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Article

A surprising finding of *Ecclisopteryx asterix* Malicky, 1979 (Insecta, Trichoptera) in Croatia with notes to DNA barcoding and new distributional data of the subfamily Drusinae

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Abstract
The subfamily Drusinae (Trichoptera, Limnephilidae) is represented in Croatia with eight (8) species, including *Ecclisopteryx asterix* Malicky, 1979 recorded for the first time in Croatian fauna. The species was collected on two occasions: March 10th 2015 and March 6th 2021 in the Šumi Spring, at the Ivanščica Mt. in Hrvatsko Zagorje (North Croatia). This new finding considerably extends the distribution of this species, previously considered a microendemic of the Alps. The distribution of the two species, *Drusus schmidi* Botosaneanu, 1960 and *D. croaticus* Marinković-Gospodnetić, 1971 has been also enlarged with new findings. Within this study, nine specimens of the aforementioned species have been DNA barcoded and sequences were entered into the BOLD database. The results of this paper contribute to the ecological and faunistic knowledge of caddisflies in Croatia, with an emphasis on the subfamily Drusinae.

Key words: Ivanščica Mt., Šumi Spring, caddisfly, new record, Limnephilidae.

Introduction

The family Limnephilidae Kolenati, 1848 is the most species-rich caddisfly (Trichoptera) family in the Palearctic region, with more than 900 species, 300 of which have been recorded in the European fauna (Holzenthal *et al.* 2007; Malicky 2004). The family includes four subfamilies: Dicosmoecinae, Drusinae, Limnephilinae and Pseudostenophylacinae (Holzenthal *et al.* 2007). Due to its specific ecology and evolutionary history resulting in high endemism, the subfamily Drusinae, distributed only in the Palearctic

region (Holznethal *et al*. 2007), has been a subject of intensive research in the past two decades (Previšić *et al.* 2012; Kučinić *et al.* 2008, 2010, 2016b; Vučković *et al.* 2016; Waringer *et al.* 2007, 2010, 2013). There are over 100 species within the Drusinae subfamily in the European fauna characterized by great genetic and morphological variability (e.g. Ibrahimi *et al*. 2015, 2016; Oláh 2010; Previšić *et al*. 2014a, 2014b; Vitecek *et al*. 2015a, 2017).

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 (Vieira-Lanero *et al*. 2005; Vitecek *et al*. 2020; Waringer *et al*. 2016). Drusinae larvae exhibit high niche specificity, mostly inhabiting 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 (AQEM Consortium 2002; Barbour *et al*. 1999). Furthermore, the subfamily has an extremely high number of microendemic species, as a consequence of allopatric speciation of disjunct mountain populations (e. g. Ibrahimi *et al.* 2016; Kučinić *et al*. 2010, 2016b; Previšić *et al*. 2014a; Vitecek *et al*. 2015a, 2017; Waringer *et al*. 2015, 2016).

Approximately 50 Drusinae species have been recorded in the Balkan Peninsula (e. g. Kučinić *et al.* 2011; Malicky 2005; Marinković-Gospodnetić 1979; Oláh *et al.* 2017; Previšić *et al*. 2014a), with an exceptionally high proportion of microendemics (Previšić *et al. ;* Vitecek *et al*. 2015a, 2015b). Previous research has documented seven Drusinae species in Croatia*: Ecclisopterax ivkae* Previšić, Graf & Vitecek 2014, *E. keroveci* Previšić, Graf & Vitecek 2014, *Drusus chrysotus* (Rambur, 1842), *D. croaticus* Marinković-Gospodnetić, 1971, *D. discolor* (Rambur, 1842), *D. schmidi* Botosaneanu, 1960 and *D. vespertinus* Marinković-Gospodnetić, 1976 (Cerjanec *et al*. 2020; Kučinić *et al*. 2014; Previšić *et al*. 2014a; Vučković *et al*. 2016). Out of seven species mentioned, two species have *locus typicus*, in Croatia: *E. ivkae*, from the spring area of the Cetina River (the Glavaš Spring) and *D. croaticus* from the area of the Plitvice Lakes (springs of the Bijela rijeka River and the Plitvica Stream) (Marinković-Gospodnetić 1971; Previšić *et al*. 2014a). Although many aspects of biodiversity, distribution and taxonomy of the Drusinae subfamily in Croatia have already been explored (Cerjanec *et al*. 2020; Kučinić *et al*. 2008, 2014, 2017b, 2020; Oláh *et al*. 2017; Marinković-Gospodnetić 1971, 1979; Previšić *et al.* 2009, 2012, 2013, 2014a, 2014b; Vučković *et al*. 2016), this research makes a very important contribution to the knowledge of Drusinae subfamily with new findings.

The DNA barcoding method was proposed in 2003 (Hebert *et al*. 2003a, 2003b) as a universal, fast and reliable system for determining different biological groups up to species level, and also as a very applicable method for detecting new, undescribed species and species that cannot be distinguished based on morphological characteristics (so-called cryptic species). The method is based on sequencing a barcode region of the cytochrome c oxidase subunit I gene (*COI*) (658 base pairs in length) (Hebert *et al*. 2003a, 2003b). Establishment of the BOLD database in 2007 (Ratnasingham & Hebert 2007) enabled the use of the obtained sequences (DNA barcodes) in the sequence analysis for taxonomic and phylogenetic research, which has both local and global value (Brehm *et al*. 2019; Cárdenas *et al*. 2013; Elías-Gutiérrez *et al*. 2008; Léger *et al*. 2020; Tyagi *et al*. 2017). In the last 10 years, a significant number of papers have been published that include DNA barcoding of Trichoptera (Geraci *et al.* 2011; Morinière *et al*. 2017; Szivák *et al*. 2017; Valladolid *et al.* 2018, 2021; Zhou *et al*. 2016), and a certain part of these also refer to the Croatian fauna (Ćukušić *et al*. 2017; Kučinić *et al*. 2013, 2016a, 2017a, 2019a, 2019b; Valladolid *et al*. 2020).

