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Larval morphology of the Western Balkans endemic caddisflies *Drusus krusniki* Malicky 1981, *D. vernonensis* Malicky 1989, and *D. vespertinus* Marinkovi 1976 (Trichoptera, Limnephilidae, Drusinae)

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Abstract

Drusinae (Trichoptera, Limnephilidae) are highland caddisflies inhabiting high-gradient, turbulent running water and spring habitats. They are disjunctly distributed over the Eurasian mountain ranges, and the majority of species is endemic to particular mountain areas. The most diverse of three main groups of the Drusinae, the grazer clade, consists of species in which larvae feed on epilithic biofilm and algae. In this paper we describe three previously unknown grazer-clade Drusinae larvae: *Drusus krusniki* Malicky 1981 (endemic to the Dinaric western Balkans), *D. vernonensis* Malicky 1989 (endemic to the Hellenic western Balkans), and *D. vespertinus* Marinkovi 1976 (endemic to the Dinaric western Balkans). The larvae of these species have toothless mandibles typical of the Drusinae grazer clade. Larvae and adults were unambiguously associated using molecular genetic data, i.e., the mitochondrial cytochrome oxidase I gene fragment (mtCOI3-P). Morphological characteristics of the larvae are described and the diagnostic features enabling species-level identification are illustrated. We further discuss the ecology and distribution of three Western Balkan endemic species.

Keywords

5th instar larva; description; identification; distribution; endemism

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Introduction

Drusinae are restricted to mountain springs and high-gradient, turbulent running waters in hard-substrate channels covering the Eurasian mountain ranges from the Iberian Peninsula to the Iranian Highlands. Three quarters of the known species are endemic to a single or very few mountain ranges, making the group an ideal model for studying evolutionary processes like speciation, diversification and cryptic species evolution (Schmid 1956; Kumanski 1973; Marinkovi -Gospodneti 1971a, b, 1976; Sipahiler 2002; Malicky 2005a; Pauls *et al.* 2006; Previši *et al.* 2014a). Mountain areas of the Western Balkans harbor particularly high numbers of Drusinae endemics (Vitecek *et al.* 2015a). The fragmented montane sky-island populations of the Drusinae are also very sensitive to global change and their species are among the most threatened by climate warming (Previši *et al.* 2009, Balint *et al.* 2011). A molecular phylogeny of the subfamily yielded three well-supported clades that reflect the morphology and feeding ecology of larvae (Pauls *et al.* 2008, Graf *et al.* 2009, Vitecek *et al.* 2015b, Waringer *et al.* 2015): omnivorous shredders, epilithic grazers, and carnivorous filter feeders. Drusinae comprises eight genera, with the genus *Drusus* Stephens containing the greatest number of species (92; e.g., Malicky 2004, 2005a; Oláh *et al.* 2015; Vitecek *et al.* 2015a).

The genus *Drusus* was further divided into particular species groups of presumably closely related species (Schmid 1956). The *Drusus bosnicus* Group was first defined by Schmid (1956) based on the morphology of genitalia, comprising species from the Balkans and Central Europe. Many new species were described from the Western Balkans in the meantime, most of them small-scale endemics with highly similar genital morphology to the *Drusus bosnicus* Group (e.g., Marinkovi -Gospodneti 1971a, b, 1976; Malicky 1989; Oláh 2010, 2011; Vitecek *et al.* 2015a).

Drusinae larvae exhibit high niche specificity, mostly prefer xenosaprobic to oligosaprobic headwater sections of streams or springs (Graf *et al.* 2008), and are comparatively easily identified. The group is therefore well-suited for water quality assessment (Barbour *et al.* 1999, Barbour & Yoder 2000, AQEM Consortium 2002). However, larval stages of many Drusinae species including some of the *Drusus bosnicus* Group are still unknown, and larvae of seven species only have been described previously [*D. bosnicus* Klapálek 1899, *D. crenophylax* Graf & Vitecek 2015a, *D. klapaleki* Marinkovi -Gospodneti 1971b, *D. medianus* Marinkovi -Gospodneti 1976, *D. radovanovici* Marinkovi -Gospodneti 1971b, *D. ramae* Marinkovi -Gospodneti 1971b and *D. septentrionis* (Marinkovi -Gospodneti 1976); Ku ini *et al.* 2008, 2010, 2011a, 2011b, 2015; Vitecek *et al.* 2015a].

In this contribution we extend the knowledge of larval taxonomy of the Drusinae by presenting descriptions of the hitherto unknown larvae of *Drusus krusniki* Malicky 1981, *D. vernonensis* Malicky 1989, and *D. vespertinus* Marinkovi 1976. The putative larvae of these three species were associated with co-occurring adults using molecular genetic sequence data [mitochondrial cytochrome oxidase *c* subunit I (mtCOI3-P)]. We also highlight the most important diagnostic features enabling separation of these larvae from those of other European Drusinae species. Additionally, we discuss the ecology and

distribution of the three Western Balkan endemic species and the *Drusus bosnicus* Group in general.

Material and methods

Sampling, collected material and taxonomy

Adults and larvae were collected by A.P., W.G. and M.K. in Bosnia and Herzegovina, Macedonia and Montenegro using a hand net and kick sampling at the following locations: *Drusus krusniki*: Montenegro: Alipaša's spring, 42°33'N, 19°49'E, 942 m, 31 May 2009, sixteen 5th instar larvae; Ibra spring, 42°48'N, 20°05'E, 1271 m, 31 May 2009, nine 5th instar larvae. *D. vernonensis*: Macedonia: Pelister Mt. Tributary of Caparska reka, 40°58'N, 21°12'E, 2323 m, 8 July 2010, two 5th instar larvae. *D. vespertinus*: Bosnia and Herzegovina: Gornji Ribnik, 44°25'N, 16°49'E, 316 m, 3 June 2008, eight 5th instar larvae. Additionally, temperature loggers (Hobo Onset) logging ambient water temperature at 0200 h and 1400 h were exposed at the same sites and recovered the following year. Material intended for sequencing was stored in 100 % ethanol, the material for morphological analyses in 70% ethanol in order to keep the specimens more flexible. Information on examined voucher specimens and other *Drusus* species larvae used as comparative material is given in Table 1 (all in collection of the first author). The larvae were studied and photographed using a Nikon SMZ 1500 binocular microscope with DS-Fi1 camera and NIS-elements D 3.1 image stacking software for combining 8 to 42 frames in one focused image. Larval morphological nomenclature follows Wiggins (1998).

Species affiliation was based on two criteria:

- 1) Larval and adult specimens were collected at the same sites, close to the type localities of the respective species where other Drusinae species are absent or already known due to existing descriptions.
- 2) Molecular sequence data from the mitochondrial cytochrome oxidase *c* subunit I (mtCOI3-P) supports conspecificity of the larvae and adults.

DNA extraction and PCR amplification

For the association of larvae and adults both published and new sequences of a 541-bp-long fragment of the mitochondrial cytochrome oxidase *c* subunit I (mtCOI3-P) were used. Whole genomic DNA was extracted from part of the thorax or abdomen of adult or larval specimens, respectively, using the DNEasy Blood and Tissue Kit or QIAamp DNA Micro Kit (Qiagen) according to the manufacturer's protocol. PCR amplifications were carried out using primers S20 and Jerry (Simon *et al.* 1994; Pauls *et al.* 2006). DNA extraction and amplification were performed as outlined by Pauls *et al.* (2008) and Previšić *et al.* (2009).

For *D. krusniki* 71 published mtCOI3-P sequences from five populations (Previšić *et al.* 2014b; Table 2) were aligned. For *D. vernonensis* mtCOI3-P sequences of 3 adults (Previšić *et al.* 2014b; Viteček *et al.* 2015a; GenBank accession nos KC881524, KP793086 and KP793087) and 3 larvae (GenBank accession nos KT598011-KT598013) from one population were aligned. For *D. vespertinus* mtCOI3-P sequences of 5 adults and 3 larvae from two populations were aligned (Previšić *et al.* 2009 and new sequences, Table 4). New

sequences were edited manually using Geneious R7 (Kearse *et al.* 2012) and all were aligned using Muscle algorithm in Mega 6.0.1 (Tamura *et al.* 2013). Intraspecific uncorrected *p*-distances were calculated in Mega 6.0.1 for each species.

Results

Association of adults and larvae of *Drusus krusniki*

Comparison of haplotypes of the mtCOI3-P segment between a total of 28 adult males and 44 larvae belonging to 5 different populations of *Drusus krusniki* supported the association of adults and larvae. Within each particular population haplotypes differed by maximally 6 nucleotides between adults and larvae (Table 2; data from Previši *et al.* 2014b).

Description of the fifth instar larva of *Drusus krusniki*

Biometry—Body length of final instar larva 7.1–12.3 mm, head width 1.60–1.83 mm (n = 25).

