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The *Rhyacophila fasciata* Group in Croatia and Bosnia and Herzegovina: *Rhyacophila f. fasciata* Hagen 1859 and the description of two new subspecies, *Rhyacophila fasciata delici* Kučinić & Valladolid (ssp. nov.) from Croatia and Bosnia and Herzegovina and *Rhyacophila fasciata viteceki* Valladolid & Kučinić (ssp. nov.) from Bosnia and Herzegovina (Trichoptera: Rhyacophilidae)

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Abstract

We present the description of two new subspecies of the *Rhyacophila fasciata* Group: *Rhyacophila fasciata delici* Kučinić & Valladolid (ssp. nov.), broadly distributed in Croatia and present also in Bosnia and Herzegovina, and *R. fasciata viteceki* Valladolid & Kučinić (ssp. nov.), found in Bosnia and Herzegovina. Our study of the morphology of adults, as well as our analysis of the barcode region of the mitochondrial cytochrome oxidase I (mtCOI) gene and geographical distribution confirm the differences of the two new subspecies with the nominal species *R. f. fasciata,* also found in both countries.

Key words: Taxonomic revision, new subspecies, identification, mitochondrial COI, distribution

Sažetak

U radu prikazujemo opis dviju novih podvrsta iz *Rhyacophila fasc*iata Grupe: *Rhyacophila fasciata delici* Kučinić & Valladolid (ssp. nov.) rasprostranjenu u Hrvatskoj i Bosni i Hercegovini i *R. fasciata viteceki* Valladolid & Kučinić (ssp. nov.), utvrđenu u Bosni i Hercegovini. Naša studija morfologije adulata, kao i barkod regije mitohondrijalnog gena citokrom oksidaze podjedinice I (mtCOI) te geografske rasprostranjenosti potvrđuje razlike dviju novih podvrsta s nominalnom vrstom *R. f. fasciata*, također utvrđenu u obje zemlje.

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Ključne riječi: Taksonomska revizija, nove podvrste, određivanje, mitohondrijalni COI, rasprostranjenost

Resumen

Se presenta la descripción de dos nuevas subespecies del Grupo *Rhyacophila fasciata*: *Rhyacophila fasciata delici* Kučinić & Valladolid (ssp. nov.), ampliamente distribuida en Croacia y presente también en Bosnia y Herzegovina y *R. fasciata viteceki* Valladolid & Kučinić (ssp. nov.), encontrada en Bosnia y Herzegovina. El estudio de la morfología de los adultos, así como el análisis de la citocromo oxidasa I mitocondrial (COImit) y la distribución geográfica confirma las diferencias de las dos nuevas subespecies con la especie nominal *R. f. fasciata*, también presente en ambos países.

Palabras clave: Revisión taxonómica, nuevas subespecies, identificación, COI mitocondrial, distribución

Introduction

In the introduction of his monograph on the genus *Rhyacophila* Pictet 1834 and the family Rhyacophilidae Stephens 1836, Schmid (1970) concluded that *Rhyacophila* is one of the most primitive and important genera of caddisflies, because it is present in almost all lotic environments in the Holarctic and Oriental Regions. This genus included 465 species at that time, a number that has increased to 792 valid species (and 30 valid subspecies) (GBIF 2020; Morse personal communication). Schmid (1970) emphasized that *Rhyacophila* represents an evolutionary stage essential for inferring the phylogeny of Trichoptera, mainly due to the male genitalia, where high variability in details is mixed with a high stability in general architecture. Furthermore, in their geographic distribution, many of the species are restricted to small areas, sometimes to only a single mountain, providing the opportunity for phylogenetic and zoogeographical inferences.

Hagen (1859), studying Stephens', Curtis', and Pictet's private collections, classified the specimens of the genus *Rhyacophila* in five different species: *R. vulgaris* Pictet 1834, *R. dorsalis* (Curtis 1834), *R. paupera* Hagen 1859, *R. ferruginea* Scopoli 1763, and *R. fasciata* Hagen 1859. In his study of the British Trichoptera, McLachlan (1865) described the species *R. septentrionis* McLachlan 1865 and said that he had doubted it was a new species, because the form of the genital appendages agreed with the figure of *R. ferruginea* sent to him by Hagen. After examining the examples of *R. septentrionis* sent by McLachlan, Hagen concluded that they were larger and darker than continental types of *R. ferruginea* and may be distinct. In 1865, McLachlan said about *R. ferruginea*: "Scopoli's name cannot be said to apply to this species with certainty," and in 1879, in his redescription of *R. septentrionis*, concluded that the species *R. ferruginea* must remain uncertain.

Malicky & Sipahiler (1993) proposed the "*R. fasciata* complex", including several subspecies, some of them degraded from species rank: *Rhyacophila f. fasciata* Hagen 1859, widely distributed over Europe, *R. fasciata denticulata* McLachlan 1879 in northern Spain and the French Pyrenees, and *R. fasciata aliena* Martynov 1916 in the Caucasus and eastern Turkey. In addition, this taxa set was complemented by *R. fasciata mysica* Malicky & Sipahiler 1993 in western Turkey, *R. fasciata kykladica* Malicky & Sipahiler 1993 in Greece, and *R. fasciata libanica* Malicky & Sipahiler 1993 in Lebanon (see also Malicky 2004 for the distributions of these subspecies). In 2018 Sipahiler described a new subspecies of this group from Turkey, *Rhyacophila fasciata ilgazica* Sipahiler 2018.

In recent studies of two subspecies of the *Rhyacophila fasciata* Group, *Rhyacophila fasciata denticulata* (Valladolid *et al.* 2018), and *R. fasciata kykladica* (Valladolid *et al.* 2019) we have proposed their promotion to species rank as *R. denticulata* and *R. kykladica*, respectively, and the resurrection of a third species, *R. sociata* Navás 1916 (Valladolid *et al.* 2018), previously synonymized with *R. fasciata denticulata* by Schmid (1970).

Systematic studies of caddisflies (biodiversity, taxonomy, ecology) in Croatia began at the end of the XX century. In the last 20 years, there have been several studies about caddisflies in different parts of Croatia (e.g., Graf *et al.* 2008; Kučinić 2002; Kučinić & Malicky 2002; Kučinić *et al.* 2008, 2010a, 2011a, 2011c, 2013, 2014, 2015, 2017; Malicky 2009; Malicky *et al.* 2007; Previšić & Popijač 2010; Previšić *et al.* 2007, 2009, 2010, 2012, 2013; Szivák *et al.* 2017; Waringer *et al.* 2009) including some parts of Bosnia and Herzegovina (e.g., Kučinić *et al.* 2008, 2010b, 2011a, 2011b; Stanić-Koštroman *et al.* 2012, 2015; Vitecek *et al.* 2015). In this paper we provide the results of our study about "*R. fasciata*" in Croatia and in Bosnia and Herzegovina. We have found morphological and genetic variability in some specimens, so we propose two more subspecies that are new for the *Rhyacophila fasciata* Group: *R. fasciata delici* Kučinić & Valladolid, from both countries, and *R. fasciata viteceki* Valladolid &

Kučinić, from Bosnia and Herzegovina, based on differences in morphology of adults and DNA analysis (genetic divergences of mitochondrial cytochrome oxidase I (mtCOI) gene) from *R. f. fasciata*. The geographical distribution and ecology of these subspecies are also provided and compared with those of *R. f. fasciata* in Croatia and Bosnia and Herzegovina.

Material and methods

Adult material examined

Specimens studied for this project are deposited in the Collection Trichoptera NIP, Croatian Natural History Museum, Zagreb, Croatia (CNHM); the D. Cerjanec Collection, Barilović, Croatia (DCC); the Trichoptera Collection of the Museo Civico di Scienze Naturali "E. Caffi," Bergamo, Italy (MCSN); the M. Kučinić Collection, Zagreb, Croatia (MKC); the Trichoptera Collection "Krka", Faculty of Science, University of Zagreb, Croatia (TCK); the Collection of Entomology, Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN); the R. Ćuk Collection, Zagreb, Croatia (RCC); the S. Stanić-Koštroman Collection, Mostar, Bosnia and Herzegovina (SSKC); and the Trichoptera Collection, University of Mostar, Bosnia and Herzegovina (UMBH).

Bosnia and Herzegovina: *R. f. fasciata*: 1 ♂ + 3♀ (BS1–BS4), river Bosna, Zenica-Doboj, Maglaj, 29–31/vii/13. Leg.: Louda. ID Data number of Access Catalog 28055, (MCSN).

R. f. fasciata (=*R. fasciata viteceki*): 1 ♂ (B1), river Bunica, upper course, 04/vi/17 (UMBH); 3 ♂ (N1–N3), river Neretva, Bačevići, 30/vi/17. Leg.: Stanić-Koštroman & M. Kučinić (N1, MNCN; N2, MKC; N3, UMBH). 2 ♂ + 1 ♀ (S1–S3), river Sturba, near Livno, 30/viii/18. Leg.: M. Kučinić (S1, S3, MNCN; S2, MKC).

Croatia: *R. f. fasciata*: 1 ♂ + 1 ♀ (K1–K2), river Kutjevačka, upper course, 16/v/05. Col. & Leg.: R. Ćuk (RCC). 3 ♂, river Voćinska, upper course, near Požega, 03/v/15. Leg.: R. Ćuk (CNHM). 4 ♂, river Vočinska, upper course, Papuk Mt., 19/v/15. Leg.: P. Crnčan (CNHM). 3 ♂ + 1 ♀, 03/viii/15; 3 ♂, 28/x/15, river Rijeka, Bastaji. Leg.: R. Ćuk (CNHM). 1 ♀, 15/v/15; 2 ♂ + 7 ♀, 05/viii/15, river Pakra, Kusonje. Leg.: A. Ćukušić & A. Delić (CNHM).

R. f. fasciata (=*R. fasciata delici*): 1 ♂ + 1 ♀ (D1–D2), spring of the river Dretulja, 09/ix/14; 2 ♂ (G1–G2), middle course of river Gacka, Otočac, 11/v/15. Leg.: D. Cerjanec & M. Kučinić (MNCN). 2 ♂ (C1–C2), 21/vi/15; 2 ♂ (C3–C4), 05/v/15, spring of the river Čabranka. Leg.: D. Cerjanec (C1–C3, MKC; C4, UMBH). 1 ♂ + 1 ♀ (V1–V2), Vukovića spring, river Cetina, 09/v/17. Leg.: A. Delić & M. Kučinić (MNCN). 1 ♂ (BI1), spring of river Bijela rijeka, National Park "Plitvice Lakes", 16/iii/18. Leg.: I. Sivec, M. Kučinić & S. Žalac (MKC). 1 ♂ + 1 ♀ (R1–R2), Roški slap (Roški waterfall), National Park "Krka", 19/v/19. Leg.: M. Kučinić & A. Delić (TCK). 1 ♂ (BR1), Brkljača channel, Udovičić, 20/vi/15. Leg.: S. Žalac & M. Kučinić (MKC). 1 ♀ (CT1), tributary of river Cetina, Civljani, 07/vi/15. Leg.: M. Kučinić & A. Delić (MKC). 1 ♂ + 1 ♀ (BT1–BT2), river Butišnica, Golubić, 06/viii/15. Leg.: A. Delić & A. Ćukušić (MKC). 1 ♀ (BU1), Brušane, Gospić, Ličko-Senjska County, Road D 25, 10–15/vii/16. Leg.: G. Galli (MCSN).

Czech Republic: *R. f. fasciata*: 1 ♂ + 1 ♀, Bohemian Switzerland National Park, brook Bílý (N Bohemia), 22/vi/10. 1 ♂, Jizerské mountains, brook Ještrabec, Rejdice (N Bohemia), 02/vi/08. 1 ♂ + 1 ♀, Krušné mountains, stream Hluboký, near Dolní Nivy (W Bohemia), 31/viii/15. 1 ♂, brook near Nové Strašecí (C Bohemia), 31/v/11, Leg.: P. Chvojka (MNCN).

Slovakia: *R. f. fasciata:* 1 ♂ + 1 ♀, High Tatras, stream Biely Váh (N Slovakia), 7/x/90. 1 ♂, Strážov Mountains, stream Sučiansky, NW Nitrianske Sučany (W Slovakia), 24/vi/15. 1 ♂ + 1 ♀, Oravské Beskydy mountains, left tributary of Bystrá stream below Babia hora mountain (N Slovakia), 15/x/91. Leg.: P. Chvojka (MNCN).

Methods

Morphology. Morphological characters of adults were observed and measured using a binocular microscope. Selected parts of genitalia from adult males (parameres and aedeagus) were photographed using a light microscope and multiple stacked images were integrated with focal stacking software (Auto-Montage Essentials, Syncroscopy©).

The following descriptions of adults of *R. fasciata* and synonymized species (*R. ferruginea*, *R. septentrionis*) were consulted: Bertrand (1954), Eidel (1974), Fotius-Jaboulet (1964), Hagen (1859), Malicky (2004), Malicky & Sipahiler (1993), McLachlan (1865, 1868, 1879), Nóvak (1963), Schmid (1970), Scopoli (1763), Tobias & Tobias (1981), Ulmer (1909). The terminology of genitalia is according to Schmid (1970).

DNA Extraction, Amplification, and Sequencing

Muscle tissue of legs and thorax from specimens was dissected. Samples were fully immersed in 0.5 ml digestion buffer (Gilbert *et al.* 2007) and incubated overnight at 55°C with gentle agitation. The buffer consisted of 5 mM $CaCl_2$, 2% sodium dodecyl sulphate (SDS), 40 mM dithiotreitol (DTT), 250 mg/ml proteinase K, 10 mM Tris buffer pH 8, 2.5 mM EDTA (Ethylene-Diamine-Tetra-Acetic acid) pH 8.0, and 10 mM $NaCl_2$ (final concentrations). After incubating, nucleic acids were extracted from the digestion buffer using a Qiaquick PCR purification kit (QIAGEN, Alda *et al.* 2007). A 508 bp region of the mitochondrial COI gene (Cytochrome Oxidase I) was amplified with the primers C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994; Simon *et al.* 1994). Three microlitres of the DNA solution were used as a template. Other components of the 25 µl PCR reaction were: 1x of the corresponding buffer (75 mM Tris HCl, pH 9.0; 50 mM KCl and 20 mM $(NH_4)_2SO_4$); 2 mM $MgCl_2$; 10 mM dNTPs mix; 0.1 µM of each primer; 0.02% BSA; and 0.125 units AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Five microlitres of PCR products were electrophoresed through a 1.5% agarose gel and visualized with SYBR Safe™ DNA Gel Stain (Invitrogen) under ultraviolet light. PCR products were purified by treatment with ExoSAP-IT (USB Amersham, Buckinghamshire, UK) and incubated at 37ºC for 45 min, followed by 80ºC for 15 min to inactivate the enzyme. Purified PCR product was then used to sequence in both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc., Foster City, USA) in a 10 µL volume, containing 15–20 ng of purified product and 3 pmol of primer. To verify that the sequences obtained came from a *Rhyacophila*, they were compared with sequences from GenBank using Blast tool (Altschul *et al.* 1997). The alignment of all *Rhyacophila* COI gene sequences generated in our lab was performed and edited manually using MEGA X (Kumar *et al*. 2018). Fine adjustments were made by eye, as the COI does not present any gaps.

