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FAUNAL FEATURES OF CADDISFLIES (INSECTA, TRICHOPTERA) IN KONAVLE REGION (CROATIA) WITH NOTES ON DNA BARCODING AND CONSERVATION BIOLOGY

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Throughout this research, 230 Trichoptera specimens were collected in the area of Konavle region, the southernmost area of Croatia. Altogether 21 species, 15 genera and 11 families were identified. DNA barcoding covered 41 specimens represented with 15 species collected in the Konavle region. The highest number of species was recorded at two localities: the spring of the River Ljuta (13) and a spring in the village Vodovađa (8). The highest number of taxa (4) was recorded within the following two families: Hydroptilidae and Hydropsychidae. From a faunistic point of view, the most interesting species in Konavle region are: *Agapetus* cf. *kampos* Oláh (the first record for Croatia), *Hydroptila martini* Marshall (the first record for the Mediterranean part of Croatia), *Oxyethira falcata* Morton (the first record for Croatia), *Tinodes andrasi* Oláh (type locality of the species is River Ljuta), *Diplectrona* cf. *atra* McLachlan (the first record from the Mediterranean part of Croatia) and *Micropterna wageneri* Malicky (the second record for Croatia). The first DNA barcoding data for the species *Agapetus* cf. *kampos* Oláh and *Tinodes andrasi* Oláh were entered into the BOLD database. The area of Konavle represents one of the most interesting regions for the fauna of Trichoptera and can be considered as a "hotspot" for caddisflies in Croatia. Thus, it is necessary to install high standards for their protection.

Keywords: fauna, aquatic insects, the River Ljuta, south Croatia, mitochondrial DNA

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U sklopu ovoga istraživanja na području Konavala, najjužnijeg područja u Hrvatskoj, prikupljeno je 230 primjeraka tulara. Utvrđena je prisutnost 21 vrste, 15 rodova i 11 porodica. DNA barkodiranje

provedeno je na 41 primjerku, odnosno na 15 vrsta prikupljenih na području Konavala. Najveći broj vrsta prikupljen je na dva lokaliteta: izvor rijeke Ljute (13) i izvor u selu Vodovađa (8). Najveći broj svojti (4) bio je zabilježen unutar sljedeće dvije porodice: Hydroptilidae i Hydropsychidae. S faunističkog gledišta najzanimljivije vrste zabilježene u Konavlima su: *Agapetus* cf. *kampos* Oláh (prvi nalaz za Hrvatsku), *Hydroptila martini* Marshall (prvi nalaz za mediteranski dio Hrvatske), *Oxyethira falcata* Morton (prvi nalaz za Hrvatsku), *Tinodes andrasi* Oláh (tipski lokalitet vrste je rijeka Ljuta), *Diplectrona* cf. *atra* McLachlan (prvi nalaz za mediteranski dio Hrvatske) i *Micropterna wageneri* Malicky (drugi nalaz za Hrvatsku). Prvi DNA barkodovi za vrste *Agapetus* cf. *kampos* Oláh i *Tinodes andrasi* Oláh uneseni su u BOLD bazu. Područje Konavala predstavlja jednu od najzanimljivijih regija za faunu Trichoptera i može se smatrati hotspot-om za tulare u Hrvatskoj. Iz tog je razloga potrebno uvođenje visokih standarda za njihovu zaštitu.

Ključne riječi: fauna, vodeni kukci, rijeka Ljuta, južna Hrvatska, mitohondrijska DNA