Head—Head capsule coarsely granulated, almost circular in shape and hypognathous (Figs. 1, 3), dorsally with dark brown to blackish brown coloration and blackish muscle attachment spots. Vertex rounded (Fig. 6). Ventral parietalia sections, submentum, maxillolabial sclerites and premandibular areas yellowish brown (Figs. 2, 3). White ring around each eye (Fig. 3). In lateral view, head capsule with straight carina (approximately 0.09 mm wide) extending from anterior eye margin to frontomedian corner of parietale (Fig. 3, arrow). Head capsule with complete set of 18 pairs of primary setae (nomenclature by Wiggins 1998) and lacking any additional spines or bristles known to occur in other Drusinae larvae (e.g., some *Ecclisopteryx* spp.). However, posterior to each eye, with oval areas (diameter approximately 0.05×0.09 mm) of spinules (= small spines approximately 0.03 mm long) surrounding bases of setae #15 and 16 (Figs. 6, 7; white ovals). Such spinule areas occur in most members of the *Drusus bosnicus* Group *sensu* Marinkovi -Gospodneti (1971a). Frontoclypeus bell-shaped, with narrow central constriction (Fig. 1). Antennae located halfway between eye and anterior head margin at small conical dorsal outcrops of lateral carina (Figs. 1, 3, 19b), each consisting of 1 short cylindrical base and 1 short flagellum. At each parietal, 10 dorsal and 2 ventral primary setae present, with primary setae # 2, 3, 7, 9, 15, and 16 long and conspicuous (Figs. 1, 3). Each side of frontoclypeus with 6 primary setae, 3 of them along anterior border. Labrum brown, with setal brush and primary setae # 1–3 at anterolateral margins; on dorsal area, setation consisting of primary setae # 4–6 (Fig. 1). Ventral apotome elongated triangular, with almost parallel sides, bell-shaped, brown, postgenal suture approximately 45% of apotome length (Fig. 2). Mandibles black with dark brown tips, lacking terminal teeth along edges as well as lacking ridges in central concavity (Figs. 2, 19b).

Thorax—Pronotum blackish brown, very coarsely granulated (Figs. 3–5); at lateral and posterior pronotal surface, adjacent series of granuli creating ribbed structures (Fig. 5). Posterior margin thickened and darkly striped (Fig. 5). In frontal view, small dorsocentral pronotal notch present (Fig. 1, arrow). Pronotal transverse groove at end of anterior 3rd lacking. Pronotum type D (Table 3) in lateral view: median hump prominent, high, with

anterior-looking crest gradually fading laterally; posterior slope straight, anterior slope concave (Fig. 5). Two setal rows along anterior border of pronotum: (1) Dense fringe of short, curved, fine, yellow setae; (2) widely-spaced, continuous row of long, straight, dark setae meeting at anterior pronotal midline (Figs. 1, 3, 5); in total, 50–60 dark setae of varying lengths distributed over each pronotal half. In addition, pronotal surface covered by high number of tiny, pale, recumbent setae (Figs. 3, 4); spines present in other Drusinae (e.g., *D. trifidus*) lacking. Prosternite pentangular, pale yellow, darker (light brown) along posterior border; prosternal horn present.

Mesonotum completely covered by 2 dark brown sclerites with black brown muscle attachment spots and slightly paler posterior and lateral sections; their anterolateral corners, lateral and posterior margins darkly sclerotized (Fig. 4). Counts for mesonotal setae are as follows: anterior setal group *sa1*: 12–15, posterior group *sa2*: 20–25, lateral group *sa3*: 25–30.

Metanotum partially covered by 3 pairs of yellowish brown sclerites. Anterior metanotal sclerites (*sa1*) very large, broadly triangular, their intermediate separation distinctly smaller than the length of each of them; each with black anterior margin; approximately 15 setae per sclerite (Fig. 4). Row of setae present between small posteromedian sclerites (*sa2*); 15–20 setae per sclerite. Small setal group present between each lateral (*sa3*) and posteromedian sclerite (*sa2*); *sa3* sclerites crescent-shaped, dark brown at center and along dorsal border, with approximately 25–30 setae per sclerite, concentrated anteriorly (Fig. 4).

Legs yellowish brown with numerous setae on coxae, trochanters and femora; tibiae and tarsi with only small number of setae; on all femora several proximodorsal setae present (Figs. 8–10). Coxa, femur and tibia of each foreleg wider than those of mid- and hind legs. Setae present only at distal sections of trochanters. Additional setae present at both anterior and posterior faces of all femora; ventral trochanteral brush present at distal sections of fore- and midtrochanters. Forefemora each with 4 yellow ventral-edge setae, mid- and hind femora each with 3–4 dark ventral edge setae. Dorsal setae only at distal third of mid- and hind tibiae (Figs. 7–9).

Abdomen—First abdominal segment with 1 dorsal and 2 lateral fleshy protuberances (Fig. 4). Dorsal setal areas *sa1*, *sa2* and *sa3* fused, thereby creating continuous transverse row of setae anterior to dorsal protuberance until dorsal section of each lateral protuberance, sharply delimited basal sclerites present for about 50% of these setae. Without setal group posterior to dorsal protuberance (Fig. 4). Posterior sclerites at lateral protuberances absent. In front of each lateral protuberance, continuous band of anterolateral setae (as Fig. 22a) linking with each dorsal and ventral *sa3* setal group (Figs. 4, 11). On 1st abdominal sternum, ventral setal areas *sa1*, *sa2* and *sa3* fused, creating continuous field of setae; setal bases at central section of 1st abdominal sternum mostly small and inconspicuous except two larger bases near midline which occasionally fuse with 1–2 neighbouring smaller setal bases (Fig. 11). On 8th abdominal dorsum, two long and two very tiny posterodorsal setae (pds) present (Fig. 12, pds). Only 1 posterolateral seta on each half of 9th abdominal dorsum (Fig. 13, arrow).

All gills single filaments. Dorsal gills present at most from 2nd segment (presegmental position) to 6th segment (postsegmental position). Ventral gills ranging from 2nd (presegmental) to 7th segment (postsegmental). Dorsolateral gills ranging from 2nd (presegmental) to 4th segment (presegmental), and ventrolateral gills ranging from 2nd (postsegmental) to 4th segment (postsegmental). Lateral fringe extending from last quarter of 2nd to middle of 8th abdominal segments (Fig. 13). Yellowish brown sclerite on 9th abdominal tergum semicircular (Fig. 12); along its posterior border, 8 long and several shorter setae present, 2 of these long setae having position of central intermediate *c* setae (Fig. 12c). Anal prolegs of limnephilid type, yellowish brown, with light brown muscle attachment spots. Ventral sole plate with black dorsal rim (Fig. 13). Tips of anal claws dark brown, each with 1 small accessory hook (Fig. 13).

Case—Larval case 10.3–13.3 mm long (n= 25), slightly curved, slightly conical (width at anterior opening 2.4–3.5 mm and at posterior opening 1.6–2.5 mm), consisting of mineral particles (sand grains of mixed size; Fig. 14).

Association of adults and larvae of *Drusus vernonensis*

Haplotypes of the mtCOI3-P segment of 3 adult males and 3 larvae of *D. vernonensis* collected at the same site were completely identical. Moreover, the two different stages were sampled in different years (i.e. larvae in 2009 and adults in 2010). This confirms the conspecificity of the larvae and adults of *D. vernonensis* collected at the Pelister Mt. in Macedonia.

Description of the fifth instar larva of *Drusus vernonensis*

Biometry—Body length of final instar larva 10.1–10.3 mm, head width 1.53–1.60 mm (n= 2). All morphological characters identical to those of *D. krusniki* except as noted below.

Head—Head capsule granulated, reddish to medium brown (Figs. 15, 18, 20), with dark brown muscle attachment spots. Large spinule field covering the dorsal sections of parietalia and frontoclypeus dorsal the eyes (Fig. 16). Antennae originating from lateral carinae (Fig. 19a). Labrum yellowish brown (Fig. 15). Ventral apotome elongated triangular, postgenal suture approximately 25–30% of apotome length (Fig. 17).

Thorax—Pronotum dark brown, without pronotal notch (Figs. 15, 18). Pronotum type A (Table 3) in lateral view: low median hump, evenly rounded; steep posterior and flat anterior slope almost straight (Fig. 20). In addition to 50–60 dark setae of varying lengths distributed over each pronotal half, pronotal surface covered by (1) tiny, pale, recumbent setae (Fig. 20), and (2) thin, long, yellowish setae concentrated at anterior center of pronotum (Fig. 18, arrows). Prosternite very weakly sclerotized, pentangular, darkest along posterior border; prosternal horn present.

Mesonotum completely covered by 2 yellowish brown sclerites. Counts for mesonotal setae are as follows: anterior setal group *sa1*: 16–20, posterior group *sa2*: 22–26, lateral group *sa3*: 35–40.

Anterior metanotal sclerites large, broadly triangular, each with brown anterior margin; approximately 20 setae per sclerite. Setae between posteromedian sclerites scarce; 12–15 setae per posteromedian sclerite; *sa3* sclerites with approximately 25–30 setae per sclerite, concentrated anteriorly (Fig. 22). Legs yellowish brown; setation as in *D. krusniki*.

Abdomen—At 1st abdominal segment a small setal group may be present posterior to dorsal protuberance. Central section of 1st abdominal sternum with two larger bases near midline which occasionally fuse with 1–2 neighbouring smaller setal bases (Fig. 21). On 8th abdominal dorsum, two long, two intermediate and up to four minute posterodorsal setae (pds) present (Fig. 23, pds). Only 1 posterolateral seta on each half of 9th abdominal dorsum (Fig. 23, arrow).

