhabit the Balkan Mountains region with partly overlapping distributions.

Table 1

PCR primers and PCR cycling conditions used in analysis of *Drusus discophorus* Group species.

Fragment	Primers & Primer Concentration	PCR Cycling conditions	Taq Kit	Additional Reagents
mtCOI5-P	HCO2198 & LCO1490 (Folmer <i>et al.</i> 1994)	0.25 µM 5' 95°C, 5 x (30" 95°C, 1' 44°C, 1' 72°C), 15x (30" 95°C, 30" 48°C, 1' 72°C), 20 x (30" 95°C, 30" 50°C, 1' + (10" * n) 72°C)	peqGOLD HotTaq	-
mtCOI3-P	Jerry & S20 (Pauls <i>et al.</i> 2006)	0.25 µM 5' 95°C, 35 x (45" 95°C, 30" 45°C, 45" 72°C), 5' 72°C	peqGOLD HotTaq	-
WG	WGbDrrev (5'-ACCCTCTCCCGCARGACTTGAG) & WGbDrfwd (5'-CTTGCTGGATGCGTCTGC C) ¹	0.5 µM 5' 95°C, 35 x (45" 95°C, 45" 60°C, 90" 72°C), 7' 72°C	Qiagen Hotstar Taq plus Master mix	-
CADH	1028r-ino & 743nF-ino (Johanson & Malm 2010)	0.25 µM 5' 95°C, 35 x (45" 95°C, 30" 50°C, 45" 72°C), 5' 72°C	peqGOLD HotTaq	-
D2 (28SnrDNA)	D1-3F (5'-CGAGTAGCGGCGAGCGA ACGGA) (Kjer <i>et al.</i> 2014) & D3-TRIC-DN (5'-ATTCCCCTGACTTCGACC TGA) ²	0.25µM 3' 95°C, 35 x (45" 95°C, 45" 60°C, 60" 72°C), 5' 72°C	peqGOLD HotTaq	2 mg BSA, 5% DMSO

¹ unpublished primer sequence by S.U. Pauls

² unpublished primer sequence by K. Kjer & X. Zhou

Table 2

Substitution models used in phylogenetic analysis of *Drusus discophorus* Group species.

Fragment	unpartitioned	codon position 1	codon position 2	codon position 3
mtCOI5-P	GTR+G+I	TN93+G	TN93+G	HKY
mtCOI3-P	GTR+G+I	TN93+G+I	K2+G	HKY
WG	T92+G	T92	JC+G	JC
CADH	T92+G+I	HKY+G	TN93	T92
D2 (28SnrDNA)	T92+G+I	-	-	-

Table 3

Collection data of specimens and length of partial gene sequences used in phylogenetic analysis of *Drusus discophorus* Group species.

Specimen ID	BOLD BIN	D2 (28SnrDNA)	COL-5P	CADH	COL-3P	Wnt1	Leg.	collection date	latitude	longitude	elevation	Species
fMelaus0101M	BOLD:ACO5932	1038[0n]	658[0n]	842[6n]	541[0n]	0	Graf	20.x.2013	46.8106	14.9931	N.A.	<i>Melanophylax austriacus</i>
fMelaus0102F	BOLD:ACO5932	1038[0n]	658[0n]	843[5n]	0[0n]	0	Graf	20.x.2013	46.8106	14.9931	N.A.	<i>Melanophylax austriacus</i>
fAns0101L	BOLD:ACO4705	1038[0n]	658[0n]	848[0n]	541[0n]	0	Graf	09.vi.2013	42.4798	2.4549	N.A.	<i>Anisogamus waitingeri</i>
fDdd0801M	BOLD:ACO6035	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Previši	10.vii.2013	42.6859	19.7364	960	<i>Drusus discolor</i>
fDdd0802F	BOLD:ACO6035	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Previši	10.vii.2013	42.6859	19.7364	960	<i>Drusus discolor</i>
fDds0110M	BOLD:ACO4561	1038[0n]	658[0n]	848[0n]	474[0n]	346[0n]	Ku ini , Kmpac & Cukusic	29.v.2013	41.267	20.521	N.A.	<i>Drusus discophorus</i>
fDds0111M	BOLD:ACO4561	1038[0n]	658[0n]	848[0n]	0[0n]	346[0n]	Ku ini , Kmpac & Cukusic	29.v.2013	41.267	20.521	N.A.	<i>Drusus discophorus</i>
fDds0112F	BOLD:ACO4561	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Ku ini , Kmpac & Cukusic	29.v.2013	41.267	20.521	N.A.	<i>Drusus discophorus</i>
fDpo0101M	BOLD:ACO5317	1038[0n]	658[0n]	0	0[0n]	0	Keresztes, Kolesar & Torok	11.vi.2012	43.114	23.1123	1304	<i>Drusus popovi</i>
fDpo0102M	BOLD:ACO5317	1037[1n]	658[0n]	848[0n]	541[0n]	0	Keresztes, Kolesar & Torok	11.vi.2012	43.114	23.1123	1304	<i>Drusus popovi</i>
fDmy0101M	BOLD:AAI9121	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Balint	10.iv.2012	41.128	25.887	650	<i>Drusus muranyorum</i>
fDmy0104M	BOLD:AAI9121	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Balint	20.iv.2012	41.128	25.887	650	<i>Drusus muranyorum</i>
fDmy0103M	BOLD:AAI9121	1038[0n]	658[0n]	848[0n]	0[0n]	346[0n]	Balint	20.iv.2012	41.128	25.887	650	<i>Drusus muranyorum</i>
fDdi0101M	BOLD:AAI9121	1038[0n]	658[0n]	840[8n]	541[0n]	346[0n]	Gordon Ramel	02.v.2008	41.3167	23.2333	1150	<i>Drusus discophoroides</i>
fDdi0102M	BOLD:AAI9121	999[1n]	658[0n]	836[12n]	490[0n]	346[0n]	Gordon Ramel	02.v.2008	41.3167	23.2333	1150	<i>Drusus discophoroides</i>
DBaI004	BOLD:AAI9121	1038[0n]	658[0n]	847[1n]	541[0n]	346[0n]	Balint & Neu	12.vi.2008	42.7734	24.6186	N.A.	<i>Drusus balcanicus</i>
DBaI003	BOLD:AAI9121	1038[0n]	658[0n]	846[1n]	541[0n]	346[0n]	Balint & Neu	12.vi.2008	42.7734	24.6186	N.A.	<i>Drusus balcanicus</i>
fDbu0105M	BOLD:AAI9121	1038[0n]	658[0n]	0	541[0n]	0	Keresztes, Kolesar & Torok	14.vi.2012	42.786	25.958	1358	<i>Drusus bureschi</i>
fDbu0102M	BOLD:AAI9121	1038[0n]	658[0n]	837[11n]	541[0n]	346[0n]	Keresztes, Kolesar & Torok	14.vi.2012	42.786	25.958	1358	<i>Drusus bureschi</i>