INTRODUCTION

Studies on caddisflies in Dalmatia, the southernmost region of Croatia, started at the beginning of the 19th century when the famous entomologist and mineralogist, Professor of Zoology and Director of the Mineralogical Museum in Halle, Prof. Ernst F. Germar, Ph.D., started collecting insects in 1811 while visiting Dalmatia. According to Nonveiller (1989), Germar included *Phryganea atrata* Fabricius (Germar, 1817) in his species list for this region. *Phryganea atrata* Fabricius is a synonym of *Notidobia ciliaris* Linnaeus (Morse, 2021), and it was probably the first caddisfly species recorded in the fauna of Croatia. Throughout later investigations on Trichoptera fauna, different areas of Dalmatia were covered, for example, the rivers Krka, Neretva, Cetina Zrmanja, Ruda, Grab and Rumin, Mt Biokovo, and the islands of Pag and Hvar. As a result of these investigations, the faunal features of Trichoptera from this region are well known (e.g. Graf *et al*., 2008a; Karaouzas *et al*., 2015; Kučinić & Ilić 1993; Kučinić *et al*., 2011, 2019a, 2019b, 2020a, 2020b; Malicky, 1979, 1980, 2005, 2014; Oláh 2010; Previšić *et al*., 2014; Vučković *et al*., 2011, 2021; Waringer *et al*., 2009). Additionally, three new-to-science Trichoptera species were discovered: *Athripsodes dalmatinus* Malicky, *Tinodes andrasi* Oláh, and *Ecclisopteryx ivkae* Previšić, Vitecek & Graf (Malicky, 1980; Oláh, 2010; Previšić *et al*., 2014); three species as yet undescribed in larval stage were also described: *Tinodes braueri* McLachlan, *Ecclisopteryx ivkae* Previšić, Vitecek & Graf and *Annitella apfelbecki* Klapálek (Graf *et al*., 2008a; Previšić *et al*., 2014; Waringer *et al*., 2009), and one undescribed female caddisfly: *Annitella apfelbecki* (Vučković *et al*., 2011).

The southernmost area of Dalmatia, as well as the southernmost county of Croatia, is Konavosko polje (plain) or Konavle (Fig. 1) (Bertić *et al*., 2001; http://www.enciklopedija.hr, Šalinović 2019). The narrow plain of Konavle is an isolated valley in south Croatia, located between Bosnia and Herzegovina in the north, Montenegro in the east, and the Croatian part of the Adriatic Sea in the south-east.

Caddisfly research in Konavle started in 2000 and has continued more or less until today. During this research period one new species, *Tinodes andrasi* Oláh was described by Hungarian researchers (Oláh, 2010). To date, four new Trichoptera species have been recorded for the area of Konavosko polje: *Rhyacophila tristis* Pictet, *Tinodes andrasi,* Oláh, *Silo nigricornis* Pictet and *Micropterna wageneri* Malicky (Kučinić *et al*., 2017a, 2020b; Oláh, 2010).

This article aims to give information on biodiversity, distribution and seasonal dynamics of Trichoptera species collected throughout field investigations conducted in Konavle during the last 20 years; (1) features of the DNA barcoded caddisfly specimens from this region, which have been a research topic in recent years (2), the protection of the aquatic insect fauna, including caddisflies, of Konavle area (3).

MATERIAL AND METHODS

Study area and field work

Konavle (Konavosko polje, plain) is the southernmost part of Croatia (Fig. 1). It is 22 km long, 6 km wide, and has a surface area of approximately 75 km². It is a valley enclosed on the northern and eastern side by mountains (these mountains being also part of the Konavle region), the southern side has its border on the Adriatic sea and the western side on the line between the town of Cavtat and Župa Dubrovačka (Bertić *et al*., 2001; Šalinović, 2019). The area extends northwest to southeast. Considering the pedological characteristics, Konavle is an area of carbonate bedrock, which represents an impermeable medium. From a hydrogeological point of view, Konavle is not an area rich in running fresh surface watercourses. Highlight is the River Ljuta which, after flowing only a few kilometers through Konavosko polje, flows into the Adriatic Sea. Besides the Ljuta – the most dominant aquatic habitat in Konavle – there are several smaller streams, springs, canals, the small river Konavočica, which dries out in the summertime, and a few anthropogenic ponds.