All gills single filaments. Dorsal gills present at most from 2nd segment (presegmental position) to 6th segment (presegmental position). Ventral gills ranging from 2nd (presegmental) to 7th segment (presegmental). Dorsolateral gills present at 3rd segment only (presegmental), and ventrolateral gills ranging from 2nd (postsegmental) to 3rd segment (postsegmental). Lateral fringe starting at last quarter of 2nd segment (Fig. 22b), followed by a gap until mid-3rd segment (Fig. 22c); from this point extending to middle of 8th abdominal segment. Pale yellowish brown sclerite on 9th abdominal tergum semicircular (Fig. 23).

Case—Larval case 10.0–10.1 mm long ($n = 2$), slightly curved, slightly conical (width at anterior opening 2.7–2.9 mm and at posterior opening 1.9–2.0 mm), consisting of a mix of mineral particles (Fig. 24).

Association of adults and larvae of *Drusus vespertinus*

Comparison of the mtCOI sequences from one adult male *D. vespertinus* and three larvae collected at the Ribnik spring reach supports the association of larvae to this species as they differed by maximally 2 nucleotide positions (Table 4). Additionally, uncorrected *p*-distances between haplotypes from two known populations of *D. vespertinus* (0.9–1.3%; Table 4) agree with variability recorded in other *Drusus* endemics in the Western Balkans (e.g. Previši *et al.* 2009, 2014b).

Description of the fifth instar larva of *Drusus vespertinus*

Biometry—Body length of final instar larva 10.9–12.1 mm, head width 1.40–1.54 mm ($n = 8$). All morphological characters identical to those of *D. krusniki* except as noted below.

Head—Head capsule coarsely granulated, broadly oval and hypognathous (Fig. 25), dorsally with dark brown to blackish brown coloration and blackish muscle attachment spots. Vertex rounded (Figs. 25, 28). In lateral view, head capsule with straight carina starting with a conical, slightly inward-curved antennal base approximately 0.09 mm high; anterior of the antenna the carina decreases in height to 0.02 mm (Figs. 25–27). Posterior to each eye, with oval areas of spinules (= small spines approximately 0.03 mm long) (Fig. 27; white oval). Ventral apotome scutiform, anterior half parallel-sided, posterior half triangular; postgenal suture approximately 33% of apotome length.

Thorax—Blackish brown pronotal surface very coarsely granulated, covered by white recumbent setae (Figs. 28–30). In frontal view, small dorsocentral pronotal notch present (Fig. 25, arrow). Pronotum type C (Table 3) in lateral view: median hump prominent, high, with edged crest gradually fading laterally; posterior slope convex, anterior slope concave (Figs. 28, 29). In total, 45–50 dark setae of varying lengths distributed over each pronotal half.

Counts for mesonotal setae are as follows: anterior setal group *sa1*: 15–20, posterior group *sa2*: 20–25, lateral group *sa3*: 20–25 (Fig. 30).

Anterior metanotal sclerites with 15–20 setae per sclerite (Fig. 30), posteromedian sclerites with 15–18 setae and posteromedian sclerites with approximately 20–25 setae per sclerite, concentrated anteriorly.

Fore femora each with 5–6 yellow ventral-edge setae, mid- and hind femora each with 5–6 ventral edge setae. Dorsal setae only at distal third of mid- and hind tibiae.

Abdomen—At first abdominal segment a small setal group may be present posterior to dorsal protuberance. Setal bases at central section of 1st abdominal sternum mostly small and inconspicuous except two larger bases near midline which occasionally fuse with 1–2 neighbouring smaller setal bases (Fig. 31). On 8th abdominal dorsum, two long posterodorsal setae (pds) present (Fig. 32, pds). Only 1 posterolateral seta on each half of 9th abdominal dorsum (Fig. 32, arrow).

All gills single filaments. Dorsal gills present at most from 2nd segment (presegmental position) to 7th segment (presegmental position). Ventral gills ranging from 2nd (praesegmental) to 7th segment (postsegmental). Dorsolateral gills ranging from 2nd (presegmental) to 4th segment (presegmental), and ventrolateral gills ranging from 2nd (postsegmental) to 4th segment (postsegmental). Lateral fringe extending from last quarter of 2nd to first quarter of 8th abdominal segments. Medium brown sclerite on 9th abdominal tergum semicircular and medium brown (Fig. 32); setation as in *D. krusniki*.

Case—Larval case 9.9–10.8 mm long (n = 8), distinctly curved, slightly conical (width at anterior opening 2.5–3.9 mm and at posterior opening 1.7–2.3 mm), consisting of mineral particles (sand grains of mixed size; Fig. 33).

Morphological separation of fifth instar larvae of *Drusus krusniki*, *D. vernonensis* and *D. vespertinus* from other European Trichoptera

Within the framework of the limnephilid key by Waringer & Graf (2011) and Waringer *et al.* (2010), *Drusus krusniki*, *D. vernonensis* and *D. vespertinus* are separable from other species by the following features:

- gills consisting of single filaments only; dorsal gills present (Fig. 22);
- metanotum covered by three pairs of small sclerites (Figs. 4, 30);
- mandibles spoon-shaped (terminal teeth and central cavity ridges lacking; Figs. 19b, 26);

- head capsule with fields of spinules (= small spines approximately 0.03 mm long; Figs. 6, 7, 16, 27);
- anterior-row setae present near dorsal midline of pronotum (Figs. 1, 15, 25);
- dorsal-edge setae restricted to distal 3rd of mid- and hind tibiae (Figs. 9, 10);
- center of first abdominal sternum without large sclerotized patches or concentrations of fused basal sclerites of setae (Figs. 11, 21, 31).

Based on this character list, *Drusus krusniki*, *D. vernonensis* and *D. vespertinus* key together with the other members of the *Drusus bosnicus* Group which have spinule fields on the head (so far, only *Drusus ramae* Marinkovi -Gospodneti 1971b, also a member of the *Drusus bosnicus* Group, was found to lack such spinule fields). These species are easily separated by differences in vertex structure of the head (Figs. 28, 34), dorsal profile (Table 3; Figs. 5, 20, 28, 29), sculpturing (Fig. 5) and setation of the pronotum (Figs. 20, 35, 36) and presence/absence of a dorsocentral pronotal notch (Figs. 1, 25). A synopsis of differentiation characters in the *Drusus bosnicus* Group with spinule fields on the head is given in Table 5.

Discussion

Larval morphology of the Western Balkans *Drusus* endemics

The three larvae described in the present paper bear morphological characters common to members of the *Drusus bosnicus* Group, such as the fields of spinules of various sizes posterior to each eye. Marinkovi -Gospodneti (1971a) assigned *D. bosnicus*, *D. klapaleki*, *D. plicatus* Radovanovi 1942, *D. radovanovici* and *D. ramae* to this group of species, based on similarities of main structures of the male genitalia. Later, *D. krusniki*, *D. medianus*, *D. septentrionis* and *D. vespertinus* were added (see discussion in Ku ini et al. 2011a). Moreover, the morphology of some *Drusus* species described in the recent years in Albania indicates close relatedness with the *Drusus bosnicus* Group (Oláh 2010, 2011) in adult morphology; larvae of these species are still unknown. Furthermore, the newly described endemic *D. crenophylax* and also *D. serbicus* Marinkovi -Gospodneti 1971a share some of the common larval morphological features of this group (Vitecek et al. 2015a, Waringer et al. 2015). In all these species, including the three described herein, the spinule fields on the larval head are only absent in *D. ramae*, but are present in *D. serbicus* (Waringer et al. 2015) and the hitherto unknown larvae of *Drusus krusniki*, *D. vernonensis* and *D. vespertinus*. Thus, the spinules most probably represent a synapomorphic character in this Group, and may also be present in the unknown larvae of other species.

D. vernonensis is unique in that the spinule field covers large areas of the parietalia and the frontoclypeus dorsally of eye level; in all other known larvae, the spinule fields are small oval areas posterior of the eyes (Ku ini et al. 2008, 2010, 2011a, 2011b, 2015, Vitecek et al. 2015a). Additionally, larval *D. vernonensis* have other unique characters (Table 3) that are inconsistent with characters usually observed in *Drusus bosnicus* Group taxa. Interestingly, these differences are consistent with differences in male adult genitalia morphology which suggest a close relationship of *D. vernonensis* with species of the *Drusus discophorus* Group (Malicky 1989). However, the larval stages of the majority of species of the latter group are still unknown. Thus, a more comprehensive discussion on the

significance of the presented larval morphological characters in “fine scale” Drusinae systematics is not possible at this point. Nevertheless, our findings are consistent with previous studies implying a close link of larval morphology, feeding ecology and phylogenetic relationships within Drusinae in general (Pauls *et al.* 2008, Graf *et al.* 2009), and more specifically within the carnivorous clade (Vitecek *et al.* 2015b). Hence, the improvement of the knowledge of the larval taxonomy presented in the current paper adds valuable information to an overall puzzle within the largest clade of Drusinae species, the epilithic grazers.