A total of 151 sequences of mtCOI were used in the study: 19 derived from specimens from Croatia (2 of *R. f. fasciata* and 17 of *R. f. delici*) and 20 from Bosnia and Herzegovina (4 of *R. f. fasciata*, 7 of *R. f. viteceki* and 9 of *R. f. delici*), together with 82 of *R. f. fasciata* from different countries, (5 from Belgium, 9 from Czech Republic, 1 from Estonia, 18 from Finland, 14 from Germany, 5 from Kosovo, 2 from North Macedonia, 6 from Norway, 12 from Poland, 7 from Russia, 2 from Slovakia and 1 from Sweden), 3 of *R. denticulata* (France) and 17 of *R. sociata* (13 from Spain and 4 from France), promoted to species rank by Valladolid *et al.* (2018, see Table 1 for references), 9 of *R. kykladica* (Greece), promoted to species by Valladolid *et al.* (2019, see Table 1 for references) and 1 of *R. relicta* (Spain) as outgroup (for more information, see Table 1). *Rhyacophila f. fasciata* has a wide area of distribution in northern and central Europe and there does not exist any GenBank entry from the type locality (Elberfeld, Germany), so we have included some sequences from localities around it (Belgium and Germany), sequences from different countries collected from GenBank and sequences prepared in our laboratory.

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. The CIPRES Science Gateway v.3.3 online server (Miller *et al.* 2010) was used to run jModeltest v.2.1.9 (Guindon & Gascuel 2003; Darriba *et al*. 2012) which was used to calculate the nucleotide substitution model and the ML with RAxML-HPC BlackBox (Stamatakis 2014), using 10,000 bootstrap replicates to assess node support. BI analyses were run in MrBayes-3.2.7 (Ronquist *et al.* 2012). We used the substitution model space with the option lset nst=mixed rates=invgamma, for 1.5×106 generations, discarding the first 25% generations as burn-in and synthesizing the results. Distances among and within species and subspecies were calculated using the maximum composite likelihood and p-distance models with MEGA X (Kumar *et al.* 2018). The maximum likelihood tree was made with MEGA X, but only a few sequences were represented in the figure. Nucleotide sequences were deposited in GenBank (2020) (accession numbers and MNCN: ADN Collection numbers in Table 1).

Geographical distribution

The analysis of the subspecies distributions was based on the localities where specimens were captured, data from the several collections [Trichoptera NIP (Croatian Natural History Museum), Trichoptera-Cerjanec, Trichoptera-Vučković, Trichoptera-Kučinić, Trichoptera Collection of the Museo Civico di Scienze Naturali "E. Caffi" (Bergamo, Italy)], and references from literature: **Bosnia and Herzegovina:** Botošâneanu (1960, 1961); Stanić-Koštroman *et al*. (2012, 2015); **Croatia:** Cerjanec (2012); Graf *et al.* (2008); Habdija *et al.* (2004); Kučinić (2002); Kučinić *et al.* (2008, 2010a, 2011c, 2017); Malicky (2009); Marinković-Gospodnetić (1979); Oláh (2010); Previšić *et al.* (2007, 2010, 2013); Previšić & Popijač (2010); Vučković (2011).

The distribution map was drawn using ArcGIS 10.3 (ESRI 2011).

TABLE 1. Data for specimens analysed by mitochondrial cytochrome oxidase I (mtCOI) gene: *R. denticulata*, *R. fasciata delici*, *R. f. fasciata*, *R. fasciata viteceki, R. kykladica*, *R. sociata* and *R. relicta*, (as outgroup). Species numbers refer to sequences included in the study and match with those selected for constructing the tree (see Fig. 11). Sex, A = adult, not specified, M = male, MP = male pupa, F = female, FP = female pupa, L = larva. N, E = latitudinal and longitudinal geographical coordinates, respectively. Alt = altitude (m a.s.l.). Accession numbers from GenBank and Tissues and the DNA Collection of the Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid. * = BIN ID, in BOLD Systems Database (specimens of *R. f. fasciata* from Republic of North Macedonia).

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
fasciata 1	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX293777	
fasciata 2		Trøgstad, Indre Østfold (Norway)	59.746	11.272	115	KX293282	
fasciata 3	F	Finnmark, Alta, Gargiaveien (Norway)	69.822	23.480	95	KX293192	
fasciata 4	L	Kuusamo, Purkuputaanoja (Finland)	66.380	29.424	225	KX292544	
fasciata 5		Eidsberg, Indre Østfold (Norway)	59.603	11.286	130	KX295452	
fasciata 6		Flateby (Norway)	59.827	11.174	124	KX291568	
fasciata 7	A	Bavaria, Ueberacker (Germany)	48.234	11.298	505	KX291079	
fasciata 8	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX294646	
fasciata 9	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX294688	
fasciata 10	A	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX292344	
fasciata 11	F?	Hessen, Schlitz, Breitenbach (Germany)	50.662	9.624	280	KX296294	
fasciata 12		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142884	
fasciata 13	M	Modrava, (Czech Republic)	48.979	13.431	1200	HQ959079	
fasciata 25	F	Trondheim, Nidelva (Norway)	63.339	10.442	70	KX294414	
fasciata 26	M	Trondheim, Nidelva (Norway)	63.339	10.442	70	KX291512	
fasciata 27	L	Paltamo, Vaarainjoki (Finland)	64.472	27.630	175	KX141034	
fasciata 28	L	Paltamo, Vaarainjoki (Finland)	64.472	27.630	175	KX143671	
fasciata 29	L	Mustjala, Moeldrioja (Estonia)	58.490	22.303	5	KX294243	
fasciata 30		Södermanland (Sweden)	59.152	17.599	20	KM225308	
fasciata 31		Vlaams-Brabant, Kappitel, (Belgium)	50.733	4.295	50	KX142866	
fasciata 32		Vlaams-Brabant, Kappitel, (Belgium)	50.733	4.295	50	KX142423	
fasciata 33		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142016	
fasciata 34		Limburg, Veurs, Voeren (Belgium)	50.749	5.814	130	KX142000	
fasciata 35	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816342	MNCN:ADN 92790
fasciata 36	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816343	MNCN:ADN 92791
fasciata 37	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816344	MNCN:ADN 92792
fasciata 38	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816345	MNCN:ADN 92793
fasciata 39	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816346	MNCN:ADN 92794

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
fasciata 40	F	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816347	MNCN:ADN 92795
fasciata 41	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816348	MNCN:ADN 92796
fasciata 42	M	Tampere, Tiikonoja (Finland)	61.720	24.031	120	MT816349	MNCN:ADN 92797
fasciata 46	A	Hesse, Breitenbach (Germany)	50.659	9,629	240	KY262289	
fasciata 47	A	Hesse, Breitenbach (Germany)	50.659	9,629	240	KY262252	
fasciata 48	A	Hesse, Breitenbach (Germany)	50.662	9,624	280	KY262207	
fasciata 50	A	Hesse, Breitenbach (Germany)	50.659	9,629	240	KY261630	
fasciata 51	A	Hesse, Breitenbach (Germany)	50.662	9,624	280	KY261529	
fasciata 52	A	Hesse, Breitenbach (Germany)	50.662	9,624	180	KY261518	
fasciata 53	A	Hesse, Breitenbach (Germany)	50.662	9,624	280	KY261298	
fasciata 54	A	Hesse, Breitenbach (Germany)	50.662	9,624	280	KY261164	
fasciata 57	MP	River Kumiela, Elblag (Poland)	54.158	19.414	15	MT816350	MNCN:ADN 94323
fasciata 58	FP	River Kumiela, Elblag (Poland)	54.158	19.414	15	MT816351	MNCN:ADN 94324
fasciata 59	MP	River Mała Wkra (Poland)	53.552	19.964	140	MT816352	MNCN:ADN 94332
fasciata 60	FP	River Mała Wkra (Poland)	53.552	19.964	140	MT816353	MNCN:ADN 94333
fasciata 61	MP	River Wałsza (Poland)	54.236	20.128	70	MT816354	MNCN:ADN 94334
fasciata 62	FP	River Wałsza (Poland)	54.236	20.128	70	MT816355	MNCN:ADN 94335
fasciata 63	MP	River Gizela, Gaznoty (Poland)	53.534	19.901	190	MT816356	MNCN:ADN 94340
fasciata 64	FP	River Gizela, Gaznoty (Poland)	53.534	19.901	190	MT816357	MNCN:ADN 94341
fasciata 65	FP	River Gizela, Zajączki (Poland)	53.560	19.882	170	MT816358	MNCN:ADN 94342
fasciata 66	FP	River Gizela, Zajączki (Poland)	53.560	19.882	170	MT816359	MNCN:ADN 94343
fasciata 67	MP	Srebrny Potok (Poland)	54.162	19.491	135	MT816360	MNCN:ADN 94344
fasciata 68	FP	Srebrny Potok (Poland)	54.162	19.491	135	MT816361	MNCN:ADN 94345
fasciata 69	M	Bílý potok, Bohemia (Czech Republic)	50.925	14.401	330	MT816362	MNCN:ADN 94391
fasciata 70	F	Bílý potok, Bohemia (Czech Republic)	50.925	14.401	330	MT816363	MNCN:ADN 94392
fasciata 71	M	Ještkrabec, Rejdice, Bohemia (Czech Republic)	50.733	15.365	700	MT816364	MNCN:ADN 94393
fasciata 72	M	Hluboký potok, Bohemia (Czech Republic)	50.236	12.604	540	MT816365	MNCN:ADN 94394
fasciata 73	F	Hluboký potok, Bohemia (Czech Republic)	50.236	12.604	540	MT816366	MNCN:ADN 94395
fasciata 74	M	Brook W Nové Strašecí (Czech Republic)	50.145	13.827	430	MT816367	MNCN:ADN 94396
fasciata 75	M	Drietomice, Moravia (Czech Republic)	48.952	17.875	390	MT816368	MNCN:ADN 94398

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
fasciata 76	F	Drietomice, Moravia (Czech Republic)	48.952	17.875	390	MT816369	MNCN:ADN 94399
fasciata 77	F	Tatras, Biely Váh (Slovakia)	49.133	20.021	1400	MT816370	MNCN:ADN 94401
fasciata 78	F	Tributary of Bystrá (Slovakia)	49.560	19.510	1200	MT816371	MNCN:ADN 94404
fasciata 80	M	River Kutjevačka (Croatia)	45.476	17.866	470	MT559339	MNCN:ADN 96487
fasciata 81	F	River Kutjevačka (Croatia)	45.476	17.866	470	MT559340	MNCN:ADN 96488
fasciata 82	M	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559341	MNCN:ADN 109896
fasciata 83	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559342	MNCN:ADN 109897
fasciata 84	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559343	MNCN:ADN 109898
fasciata 85	F	River Bosna (Bosnia and Herzegovina)	44.550	18.105	170	MT559344	MNCN:ADN 109899
fasciata 86	L	Bruchanicha stream, Ryjiy Ovrag, (Russia)	56.036	36.440	270	MT816372	MNCN:ADN 109948
fasciata 87	L	Bruchanicha stream, Ryjiy Ovrag, (Russia)	56.036	36.440	270	MT816373	MNCN:ADN 109949
fasciata 88	L	Stream near Volgusha river (Russia)	56.242	37.405	150	MT816374	MNCN:ADN 109952
fasciata 89	L	Stream Medvejiy, Murmansk (Russia)	67.136	32.702	150	MT816375	MNCN:ADN 109954
fasciata 90	MP	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816376	MNCN:ADN 109955
fasciata 91	FP	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816377	MNCN:ADN 109956
fasciata 92	L	Spring brook near Moskva river (Russia)	55.627	36.386	170	MT816378	MNCN:ADN 109957
fasciata 93	M	Kuhmo, Viiksimonjoki (Finland)	64.311	30.299	195	MT816379	MNCN:ADN 110209
fasciata 94	F	Kuhmo, Viiksimonjoki (Finland)	64.311	30.299	195	MT816380	MNCN:ADN 110210
fasciata 95	L	Obb Simo, Kuivasoja brook (Finland)	66.005	25.459	110	MT816381	MNCN:ADN 110211
fasciata 96	L	Obb Simo, Kuivasoja brook (Finland)	66.005	25.459	110	MT816382	MNCN:ADN 110212
fasciata 97	L	Kuusamo, Oulanka brook (Finland)	66.326	29.537	190	MT816383	MNCN:ADN 110213
fasciata 98	M	Kuusamo, Putaanoja brook (Finland)	66.380	29.424	225	MT816384	MNCN:ADN 110214
fasciata 99	L	Kuusamo, Uopajanpuro brook (Finland)	66.339	29.520	180	MT816385	MNCN:ADN 110216
fasciata 100	M	Shushtë (Kosovo)	42.281	21.359	570	MT816386	MNCN:ADN 110218
fasciata 101	M	Shushtë (Kosovo)	42.281	21.359	570	MT816387	MNCN:ADN 110219
fasciata 102	M	Shushtë (Kosovo)	42.281	21.359	570	MT816388	MNCN:ADN 110220
fasciata 103	M	Shushtë (Kosovo)	42.281	21.359	570	MT816389	MNCN:ADN 110221
fasciata 104	M	Lugu i Kopilaqës (Kosovo)	42.246	21.431	1175	MT816390	MNCN:ADN 110225
fasciata 105	M	River Trnovska (Republic of North Macedonia)	42.258	22.361	820	*AAD5716**	
fasciata 106	M	River Kriva (Republic of North Macedonia)	42.231	22.636	710	*AAD5716**	