With all this in mind, we aimed to: review the new findings of the subfamily Drusinae (1), confirm the presence of these species using DNA barcoding (2), and to state certain aspects of the protection of potentially endangered species of the genera *Ecclisopteryx* and *Drusus* (3).

Material and Methods

Sampling and research area

Several remarkable Trichoptera species were collected during the field research of stonefly fauna (Plecoptera) within the NIP project (EU Natura 2000 Integration Project) (2014-2016) (Popijač, 2016). The specimens were collected by handpicking, hand net and kick sampling. Through three years, the field work was done in three periods (autumn, winter, spring). All collected specimens were stored in absolute EtOH and are an integral part of the Trichopteran Popijač Collection stored in the Croatian Museum of Natural History in Zagreb. This paper presents the analysis of larvae in the abovementioned collection from the

subfamily Drusinae (family Limnephilidae) (Table 1). At the Šumi Spring (Ivanščica Mt.), additional larvae of *Ecclisopteryx asterix* were collected on March 6th 2021 (Figure 1).

Table 1. List of six (6) Drusinae species recorded in Croatia within this study.

Locality	Date: nr. of larvae	x	y	Altitude(m)
Ecclisopteryx asterix				
Šumi Spring, Ivanščica	10.3.2015: 1; 6.3.2021: 2	473425	5116652	397
Ecclisopteryx ivkae				
Rumin, Rumin Spring	18.11.2015: 4	512295	4848651	311
Ecclisopteryx keroveci				
Krka River, upstream counfluence with the Una River	28.10.2014: 1	471761	4920104	382
Krka River, counfluence with the Una River	28.10.2014: 1	471518	4920588	380
Drusus croaticus				
Vitunjčica Spring	30.10.2014: 3; 30.3.2015: 2	393365	5017714	346
Jasenačka rijeka Spring, Bjelolasica	13.6.2014: 15; 30.10.2014: 13; 7.4.2015: 24	382815	5014029	648
Dretulja Spring	30.3.2015: 1	408909	4993378	383
Plitvica Spring, Plitvice Lakes	23.6.2014: 6; 19.10.2014: 1; 10.4.2015: 16	426837	4973877	666
Mlinac Spring, Plitvice Lakes	26.6.2014: 3; 9.4.2015: 5	433351	4960491	699
Krasulja upper reach, Mirići	13.10.2014: 1	430765	4953482	696
Gacka, Tonkovića Spring	29.10.2014: 1; 1.4.2015: 16	410331	4961517	470
Gacka, Majer Spring	29.10.2014: 7; 1.4.2015: 16	409717	4964491	470
Čabranka Spring, Gorski kotar	23.4.2016: 4	354934	5052951	561
Drusus schmidi				
Rikino Spring, Krndija Mt.	14.4.2016: 1	606261	5038947	560
Veličanka Stream, Papuk Mt.	20.4.2016: 2	589727	5039621	420
Dubočanka River, Forest University, Papuk Mt.	20.4.2016: 2	594570	5040102	618
Dubočanka River, Papuk Mt.	20.4.2016: 3	592144	5039231	494
Kovačica Stream, Papuk Mt.	25.6.2015: 1	590946	5042755	539
Drusus discolor				
Čabranka Spring, Gorski kotar	1.7.2015: 2; 22.4.2016: 1	354934	5052951	561

Identification of larvae was performed following: Kučinić *et al.* 2008 and Waringer & Graf 2011. In order to confirm identification based on morphological characters, DNA barcoding was performed on several specimens (Table 2). A systematic review is presented according to Morse (2021).