Distribution and ecology of *Drusus krusniki*, *D. vernonensis* and *D. vespertinus*

Species included in to the *Drusus bosnicus* Group are all endemic to the Dinaric Balkans, i.e. to the ecoregions ER5 (Dinaric Western Balkan) and ER6 (Hellenic Western Balkan) (*sensu* Illies 1978; Table 5). *Drusus krusniki* is endemic to the Western Balkans, inhabiting the southeastern parts of the Dinaric Alps in Montenegro and Kosovo (Graf *et al.* 2008, Previši *et al.* 2014b, Gashi *et al.* 2015) and the Prokletje Mts. in Albania (Oláh 2010). *D. vespertinus* is a (micro-) endemic to the Dinaric Alps, (ER 5), known from the western part of Bosnia and Herzegovina (Marinkovi -Gospodneti 1976) and Una River spring in Croatia (Kušini *et al.* 2014). *D. vernonensis* was considered endemic to the Vernon Mountains in Greece (Malicky 2005b), but the current data show that its distribution extends northwards to the Pelister Mt. in Macedonia. However, it is an endemic species of the ecoregion 6 (Graf *et al.* 2008).

All these species prefer spring areas and the headwaters of cold, oxygen-rich streams with high to moderate currents at high altitudes in the mountains. *D. vespertinus* occurs in large karstic springs, like the spring of the river Ribnik, a tributary of the river Sana (Marinkovi -Gospodneti, 1976), as well as the Una River spring (Kušini *et al.* 2014). Mean annual water temperatures obtained by permanently exposed data loggers for sites inhabited by *D. vernonensis* (e.g., Pelister, 1805 m a.s.l, Macedonia) were 3.78°C (annual range 2.07–6.84°C) and for *D. krusniki* (e.g., Montenegro: Ibar spring, 1256 m a.s.l.; Alipaša's springs, 932 m a.s.l.) 6.22°C (annual range 5.88–8.20°C) and 6.13°C (annual range 3.60–14.35°C), respectively.

Mandible morphology of *Drusus krusniki*, *D. vernonensis* and *D. vespertinus* larvae suggests a grazing lifestyle, with larvae feeding on biofilms and epilithic algae. The adults and larvae of *Drusus krusniki*, *D. vernonensis* and *D. vespertinus* were collected in late May in 2009, June and in early July. The latter species may be on the wing as early as late March since the holotype, paratypes and allotypes were collected on 26 March 1968 (Marinkovi -Gospodneti, 1976). The holotype of *D. krusniki* was collected at the end of May (Malicky 1981) whereas only few samples of *D. vernonensis* exist which were obtained in June and July (Malicky 1989, 2005b). This is in accordance with the reported short summer flight period of *D. vernonensis* by Graf *et al.* (2008).

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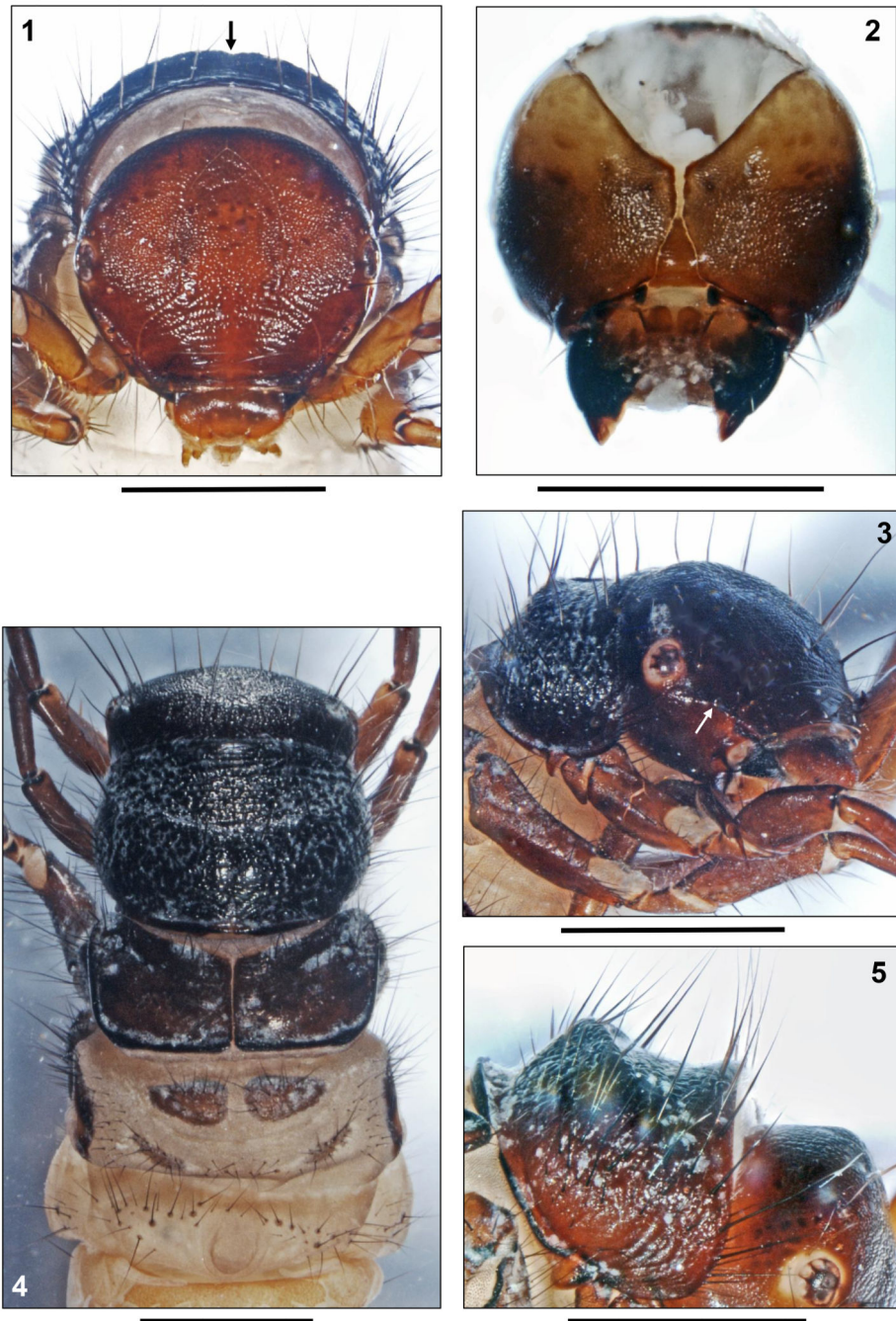
also funded by the Croatian Ministry of Science, Education and Sports (Project Nos. 119–1193080–1206 and 119–1193080–3076) and the University in Zagreb.