Specimen ID	BOLD BIN	D2 (28SnrDNA)	COI-5P	CADH	COI-3P	Wnt1	Leg.	collection date	latitude	longitude	elevation	Species
fDos0118F	BOLD:AAI9121	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Keresztes, Kolesar & Torok	11.vi.2012	42.1967	22.6231	1639	<i>Drusus osogovicus</i>
fDos0102M	BOLD:AAI9121	1037[1n]	658[0n]	810[0n]	541[0n]	346[0n]	Keresztes, Kolesar & Torok	11.vi.2012	42.1967	22.6231	1639	<i>Drusus osogovicus</i>
fDos0108M	BOLD:AAI9121	1038[0n]	658[0n]	848[0n]	541[0n]	346[0n]	Keresztes, Kolesar & Torok	11.vi.2012	42.1967	22.6231	1639	<i>Drusus osogovicus</i>
fDsp4302M	BOLD:AAI9121	1000[0n]	658[0n]	850[0n]	541[0n]	345[0n]	Ibrahimi	11.v.2014	43.118159	20.99330	1335	<i>Drusus dardanicus</i> sp. nov.
fDsp4301M	BOLD:AAI9121	0	658[0n]	850[0n]	541[0n]	345[0n]	Ibrahimi	11.v.2014	43.118159	20.99330	1335	<i>Drusus dardanicus</i> sp. nov.

Table 4

An overview of the distribution of caddisfly taxa found in investigated stations.

Taxa	Stream above Bollosicë Village, 1330 m a.s.l.	Stream in Mazhiq Village, 850 m a.s.l.	Side spring of Llap River in Brecë Village, 838 m a.s.l.	Side spring of Kaçandoll River in Bajgorë, 1262 m a.s.l.	Llap River in Zakut Village, 673 m a.s.l.	Stream in Keqekollë Village, 715 m a.s.l.	Stream in Mramor Village, 667 m a.s.l.	Lloqan River above Lloqan Village, 1333 m a.s.l.
<i>Rhyacophila loxias</i> Schmid 1970		*	*					
<i>Rhyacophila polonica</i> McLachlan 1879		*		*		*		
<i>Philopotamus montanus</i> (Donovan 1813)	*							
<i>Wormaldia occipitalis</i> (F.J. Pictet 1834)			*			*		
<i>Hydropsyche</i> sp.	*	*		*				
<i>Thremma anomalum</i> McLachlan 1876	*							
<i>Silo graellsii</i> A.E. Pictet 1865		*		*				
<i>Drusus botosaneanui</i> Kumanski 1968		*		*				
<i>Drusus dardanicus</i> sp.nov.	*							
<i>Chaetopteryx bosniaca</i> Marinkovi -Gospodneti 1959	*		*	*				
<i>Ecclisopteryx keroveci</i> Previši , Graf & Vitecek 2014								*
<i>Halesus digitatus</i> (Schränk 1781)		*						
<i>Halesus tessellatus</i> (Rambur 1842)					*			
<i>Potamophylax pallidus</i> (Klapálek 1899)		*	*	*				
<i>Lepidostoma basale</i> (Kolenati 1848)			*	*				
<i>Adicella altandroconia</i> Botosaneanu & Novak 1965							*	
<i>Oecismus monedula</i> (Hagen 1859)		*						