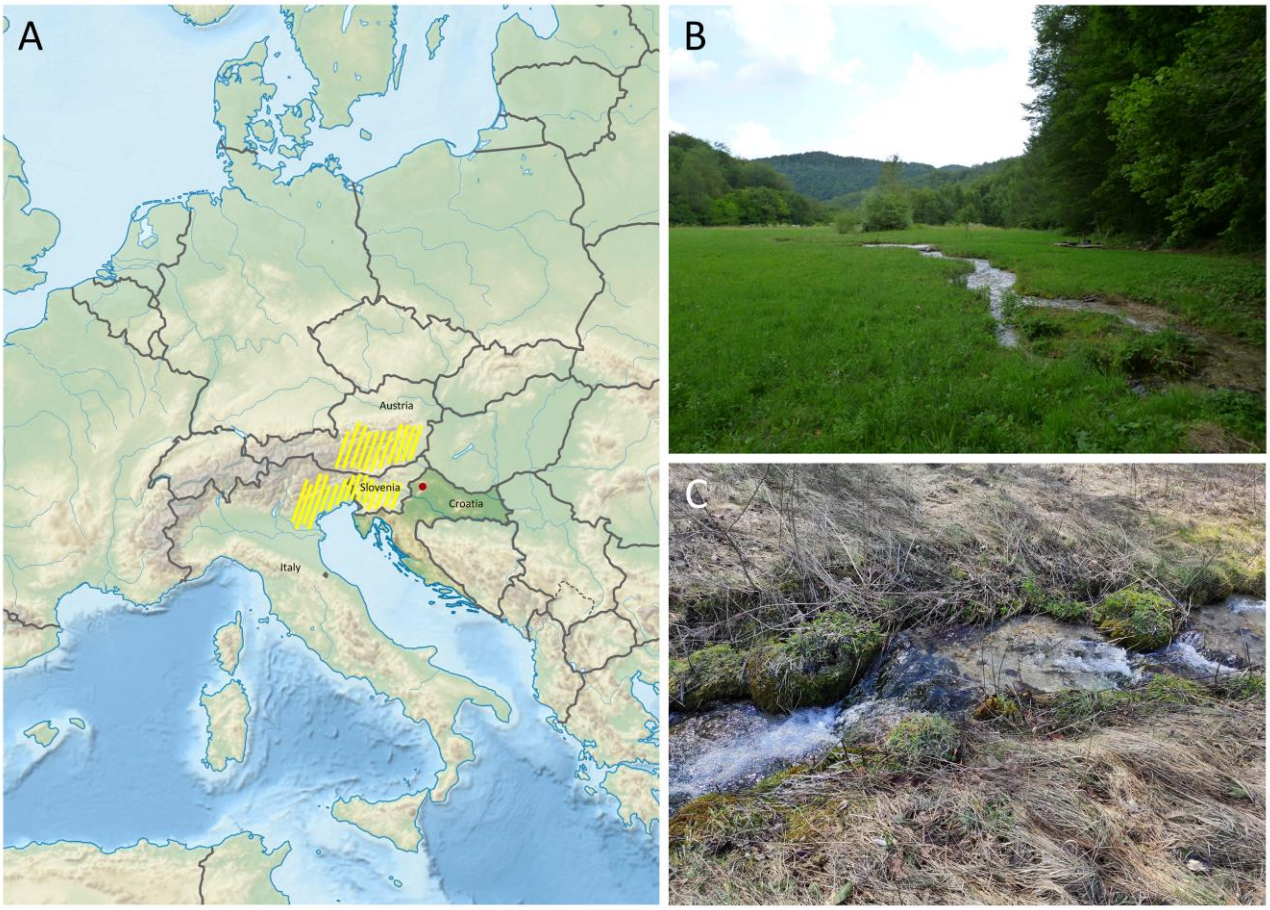

Figure 1. **A** New record of *Ecclisopteryx asterix* Malicky, 1979 in Croatia (red dot) and hitherto known range in Austria, Slovenia and Italy (yellow area) (Robert, 2015); **B** the crenal reach of the Šumi Spring, and **C** a close up of the habitat where *E. asterix* larvae were collected.

DNA extraction and gene amplification

Genomic DNA was extracted from the single leg of each specimen (*E. asterix*, 3 larvae; *E. keroveci*, 1 larva; *D. schmidi*, 5 larvae) using GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, Germany) following the manufacturer's protocol and eluted in 50 µL of elution buffer. The standard DNA barcode region of the mitochondrial cytochrome c oxidase subunit I *(COI)* gene was amplified using standard PCR-protocols and the universal primer pair LCO-1490/HCO-2198 (Folmer *et al*. 1994) in 20 µL reaction mixture. Polymerase chain reactions (PCRs) were carried out using: 1 x DreamTaq™ reaction buffer with 2 mM $MgCl_2$ (Thermo Fisher Scientific Inc., US), 0.2 mM dNTPs, 0.4 µM of each primer, 0.025 U/µL of DreamTaq polymerase (Thermo Fisher Scientific Inc., US) and 1 µl of eluted DNA. The PCR cycling protocol included: initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. Purification and sequencing were performed by Macrogen Inc. (Amsterdam, Netherlands) using the same amplification primers. Sequences obtained in this study were deposited in the Barcode of Life Database (Ratnasingham & Hebert 2007) under the accession numbers listed in Table 2.

Sequence data

Sequences were checked, edited and inspected manually for base-pair ambiguities, stop codons, indels or double peaks in chromatograms in the programs Geneious R6 (https://www.geneious.com) and MEGA-X (Kumar *et al.* 2018). Sequences were collapsed to haplotypes using the online tool FaBox v.1.5 (Villesen 2007). Uncorrected *p*-distances between haplotypes were calculated using MEGA-X (Kumar *et al*. 2018). An ML analysis was performed in same program. BOLD Identification Engine (accessed 16/05/2021) was used to compare obtained DNA sequences with sequences available in the BOLD database.

Table 2. Collection data and BOLD IDs of obtained sequences in this study.