The area of Konavle was visited eight times from 2000 until 2020 for a total of 21 field days. The Trichoptera species were collected at altogether eight different locali-

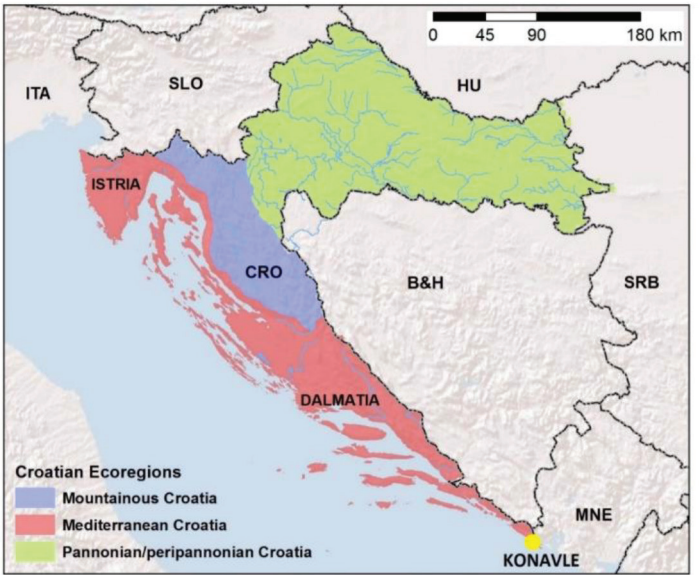

Fig. 1. Three biogeographical regions in Croatia, according to Bertić *et al*. (2001): Pannonian-peripannonian Croatia – continental area (green), Mountainous Croatia – central mountain area (blue) and Mediterranean Croatia (pink), with the Konavle region (marked with yellow circle).

ties: spring of the River Ljuta (1), the River Ljuta, upper part (2), the River Ljuta, middle part (3), spring in the village of Vodovađa (4), spring next to the Villa Marija (second spring in the village of Vodovađa) (5), stream in the village of Palje Brdo (6), stream in the village of Lovorno (7), and stream in the village of Pridvorje (8).

For each locality a short preview of the main hydrological features is given:

1. Spring of the River Ljuta (Fig. 2A) consists of several separate springs, which together form one of the strongest spring areas in the Mediterranean part of Croatia. The River Ljuta is the only liquid surface fresh water in Konavle with a whole spectrum of microhabitats that feature a bottom of huge to small rocks, gravel, sand, and habitats that are covered with moss. The spring of the River Ljuta is a typical strong karst rheocrene spring. It is located 100 meters above sea level within forest vegetation, which is dominated by laurel and holm oak. The spring never dries up.

2. The River Ljuta, upper part (Fig. 2B-C). The locality is located 150 meters downstream from the spring of the River Ljuta. This is the place where the river divides into the main stream and several side streams (brooks), near the restaurant Konavoski dvori. The benthos covers a wide spectrum of microhabitats like pebble, gravel, or moss. The locality is situated within very well developed forest vegetation, which is dominated mainly by holm oak and less by laurel. It is located 61 meters above sea level.

3. The River Ljuta, middle part (Fig. 2D) is located about 400 meters away from the spring of the River Ljuta. At this point, the river flows is much slower and the river bed is some seven to eight meters wide. The bottom consists of pebble, gravel, and some moss-covered areas. The locality is located at 57 meters above sea level on the left side of the tarmac road leading to the village of Grude, and it is not located within forest vegetation.

4. The karst rheocrene spring in the village of Vodovađa (Fig. 2E) is partially artificial and partially emerges from a cave. It has less power but never dries up. On the bottom part and the upper part, which has a width of 70 cm and a length of about 14 m, pebble, moss, and gravel dominate. The rest of the upper part of the stream is canalized on a hillslope and is very fast-flowing. This is the easternmost research locality in the area of Konavosko plain and one of the easternmost springs in Croatia. It is located on the eastern slopes of Mt Snježnica at an altitude of 305 meters above sea level, without forest vegetation.