References

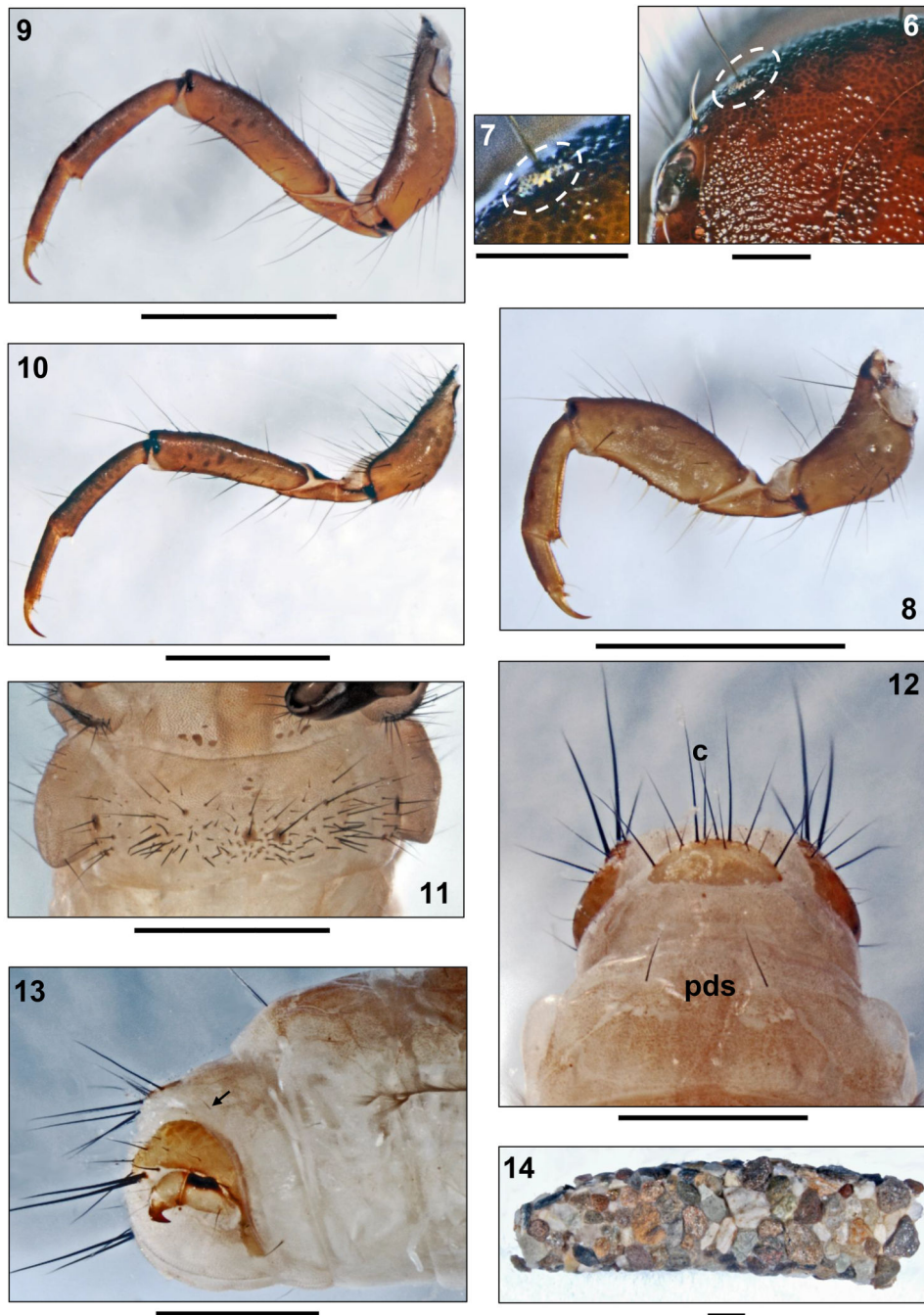
- AQEM Consortium. Manual for the Application of the AQEM System. A Comprehensive Method to Assess European Streams Using Benthic Macroinvertebrates, Developed for the Purpose of the Water Framework Directive. 2002. Version 1_0
- Bálint M, Domisch S, Engelhardt CHM, Haase P, Lehrian S, Sauer J, Theissing K, Pauls SU, Nowak C. Cryptic biodiversity loss linked to global climate change. *Nature Climate Change*. 2011; 1:313–318.
- Barbour, MT.; Gerritsen, J.; Snyder, BD.; Stribling, JB. Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, benthic macroinvertebrates and fish. 2. U.S. Environmental Protection Agency; Washington, D.C.: 1999. xii + 326 pp.
- Barbour, MT.; Yoder, CO. The multimetric approach to bioassessment, as used in the United States. In: Wright, JF.; Sutcliffe, DW.; Furse, MT., editors. *Assessing the Biological Quality of Fresh Waters: RIVPACS and Other Techniques*. Freshwater Biological Association; Ambleside, UK: 2000. p. 281–292.
- Gashi A, Ibrahim H, Grapci-Kotori L, Sejdiu N, Bislimi K. New Records of *Drusus siveci* Malicky, 1981 (Trichoptera, Limnephilidae, Drusinae) from the Balkan Peninsula, with Ecological Notes. *Acta Zoologica Bulgarica*. 2015; 67:259–264.
- Graf, W.; Murphy, J.; Dahl, J.; Zamora-Muñoz, C.; López-Rodríguez, MJ. Trichoptera. In: Schmidt-Kloiber, A.; Hering, D., editors. *Distribution and Ecological Preferences of European Freshwater Organisms*. Vol. 1. Pensoft Publishers; Sofia, Moscow: 2008. p. 388
- Graf W, Waringer J, Pauls SU. A new feeding group within larval Drusinae (Trichoptera: Limnephilidae): The *Drusus alpinus* Group sensu Schmid, 1956, including larval descriptions of *Drusus franzi* Schmid, 1956, and *Drusus alpinus* (Meyer-Dür, 1875). *Zootaxa*. 2009; 2031:53–62. [PubMed: 27069350]
- Illies, J. *Limnofauna Europaea. A Checklist of the Animals Inhabiting European Inland Waters, with an Account of their Distribution and Ecology*. 2nd Edition. Gustav Fischer Verlag; Stuttgart: 1978. p. 552
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 2012; 28:1647–1649. [PubMed: 22543367]
- Klapálek F. Prilozi k poznavanju faune Trichoptera i Neuroptera Bosne i Hercegovine. *Glasnik zemaljskog muzeja u Sarajevu*. 1899; 11:323–337.
- Kuini M, Previši A, Gottstein-Matoec S, Hrašovec B, Stani-Koštroman S, Pernek M, Deli A. Description of the larvae of *Drusus radovanovici septentrionis* Marinkovi-Gospodneti, 1976 and *Drusus croaticus* Marinkovi-Gospodneti, 1971 (Trichoptera: Limnephilidae) from Bosnia and Herzegovina, and Croatia. *Zootaxa*. 2008; 1783:1–17.
- Kuini M, Previši A, Stani-Koštroman S, Franjevi M, Šeri Jelaska L, Deli A, Posilovi H. Description of the larvae of *Drusus ramae* Marinkovi-Gospodneti, 1971 and *Drusus medianus* Marinkovi-Gospodneti, 1976 (Trichoptera: Limnephilidae) with some genetic data, distribution, ecological, faunal and conservation notes. *Zootaxa*. 2010; 2484:1–24.
- Kuini M, Previši A, Graf W, Šeri Jelaska L, Stani-Koštroman S, Waringer J. Larval description, genetic and ecological features of *Drusus radovanovici radovanovici* Marinkovi-Gospodneti, 1971 (Trichoptera: Limnephilidae) with some phylogenetic and taxonomic data on the *bosnicus* group in the Balkan Peninsula. *Deutsche Entomologische Zeitschrift*. 2011a; 58:136–153.
- Kuini M, Previši A, Stani-Koštroman S, Graf W, Franjevi M, Posilovi H, Waringer J. Morphological and ecological features of *Drusus* larvae from the *bosnicus* group on the Balkan Peninsula with description of the larva of *Drusus klapaleki* Marinkovi-Gospodneti, 1976. *Zoosymposia*. 2011b; 5:244–254.
- Kuini M, Deli A, Uk R, Previši A, Mihoci I, Žganec K, Cerjanec D, Vučković I. The first finding of *Drusus bosnicus* Group (Insecta, Trichoptera, Limnephilidae) in Croatia with some notes on

- diversity, distribution and ecology of genus *Drusus* in Croatia and in Dinaric karst of the Balkan Peninsula. *Natura Croatia*. 2014; 23:265–377.
- Ku ini M, Previši A, Graf W, Mihoci I, Šoufek M, Stani -Koštroman S, Suvad L, Vitecek S, Waringer J. Larval description of *Drusus bosnicus* Klapálek 1899 (Trichoptera: Limnephilidae), with distributional, molecular and ecological features. *Zootaxa*. 2015; 3957:85–97. [PubMed: 26249056]
- Kumanski K. Die Unterfamilie Drusinae (Trichoptera) in Bulgarien. *Tijdschrift voor Entomologie*. 1973; 116:107–121.
- Malicky H. Weiteres Neues über Köcherfliegen aus dem Mediterrangebiet (Trichoptera). *Entomofauna*. 1981; 2:335–355.
- Malicky H. Ein neuer *Drusus* aus Nord-Griechenland (Trichoptera, Limnephilidae). *Entomologische Zeitung*. 1989; 99:303–304.
- Malicky, H. Atlas of European Trichoptera. 2nd Edition. Springer; 2004. p. 359
- Malicky H. Ein kommentiertes Verzeichnis der Köcherfliegen (Trichoptera) Europas und des Mediterrangebietes. *Linzer biologische Beiträge*. 2005a; 37:533–596.
- Malicky H. Die Köcherfliegen Griechenlands. *Denisia*. 2005b; 17:1–240.
- Marinkovi -Gospodneti M. The species of the genus *Drusus* in Yugoslavia. *Godišnjak biološkog Instituta Univerziteta u Sarajevu*. 1971a; 24:105–109.
- Marinkovi -Gospodneti M. New species of Trichoptera from a Bosnia and Herzegovina. *Bulletin Scientifique (Section A)*. 1971b; 15(5–6):144–145.
- Marinkovi -Gospodneti M. In: Malicky, H., editor. The differentiation of *Drusus* species of the group *bosnicus*; Proceedings of the First International Symposium on Trichoptera; 1976; The Hague: Dr. W. Junk; p. 77–85.
- Oláh J. New species and new records of Palaearctic Trichoptera in the material of the Hungarian Natural History Museum. *Annales historico-naturales Musei nationalis hungarici*. 2010; 102:65–117.
- Oláh J. New species and records of Balkan Trichoptera. *Folia historico-naturalia Musei Matraensis*. 2011; 35:111–121.
- Oláh J, Chvojka P, Coppa G, Godunko JR, Lodovici O, Majecka K, Majecki J, Szcz sny B, Urbani G, Valle M. Limnephilid taxa revised by speciation traits: *Rhadicoleptus*, *Isogamus*, *Melampophylax* genera, *Chaetopteryx rugulosa*, *Psilopteryx psorosa* species groups, *Drusus bolivari*, *Annitella kosciuszki* species complexes (Trichoptera: Limnephilidae). *Opuscula Zoologica Budapest*. 2015; 46:3–117.
- Pauls SU, Lumbsch HT, Haase P. Phylogeography of the montane caddisfly *Drusus discolor*: Evidence for multiple refugia and periglacial survival. *Molecular Ecology*. 2006; 15:2153–2169. [PubMed: 16780432]
- Pauls SU, Graf W, Haase P, Lumbsch HT, Waringer J. Grazers, shredders and filtering carnivores – The evolution of feeding ecology in Drusinae (Trichoptera: Limnephilidae): Insights from a molecular phylogeny. *Molecular Phylogenetics and Evolution*. 2008; 46:776–791. [PubMed: 18171625]
- Previši A, Walton C, Ku ini M, Mitrikeski PT, Kerovec M. Pleistocene divergence of Dinaric *Drusus* endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. *Molecular Ecology*. 2009; 18:634–647. [PubMed: 19175506]
- Previši A, Graf W, Vitecek S, Ku ini M, Bálint M, Keresztes L, Pauls SU, Waringer J. Cryptic diversity of caddisflies in the Balkans: the curious case of *Ecclisopteryx* species (Trichoptera: Limnephilidae). *Arthropod Systematics and Phylogeny*. 2014a; 72:309–329. [PubMed: 25810791]
- Previši A, Schnitzler J, Ku ini M, Graf W, Ibrahim H, Kerovec M, Pauls SU. Microscale vicariance and diversification of western Balkan caddisflies linked to karstification. *Freshwater Science*. 2014b; 33:250–262. [PubMed: 27042385]
- Radovanovi M. Über zwei neue Trichopteren-Arten aus Mazedonien. *Zoologischer Anzeiger*. 1942; 140:183–190.
- Schmid F. La sous-famille des Drusinae (Trichoptera, Limnephilidae). *Memoires de l'Institut Royal des Sciences Naturelles de Belgique*, 2. Serie. 1956; 55:1–92.