Morphologically identified taxon	BOLD ID	Collection site	Collection date	BOLD Identification Engine
Ecclisopteryx asterix	CROTR341-21	Ivanščica Mt., Šumi Spring	06.03.2021	*E. asterix* (98.32)
Ecclisopteryx asterix	CROTR340-21	Ivanščica Mt., Šumi Spring	06.03.2021	*E. asterix* (98.32)
Ecclisopteryx asterix	CROTR339-21	Ivanščica Mt., Šumi Spring	10.03.2015	*E. asterix* (98.17)
Ecclisopteryx keroveci	CROTR338-21	Krka River	28.10.2014	*E. keroveci* (99.85)
Drusus schmidi	CROTR337-21	Papuk Mt., Kovačica Stream	15.04.2016	*D. schmidi* (100)
Drusus schmidi	CROTR336-21	Papuk Mt., Veličanka Stream, near Velika	20.04.2016	*D. schmidi* (100)
Drusus schmidi	CROTR335-21	Papuk Mt., Dubočanka Stream, near Velika	20.04.2016	*D. schmidi* (100)
Drusus schmidi	CROTR334-21	Papuk Mt., Dubočanka Stream	20.04.2016	*D. schmidi* (100)
Drusus schmidi	CROTR333-21	Krndija Mt., Rikino vrelo Stream, bridge 4030	14.04.2016	*D. schmidi* (100)

Results and Discussion

In this paper, we present new faunal and DNA barcoding data and notes on the distribution and ecology of the subfamily Drusinae in Croatia. During this study, 155 specimens of Drusinae larvae were collected at 18 localities. Six species were confirmed by morphological characters: *Drusus croaticus, D. discolor, D. schmidi, Ecclisopteryx keroveci, E. ivkae* and *Ecclisopteryx* sp. To confirm morphological identification, DNA barcoding was performed on several specimens of *E. keroveci, D. schmidi* and *Ecclisopteryx* sp., which had weakly expressed morphological characters, making morphological determination difficult.

These records and their morphological identifications were also confirmed by the application of DNA barcoding on three larval specimens (Table 2). Thus, our study further indicates that the DNA barcoding method is a valuable tool in faunistic, taxonomic and biodiversity research, as already implemented in other studies in Croatia (e.g. Cerjanec *et al.* 2020; Kučinić *et al.* 2020; Szivák *et al*. 2017; Valladolid *et al*. 2020; Vučković *et al.* 2021) and other countries (e. g. Ibrahimi *et al*. 2021; Morinière *et al*. 2017; Valladolid *et al*. 2019, 2021; Zhou *et al*. 2007, 2016).

1. *Ecclisopteryx asterix* Malicky, 1979: An Alpine endemic lives in Croatia too

In the current study, we found larvae of *Ecclisopteryx asterix* from northern Croatia, at the Ivanščica Mt. (i.e. three larvae of species *E. asterix* were collected at the Šumi Spring during two visits, on March 10th 2015 and on March 6th 2021; Table 1, Figure 1). Up till now, *E. asterix* was considered a stenoendemic species of the southeastern Alps, distributed in the limnoecoregion 4 only (the Alps; Figure 1A, Graf *et al*. 2021). Thus, by adding one more Drusinae species to the list of caddisfly species in Croatia (Kučinić *et al.* 2012), our findings bring new insights into the distribution of this species. This suggests that *E. asterix* has a broader distribution, or at least that some additional disjunct populations exist in the area.

Even though we only found larvae, these could be identified after morphological examination and comparison with *Ecclisopteryx malickyi* Moretti, 1991, *Drusus annulatus* (Stephens, 1837) and *Drusus biguttatus* (Pictet, 1834). These species differ in the contours of the pronotum in lateral view, the presence/absence of the pronotal transverse groove, the shape of the median notch of the pronotum (in

anterior view), pronotal sculpturing, presence/absence of the lateral carina of the head capsule, the number of proximo-dorsal setae on the mid-and hind femora, where the lateral fringe starts on the abdomen, and also in distribution (Graf *et al*. 2011).

Larva of *E. asterix* look similar to *D. annulatus* and *D. biguttatus*. It is characterized by the shape of the case, which is smooth, slightly curved and consists completely of mineral particles (Figure 2A). The dorsal line of the pronotum is rounded in the last third, creating a small dorsal hump, and a step-like interruption of the dorsal silhouette (Figure 2B). The metanotum is partially covered by three pairs of sclerites of which the anterior pair is large, ovoid and has a very narrow median separation (Figure 2C). The lateral fringe starts on the last third of the third abdominal segment (Figure 2D).