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
f. delici G1	M	River Gacka, Otočac (Croatia)	44.787	15.368	475	MT559345	MNCN:ADN 96483
f. delici G2	M	River Gacka, Otočac (Croatia)	44.787	15.368	475	MT559346	MNCN:ADN 96484
f. delici V1	M	Vukovića spring, river Cetina (Croatia)	43.965	16.413	375	MT559347	MNCN:ADN 96485
f. delici V2	F	Vukovića spring, river Cetina (Croatia)	43.965	16.413	375	MT559348	MNCN:ADN 96486
f. delici D1	M	River Dretulja, spring (Croatia)	45.074	15.343	390	MT559349	MNCN:ADN 96489
f. delici D2	F	River Dretulja, spring (Croatia)	45.074	15.343	390	MT559350	MNCN:ADN 96490
f. delici C1	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559351	MNCN:ADN 96491
f. delici C2	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559352	MNCN:ADN 96492
f. delici C3	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559353	MNCN:ADN 103406
f. delici C4	M	River Čabranka, spring (Croatia)	45.601	14.640	560	MT559354	MNCN:ADN 103407
f. delici BI1	M	River Bijela rijeka, NP Plitvice Lakes (Croatia)	44.833	15.557	750	MT559355	MNCN:ADN 103408
f. delici R1	M	Roški slap (waterfall), NP Krka (Croatia)	43.906	15.975	80	MT559356	MNCN:ADN 103409
f. delici R2	F	Roški slap (waterfall), NP Krka (Croatia)	43.906	15.975	80	MT559357	MNCN:ADN 103410
f. delici BR1	M	Brkljača channel, Udovičić (Croatia)	43.656	16.732	290	MT559358	MNCN:ADN 103411
f. delici CT1	F	River Cetina, tributary, Civljani (Croatia)	43.948	16.401	375	MT559359	MNCN:ADN 103412
f. delici BT1	M	River Butišnica, Golubić (Croatia)	44.111	16.227	350	MT559360	MNCN:ADN 103413
f. delici BU1	F	Brušane (Gospić) (Croatia)	44.505	15.236	620	MT559361	MNCN:ADN 109891
f. delici M6	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765286	
f. delici M8	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765287	
f. delici M7	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT765290	
f. delici B2	L	River Bosna, Vrelo Bosne (Bosnia and Herzegovina)	43.816	18.273	560	MT772015	
f. delici B1	L	River Bosna, Vrelo Bosne (Bosnia and Herzegovina)	43.816	18.273	560	MT772017	
f. delici M4	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	560	MT772020	
f. delici M5	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772022	
f. delici M1	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772028	
f. delici M3	L	River Miljacka, Dariva (Bosnia and Herzegovina)	43.858	18.442	580	MT772029	
denticulata 1	M	River Balameth, Bethmale (France)	42.865	1.092	1000	MF347380	MNCN:ADN 49487
denticulata 2	M	River Balameth, Bethmale (France)	42.865	1.092	1000	MF347381	MNCN:ADN 49488
denticulata 3	F	River Balameth, Bethmale (France)	42.865	1.092	1000	MF683825	MNCN:ADN 96497
kykladica 1	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422501	MNCN:ADN 94016
kykladica 2	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422502	MNCN:ADN 94017

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TABLE 1. (Continued)

Species	Sex	Locality	N	E	Alt	GenBank	MNCN Collection
kykladica 3	M	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422503	MNCN:ADN 94018
kykladica 4	L	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422504	MNCN:ADN 94424
kykladica 5	L	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422505	MNCN:ADN 94425
kykladica 6	MP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422506	MNCN:ADN 94426
kykladica 7	FP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422507	MNCN:ADN 94427
kykladica 8	FP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422508	MNCN:ADN 94428
kykladica 9	MP	Platanistos, South Euboea (Greece)	38.013	24.511	214	MK422509	MNCN:ADN 94429
sociata 1	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347382	MNCN:ADN 57431
sociata 2	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347383	MNCN:ADN 57433
sociata 3	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347384	MNCN:ADN 57436
sociata 4	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347385	MNCN:ADN 57440
sociata 5	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347386	MNCN:ADN 57441
sociata 6	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347387	MNCN:ADN 57442
sociata 7	F	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347388	MNCN:ADN 57443
sociata 8	M	River Oja, La Rioja (Spain)	42.547	-2.915	490	MF347389	MNCN:ADN 57444
sociata 9	M	River Oja, La Rioja (Spain)	42.953	-2.915	490	MF347390	MNCN:ADN 57445
sociata 10	M	River Oja, La Rioja (Spain)	42.953	-2.915	490	MF347391	MNCN:ADN 57434
sociata 11	M	River Lez, Audressein (France)	42.930	1.023	500	MF347392	MNCN:ADN 49486
sociata 12	M	River Aude, Languedoc-Roussillon (France)	42.954	2.449	500	KX141525	
sociata 13	M	River Vernal, Aragón (Spain)	42.811	-0.833	970	HM395984	
sociata 14	M	River Cares, Asturias (Spain)	43.193	-4.908	570	MF347393	MNCN:ADN 92806
sociata 15	M	River Cares, Asturias (Spain)	43.193	-4.908	570	MF347394	MNCN:ADN 92807
sociata 16	M	R. Correc de la Sogueda, Sorède (France)	42.500	2.958	390	MF683826	MNCN:ADN 96499
sociata 17	F	R. Correc de la Sogueda, Sorède (France)	42.500	2.958	390	MF683827	MNCN:ADN 96500
f. viteceki N1	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559365	MNCN:ADN 96493
f. viteceki N2	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559366	MNCN:ADN 96494
f. viteceki N3	M	River Neretva, Bačevići (Bosnia and Herzegovina)	43.254	17.825	35	MT559367	MNCN:ADN 96495
f. viteceki B1	M	River Bunica (Bosnia and Herzegovina)	43.243	17.855	30	MT559368	MNCN:ADN 96496
f. viteceki S1	M	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559362	MNCN:ADN 94430
f. viteceki S2	M	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559363	MNCN:ADN 94431
f. viteceki S3	F	River Sturba, Livno (Bosnia and Herzegovina)	43.762	17.030	755	MT559364	MNCN:ADN 94432
relicta	M	R. Miera, Solares, Cantabria (Spain)	43.381	-3.721	40	MG132072	MNCN:ADN 96504

Results

Rhyacophila fasciata delici Kučinić & Valladolid (ssp. nov.)

This subspecies has been found widely distributed in Croatia and it is also present in Bosnia and Herzegovina.

Etymology. The specific name is the genitive singular of Delić, given in honour of Dr. Antun Delić, naturalist and biologist, retired professor of the Teachers Faculty, University of Zagreb.

Type material. Holotype ♂: CROATIA, Roški slap (Roški waterfall), National Park "Krka" (43.90625º N 15.975º E, 80 m a.s.l.), 10/v/19 (M. Kučinić & A. Delić) [R1, no. 4444 (TCK)].

Paratypes: 1 ♀, same locality as holotype, 19/v/19, (M. Kučinić & A. Delić) [R2, no. 4445 (TCK)]. 2 ♂, spring of the river Čabranka, 21/vi/15 (D. Cerjanec) [C1–C2, nos. 4446–4447 (MKC)] 2 ♂, same locality, 05/v/15 (D. Cerjanec) [C3, no. 4448 (MKC), C4, no. 1110 (UMBH)]. 1 ♂, spring of river Bijela rijeka, National Park "Plitvice Lakes", 16/iii/18 (I. Sivec, M. Kučinić & S. Žalac) [BI1, no. 4449 (MKC)]. 1 ♂, Brkljača channel, Udovičić, 20/vi/15 (S. Žalac & M. Kučinić) [BR1, no. 4450 (MKC)]. 1 ♀, tributary of river Cetina, Civljani, 07/vi/15 (M. Kučinić & A. Delić) [CT1, no. 4451 (MKC)]. 1 ♂ and 1 ♀, river Butišnica, Golubić, 06/viii/15 (A. Delić & A. Ćukušić) [BT1–BT2, nos. 4452–4453 (MKC)]. 1 ♀, Brušane, Gospić, Ličko-Senjska county, Road 25, 10-15/vii/16 (G. Galli) [BU1, ID Data number of Access Catalog 33789 (MCSN)]. 1 ♂ and 1 ♀, spring of the river Dretulja, 09/ix/14 (D. Cerjanec & M. Kučinić) [D1–D2, MNCN_Ent 269370, MNCN_Ent 269371 (MNCN)]. 1 ♂ and 1 ♀, Vukovića spring, river Cetina, 09/v/17 (A. Delić & M. Kučinić) [V1–V2, MNCN_Ent 269372, MNCN_Ent 269373, (MNCN)]. 2 ♂, middle part of river Gacka, Otočac, 11/v/15 (D. Cerjanec & M. Kučinić) [G1–G2, MNCN_Ent 269374, MNCN_Ent 269375, (MNCN)].

Description of the imago. Holotype (R1): Length from front of head to distal edge of segment IX 8.82 mm, each forewing 11.42 mm, each hind wing 9.65 mm.

Males: Length 7.21–9.92 mm ($\overline{x}$ = 8.61, n = 12), each forewing 10.07–13.09 ($\overline{x}$ = 11.15, n = 12), each hind wing 8.29–11.17 mm ($\overline{x}$ = 9.66, n = 12).

Females: Length from front of head to distal edge of segment VIII 8.96–11.61 mm ($\overline{x}$ = 10.69, n = 6), each forewing 10.48–14.15 mm ($\overline{x}$ = 12.28, n = 6), each hind wing 9.77–12.7 mm ($\overline{x}$ = 10.93, n = 6).

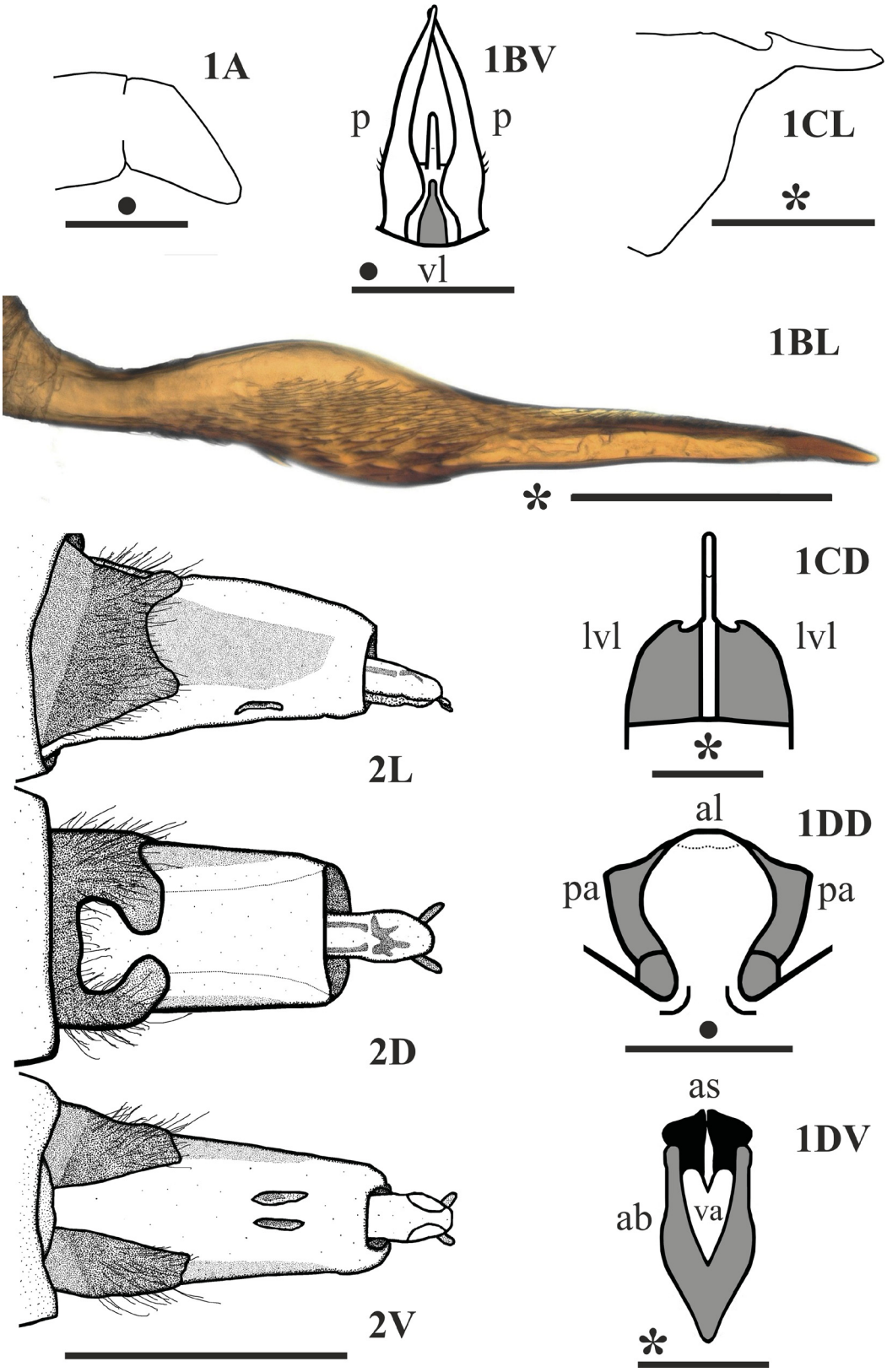
In ethanol-preserved specimens colour generally pale brown and yellowish, with golden brown setae. Head, thorax, and abdomen dorsally pale brown, with small black and irregular darker brown spots in dorsal area, abdomen ventrally yellowish; females generally darker than males, anterior 2/3 of abdominal segments pale brown or brown in darker specimens. Legs light, with spurs reddish brown. Forewings brown, spotted lighter than background; hind wings pale.

Male genitalia (Figs 1, 5a–5e): Apical segment of each inferior appendage (Figs 1A, 5a) with basal and distal edges diverging, posterior edge oblique straight or slightly convex, ventral edge slightly concave, at least two times longer that dorsal edge. Apicodorsal vertex slightly angular, apicoventral angle projecting as thick lobe narrowing progressively to round apex (Fig 5a).