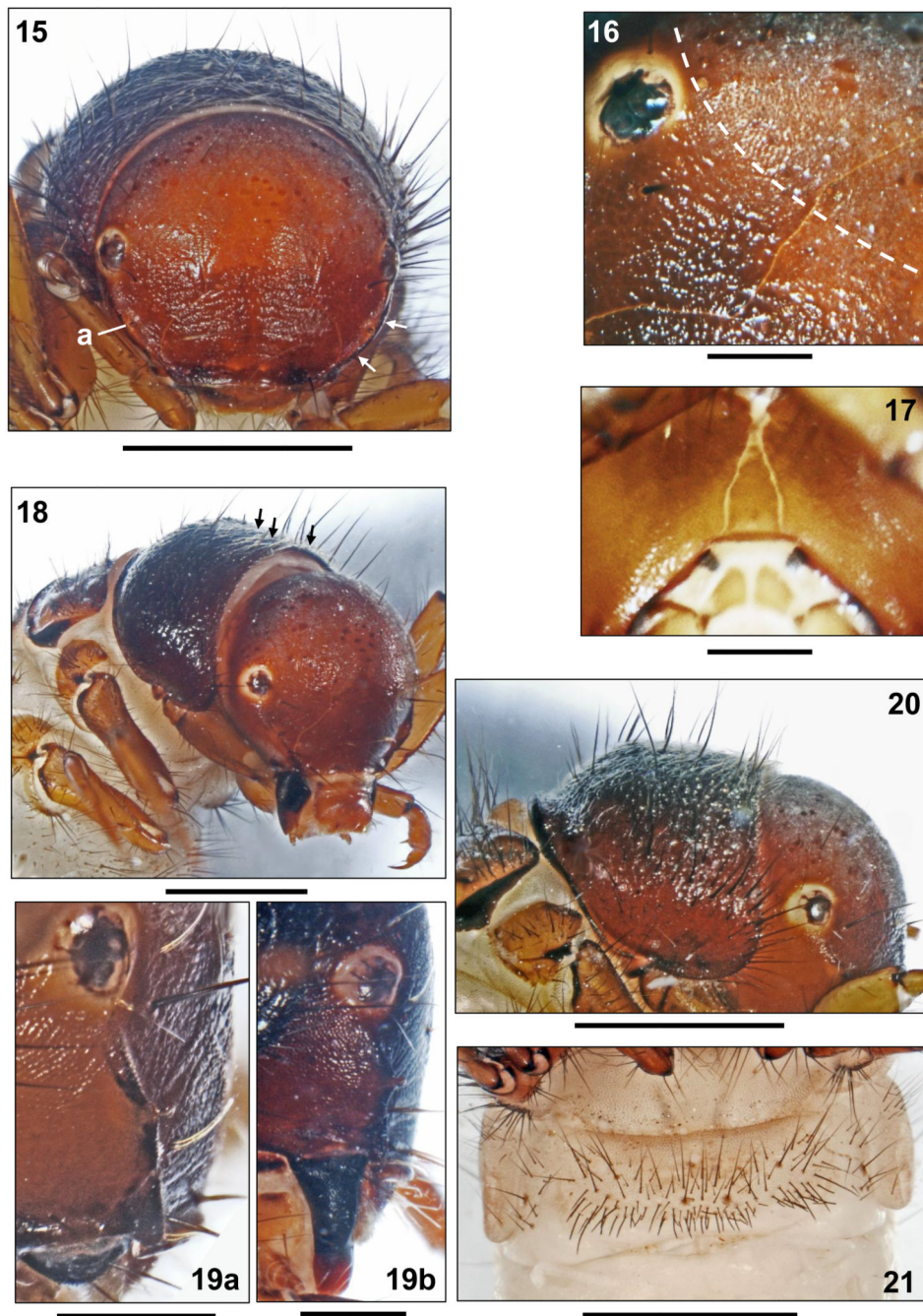
- Simon C, Frati F, Beckenbach A. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*. 1994; 87:651–701.
- Sipahiler, F. *Hadimina torosensis*, new genus and new species of Drusinae from southern Turkey (Trichoptera: Limnephilidae). (Proceedings of the 10th International Symposium on Trichoptera); 2002; Nova Supplementa Entomologica; p. 239-248.
- Stephens, JF. Illustrations of British entomology; or, a synopsis of indigenous insects: containing their generic and specific distinctions; with an account of their metamorphoses, times of appearance, localities, food, and economy, as far as practicable. Vol. VI. Mandibulata. Baldwin & Cradock; London: 1836-1837. p. 240
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*. 2013; 30:2725–2729. [PubMed: 24132122]
- Vitecek S, Previši A, Kušini M, Bálint M, Keresztes L, Waringer J, Pauls SU, Malicky H, Graf W. Description of a unique *Wormaldia* from Sardinia and a new *Drusus* from the Western Balkans (Trichoptera: Philopotamidae; Limnephilidae). *ZooKeys*. 2015a; 496:85–103. [PubMed: 25931956]
- Vitecek S, Graf W, Previši A, Kušini M, Oláh J, Bálint M, Keresztes L, Pauls SU, Waringer J. A hairy case: The evolution of filtering carnivorous Drusinae (Limnephilidae, Trichoptera). *Molecular Phylogenetics and Evolution*. 2015b; 93:249–260. [PubMed: 26265260]
- Waringer J, Graf W, Pauls SU, Previši A, Kušini M. A larval key to the Drusinae species of Austria, Germany, Switzerland and the dinaric western Balkan. *Denisia*. 2010; 29:383–406. [PubMed: 26985170]
- Waringer, J.; Graf, W. Atlas of Central European Trichoptera Larvae. Erik Mauch Verlag; Dinkelscherben: 2011. p. 468
- Waringer J, Graf W, Balint M, Kušini M, Pauls SU, Previši A, Keresztes L, Ibrahimi H, Živi I, Bjelanovi K, Krpa V, Vitecek S. Larval morphology and phylogenetic position of *Drusus balcanicus*, *D. botosaneanui*, *D. serbicus* and *D. tenellus* (Trichoptera: Limnephilidae: Drusinae). *European Journal of Entomology*. 2015; 112:344–361. [PubMed: 26997882]
- Wiggins, GB. Larvae of the North American Caddisfly Genera (Trichoptera). 2nd Edition. University of Toronto Press; Toronto: 1998. p. 457