5. The spring of Marija (Fig. 2F), close to the Villa Marija in the village of Vodovađa is a smaller spring that is partially covered with stones. It belongs to small karst rheocrene springs and it never dries up. There are small pebbles covered with moss, gravel, and at some places sand on the bottom. The spring is located outside of the forest vegetation in an open area at an altitude of 227 meters above sea level.

6. The stream in the village of Palje Brdo is a locality on a bridge just outside the village of Palje Brdo. The brook is fast-flowing and partially on a hillslope. The locality is covered by Mediterranean scrub vegetation. There are small pebbles and gravel at the bottom. The locality is located at an altitude of 149 meters above sea level.

7. The stream in the village of Lovorno (Fig. 2G-H) is a smaller watercourse with a width of about 1 meter and a depth of 20 to 30 centimeters. At the bottom, there is sand, small and mid-sized pebbles. The locality is on an open area outside of the forest vegetation at an altitude of 85 meters above sea level.

Fig. 2 A-H. Spring of the River Ljuta (A), the River Ljuta, upper part (B-C), the River Ljuta, middle part (D), the spring in the village of Vodovađa (E), the spring of Marija (village of Vodovađa) (F), the stream in the village of Lovorno (G-H).

8. The stream in the village of Pridvorje is a smaller watercourse similar to the brook in the village of Pridvorje. It is not a fast-flowing brook. The width is about 1.5 meters and the bottom is covered by pebbles, gravel, and sand. The locality is also located on an open area outside the forest vegetation at an altitude of 61 meters above sea level.

The collecting of Trichoptera was done at all localities with portable 12 V UV lamps. The collection period was one hour at each locality. As well as at night, the caddisflies were also collected during daytime from the surrounding vegetation with an entomological net. The collected material was preserved in 80% or absolute alcohol for morphological and molecular analysis, respectively.

The collected specimens are all part of four collections: the NIP Trichoptera collection deposited in the Croatian Natural History Museum in Zagreb, the Trichoptera collection of the Faculty of Science at the University of Zagreb, "Pogledi Nova – Trichoptera DNA barcoding collection" deposited in the Croatian Natural History Museum in Zagreb", and the first author's research collection.

DNA extraction and gene amplification

Genomic DNA was extracted from a single leg of each specimen 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 mitochondrial cytochrome oxidase subunit I gene *(COI)* was amplified using standard PCR-protocols and two primer sets LCO-1490/HCO-2198 (Folmer *et al*., 1994) and C_LepFolF/C_LepFolR (Hebert *et al*., 2004) 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 (Qiagen), 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) (Tab. 4).

Sequence data

Sequences were checked, edited, and inspected manually for base pair ambiguities and stop codons in the program BioEdit v.7.2.5. (Hall, 1999). BOLD Identification Engine (accessed 14/06/2021) was used for comparison of obtained DNA sequences with sequences available in BOLD database.

Systematic presentation

Determination of all species was done according to Kumanski (1985, 1988) and Malicky (2004). An exception was the determination of *Tinodes andrasi* and *Agapetus* cf. *kampos* which was done according to Oláh (2010) and Oláh & Kovács (2013). Systematics followed Morse (2021). On the basis of our research, a degree thesis was prepared (Šalinović, 2019), the results of which were partially corrected in this paper.

RESULTS

Throughout this research, 230 caddisflies specimens were collected in the plain of Konavle. Altogether 21 species, 15 genera and 11 families were identified (Tabs 1–2). The highest number of taxa (4) was recorded within the families Hydroptilidae, and Hydropsychidae (Tab. 1). Seven families were represented only by one species (Tab. 1). The most numerous genera, represented by three species per genus, are *Hydroptila* Dalman, *Tinodes* Curtis and *Hydropsyche* Pictet. Altogether 12 out of 15 genera are represented only by one species per genus (Tab. 2).

The highest number of species was recorded on the localities: spring of the River Ljuta (13 species) and spring in the village of Vodovađa (8 species) (Tab. 2). The presence of only 3 species was confirmed at 2 localities: the spring of Marija and the stream in the village of Lovorno (Tab. 2).