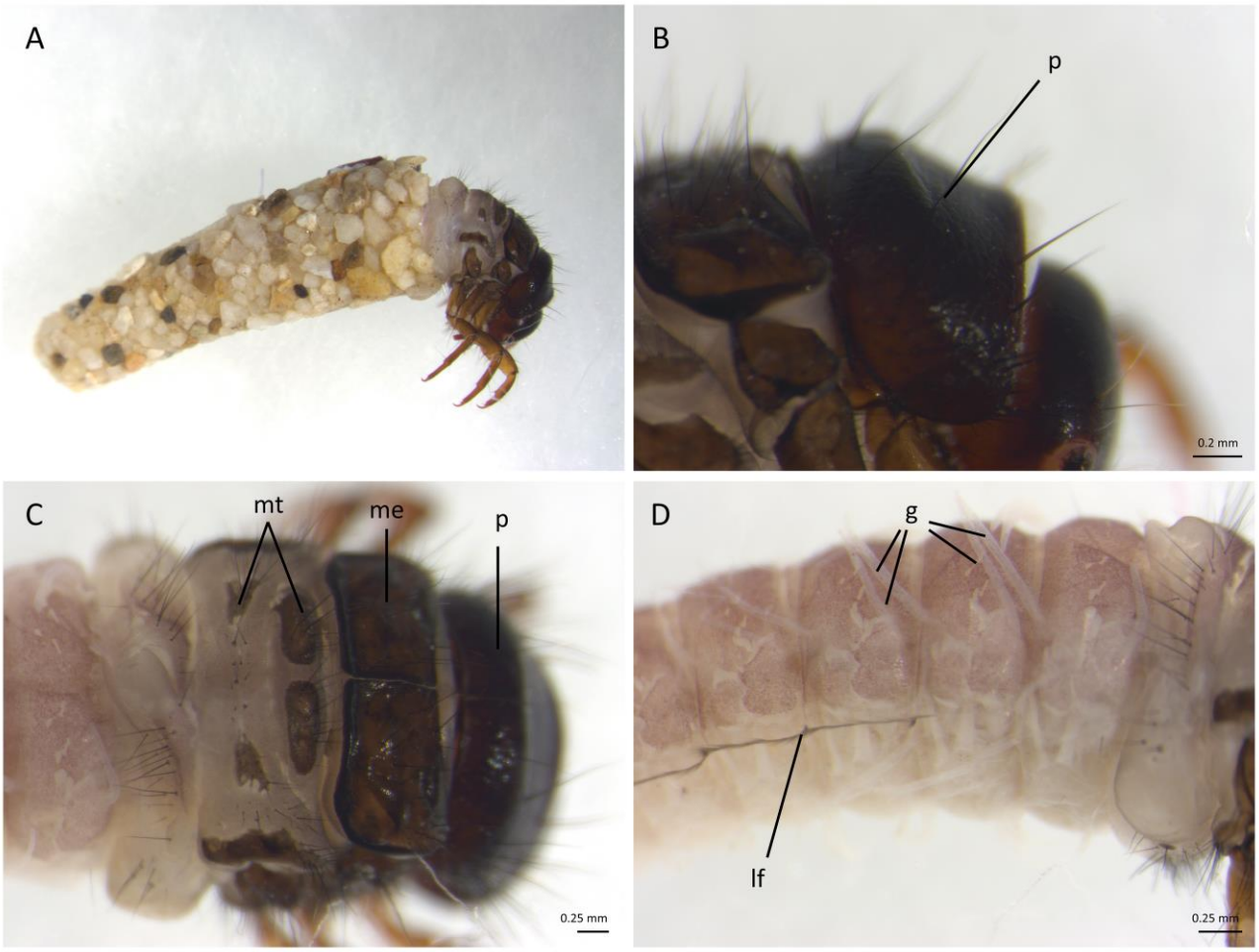

Figure 2. **A** Larva of *Ecclisiopteryx asterix* in its case; **B** morphological character important for identification: shape of the pronotum (p); **C** pronotum (p), mesonotum (me), and sclerites at metanotum (mt) in dorsal view; **D** abdominal segments I-VI with gills (g) and lateral fringe (lf), right lateral view.

Additionally, molecular analyses supported our morphological identification as obtained sequences were identified as the same species we identified using morphological characters. The new findings of *E. asterix* were confirmed with the use of BOLD Identification Engine (98.17 – 98.32 %, private sequence, sample from Austria). Obtained *COI* gene sequences were 658 bp long and formed two *COI* haplotypes with low sequence divergence (0.00153 uncorrected *p*-distance) and with low sequence divergence between the single *E. asterix* sequence available on BOLD (Table 2). The topology was congruent during two conducted analyses (Figure 3). Sequences of *E. asterix* from Croatia group together with the single sequence of *E. asterix* (HMKKT864-11) available in the BOLD database.

A separate taxonomic status of *E. asterix* is possible for populations in Croatia. For now, this is indicated by DNA barcoding data, as well as the separation of populations from the Ivanščica Mt. However, for any taxonomic study it is hard to make a conclusion with only three collected larvae. Future

investigations are needed and planned as it is necessary to collect more larvae and adults of both sexes for detailed morphological analyses.