**FIGURES 1-5.**

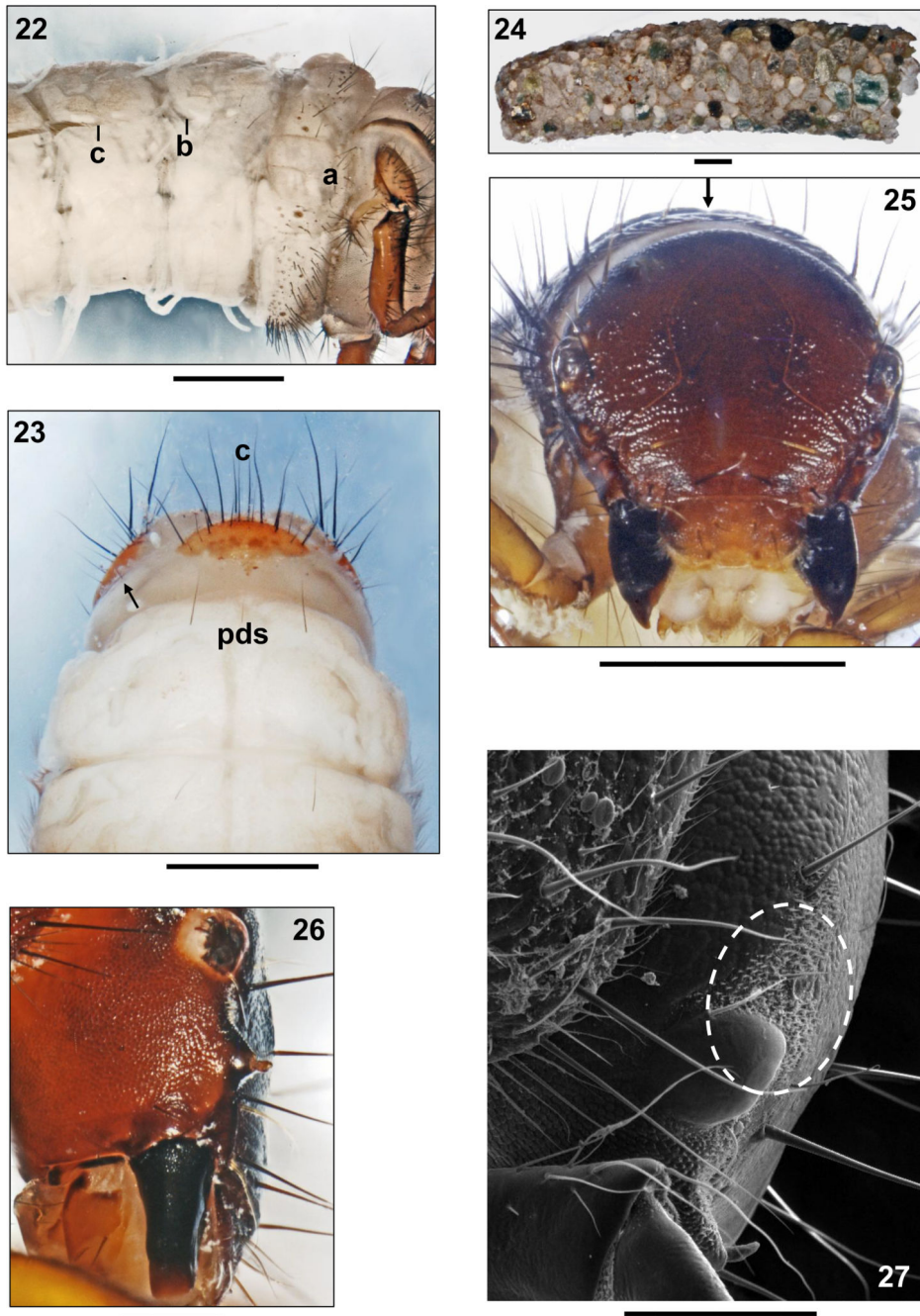
Drusus krusniki Malicky 1981, 5th instar larva. **1**, Head, dorsal (arrow: pronotal notch). **2**, Head, ventral. **3**, Head and prothorax, right anterolateral (arrow: antenna on lateral carina). **4**, Head, thorax and first abdominal segment, dorsal. **5**, Pronotum, right lateral. Scale bars: 1 mm.

**FIGURES 6–14.**

Drusus krusniki Malicky 1981, 5th instar larva. **6**, Head, frontal (dotted oval: spinule field). **7**, Head, detail of spinule field (dotted oval). **8**, Right foreleg, anterior. **9**, Right mid leg, anterior. **10**, Right hind leg, anterior. **11**, First abdominal sternum, ventral. **12**, 8th and 9th abdominal terga, dorsal (pds: posterodorsal setae; c: position of c setae). **13**, Tip of abdomen, right lateral view (arrow: posterolateral seta). **14**, Larval case, right lateral. Scale bars: 1 mm (except Figs. 6, 7: 0.25mm).

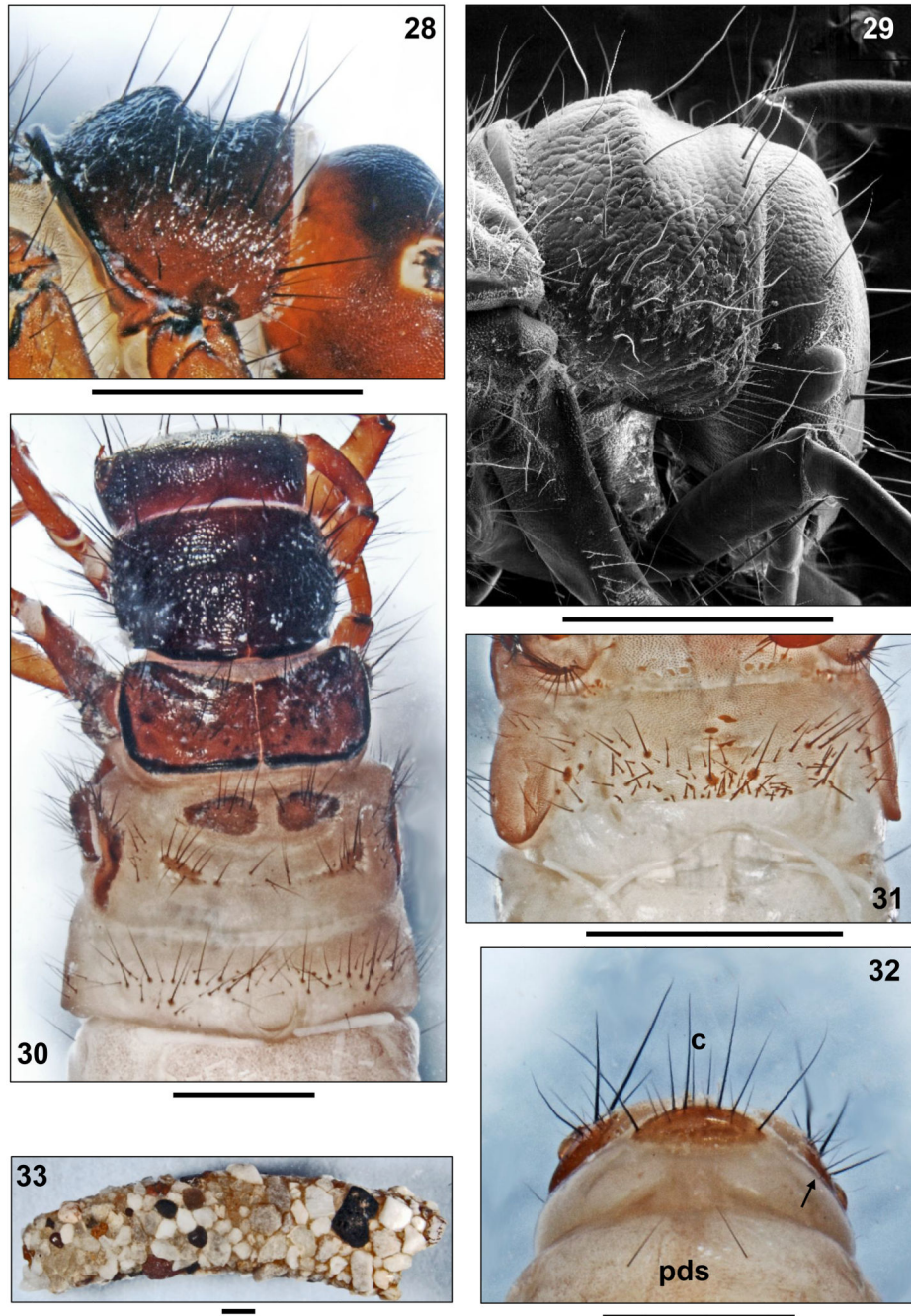
**FIGURES 15–21.**

Drusus vernonensis Malicky 1989, 5th instar larva. **15**, Head, dorsal (arrows: left carina; a: antenna). **16**, Head, detail of spinule field (dotted oval). **17**, Head, ventral, submentum. **18**, Head, pro- and mesothorax, right anterolateral (arrows: thin, long, yellowish setae). **19a**, Head, lateral, carina with antenna. **19b**, *Drusus krusniki* Malicky 1981, 5th instar larva. Head, lateral, carina with antenna. **20**, *Drusus vernonensis* Malicky 1989, 5th instar larva. Head and pronotum, right lateral. **21**, First abdominal sternum, ventral. Scale bars: 1 mm (except Figs. 16, 17, 19: 0.25mm).

**FIGURES 22–27.**

FIGURES 22–24. *Drusus vernonensis* Malicky 1989, 5th instar larva. **22**, Metathorax and anterior half of abdomen, right lateral (a: row of setae anterior of lateral protuberance; b: small portion of lateral fringe on 2nd segment, c: start of lateral fringe on 3rd segment). **23**, 8th and 9th abdominal terga, dorsal (pds: posterodorsal setae; c: position of c setae; arrow: posterolateral seta). **24**, Larval case, right lateral. **FIGURES 25–27.** *Drusus vespertinus* Marinkovi 1976, 5th instar larva. **25**, Head, dorsal (arrow: pronotal notch). **26**, Head,

lateral, carina with antenna. **27**, Head, detail of spinule field (dotted oval). Scale bars: 1 mm (except Figs. 26, 27: 0.25 mm).

**FIGURES 28–33.**

Drusus vespertinus Marinkovi 1976, 5th instar larva. **28, 29**, Pronotum, right lateral. **30**, Head, thorax and first abdominal segment, dorsal. **31**, First abdominal sternum, ventral. **32**, 8th and 9th abdominal terga, dorsal (pds: posterodorsal setae; c: position of c setae; arrow: posterolateral seta). **33**, Larval case, right lateral. Scale bars: 1 mm.