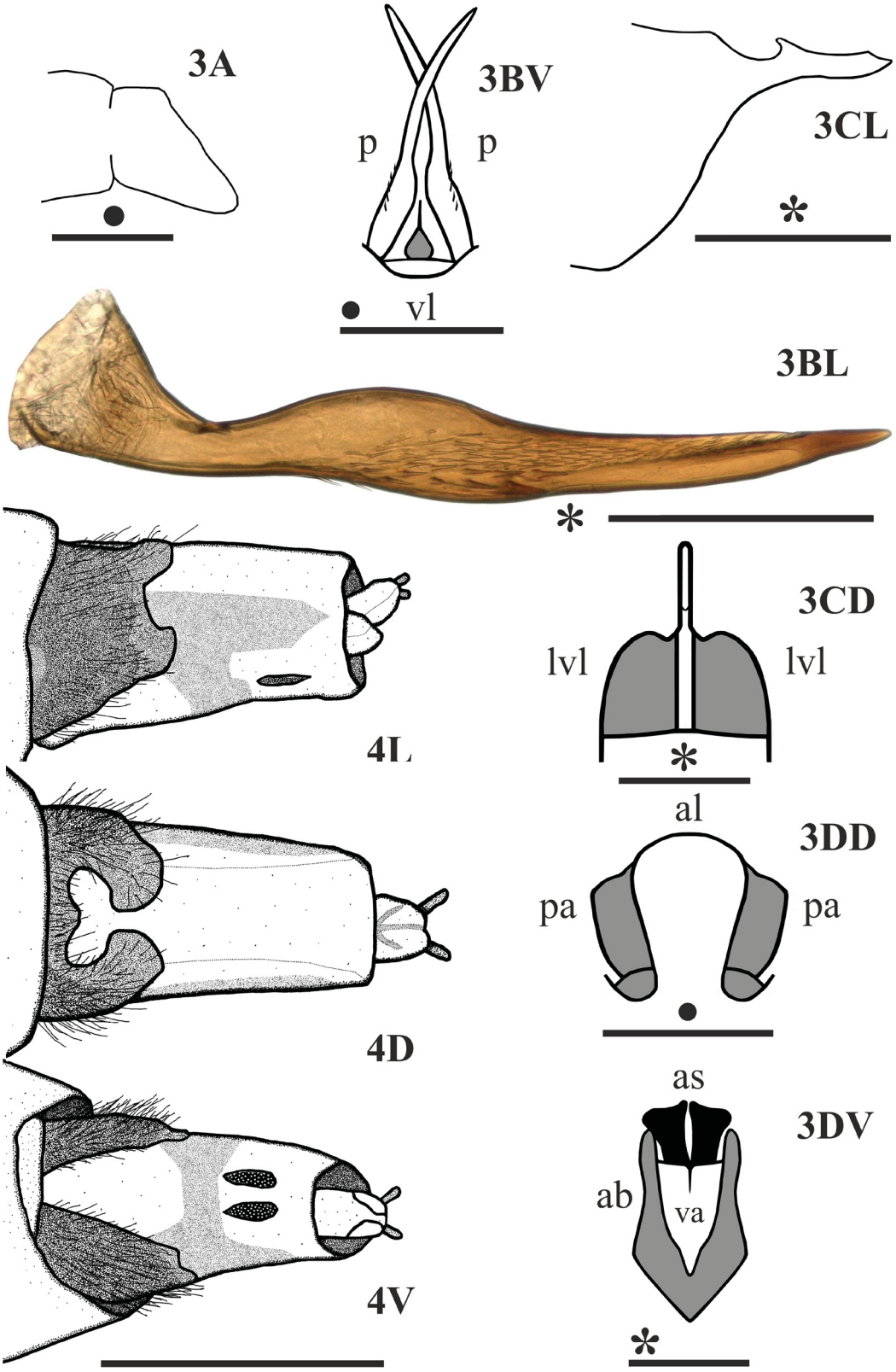
Parameres (Figs 1BV–1BL, 5c, 5d) in ventral view curved posteromesad in apical half (Figs 1BV p, 5c). In lateral view (Figs 1BL, 5d) each slender at base, dilated in middle, with almost parallel dorsal and ventral margins in central area, pointed at apex; few thick spines on midventral margin and posterior midventral area, parallel to surface (Figs 5c, 5d); midlateral surface almost completely covered by thin spicules or setae, reaching from anteroventral edge to posterodorsal edge of paramere, decreasing in size from ventral to dorsal margin, absent on middorsal edge.

Aedeagus (phallicata) in lateral view (Figs 1CL, 5e) with anterodorsal margin straight, then slightly concave with posterior corner of concavity hooked anterad, upper posterior edge slightly concave, then straight caudad, ventral apex round, ventral edge curved upward anteriorly to slight angle below hook. Lateroventral lobes of phallus (Fig 1CD lvl) convex, narrowing progressively towards apex, apicolateral margins slightly pointed to inside, posterior edge of each lobe concave. Ventral lobe of aedeagus subtriangular, pointed progressively backwards (Fig 1BV vl).

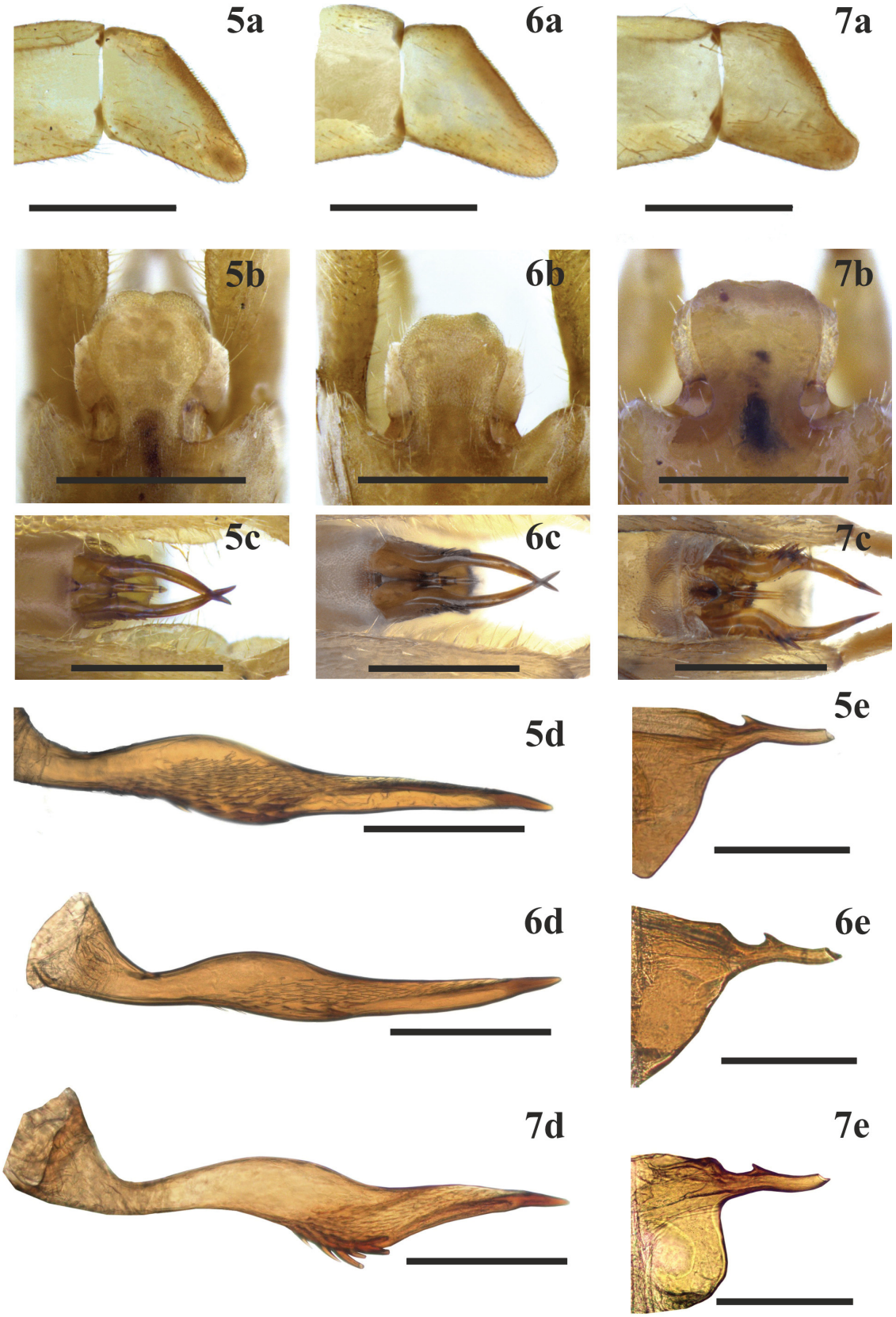
Apicodorsal lobe of segment IX (Figs 1DD, 5b) dilated subapicolaterally, nearly circular, ratio of posterior maximum width to anterior width 2:1; preanal appendages (Fig 1DD pa) shorter than apicodorsal lobe (Fig 1DD al) of segment IX laterally, with concave posterior edges. In ventral view (Fig 1DV), apical band V-shaped, inner and outer edges almost parallel in posterior third, rounded apically, longer than wide (Fig 1DV ab); posterior edge of non-sclerotized ventral area round, with apicomesal incision (Fig 1DV va), anal sclerites triangular (Fig 1DV as).



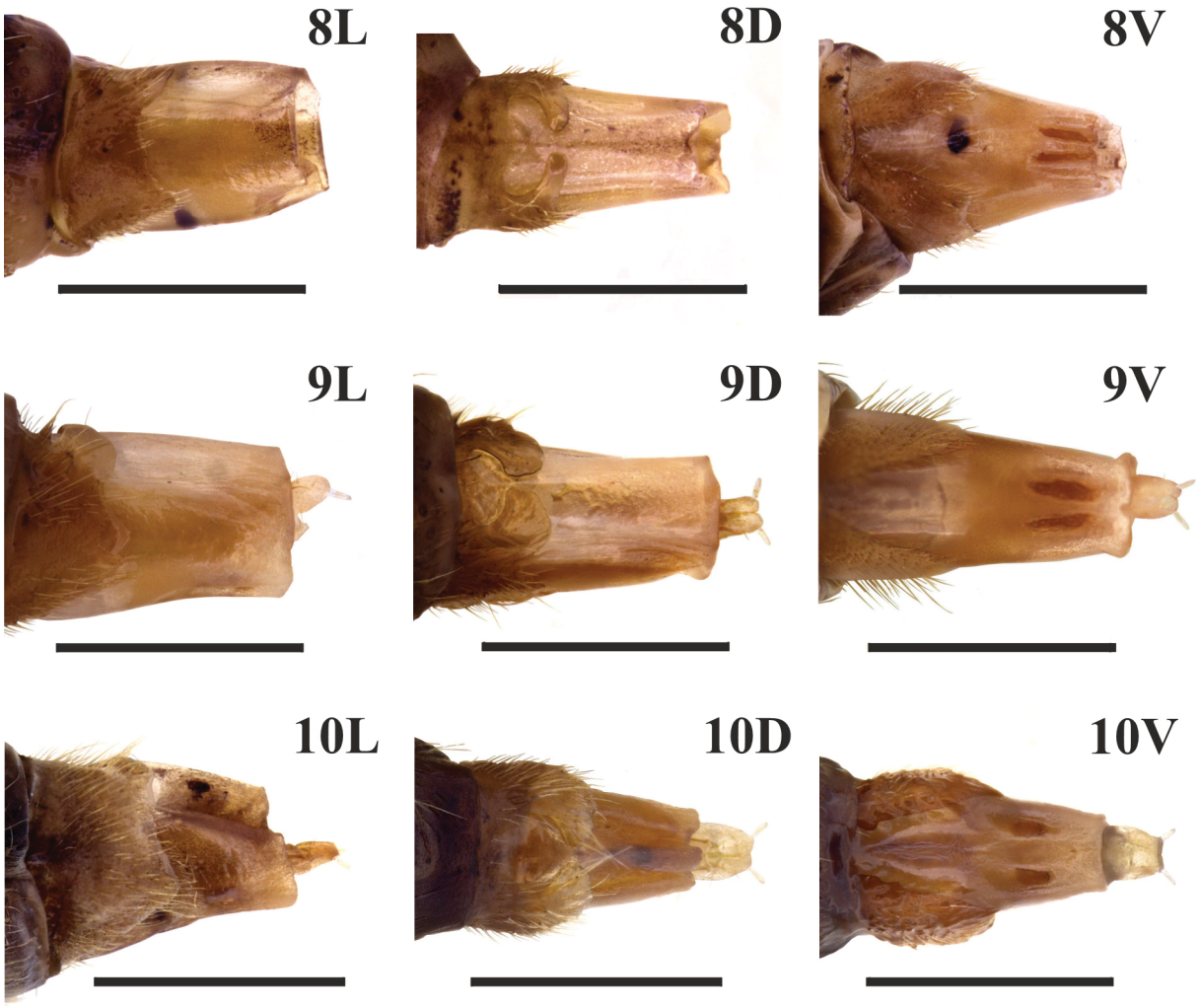
FIGURES 1, 2. Male and female genitalia of *Rhyacophila fasciata delici* Kučinić & Valladolid ssp. nov. **1**, male: **1A**, 2nd segment of left inferior appendage, left lateral; **1BV–1BL**, parameres: **1BV**, parameres (p) and ventral lobe of aedeagus (phallicata) (vl), ventral; **1BL**, left paramere, left lateral. **1CL–1CD**, aedeagus (phallicata) and lateroventral lobes: **1CL**, aedeagus and ventral lobe, lateral; **1CD**, aedeagus and lateroventral lobes (lvl), dorsal. **1DD**, apicodorsal lobe of segment IX (al) and preanal appendages (pa); **1DV**, segment X, ventral, ab = apical band, as = anal sclerites, va = non-sclerotized ventral area. **2**, female segments VIII–XI: **2L**, left lateral; **2D**, dorsal; **2V**, ventral. Scale bars: unlabelled = 1 mm; circle = 0.5 mm; asterisk = 250 µm.



FIGURES 3, 4. Male and female genitalia of *Rhyacophila fasciata viteceki* Valladolid & Kučinić ssp. nov. **3**, male: **3A**, 2nd segment of left inferior appendage, left lateral; **3BV–3BL**, parameres: **3BV**, parameres (p) and ventral lobe of aedeagus (phallicata) (vl), ventral; **3BL**, left paramere, left lateral. **3CL–3CD**, aedeagus (phallicata) and lateroventral lobes: **3CL**, aedeagus and ventral lobe, left lateral; **3CD**, aedeagus and lateroventral lobes (lvl), dorsal. **3DD**, apicodorsal lobe of segment IX (al) and preanal appendages (pa); **3DV**, segment X, ventral, ab = apical band, as = anal sclerites, va = non-sclerotized ventral area. **4**, female segments VIII–XI: **4L**, left lateral; **4D**, dorsal; **4V**, ventral. Scale bars: unlabelled = 1 mm; circle = 0.5 mm; asterisk = 250 µm.



FIGURES 5–7. Male genitalia of *Rhyacophila* spp. **5**, *R. fasciata delici* Kučinić & Valladolid ssp. nov. **6**, *R. fasciata viteceki* Valladolid & Kučinić ssp. nov. **7**, *R. f. fasciata* Hagen, 1859. **5a–7a**, 2nd segment of left inferior appendage, left lateral; **5b–7b**, apicodorsal lobe and preanal appendages, dorsal; **5c–7c**, parameres and aedeagus (phallicata), ventral; **5d–7d**, left paramere, left lateral; **5e–7e**, aedeagus (phallicata) and ventral lobe, left lateral. Scale bars: a–c = 0.5 mm, d–e = 250 µm.



FIGURES 8–10. Segments VIII–XI of females of *Rhyacophila* spp. **8**, *R. fasciata delici* Kučinić & Valladolid ssp. nov. **9**, *R. fasciata viteceki* Valladolid & Kučinić ssp. nov. **10**, *R. f. fasciata* Hagen, 1859. **8L–10L**, left lateral; **8D–10D**, dorsal; **8V–10V**, ventral. Scale bar: 1 mm.