From a faunistic point of view, the most interesting species in the Konavle region are: *Agapetus* cf. *kampos* Oláh (the first record for Croatia, the second record for this species after the type locality), *Hydroptila martini* Marshall (the first record for the Mediterranean part of Croatia), *Oxyethira falcata* Morton (the first record for Croatia), *Tinodes andrasi* Oláh (type locality of the species is River Ljuta), *Diplectrona* cf. *atra* McLachlan (the first record in the Mediterranean part of Croatia), and *Micropterna wageneri* Malicky (the second record for Croatia).

The highest number of recorded species, 10, was collected in June, August and October, and only two species were recorded in May and three in April and November (Tab. 3). During the winter months, no specimens were collected. Three species have the longest flying period in the area of Konavle. *Hydropsyche instabilis* Curtis was recorded during six and *Agapetus* cf. *kampos* Oláh and *Diplectrona* cf. *atra* McLachlan during five months (Tab. 3).

DNA barcoding covered 15 species represented by 41 specimens collected in the Konavle region (Tab. 4). The first DNA barcoding data for the species *Agapetus* cf. *kampos* Oláh and *Tinodes andrasi* Oláh were entered into the BOLD database. The results of this research indicate interesting genetic characteristics from the populations of *Agapetus* cf. *kampos* Oláh, *Tinodes andrasi* Oláh and *Diplectrona* cf. *atra* McLachlan. The results of DNA barcoding also enable a more precise identification of the species from the family Hydroptilidae (Tab. 4).

Systematic presentation of caddisflies collected in the Konavle region with data of collection dates and number of collected females and males follows.

Family Rhyacophilidae

Rhyacophila tristis Pictet, 1834
spring of the River Ljuta, 20.06.2020, 1 ♀, spring in the village of Vodovađa, 31.10.2015, 2 ♂♂

Family Glossosomatidae

Agapetus cf. *kampos* (Fig. 3 A-C)
spring of the River Ljuta, 24.09.2004, 2 ♀♀, 13 ♂♂, 3.07.2007 1 ♂, 17.08.2016, 1 ♀, 2 ♂♂, 25.04.2019, 1 ♀, 6 ♂♂, 1.06.2020, 2 ♂♂, the River Ljuta, upper part, 17.08.2016, 1 ♀

Family Hydroptilidae

Hydroptila martini Marshall, 1977
stream in the village of Pridvorje, 17.08.2016, 1 ♂

Hydroptila sparsa Curtis, 1834
stream in the village of Pridvorje, 17.08.2016, 1 ♂

Hydroptila vectis Curtis, 1834
stream in the village of Pridvorje, 17.08.2016, 1 ♂

Oxyethira falcata Morton, 1893
stream in the village of Pridvorje, 17.08.2016, 3 ♂♂

Hydroptilidae sp. (female)
stream in the village of Pridvorje, 17.08.2016, 10 ♀♀

Family Polycentropodidae

Polycentropus excisus Klapálek, 1894
spring of the River Ljuta, 24.09.2004, 2 ♂♂

Family Psychomyiidae

Tinodes andrasi Oláh, 2010 (Fig. 4)
spring of the River Ljuta, 3.07.2007, 3 ♂♂, 21.06.2020 2 ♂♂ (DNA barcoded), the River Ljuta, upper part, 5.10.2020, 1 ♂