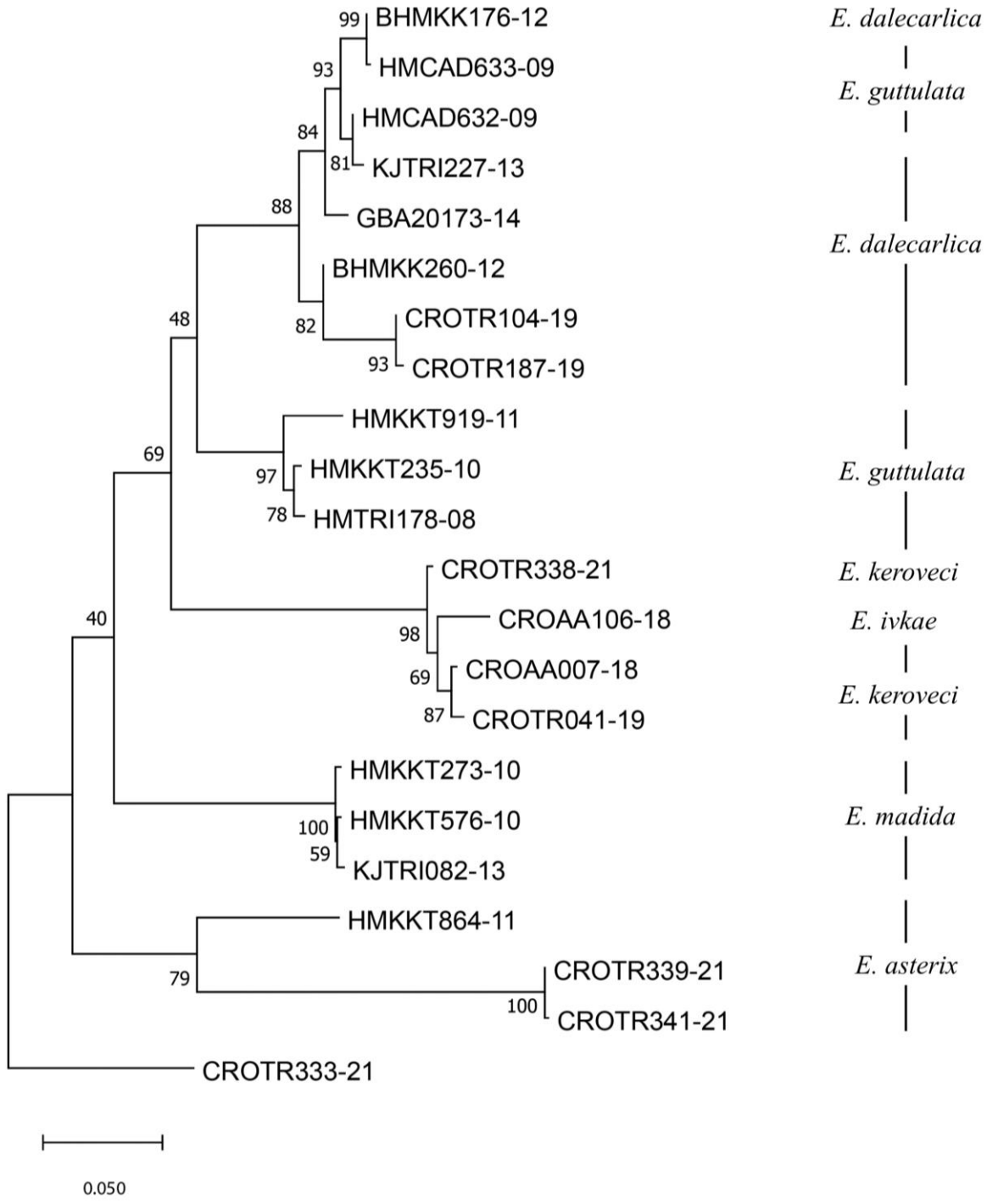

Figure 3. Maximum likelihood phylogram based on the *COI* sequences of *Ecclisopteryx asterix* from Croatia and haplotypes of *Ecclisopteryx* species available in BOLD database. Numbers at the nodes indicate maximum likelihood (ML) bootstrap support values (BS). Terminal codes represent BOLD Process IDs.

Ecclisopteryx asterix is mainly montane species, distributed from 400 to 1100 m above sea level (Urbanič *et al*. 2003). It inhabits springs, spring reaches and upper stream reaches, and is considered as a lithal habitat specialist and a cold water stenotherm (Graf *et al.* 2021). Because of these characteristics, it is also highly vulnerable to climate changes (Graf *et al.* 2021). According to the morphology of the mouth parts, larvae of

this species are scrapers, caddisflies that feed on periphyton on stone pebbles and aquatic plants. The location at the Šumi Spring is at an altitude of ca. 400 m a.s.l., in line with *E. asterix* being a submontane species (Graf *et al.* 2021). Generally, the habitat where it was collected is similar to the ecology from the Alpine region (Graf *et al.* 2021). We collected larvae in a small (ca. 1 m wide, average 15 cm deep), fast flowing brook, around 200 m downstream of the spring (spring is partially captured). This is typical of the habitat preference of this species, as it mainly inhabits hypocrenal, but also eucrenal and epirithral areas, and zones with higher current velocity (Graf *et al.* 2021). The substrate consists of bare microlithal (2-6 cm) (60%) and mesolithal (6-20 cm) covered by moss (35%) with only 5% of akal (0,2-2 cm), what is also in accordance with species substrate preferences. The temperature in March 2015 at 3:00 PM was 9.3°C, which corresponds with cold stenotherm temperature preferences according to Graf *et al.* (2021). Electric conductivity was 429 μS/cm. Hence, we found *E. asterix* in a typical habitat, but geographically dislocated. It is therefore highly likely that the species inhabits additional similar habitats in the vicinity of the Šumi Spring, but also in areas in between the known distribution in the southeastern Alps and the current finding.

2. Additional new faunistic data on Drusinae in Croatia

In this study, three (3) species were recorded from the genus *Drusus*: *D. croaticus, D. discolor, D. schmidi.* Besides *E. asterix* we found two other species in the genus *Ecclisopteryx*: *E. keroveci* and *E. ivkae* (Figure 4).

2.1. New data on the distribution of *Drusus croaticus* in Croatia

Drusus croaticus was recorded from the highest number of localities surveyed in the study, nine (50%) out of 18 (Table 1). It is a species whose type locality is the spring of the Crna Rijeka River and the Plitvica Stream within the Plitvice Lakes National Park. The species was found and described in the early 1970s by biology professor Mara Marinković-Gospodnetić from the University of Sarajevo, Bosnia & Herzegovina (Marinković-Gospodnetić 1971). Later studies recorded this species in about 20 springs in Croatia (Cerjanec *et al*. 2020; Kučinić *et al*. 2008, 2014; Marinković-Gospodnetić 1979; Previšić *et al*. 2007, 2009; Previšić & Popijač 2010), and with this research the species was found for the first time in the Krasulja River near the settlement of Mirići in Krbavica, where the spring of the Krasulja River is situated, while its sinkhole is in Krbavičko polje (Figure 4). Regarding feeding behaviour, *D. croaticus* is also a grazer (Kučinić *et al*. 2008), and emerges in two peaks, spring and autumn, most likely as two one-year generations. The flight period is quite long: about 7 months (Kučinić *et al*. 2017b), as is often the case with species living in springs or crenal sections of mountain and mountain streams. Both sexes and larval stages of this species are well known and described (Kučinić *et al*. 2008; Marinković-Gospodnetić 1971).