Morphological characters diagnosing males of *R. fasciata delici* and *R. f. fasciata*

i) In *R. fasciata delici*, apical segment of each inferior appendage with posterior edge straight or slightly convex (Figs 1A, 5a), ventral edge at least two times longer that dorsal edge. In *R. fasciata fasciata*, apical segment of each inferior appendage with posterior edge slightly convex dorsally and slightly concave subapicoventrally (Fig 7a), ventral edge less than two times as long as dorsal edge.

ii) In *R. fasciata delici*, parameres in lateral view (Figs 1BL, 5d) with few thick spines on midventral margin and posterior midventral area, parallel to surface (Figs 5c, 5d); midlateral surface almost completely covered by very fine spicules or setae, reaching from anteroventral to posterodorsal edges, decreasing in size from ventral to dorsal margin, absent on middorsal edge. In *R. fasciata fasciata*, (Fig 7d) parameres in lateral view each with two rows of thick spines diverging from midventral margin (Figs 7c, 7d); midlateral surface covered partially by fine spicules or setae, reaching from middle posteroventral edge almost to posterodorsal edge, absent from anteroventral and dorsal edges.

iii) In *R. fasciata delici*, dorsal margin of aedeagus straight; upper posterior edge of aedeagus concave, then straight caudad, ventral apex truncate (Figs 1CL, 5e), subtriangular and pointed backward in ventral view (Fig 1BV vl); lateroventral lobes of phallus (Fig 1CD lvl) convex, narrowing progressively towards apex, apicolateral margins slightly pointed mesad, posterior edge of each lobe concave. In *R. fasciata fasciata* anterodorsal margin of aedeagus shallowly concave; posterior edge of aedeagus straight, projected posteroventrally, ventral subapical margin round, sligthly truncate (Fig 7e), triangular in ventral view; lateral margins of lateroventral lobes of phallus almost parallel, posterolateral edges almost straight, apicolateral margins round.

iv) In *R. fasciata delici*, apicodorsal lobe of segment IX subcircular, posterior half maximum width about two times width of base (Figs 1DD al, 5b); preanal appendages with concave posterior edges (Figs 1DD pa, 5b); in ventral view, apical band (Fig 1DV ab) longer that wide, inner and outer edges almost parallel in posterior third, non-sclerotized ventral area with apicomesal excision, posterior edges rounded (Fig 1DV va). In *R. fasciata fasciata*, apicodorsal lobe of segment IX uniformly rounded, almost circular (Fig. 7b), preanal appendages with convex posteromesal edges; in ventral view, apical band almost as wide as long, posterior edges converging posterad, posterior edge of non-sclerotized ventral area with V-shaped posteromesal excision.

Description of the female of *R. fasciata delici* and morphological character diagnosing female genitalia of *R. fasciata delici* and *R. fasciata fasciata*

i) *R. fasciata delici,* in lateral view (Figs 2L, 8L), posterodorsal margin of segment VIII valves on each side concave, with dorsolateral projection, posteroventral margin pointed or round; triangular yellow sclerotized tissue pointed backwards at midheight on segment IX. In *R. fasciata fasciata* (Fig. 10L), with posterior margins of segment VIII valves on each side irregular, with dorsolateral, ventrolateral, and mid-height projections of different sizes and shapes.

ii) *R. fasciata delici* in dorsal view (Figs 2D, 8D), with indentation between segment VIII valves, apicodorsal ends of valves narrow with round apical corners, almost parallel anterior and posterior edges, delimiting oval space. In *R. fasciata fasciata* (Fig 10D), apicodorsal ends of valves with round apical corners, wider than in *R. fasciata delici*, in some specimens with small projection in subapical corners, anterior and posterior edges not parallel.

iii) *R. fasciata delici* in ventral view (Figs 2V, 8V), segment VIII valves with proximal edges diverging, slightly concave, posterior edges truncate, intersegmental membrane with pair of elongate sclerites, more or less sclerotized near apex. Band of yellow sclerotized tissue along each lateral margin of segment, visible in lateral, dorsal and ventral views (Figs 2L, 2D, 2V, 8L, 8D, 8V). In *R. fasciata fasciata* (Fig. 10V) segment VIII valves with proximal edges nearly parallel, posterior edges irregular, intersegmental membrane with pair of oval sclerites near valves. Narrow lateral bands of yellow sclerotized tissue at midheight on segment IX, sometimes not conspicuous.

***Rhyacophila fasciata viteceki* Valladolid & Kučinić (ssp. nov.)**

This subspecies has been found in Bosnia and Herzegovina.

Etymology. The specific name is the genitive of Vitecek, given in honour of Dr. Simon Vitecek, biologist and trichopterologist of Wasser Cluster Lunz, Lunz am See, Austria.

Type material. Holotype ♂: BOSNIA AND HERZEGOVINA, river Neretva, Bačevići (43.254154º N 17.824627º E, 35 m a.s.l.), 30/vi/17 (Stanić-Koštroman & M. Kučinić) [N1, MNCN_Ent 269367 (MNCN)].

Paratypes: 2 ♂, same locality as holotype, 30/vi/17 (Stanić-Koštroman & M. Kučinić) [N2, no. 4455, (MCK) and N3, no. 1111 (UMBH)]. 2 ♂ and 1 ♀, river Sturba, 30/viii/18 (M. Kučinić) [1 ♂, S1, MNCN_Ent 269368 (MNCN); 1 ♀, S3, MNCN_Ent 269369 (MNCN); 1 ♂ S2, no. 4456 (MKC)]. 1 ♂, river Bunica, 04/vi/17 (Stanić-Koštroman & M. Kučinić) [B1, no. 1112 (UMBH)].

Description of the imago. Holotype (N1): Length from front of head to distal edge of segment IX 8.91 mm, each forewing 12.55 mm, each hind wing 10.54 mm.

Males: Length 8.29–9.21 mm ($\bar{x}$ = 8.72, n = 6), each forewing 9.83–12.55 ($\bar{x}$ = 11.34, n = 6), each hind wing 8.85–10.54 mm ($\bar{x}$ = 9.79, n = 6).

Female: Length from front of head to distal edge of segment VIII 11.26 mm (n =1), forewing 14.52 mm (n = 1), hind wing 12.15 mm (n = 1).

In ethanol-preserved specimens colour generally pale brown, yellowish, with golden brown setae. Head, thorax, and abdomen dorsally pale brown, with small dark brown spots in dorsal area, some specimens in lateral view with long black spots marking borders between dorsal and ventral areas, abdomen ventrally yellowish; female darker than male, anterior 2/3 of abdominal segments pale brown or brown in darker specimens. Legs light, with spurs reddish brown. Forewings brown, spotted; hind wings pale.

Male genitalia (Figs 3, 6a–6e): Apical segment of each inferior appendage (Figs 3A, 6a) with basal and distal edges diverging, posterior edge oblique and slightly convex, ventral edge straight to slightly concave, at least two times longer that dorsal edge. Apicodorsal vertex angular, apicoventral angle projecting as thick lobe narrowing progressively to round apex (Fig 6a).

Parameres (Figs 3BV–3BL, 6c–6d) in ventral view curved posteromesad in apical 2/3 (Figs. 3BV p, 6c). In lateral view (Figs. 3BL, 6d) each slender at base, slightly dilated in middle, with rounded dorsal margin before midlength and almost straight ventral margin at midlength, pointed at apex; row of few thick spines on midventral margin (Figs 6c–6d); midlateral surface covered by very fine spicules or setae, reaching from anteroventral to posterodorsal edges of paramere, absent from dorsal edge.

Aedeagus (phallicata) in lateral view (Figs 3CL, 6e) with dorsal margin deeply concave and posterior corner of concavity hooked anterad; upper posterior edge concave to obliquely truncate apex, ventral edge sinous. Lateroventral lobes of phallus (Fig. 3CD lvl) convex, narrowing progressively towards apex, apicolateral margins round, posteromesal edge of each lobe concave. Ventral lobe of aedeagus nearly round, tapering and acute caudally (Fig 3BV vl).

Apicodorsal lobe of segment IX (Figs 3DD, 6b) slightly dilated subapicolaterally, posterior edge semicircular, lateral edges converging anterad; preanal appendages (fig 3DD pa) shorter than apicodorsal lobe (Fig 3DD al), obliquely subtruncate apicolaterally, with slightly concave posteromesal edges (Figs. 3DD, 6b). In ventral view, apical band V-shaped, inner and outer edges converging in posterior third, pointed apically, longer than wide (Fig 3DV ab); posterior edge of non-sclerotized ventral area slightly concave, with small mesal incision (Fig 3DV va), anal sclerites subtriangular, concave apically (Fig 3DV as).

Morphological characters diagnosing males of *R. fasciata viteceki* and *R. fasciata fasciata*

i) In *R. fasciata viteceki*, apical segment of each inferior appendage with posterior edge slightly convex (Figs 3A, 6a), ventral edge at least two times as long as dorsal edge. In *R. fasciata fasciata*, apical segment of each inferior appendage with posterior edge slighly convex dorsally and slightly concave subapicoventrally (Fig 7a), ventral edge less than two times as long as dorsal edge.

ii) In *R. fasciata viteceki*, parameres in lateral view (Figs 3BL, 6d) with row of few thick spines on midventral margin (Figs 6c, 6d); midlateral surface covered by very fine spicules or setae, reaching from anteroventral to posterodorsal edges of paramere, absent from dorsal edge. In *R. fasciata fasciata*, (Fig. 7d) parameres in lateral view each with two rows of thick spines diverging from midventral margin (Figs 7c, 7d); midlateral surface covered partially by fine spicules or setae, reaching from middle posteroventral edge to almost posterodorsal edge, absent from anteroventral and dorsal edge.

iii) In *R. fasciata viteceki*, anterodorsal margin of aedeagus deeply concave; posterodorsal edge concave, apex obliquely truncate, ventral subapical margin round (Figs 3CL, 6e), subcircular, tapering and acute caudally in ventral view (Figs. 3BV vl); lateroventral lobes of phallus (Fig 3CD lvl) convex, narrowing progressively towards apex, apicolateral margins round. In *R. fasciata fasciata*, anterodorsal margin of aedeagus shallowy concave; posterior edge of aedeagus straight, projected posteroventrally, ventral subapical margin round (Fig 7e), triangular in ventral view; lateral margins of lateroventral lobes of phallus almost parallel, posterolateral edges almost straight, apicolateral margins round.

iv) In *R. fasciata viteceki*, apicodorsal lobe of segment IX slightly dilated subapicolaterally, posterior edge semicircular, lateral edges converging anterad (Figs 3DD al, 6b); preanal appendages obliquely subtruncate apicolaterally, with slightly concave posteromesal edges (Figs 3DD pa, 6b); in ventral view, apical band (Fig 3DV ab) longer than wide, posterior edge of non-sclerotized ventral area slightly concave, with small mesal incision (Fig 3DV va). In *R. fasciata fasciata*, apicodorsal lobe of segment IX uniformly round, almost circular (Fig 7b), preanal appendages with convex posteromesal edges; in ventral view, apical band almost as wide as long, posterior edges converging posterad, posterior edge of non-sclerotized ventral area with V-shaped posteromesal excision.

Description of the female of *R. fasciata viteceki* and morphological character diagnosing female genitalia of *R. fasciata viteceki* and *R. fasciata fasciata* (1 specimen)

i) *R. fasciata viteceki,* in lateral view (Figs 4L, 9L), with posterior margins of segment VIII valves on each side concave, with dorsolateral and ventrolateral projections of similar size; triangular yellow sclerotized tissue pointed caudad at mid-height of segment IX. In *R. fasciata fasciata* (Fig 10L) posterior margin of segment VIII valves on each side irregular, with dorsolateral, ventrolateral, and mid-height projections of different sizes and shapes.

ii) *R. fasciata viteceki* in dorsal view (Figs 4D, 9D) with indentation between segment VIII valves, apicodorsal ends of valves dilated, fan shaped. In *R. fasciata fasciata* (Fig 10D), apicodorsal ends of valves with round apical corners, in some specimens with small projection in subapical corners.

iii) *R. fasciata viteceki* in ventral view (Figs 4V, 9V), segment VIII valve proximal edges slightly convex, posterior edges sinuous; intersegmental membrane with pair of elongate sclerites, reddish brown with pale spots inside. Two bands of yellow sclerotized tissue along lateral surfaces of segment IX, visible in lateral and ventral views, connected ventrally by band of yellow tissue anterior of pair of sclerites (Figs 4L–4V, 9L–9V). In *R. fasciata fasciata* (Fig 10V) segment VIII valves with proximal edges nearly parallel, posterior edges irregular, intersegmental membrane with pair of oval sclerites near valves without pale spots. Narrow lateral bands of yellow sclerotized tissue at midheight on segment IX, sometimes not conspicuous.

Genetic analysis

The mitochondrial sequences of the cytochrome oxidase gene subunit I (mtCOI) analysed show clear differences between the nominal *R. fasciata fasciata* specimens and those of *R. fasciata delici, R. fasciata viteceki, R. denticulata, R. sociata*, and *R. kykladica*. Based on the divergence of sequences, there are six well-supported branches in maximum likelihood tree, corresponding to *R. fasciata delici* (bootstrap value of 92) (Fig. 11a), *R. fasciata fasciata* (b.v. of 99) (Fig. 11c), *R. denticulata* (b.v. of 98) (Fig. 11d), *R. sociata* (b.v. of 98) (Fig. 11e), *R. kykladica* (b.v. of 100) (Fig. 11f), all clearly different from the *R. relicta* branch (b.v. of 99) (Fig. 11g). *R. fasciata viteceki* (Fig. 11b) appears as an unresolved paraphyletic group in this analysis (b.v. of 72). Intraspecific distances (maximum composite likelihood model) are lower than 1% (Table 2a), being the lowest in *R. f. viteceki* (0.0017), and interspecific distances are mostly higher than 4.35% (Table 2b) among all of these species. The lower distances are between species *R. denticulata* and *R. sociata* (0.0430) and between subspecies *R. f. fasciata* & *R. f. delici* (0.0435) and *R. f. fasciata* & *R. f. viteceki* (0.0239) and *R. f. delici* & *R. f. viteceki* (0.0203).

TABLE 2. Genetic distances (maximum composite likelihood model). 2a: intraspecific distances, n/c = not computed (1 specimen only). 2b: interspecific distances.