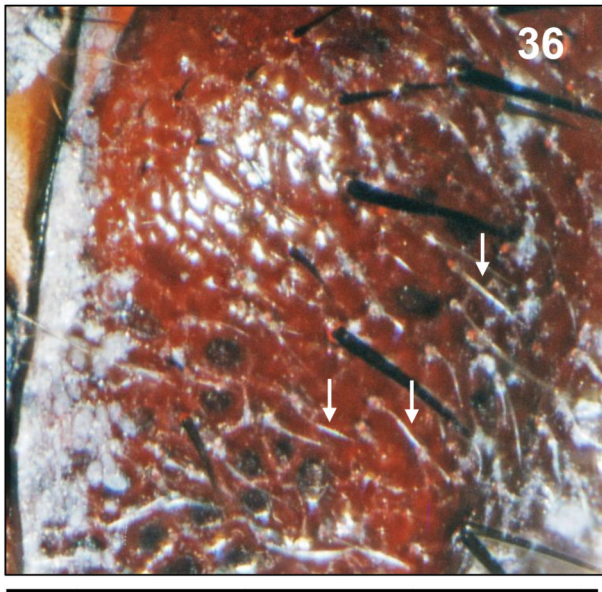
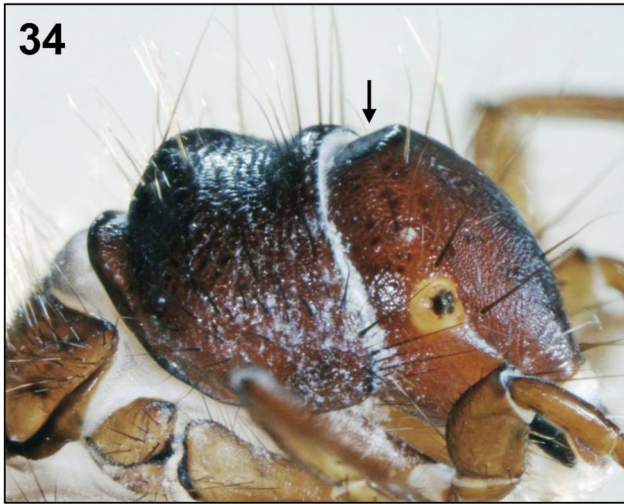


FIGURE 34–36.

FIGURE 34. *Drusus bosnicus* Klapálek 1899, 5th instar larva. Head and pronotum, right lateral (arrow: vertex flattened). **FIGURE 35.** *Drusus radovanovici* Marinkovi 1970, 5th instar larva. Pronotum, right lateral (arrows: thin long yellowish setae). **FIGURE 36.** *Drusus medianus* Marinkovi 1976, 5th instar larva. Detail of pronotum, right lateral (arrows: white recumbent setae). Scale bars: 1 mm (except Fig. 36: 0.5 mm).

Table 1
Information on larval *Drusus* specimens (fifth instar larvae) from the Western Balkans used in this paper.

Species	Locality (country, name)	Latitude (°)	Longitude (°)	No of larvae examined	Collector
<i>Drusus bosniacus</i>	(BIH) Paljanska Miljačka spring	N 43.9234	E 18.5973	1	Ku ini & Previši
<i>Drusus erenophylax</i>	(BIH) Cvreka river	N 44.5489	E 17.3927	2	Dmitrovi & Šukalo
<i>Drusus klapaleki</i>	(BIH) Toplica spring	N 43.5943	E 18.4949	2	Graf & Previši
<i>Drusus krusniki</i>	(MNE) Alipaša's springs, Gusinje	N 42.5501	E 19.8259	16	Previši
<i>Drusus krusniki</i>	(MNE) Ibar spring	N 42.7975	E 20.0904	9	Previši
<i>Drusus medianus</i>	(BIH) Plava voda spring	N 44.2303	E 17.6717	1	Ku ini & Previši
<i>Drusus radovanovici</i>	(BIH) Suijeska NP, stream close to ememo	N 43.2650	E 18.5928	1	Graf & Previši
<i>Drusus septentrionis</i>	(BIH) Bistrica spring, Livno	N 43.8325	E 17.0084	2	Ku ini
<i>Drusus serbicus</i>	(SRB) Golija Mt, spring Ilinac	N 43.3333	E 20.2819	3	Bjelanovi, Ku ini & Živi
<i>Drusus vernonensis</i>	(MKD) Pelister, tributary of Caparska reka	N 41.0148	E 21.1741	2	Previši
<i>Drusus vespertinus</i>	(BIH) Ribnik, spring reach	N 44.4655	E 16.8380	8	Ku ini & Previši

Table 2

Minimal and maximal values of nucleotide differences and uncorrected *p*-distance of the mtCOI3-P gene segment within populations of *Drusus krusniki* (data from Previšić *et al.* 2014b).

<i>Drusus krusniki</i> populations	Number of adult males	Number of larvae	Number of nucleotide difference (min-max)	Uncorrected <i>p</i> -distance (min-max)	GenBank accession nos
Alipaša springs	11	2	0-1	0-0.002	KC881401 - KC881413
Biogradska rijeka	8	5	0-6	0-0.011	KC881414 - KC881426
Bukovica spring	2	14	0-2	0-0.004	KC881427 - KC881442
Ibar spring	1	14	0-4	0-0.004	KC881443 - KC881457
Murinska rijeka	6	8	0	0	KC881458 - KC881471

Table 3Pronotum types observed in the *Drusus bosnicus* group.


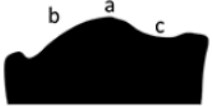
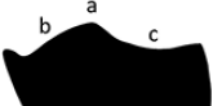

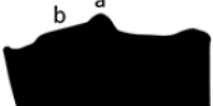
Pronotum type	Lateral right profile of pronotum	Description
Type A		Low median hump, evenly rounded (a); posterior (b) and anterior slope (c) almost straight.
Type B		Median hump prominent, high, smoothly rounded (a); posterior slope convex (b); anterior slope concave (c).
Type C		Median hump prominent, high, with crest gradually fading laterally (a); posterior slope convex (b); anterior slope concave (c).
Type D		Median hump prominent, high, with crest gradually fading laterally (a); posterior slope straight (b); anterior slope concave (c).
Type E		Median hump annular, crest-like, highest at dorsal center, gradually fading laterally (a); with semicircular step (b) directly posterior of crest center and in front of posterior pronotal rim.

Table 4

Number of nucleotide differences (below diagonal) and uncorrected p -distance (above diagonal) of the mtCOI3-P gene segment within and between populations of *Drusus vespertinus*. For details on specimen sampling sites see Table 1.

Population	DvRIM1	DvRL1	fDvs0103L	fDvs0104L	fDvs0202M	fDvs0203F	fDvs0205F	fDvs0206F	GenBank access. nos
Ribnik spring, BIH	DvRIM1	0.002	0.004	0.004	0.013	0.013	0.013	0.013	KT598004
Ribnik spring, BIH	DvRL1	1	0.002	0.002	0.011	0.011	0.011	0.011	FJ002685
Ribnik spring, BIH	fDvs0103L	2	1	0.004	0.013	0.013	0.013	0.013	KT598005
Ribnik spring, BIH	fDvs0104L	2	1	2	0.013	0.013	0.009	0.009	KT598006
Una spring, HRV	fDvs0202M	7	6	7	7	0	0.004	0.007	KT598007
Una spring, HRV	fDvs0203F	7	6	7	7	0	0.004	0.007	KT598008
Una spring, HRV	fDvs0205F	7	6	7	7	2	2	0.007	KT598009
Una spring, HRV	fDvs0206F	7	6	7	7	4	4	4	KT598010

Table 5

Synopsis of characters separating the currently known Drusinae larvae (5th instars) which share the following group morphomatrix: spoon-shaped mandibles; with spinule areas at the head capsule; anterior-row setae present near dorsal pronotal midline; dorsal gills present; dorsal edge setae restricted to distal third of mid- and hind tibiae; basal sclerites of setae at first abdominal sternum not fusing into sclerotized plates or multilobed patterns. Pronotum types are defined in Table 3. Distribution in ecoregions according to Illies 1978; ER5 – Dinaric Western Balkans, ER6 – Hellenic Western Balkans.

Species/character	Head with flat vertex (Fig. 34)?	Pronotum with thin, long, yellowish setae (Figs. 20, 35)?	Pronotum with numerous white, recumbent setae (Fig. 36)?	Pronotum type (Table 3)	Dorsocentral pronotal notch present (Figs. 1, 25)?	Distribution (ecoregions sensu Illies 1978)	References
<i>Drusus bosniacus</i>	yes	no	yes	C	yes	ER5	Ku ini et al. 2015
<i>Drusus crenophylax</i> ¹	no	no	yes	B	yes	ER5	Vitecek et al. 2015a
<i>Drusus klapaleki</i> ¹	no	no	yes	B	yes	ER5	Ku ini et al. 2011b
<i>Drusus krusniki</i>	no	no	yes	D	yes	ER5, ER6	this paper
<i>Drusus medianus</i>	no	no	yes	B	no	ER5	Ku ini et al. 2010
<i>Drusus radovanovici</i>	no	yes	no	B	no	ER5	Ku ini et al. 2011a
<i>Drusus septentrionis</i>	no	no	no	C	yes	ER5	Ku ini et al. 2008
<i>Drusus serbicus</i>	no	no	yes	E	yes	ER5	Waringer et al. 2015
<i>Drusus vernonensis</i>	no	yes	yes	A	no	ER6	this paper
<i>Drusus vespertinus</i>	no	no	yes	C	yes	ER5	this paper

¹ In *Drusus klapaleki*, white recumbent setae are distributed over the whole pronotal surface (Ku ini et al. 2011b) whereas in *D. crenophylax* those setae are lacking in a semicircular area anterior of the pronotal dorsal hump (Vitecek et al. 2015a).