2.2. New data on the distribution of *Drusus discolor* in Croatia

The second recorded species from the genus *Drusus* in this study was *D. discolor* at the spring site of the Čabranka River (Figure 4). This finding only confirmed the presence of this species at the investigated site, as it was recorded in previous research (Previšić & Popijač 2010). Apart from the spring area of the Čabranka River, *D. discolor* was first found in Croatian fauna in the larval stage in the Mrežnica River (Habdija 1979) and in the adult stage at the spring of the Dobra River (Cerjanec *et al.* 2020). According to the distribution, *D. discolor* most probably has the widest distribution (Graf *et al.* 2021) among all species of the genus *Drusus.* The larva is characterized by a very pronounced area with small, thick, yellow hairs on the head in which it collects some organic matter and small invertebrates which it "removes" with especially adapted forelegs with large and strong hairs. According to the feeding type, it belongs to the carnivorous caddisflies (Vitecek *et al*. 2015a). Both sexes of the adult stage, as well as the larva, are well known and described (Malicky 2004; Vitecek *et al*. 2015a; Waringer & Graf 2011).

2.3. New data on the distribution of *Drusus schmidi* in Croatia

The third species, *D. schmidi* Botosaneanui, 1953, was recorded during this research at four localities in the area of the Papuk Mt. and at one locality in the area of the Krndija Mt. in the continental-lowland part of Croatia (Figure 4). Previous research has recorded this species in the Papuk Mt. area at two locations, the Dubočanka Stream and the Jankovac Spring (Previšić *et al.* 2013), and our research has significantly expanded its distributional range in Croatia (Figure 4). Morphological identification of larvae was also confirmed by DNA barcoding (Table 2). Analysis of DNA barcoding data indicated an association of larvae

and adults of *D. schmidi*, which has not been done so far. Adult forms of both males and females are well known (Malicky 2004), and for morphological characteristics of larva there are only preliminary data (Waringer *et al*. 2015) because the description of the larva has only recently been completed (Kučinić *et al*. in prep.). *D. schmidi* belongs to the group of so-called "yellow" caddisflies (at the adult stage). Unlike the "black" caddisflies from the *Drusus bosnicus* Group, which are active during the day and have smaller distributions (Kučinić *et al*. 2014), "yellow" caddisflies have a more pronounced flight and are active at night. In a certain number of "yellow" caddisfly species, significantly larger distributional areas have been recorded (Marinković-Gospodnetić 1979; Pauls *et al*. 2006; Previšić *et al*. 2009) compared to microendemics of the *Drusus bosnicus* Group (Kučinić *et al*. 2014; Marinković-Gospodnetić 1979).