Table 2a

Intraspecific	
R. relicta	n/c
R. kykladica	0.0019
R. sociata	0.0062
R. denticulata	0.0053
R. f. fasciata	0.0041
R. f. delici	0.0087
R.f. viteceki	0.0017

Table 2b

Interspecific	***R. relic.***	***R. kykla.***	***R. soc.***	***R. den.***	***R. f. fas.***	***R. f. delici***
R. relicta						
R. kykladica	0.0699					
R. sociata	0.0870	0.0773				
R. denticulata	0.0935	0.0669	0.0430			
R. f. fasciata	0.0717	0.0655	0.0678	0.0619		
R. f. delici	0.0719	0.0551	0.0614	0.0579	**0.0435**	
R. f. viteceki	0.0585	0.0506	0.0474	0.0470	**0.0239**	**0.0203**

Discussion

Mitochondrial DNA (mtDNA) sequences can be useful as a first indicator in species delineation, and, combined with careful morphological, behavioural, and ecological analysis, can help to establish accurate species boundaries (Bickford *et al.* 2007; Dasmahapatra & Mallet 2006; Galtier *et al.* 2009). Most recent studies show the importance of mtDNA in the creation of new species, through mitochondrial-nuclear (mitonuclear) coevolution: Some changes in mitochondrial DNA are selected and lead to cyto-nuclear incompatibility between populations that become new species (Burton *et al.* 2013; Galtier *et al.*, 2009; Chou & Leu 2015; Healya & Burton 2020; Hill 2016, 2017). Hill (2016) proposed a new definition for bisexual species: *A species is a population that is reproductively isolated from other populations by incompatibilities in uniquely coadapted mt and N-mt genes. Species boundaries are the boundaries of coadapted mitochondrial and nuclear genes, which will be boundaries already deduced from the mtCOI barcode gap*.

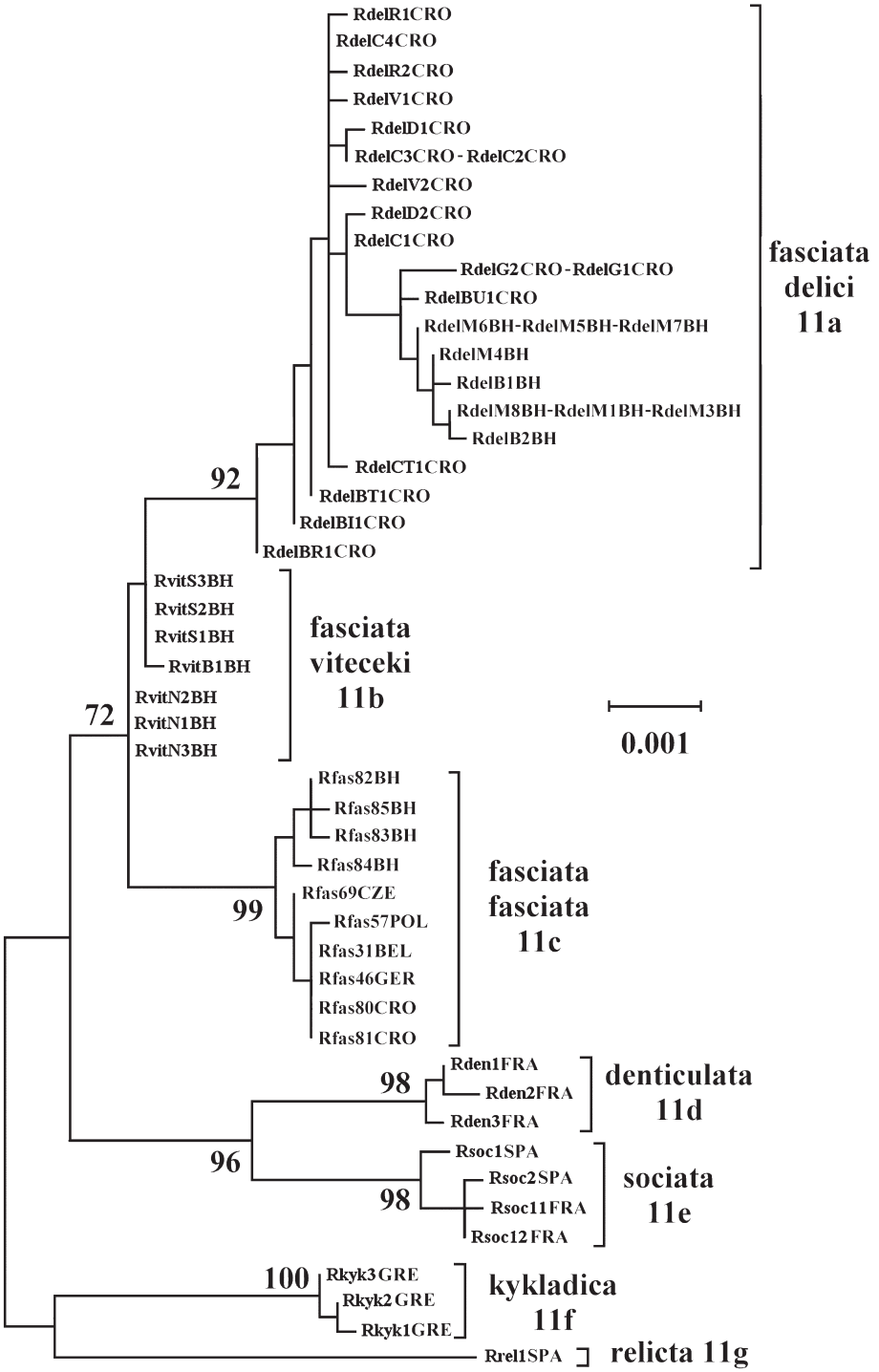


FIGURE 11. Maximum likelihood tree of some species and subspecies of the *Rhyacophila fasciata* Group: *Rhyacophila fasciata delici* (Rdel) (**11a**), *Rhyacophila fasciata viteceki* (Rvit) (**11b**), *Rhyacophila f. fasciata* (Rfas) (**11c**), *Rhyacophila denticulata* (Rden) (**11d**), *Rhyacophila sociata* (Rsoc) (**11e**), *Rhyacophila kykladica* (**11f**) together with *Rhyacophila relicta* (Rrel) (**11g**) as outgroup, based on mitochondrial COI sequence data. Bootstrap values of main branches are shown; data for specimens are summarized in Table 1. RdelBH = *R. fasciata delici* specimens from Bosnia and Herzegovina, RdelCRO = *R. fasciata delici* specimens from Croatia; RdenFRA = *R. denticulata* specimens from France; RfasBEL = *R. f. fasciata* specimen from Belgium, RfasBH = *R. f. fasciata* from Bosnia and Herzegovina, RfasCRO = *R. f. fasciata* specimens from Croatia, RfasCZE = *R. f. fasciata* specimen from Czech Republic, RfasGER = *R. f. fasciata* specimen from Germany, RfasPOL = *R. f. fasciata* specimen from Poland; RkykGRE = *R. kykladica* specimens from Greece; RrelSPA = *R. relicta* specimen from Spain; RsocFRA = *R. sociata* specimens from France, RsocSPA = *R. sociata* specimens from Spain; RvitBH = *R. fasciata viteceki* specimens from Bosnia and Herzegovina. Scale bar: 1% divergence.

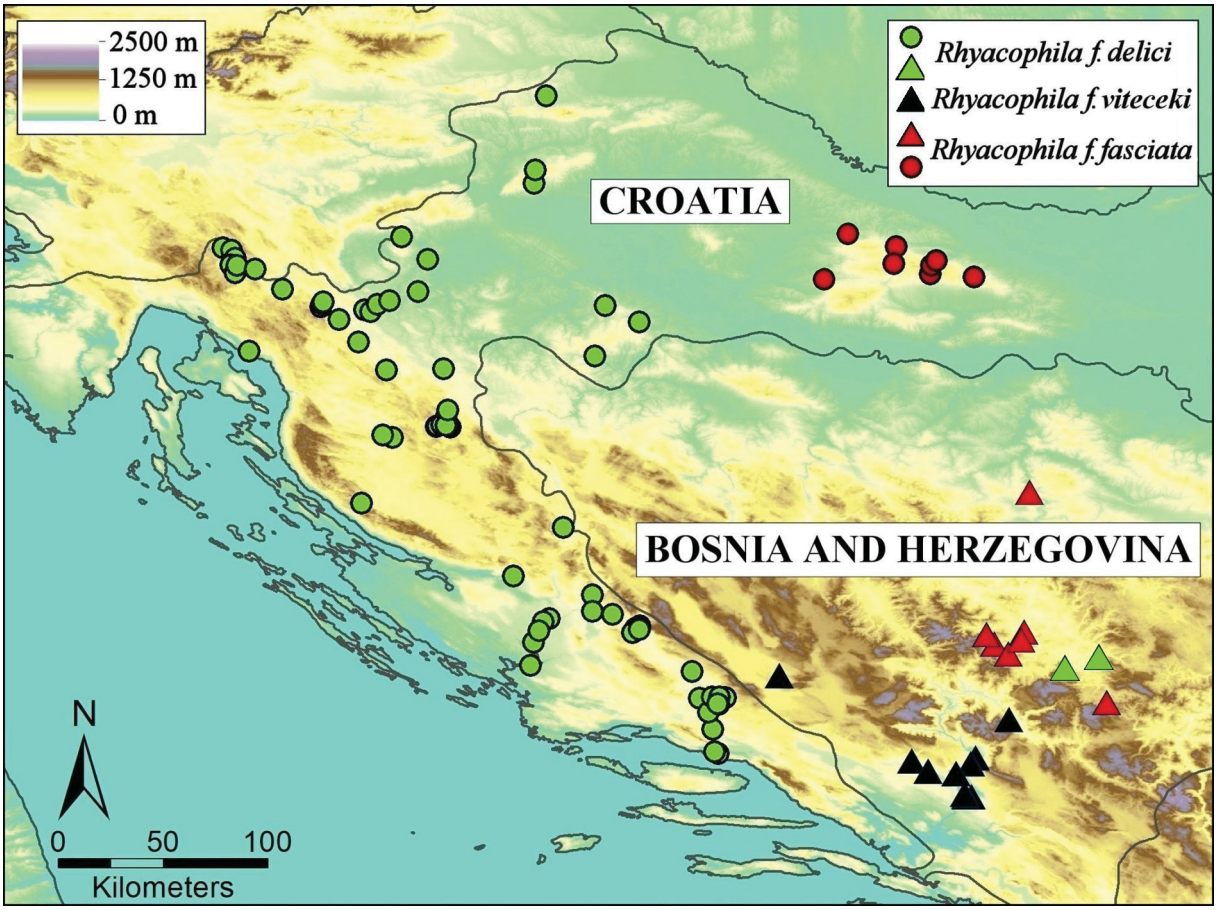


FIGURE 12. Distribution of *Rhyacophila fasciata delici* Kučinić & Valladolid ssp. nov., *R. fasciata viteceki* Valladolid & Kučinić ssp. nov., and *R. f. fasciata* Hagen 1859 in Croatia and in Bosnia and Herzegovina. Colour scale: altitude (m a.s.l.). Circle: species and subspecies present in Croatia, Triangle: species and subspecies present in Bosnia-Herzegovina. Green: *R. fasciata delici*, black: *R. fasciata viteceki*, red: *R. f. fasciata.*

Among the mitochondrial DNA, the nucleotide sequence of the gene cytochrome c oxidase subunit 1 has been established as a highly effective DNA barcode for diagnosing the species boundaries of animals (Hill 2016; Hebert *et al*. 2003b; Dasmahapatra & Mallet 2006; Bucklin *et al.* 2011). In a majority of metazoan populations, there is a correspondence between the species designated by mtCOI and species previously described morphologically (Tavares & Baker 2008; Tavares *et al.* 2011) and discovery of barcode gaps often leads to the recognition of new species upon further study (Hebert *et al.* 2004; Bickford *et al.* 2007; Hill, 2016).

In this study, the differences observed at genetical and morphological levels between specimens corroborate the hypothesis that in Croatia and Bosnia and Herzegovina there are at least three different subspecies: *R. f. fasciata* is found in northern Croatia and Bosnia and Herzegovina and is genetically close and morphologically similar to specimens of other European countries, *R. fasciata delici* is a new subspecies present in Croatia and in Bosnia and Herzegovina and *R. fasciata viteceki*, is a new subspecies found in Bosnia and Herzegovina. Morphological and genetic results highlight the new subspecies status: The intraspecific distances were always lower than 1% divergence, and interspecific distances were usually higher than 4.35% (Table 2), in a similar way that we found in specimens of *R. denticulata* and *R. sociata* (Valladolid *et al.* 2018), and *R. kykladica* (Valladolid *et al.* 2019). Other authors that have analysed the divergence of mtCOI sequences between different species of several taxonomic groups found that more than 98% of the species pairs show divergences greater than 2% (e.g., Hebert *et al*. 2003b), and that the intraspecific divergences usually are less than 1% (Avise 2000). We also agree with the premise of Hebert *et al.* (2003a) that we can recognize species by their genetic divergences from known species assemblages. In this case, both subspecies were identified originally as *R. f. fasciata.* Based on our morphological and genetic evidence and the geographical isolation of the populations (allopatric populations), we propose conservatively the creation of two new subspecies: *R. fasciata delici* Kučinić & Valladolid, from Croatia and Bosnia and Herzegovina, and *R. fasciata*

viteceki Valladolid & Kučinić, from Bosnia and Herzegovina. Possible future discovery of co-occurrence of two or all three of these haplotypes/morphotypes may demonstrate their reproductive isolation and justify their elevation as distinct species according to Hill's (2016) definition.

Distribution and Ecology. The Balkan Peninsula is known for its high species diversity of aquatic insects (Kumanski 1985; 1988; Kvifte *et al*. 2013; Malicky 2005; Petrović *et al*. 2014; Vitecek *et al*. 2015; Vilenica *et al*. 2017). In this part of Europe, many aquatic species have been found and described recently, showing small distribution areas [microendemic species (e.g., Graf *et al.* 2012; Kvifte *et al.* 2013; Mičetić Stanković *et al.* 2015; Murányi 2011; Vitecek *et al.* 2017)]. Together with the Alps, the Iberian Peninsula, the Apennine Peninsula, and the Carpathian Mountains, the Balkan Peninsula is one of the most interesting areas in Europe concerning biodiversity and endemisms.

The distributions of these three subspecies of the *Rhyacophila fasciata* Group in Croatia and Bosnia and Herzegovina, *Rhyacophila f. fasciata, R. fasciata delici*, and *R. fasciata viteceki*, are shown in Fig. 12.

Rhyacophila f. fasciata was found in northeastern Croatia and northern Bosnia and Herzegovina. In Croatia, the subspecies inhabits the rivers of the Papuk Nature Park: Dubočanka, Kovačica, Djedovica, Brzaja, and Jankovac spring (Previšić *et al*. 2013), and the upper part of the Kutjevačka, a 13 km long river situated on the slopes of the Krndija Mountain, in the eastern part of the Park. This area is mostly covered by forests and harbours a large network of streams and a variety of different freshwater areas.

In Bosnia and Herzegovina we found this subspecies in Bosnia: Trnovo River (Botošâneanu 1960, 1961) and Bosna, Fojnička, Kreševka and Željeznica Rivers (Stanić-Koštroman *et al.* 2015). This last area belongs to a mountainous region of Bosnia and Herzegovina characterized by moderate continental climate with strong influences of mountain and subalpine climates, with harsh snowy winters and moderately warm summers. The rivers belong to the Bosna Basin and the Black Sea watershed (Stanić-Koštroman *et al.* 2015).

Rhyacophila fasciata delici is present in all ecological areas of Croatia, in the lowland continental part (Kučinić *et al.* 2010a; Previšić *et al.* 2007, 2013), the central-mountain part (Cerjanec 2012; Kučinić 2002, Kučinić *et al.* 2008, 2017; Previšić *et al.* 2010), and in the Mediterranean part (Graf *et al.* 2008; Kučinić *et al.* 2011c; Vučković 2011). In these areas this subspecies was previously identified as *R. fasciata.*

In our investigation of caddisflies, we usually found *R. fasciata delici* in springs, streams and rivers but almost never in lake habitats (Kučinić *et al*. 2017). For example, in the NP Plitvice Lakes in the central-mountain area we recorded this subspecies in springs, streams, and tuffa barrier, but never in lakes (Kučinić 2002; Kučinić *et al.* 2017; Previšić *et al.* 2007).

In the continental part of Croatia (NP Plitvice Lakes) in emergence pyramid traps we found adults with long winter flight period in all months except February (Previšić *et al.* 2007). In the Mediterranean part (Krka River), we collected adults of *R. fasciata delici* in the period between March to December and in the Cetina River in all months except March. These results are in accordance with the findings of Otto (1981) and demonstrate that caddisflies with predatory larvae have food resources during all periods of the year and long emergence of adults (Previšić *et al.* 2007) without well-separated generations.

In Croatia we found this subspecies in altitudes from 53 m a.s.l. to about 720 m a.s.l. (Kučinić *et al.* 2011c, 2017).

In Bosnia and Herzegovina, *R. fasciata delici* has been found in two localities near Sarajevo: the Miljacka River in Dariva, and the Bosna River, in Vrelo Bosne, about 560–580 m a.s.l. (Kalamujić, personal communication).

Rhyacophila fasciata viteceki is a common subspecies in Bosnia and Herzegovina, widespread in the mountainous, sub-mountainous, and Mediterranean regions of Herzegovina. It inhabits faster-flowing streams and rivers, from the crenal to the metarhithral zone, and the larvae are most numerous on the boulders, cobbles, and pebbles. In comparative studies undertaken during 2003 and 2004, *R. fasciata viteceki* was very common in Herzegovina, while it was less prevalent at stations in central Bosnia where *Rhyacophila nubila* Zetterstedt 1840 occurs (Stanić-Koštroman 2009; Stanić-Koštroman *et al.* 2015).

The specimens studied come from the Neretva Basin and Sturba River. The Neretva River is located in the karst region of Bosnia and Herzegovina, characterized by modified Mediterranean climate with maritime influences: Relatively low humidity and cloudiness, long hot summers and more frequent rainfall during winter. This basin belongs to Adriatic Sea watershed.

The Sturba is a karstic river situated in the western part of Bosnia and Herzegovina, near the town of Livno, with a spring in the village Sturba. It is a short river (15 km long) that flows slowly through the largest karst field in Europe, Livanjsko Polje, and belongs to the Adriatic Sea watershed. The adult caddisflies were collected near the

spring in the upper part of the river (400 m from the spring), in the village of Sturba. In this location, the substrate consisted of stones, pebbles, moss, and aquatic vegetation.

Specimens of *R. fasciata viteceki* have been found from 30 m a.s.l. to 755 m a.s.l.

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References

Alda, F., Rey, I. & Doadrio, I. (2007) An improved method of extracting degraded DNA samples from birds and other species. *Ardeola*, 54 (2), 331–334.
https://www.ardeola.org/uploads/articles/docs/1329.pdf

Altschul, S.F., Madden, T.L., Schaeffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Gapped blast and psi-blast: A new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402.
https://doi.org/10.1093/nar/25.17.3389

Avise, J.C. (2000) *Phylogeography. The History and Formation of Species.* University Press, Cambridge, Massachusetts, 447 pp.

Bertrand, H. (1954). Les Trichopteres. *In*: Lechevalier, P. (Ed.), *Les insectes aquatiques d'Europe*. *Encyclopédie Entomologique*, Serie A, XXXI (2), pp. 1–548.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22, 148–155.
https://doi.org/10.1016/j.tree.2006.11.004

Botoşăneanu, L. (1960) Trichoptères de Yougoslavie recueillis en 1955 par le Dr. F. Schmid. *Deutche Entomologische Zeitschrift*, 7 (3), 261–293.
https://doi.org/10.1002/mmnd.19600070304

Botošâneanu, L. (1961) Materiaux pour servir à la connaissance des Trichoptères d'Europe Orientale et Centrale. *Folia Entomologica Hungarica*, Series Nova, 14 (2), 11–91.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. (2011) DNA barcoding of marine Metazoa. *Annual Review of Marine Science*, 3, 471–508.
https://doi.org/10.1146/annurev-marine-120308-080950

Burton, R.S., Pereira, R.J. & Barreto, F.S. (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution and Systematics*, 44, 281–302.
https://doi.org/10.1146/annurev-ecolsys-110512-135758

Cerjanec, D. (2012) *Ekološke i biogeografske značajke faune tulara (Insecta: Trichoptera) u različitim tipovima staništa sliva rijeke Dobre.* Doktorska disertacija (PhD Thesis), Faculty of Science, University of Zagreb, Zagreb, 172 pp.

Chou, J.-Y. & Leu, J.-Y. (2015) The Red Queen in mitochondria: cyto-nuclear co-evolution, hybrid breakdown and human disease. *Frontiers in Genetics*, 6, 1–29.
https://doi.org/10.3389/fgene.2015.00187

Curtis, J. (1834) Description of some hitherto nondescript British species of mayflies of anglers. *The London and Edinburgh Philosophical Magazine and Journal of Science*, 3 (4), 120–125 + 212–218.
https://doi.org/10.1080/14786443408648304

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9 (8), 772.
https://doi.org/10.1038/nmeth.2109

Dasmahapatra, K. & Mallet, J. (2006) DNA barcodes: Recent successes and future prospects. *Heredity*, 97, 254–255.
https://doi.org/10.1038/sj.hdy.6800858

Eidel, K. (1974) Die Köcherfliegen (Trichoptera) des Wutachgebietes. *Mitteilungen des badischen Landesvereins für Naturkunde und Naturschutz*, 11 (2), 181–195.

[https://www.zobodat.at/pdf/Mitt-Bad-Landesver-Natkde-Natschutz-Freiburg_NF_11_0181-0195.pdf]
ESRI (2011) *ArcGIS Desktop: Release 10*. Environmental Systems Research Institute. Redlands, California. [program]
Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome C oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.
Fotius-Jaboulet, M.C. (1964) Description de la larve de *Rhyacophila praemorsa* Mcl. et comparaison de cette larve à celle de *Rhyacophila septentrionis* Mcl. *Travaux du Laboratoire de Zoologie et de la Station Aquicole Grimaldi de la Faculté des Sciences de Dijon*, 62, 1–13.
Galtier, N., Nabholz, B., Glemin, S. & Hurst, G.D.D. (2009) Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecology*, 18, 4541–4550.
https://doi.org/10.1111/j.1365-294x.2009.04380.x
GenBank (2020) Genetic Sequence Database. National Center for Biotechnology Information.
https://www.ncbi.nlm.nih.gov/genbank/
GBIF (2020) The Global Biodiversity Information Facility. Available from: https://www.gbif.org/es/species/search?q=Rhyacophila&rank=SPECIES&rank=SUBSPECIES&highertaxon_key=216&highertaxon_key=1430783&qField=SCIENTIFIC (accessed August 2020)
Gilbert, M.T.P., Moore, W., Melchior, L. & Worobey, M. (2007) DNA extraction from dry museum beetles without conferring external morphological damage. *PLOS ONE*, 2 (3), e272.
https://doi.org/10.1371/journal.pone.0000272
Graf, W., Kučinić, M., Previšić, A., Vučković, I. & Waringer, J. (2008) The larva, ecology and distribution of *Tinodes braueri* McLachlan, 1878 (Trichoptera: Psychomyiidae). *Aquatic Insects*, 30 (4), 295–299.
https://doi.org/10.1080/01650420802331158
Graf, W., Popijač, A., Previšić, A., Gamboa, M. & Kučinić, M. (2012) Contribution to the knowledge of *Siphonoperla* in Europe (Plecoptera: Chloroperlidae): *Siphonoperla korab* sp. n. *Zootaxa*, 3164 (1), 41–48.
https://doi.org/10.11646/zootaxa.3164.1.4
Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, 52, 696–704.
https://doi.org/10.1080/10635150390235520
Habdija, I., Primc Habdija, B., Matoničkin, R., Kučinić, M., Radanović, I., Miliša, M. & Mihaljević, Z. (2004) Current velocity and food supply as factors affecting in bryophyte habitats in karst running water. *Biologia, Bratislava*, 59 (5), 577–593. [https://pdfs.semanticscholar.org/1847/ef550e02a05d3f2d9e6ba96793606dff9831.pdf]
Hagen, H. (1859) Die Phryganiden Pictet's. *Entomologische Zeitung*, 20 (4–6), 131–170.
[https://www.biodiversitylibrary.org/item/35933#page/141/mode/1up]
Healy, T.M. & Burton, R.S. (2020) Strong selective effects of mitochondrial DNA on the nuclear genome. *Proceedings of the National Academy of Sciences of the United States of America*, 117 (12), 6616–6621.
https://doi.org/10.1073/pnas.1910141117
Hebert, P.D.N., Cywinska, A., Ball, S.L. & de Waard, J.R. (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London*, Series B, 270 (1512), 313–321.
https://doi.org/10.1098/rspb.2002.2218
Hebert, P.D.N., Ratnasingham, S. & de Waard, J.R. (2003b) Barcoding animal life: Cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London*, Series B, 270 (Supplement 1), S96–S99.
https://doi.org/10.1098/rsbl.2003.0025
Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.
https://doi.org/10.1073/pnas.0406166101
Hill, G.E. (2016) Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap. *Ecology and Evolution*, 6 (16), 5831–5842.
https://doi.org/10.1002/ece3.2338
Hill, G.E. (2017) The mitonuclear compatibility species concept. *The Auk: Ornithological Advances*, 134, 393–409.
https://doi.org/10.1642/auk-16-201.1
Kučinić, M. (2002) *Diversity and distribution of caddisfles in Plitvička Lakes*. PhD Thesis, University of Zagreb, Zagreb, 139 pp. [in Croatian]
Kučinić, M. & Malicky, H. (2002) *Rhyacophila dorsalis plitvicensis*, new subspecies from Croatia. *In*: Mey, W. (Ed.), *Proceedings of the 10th International Symposium on Trichoptera. Nova Supplementa Entomologica*, 15, pp. 145–147.
Kučinić, M., Previšić, A., Gottstein, A., Hrašovec, B., Stanić-Koštroman, S., Pernek, M. & Delić, A. (2008) Description of the larvae of *Drusus radovanovici septentrionis* Marinković-Gospodnetić, 1976 and *Drusus croaticus* Marinković-Gospodnetić, 1971 (Trichoptera: Limnephilidae) from Bosnia and Herzegovina and Croatia. *Zootaxa*, 1783 (1), 1–17.
https://doi.org/10.11646/zootaxa.1783.1.1
Kučinić, M., Bučar, M. & Delić, A. (2010a) Prvi prilog poznavanju faune tulara (Insecta, Trichoptera) na području Banovine [The first contribution to the knowledge of caddisflies (Insecta, Trichoptera) in the Banovina area]. *Zbornik radova: Zrinska gora: regionalni park prirode / Bučar.* Matija (ur.), Matica hrvatska, pp. 226–235. [in Croatian]

Kučinić, M., Previšić, A., Stanić-Koštroman S., Franjević, M., Šerić Jelaska L., Delić, A. & Posilović, H. (2010b) Description of the larvae of *Drusus ramae* Marinković-Gospodnetić and *Drusus medianus* Marinković-Gospodnetić (Trichoptera: Limnephilidae) with some genetic, distributional, ecological, faunal and conservation notes. *Zootaxa*, 2484 (1), 1–24. https://doi.org/10.11646/zootaxa.2484.1.1

Kučinić, M., Previšić, A., Graf, W., Šerić Jelaska, L., Stanić-Koštroman, S. & Waringer, J. (2011a) Larval description, genetic and ecological features of *Drusus radovanovici radovanovici* Marinković-Gospodnetić, 1971 (Trichoptera, Limnephilidae) with some phylogenetic and taxonomic data on the *bosnicus* group in the Balkan Peninsula. *Deutsche Entomologische Zeitschrift*, 58 (1), 135–153. https://doi.org/10.1002/mmnd.201100010

Kučinić, M., Previšić, A., Stanić-Koštroman, S., Graf, W., Franjević, M., Posilović, H. & Waringer, J. (2011b) Morphological and ecological features of *Drusus* larvae from the *D. bosnicus* Group on the Balkan Peninsula with description of the larva of *Drusus klapaleki* Marinković-Gospodnetić, 1971. *Zoosymposia*, 5, 244–254. https://doi.org/10.11646/zoosymposia.5.1.18

Kučinić, M., Vučković, I., Kutnjak, H., Šerić Jelska, L. & Marguš, D. (2011c) Diversity, distribution, ecology and biogeography of caddisflies (Insecta, Trichoptera) in the Krka River (National Park "Krka", Croatia). *Zoosymposia*, 5, 255–268. https://doi.org/10.11646/zoosymposia.5.1.19

Kučinić, M., Szivák, I., Pauls, S.U., Bálint, M., Delić, A. & Vučković, I. (2013) *Chaetopteryx bucari* sp. n., a new species from the *Chaetopteryx rugulosa* group from Croatia (Insecta, Trichoptera, Limnephilidae) with molecular, taxonomic and ecological notes on the group. *Zookeys*, 320, 1–28. https://doi.org/10.3897/zookeys.320.4565

Kučinić, M., Delić, A., Ćuk, R., Previšić, A., Mihoci, I., Žganec, K., Cerjanec, D. & Vučković, I. (2014) The first finding of the *Drusus bosnicus* Group (Insecta, Trichoptera) in Croatia with some notes on diversity, taxonomy, distribution and ecology of genus *Drusus* in Croatia and in Dinaric karst of the Balkan Peninsula. *Natura Croatia*, 23 (2), 265–377. https://hrcak.srce.hr/130945

Kučinić, M., Cerjanec, D., Vučković, I., Mihoci, I., Perović, F., Kutnjak, H., Ibrahimi, H., Pelić Fixa, D., Žalac, S., Mrnjavačić Vojvoda, A. & Plantak, M. (2015) Some new and interesting species of caddisflies (Insecta, Trichoptera) found in] Croatia. *Natura Croatica*, 24 (2), 293–310. https://hrcak.srce.hr/149943

Kučinić, M., Previšić, A., Vajdić, M., Tunjić, M., Mihoci, I., Žalac, S., Sviben, S., Vučković, I., Trupković, M. & Habdija, I. (2017) First systematic investigation of adults and second checklist of caddisflies of the Plitvice Lakes National Park with notes on research history, biodiversity, distribution and ecology. *Natura Croatica*, 26 (2), 225–260. https://doi.org/10.20302/nc.2017.26.19

Kumanski, K. (1985) *Trichoptera, Annulipalpia*. Fauna Bulgarica 15, Bulgarska Akademi na Naukite, Sofia, 243 pp.