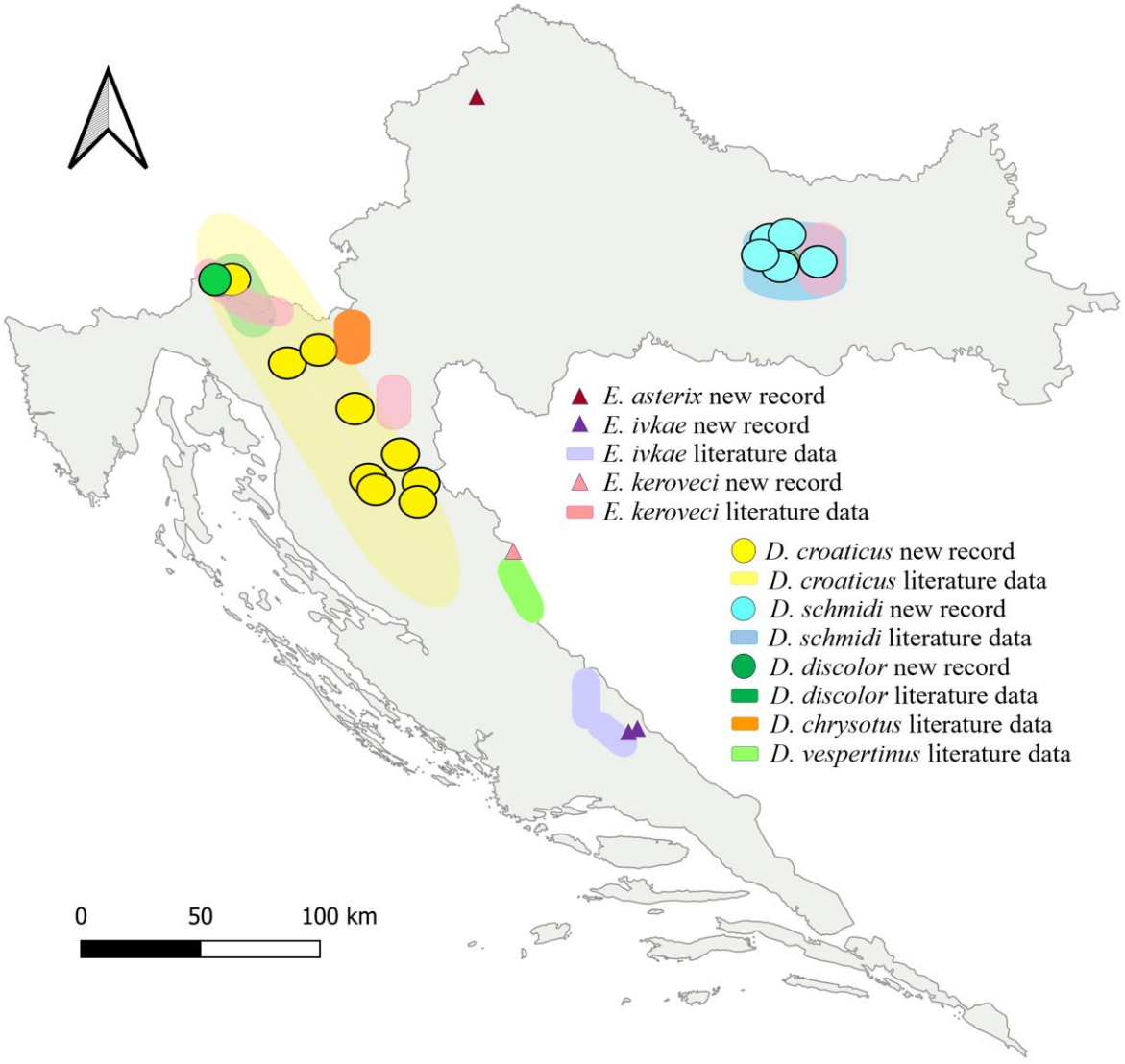

Figure 4. Spatial distribution of the Drusinae species in Croatia, literature data supplemented with new records.

2.4. New data on the distribution of *Ecclisopteryx ivkae* and *E. keroveci* in Croatia

Previous studies of the genus *Ecclisopteryx* have identified two species in Croatia: *E. ivkae* and *E. keroveci* (Previšić *et al*. 2014a). The species *E. ivkae* is the only endemic species of the subfamily *Drusinae* in the Croatian fauna, described from the Cetina River spring reach (Previšić *et al*. 2014a). In later research, this species was also recorded in the spring area of the Rumin River, a tributary of the Cetina River (Vučković *et al*. 2016). Within this study, we collected specimens of *E. ivkae* from localities where it was previously recorded.

In this study, we present a new record of *E. keroveci* from the confluence of the Una and Krka Rivers. This record is in line with the previously known range of this species that stretches from Gorski kotar in Croatia to the south-eastern area of Northern Macedonia (Oláh *et al*. 2017; Previšić *et al.* 2014a; Vučković *et al.* 2016). Larvae of *E. keroveci* are scrapers, feeding on periphyton on the surface of stone pebbles and aquatic vegetation. Previšić *et al*. (2014a) provide a description of this species with a type locality in the area of the Sutjeska National Park in Bosnia and Herzegovina, with a detailed presentation of the morphology of adult forms of both sexes and a detailed description of the larva. The findings of *E. keroveci* on the Papuk Mt. indicate a similar distribution as *D. schmidi*, and also certain similarities in the morphology of the larvae between the two species.

The distribution of *Drusus schmidi* and *E. keroveci* (disjunct ranges) indicates the faunal similarity of the Slavonian mountains with the Dinarides. The area of *D. schmidi* covers the continental parts of Croatia, and the area of the Dinarides. It stretches from Livno (Bosnia and Herzegovina) in the west, to part of Kosovo in the east (Ibrahimi *et al*. 2014; Marinković-Gospodnetić, 1979). A similar case is observed in the distribution of *E. asterix* at the Ivanščica Mt. and the Eastern Alps, where this species is considered stenoendemic. In the abovementioned cases, geological and hydrological processes in the past, together with the biological characteristics of the species, cause such a distributional pattern (Kučinić *et al*. 2014; Previšić *et al.* 2013, 2014a).

Conclusion

The current paper is a modern contribution to the knowledge on fauna of Trichoptera in Croatia, as it integrates classical approach and the DNA barcoding method, a valuable tool in taxonomic and faunistic research (e.g. Kučinić *et al.* 2020; Vučković *et al.* 2021). Today, the finding of each new species in the fauna of Croatia is challenging. This is especially true for the Drusinae subfamily, which is recognizable for its endemism and is of interest to many faunal, phylogenetic, and taxonomic studies (Pauls *et al.* 2006; Previšić 2014a, 2014b; Waringer *et al*. 2015, 2016). Precisely for this reason, when it comes to caddisflies studied in this work, faunistically, and potentially taxonomically, the most interesting finding is the record of *E. asterix* at the Ivanščica Mt., almost 100 km away from the area of the Eastern Alps, where this species is widespread (Figure 1; Malicky 1979; Urbanič *et al*. 2003). Therefore, the current distribution of *E. asterix* supports the biogeographic link between the southeastern Alps and the Dinaric Alps (review in Schmitt 2009). Moreover, such new insights into distribution provoke additional questions on evolutionary history and resulting biogeography of Drusinae (e.g. distribution of *E. keroveci* and *D. schmidi* indicates potential linkage of the Slavonian mountains in Croatia and the Dinaric Alps in Bosnia and Herzegovina), but also of caddisfly fauna in general.

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