Kumanski, K. (1988) *Trichoptera, Integripalpia*. Fauna Bulgarica 19, Bulgarska Akademi na Naukite, Sofia, 354 pp.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kvifte, M.G., Ivković, M. & Klarić, A. (2013) New records of moth flies (Diptera: Psychodide) from Croatia, with the description of *Berdeniella keroveci* sp. nov. *Zootaxa*, 3737 (1), 57–67. https://doi.org/10.11646/zootaxa.3737.1.4

Malicky, H. (2004) *Atlas of European Trichoptera.* Springer, Dordrecht, 359 pp. https://doi.org/10.1007/978-1-4020-3026-0

Malicky, H. (2005) Die Köcherfliegen Griechenlands. *Denisia*, 17, 1–240. https://www.zobodat.at/pdf/DENISIA_0017_0001-0240.pdf

Malicky, H. (2009) Die Köcherfliegen (Insecta, Trichoptera) der Sammlung von Franjo Koscec im Museum Varazdin, Kroatien. *Natura Croatica*, 18 (1), 129–134. https://hrcak.srce.hr/38477

Malicky, H., Previšić, A. & Kučinić, M. (2007) *Rhyacophila cabrankensis* nov. spec. from Croatia. *Braueria*, 34, 14. https://www.zobodat.at/pdf/BRA_34_0014.pdf

Malicky, H. & Sipahiler, F. (1993) Köcherfliegen (Trichoptera) aus der túrkei, mit Bemerkungen zu weiteren mediterranean Köcherfliegen. *Bulletin de la Société Entomologique Suisse*, 66, 457–468.

Marinković-Gospodnetić, M. (1979) Trichoptera (Insecta) velikih karstnih izvora u Dinaridima. *In*: Rauš, Đ. (Ed.), *Drugi kongres Ekologa Jugoslavije (Second Congress of Ecologists of Yugoslavia)*. Savez društava ekologa Jugoslavije, Zagreb, pp. 1837–1849.

Martynov, A.V. (1916) Notes sur quelques nouveaux Trichopteres du Musee du Caucase. *Bulletin du Musee du Caucase, Tiflis*, 9, 186–202

McLachlan, R. (1865) Trichoptera Britannica; a monograph of the British species of caddis-flies. *Transactions of the Entomological Society of London*, Series 3, 5, 1–183. https://doi.org/10.1111/j.1365-2311.1967.tb01433.x

McLachlan, R. (1868) Contributions to a knowledge of European Trichoptera. *Transactions of the Entomological Society of London*, Series 4, 1, 289–308.

https://doi.org/10.1111/j.1365-2311.1868.tb00629.x
McLachlan, R. (1879) *A Monographic Revision and Synopsis of the Trichoptera of the European Fauna. Part VIII*. John Van Voorst, London, pp. 429–500, pls. 45–51. [1874–1880]
https://doi.org/10.5962/bhl.title.28556
Mičetić Stanković, V., Jäch, M.A. & Kučinić, M. (2015) Annotated checklist of Croatian riffle beetles (Insecta: Coleoptera: Elmidae). *Natura Croatica*, 24 (1), 93–109.
https://hrcak.srce.hr/140218
Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: *Proceedings of The Gateway Computing Environments Workshop* (*GCE*), *New Orleans*, *Louisiana*, 14 November 2010, pp. 45–52.
https://doi.org/10.1109/GCE.2010.5676129
Murányi, D. (2011) Balkanian species of the genus *Isoperla* Banks, 1906 (Plecoptera: Perlodidae). *Zootaxa*, 3049, 1–46.
Navás, L. (1916) Tricópteros nuevos de España. 1ª Serie. *Broteria*, *Serie Zoológica*, 14, 5–11.
Nóvak, K. (1963) Beschreibung und bestimmungstabelle der weibchen böhmischer arten der gattung *Rhyacophila*. *Acta Societatis Entomologicae Cechosloveniae*, 60 (4), 304–311.
[http://www.digitalniknihovna.cz/knav/view/uuid:afcf27c3-bac8-11e1-1726-001143e3f55c?page=uuid:afcf290a-bac8-11e1-1726-001143e3f55c]
Oláh, J. (2010) New species and new records of Palaearctic Trichoptera in the material of the Hungarian Natural History Museum. *Annales Historico-Naturales Musei Nationalis Hungarici*, 102, 65–117.
[http://www.nhmus.hu/sites/default/files/balkan/literature/Trichoptera/olah_2010.pdf]
Otto, C. (1981) Why does duration in flight period differ in caddisflies? *Oikos*, 37, 383–386.
https://doi.org/10.2307/3544133
Petrović, A., Simić, V., Milošević, D.J., Paunović, M. & Sivec, I. (2014) Diversity and distributional patterns of stoneflies (Insecta: Plecoptera) in the aquatic ecosystems of Serbia (Central Balkan Peninsula). *Acta Zoologica Bulgarica*, 66 (4), 517–526.
[http://acta-zoologica-bulgarica.eu/downloads/acta-zoologica-bulgarica/2014/66-4-517-526.pdf]
Pictet, F.J. (1834) *Recherches pour Servir à l'Histoire et à l'Anatomie des Phryganides*. A. Cherbuliez, Genève [Geneva] and J.-B. Baillière, Paris, iii + 235 pp., 20 coloured pls.
https://doi.org/10.5962/bhl.title.66017
Previšić, A. & Popijač, A. (2010) Caddisfly (Insecta: Trichoptera) fauna of Kupa and Cabranka rivers and their tributaries, Gorski Kotar, W Croatia. *Natura Croatica*, 19 (2), 357–368.
https://hrcak.srce.hr/index.php?show=clanak&id_clanak_jezik=94079
Previšić, A., Kerovec, M. & Kučinić, M. (2007) Emergence and composition of Trichoptera from karst habitats, Plitvice Lakes Region, Croatia. *International Review of Hydrobiology*, 92 (1), 61–83.
https://doi.org/10.1002/iroh.200510921
Previšić, A., Walton, C., Kučinić, M., Mitrikeski, P.T. & Kerovec, M. (2009) Pleistocene divergence of Dinaric *Drusus* endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. *Molecular Ecology*, 18 (4), 634–647.
https://doi.org/10.1111/j.1365-294x.2008.04046.x
Previšić, A., Graf, W. & Kučinić, M. (2010) Caddisfly (Trichoptera) fauna of the Plitvice Lakes National Park, Croatia. *Denisia*, 29, 287–294.
Previšić, A., Cerjanec D., Graf, W. & Kučinić, M. (2012) *Drusus chrysotus* (Rambur, 1842) (Trichoptera: Limnephilidae: Drusinae): A new caddisfly species in the Croatian fauna. *Natura Croatica*, 21, 419–425.
https://hrcak.srce.hr/94464
Previšić, A., Ivković, M., Miliša, M. & Kerovec, M. (2013) Caddisfly (Insecta: Trichoptera) fauna of Papuk Nature Park, Croatia. *Natura Croatica*, 22 (1), 1–13.
[https://hrcak.srce.hr/index.php?show=clanak&id_clanak_jezik=153540]
Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
https://doi.org/10.1093/sysbio/sys029
Schmid, F. (1970) Le genre *Rhyacophila* et la famille des Rhyacophilidae (Trichoptera). *Mémoires de la Société Entomologique du Canada*, 66, 1–230.
https://doi.org/10.4039/entm10266fv
Scopoli, I.A. (1763) *Entomologia Carniolica Exhibens Insecta Carnioliae Indigena et Distributa in Ordines, Genera, Species, Varietates. Methodo Linnaeana*. I.T. Trattner, Vindobonae [Vienna], xxxvi + 420 pp.
https://doi.org/10.5962/bhl.title.119976
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) 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*, 87 (6), 651–701.
https://doi.org/10.1093/aesa/87.6.651
Sipahiler, F. (2018) A new subspecies of *Rhyacophila fasciata* HAGEN from northern Turkey with a description of the unknown

female of *Rhyacophila fasciata mysica* MALICKY & SIPAHILER (Trichoptera, Rhyacophilidae). *Braueria*, 45, 15–18.
Stamatakis, A. (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30 (9), 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033
Stanić-Koštroman, S. (2009) *Faunal, ecological and biogeographical features of caddisflies (Insecta: Trichoptera) in the Bosnia and Herzegovina*. Ph.D. Thesis, Faculty of Science, University of Zagreb, Zagreb, 151 pp.
Stanić-Koštroman, S., Kučinić, M., Kolobara, A., Škobić, D., Knezović, L. & Durbešić, P. (2012) Light-trapped caddisflies (Insecta: Trichoptera) as indicators of the ecological integrity of the Lištica river, Bosnia and Herzegovina. *Entomologia Croatica*, 16 (1–4), 21–36.
https://hrcak.srce.hr/95189
Stanić-Koštroman, S., Previšić, A., Planinić, A., Kučinić, M., Škobić, D., Dedić, A. & Durbešić, P. (2015) Environmental determinants of contrasting caddisfly (Insecta, Trichoptera) biodiversity in the Neretva and Bosna river basins (Bosnia and Herzegovina) under temperate and Mediterranean climates. *International Review of Hydrobiology*, 100 (2), 79–95.
https://doi.org/10.1002/iroh.201301631
Stephens, J.F. (1836) *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, with Coloured Figures (from Westwood) of the Rarer and More Interesting Species. Mandibulata. Vol. VI* [1836–1837]. Baldwin and Cradock, London.
[https://www.biodiversitylibrary.org/item/36352#page/5/mode/1up]
Szivák, I., Mikes, T., Szalontai, B., Kučinić, M., Vučković, I., Vadkerti, E., Kisfali, P., Pauls, S.U. & Bálint, M. (2017) Ecological divergence of *Chaetopteryx rugulosa* species complex (Insecta, Trichoptera) linked to climatic niche diversification. *Hydrobiologia*, 794, 31–47.
https://doi.org/10.1007/s10750-016-3068-0
Tavares, E.S. & Baker, A.J. (2008) Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. *BMC Evolutionary Biology*, 8 (Art. No. 81), 1–14.
https://doi.org/10.1186/1471-2148-8-81
Tavares, E.S., Gonçalves, P., Miyaki, C.Y. & Baker, A.J. (2011) DNA barcode detects high genetic structure within Neotropical bird species. *PLOS One*, 6, e28543.
https://doi.org/10.1371/journal.pone.0028543
Tobias, W. & Tobias, D. (1981): *Trichoptera Germanica. Bestimmungstafeln für die deutschen Köcherfliegen. Teil I: Imagines*. *Courier Forschungs Institut Senckenberg, Frankfurt*, 49, 1–672.
Ulmer, G. (1909) *Die Süsswasserfauna Deutschlands. Heft 5 & 6. Trichoptera.* Verlag, Gustav Fischer, Jena, 326 pp.
https://doi.org/10.5962/bhl.title.8526
Valladolid, M., Arauzo, M., Basaguren, A., Dorda, B.A. & Rey, I. (2018) *The Rhyacophila fasciata* Group in Western Europe: Confirmation of *Rhyacophila denticulata* McLachlan 1879 (*stat. prom*.) and *Rhyacophila sociata* Navás 1916 (*stat. res*.), based on morphological and molecular genetic evidence (Trichoptera: Rhyacophilidae), *Zootaxa,* 4418 (6), 526–544.
https://doi.org/10.11646/zootaxa.4418.6.2
Valladolid, M., Karaouzas, I., Arauzo, M., Dorda, B.A. & Rey, I. (2019) The *Rhyacophila fasciata* Group in Greece: *Rhyacophila kykladica* Malicky & Sipahiler 1993 (*stat. prom.*) (Trichoptera: Rhyacophilidae). Morphological description, genetic and ecological features. *Zootaxa,* 4657 (3), 503–522.
https://doi.org/10.11646/zootaxa.4657.3.5
Vilenica, M., Mičetić Stanković, V., Sartori, M., Kučinić, M. & Mihaljević, Z. (2017) Environmental factors affecting mayfly assemblages in tufa-depositing habitats of the Dinaric Karst. *Knowledge and Management of Aquatic Ecosystems*, 418, 1–14.
https://doi.org/10.1051/kmae/2017005
Vitecek, S., Previšić, A., Kučinić, M., Bálint, M., Keresztes, L., Waringer, J., Paulus, S.U., Malicky, H. & Graf, W. (2015) Description of a new species of *Wormaldia* from Sardinia and a new *Drusus* species from the Western Balkans (Trichoptera, Philopotamidae, Limnephilidae). *Zookeys*, 496, 85–103.
https://doi.org/10.3897/zookeys.496.9169
Vitecek, S., Kučinić, M., Previšić, A., Živić, I., Stojanović, K., Keresztes, L., Bálint, M., Hoppeler, F., Waringer, J., Graf, W. & Pauls, S.U. (2017) Integrative taxonomy by molecular species delimitation: multi-locus data corroborate a new species of Balkan Drusinae micro-endemics. *BMC Evolutionary Biology*, 17 (Art. No. 129), 1–18.
https://doi.org/10.1186/s12862-017-0972-5
Vučković, I. (2011) *Faunal, taxonomical and ecological characters of caddisflies (Insecta, Trichoptera) in the drainage of the River Cetina*. PhD Thesis, University of Zagreb, Zagreb, 172 pp. [in Croatian]
Waringer, J., Graf, W., Kučinić, M., Previšić, A. & Vučković, I. (2009) The larva and life cycle of *Annitella apfelbecki* (Klapalek, 1899), including a redescription of *Melampophylax nepos* (McLachlan, 1880) (Trichoptera: Limnephilidae). *Aquatic insects*, 31 (1), 71–80.
https://doi.org/10.1080/01650420802616327
Zetterstedt, J.W. (1840) *Insecta lapponica.* Voss, Lipsiae, 1068 pp.
[https://www.biodiversitylibrary.org/item/34332#page/548/mode/1up]