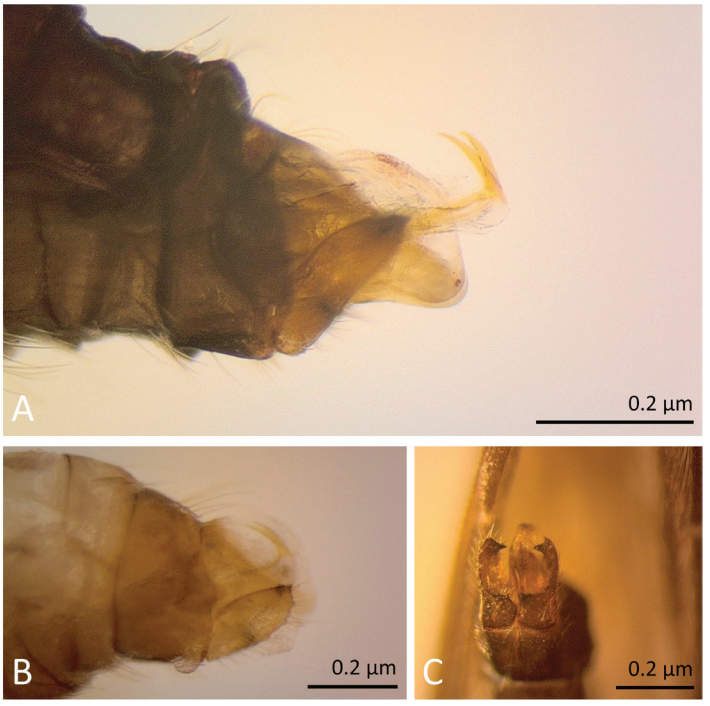

Fig. 3 A–C. *Agapetus* cf. *kampos* Oláh, male imago, lateral view, left side (A-C), ventral side (C) (imago collected in the spring of the River Ljuta, 3.07.2007) (photo Ana Mrnjavčić Vojvoda).

Fig. 4. *Tinodes andrasi* Oláh, male imago, lateral view, right side (imago collected in the spring of the River Ljuta, 3.07.2007) (photo Ana Mrnjavčić Vojvoda).

Tinodes braueri McLachlan, 1878
spring of the River Ljuta, 1.11.2015, 2 ♂♂, 4.10.2020, 1 ♂, 21.06.2020, the River Ljuta, upper part, 12.10.2008, 5 ♂♂, 20.06.2020, 1 ♂, the River Ljuta, middle part, 31.10.2015, 1 ♂

Tinodes pallidulus McLachlan, 1878
spring of the River Ljuta, 20.06.2020, 1 ♂

Tinodes sp.
spring of the River Ljuta, 24.09.2004, 1 ♀, 28.06.2007, 1 ♀, 4.10.2020, 2 ♀♀, the River Ljuta, upper part, 12.10.2008, 1 ♀, the River Ljuta, middle part, 2.04.2015, 1 ♀, 4.10.2020, 2 ♀♀

Family Hydropsychidae

Diplectrona cf. *atra* McLachlan, 1878 (Fig. 5 A-B)
spring of the River Ljuta, 21.05.2000, 1 ♂, 28.06.2007, 1 ♂, 3.07.2007, 1 ♂, 6 ♂♂, 20.07.2015, 6 ♂♂, 20.06.2020, 1 ♂, 21.06.2020, 1 ♂, 4.10.2020, 3 ♂♂, the River Ljuta, upper part, 12.10.2008, 1 ♂, 20.06.2020, 3 ♂♂, the River Ljuta, middle part, 1.04.2015, 1 ♂, spring in the village, Vodovađa, 21.07.2015, 3 ♂♂

Hydropsyche angustipennis Curtis, 1834
stream in the village of Lovorno, 17.08.2016, 1 ♂

Hydropsyche fulvipes Curtis, 1834
stream in the village of Palje Brdo, 21.07.2015, 1 ♂

Hydropsyche instabilis (Curtis, 1834)
spring of the River Ljuta, 24.09.2004, 1 ♂, 28.06.2007., 2 ♂♂, 20.07.2015, 1 ♂, 1.11.2015, 1 ♂, 17.08.2016, 1 ♂, 20.06.2020, 1 ♂, 4.10.2020, 1 ♂, the River Ljuta, middle part, 1.11.2015, 3 ♂♂, spring in the village of Vodovađa, 21.07.2015, 1 ♂, stream in the village of Palje Brdo, 21.07.2015, 6 ♂♂, stream in the village of Lovorno, 17.08.2016, 2 ♂♂, stream in the village of Pridvorje, 17.08.2016, 2 ♂♂

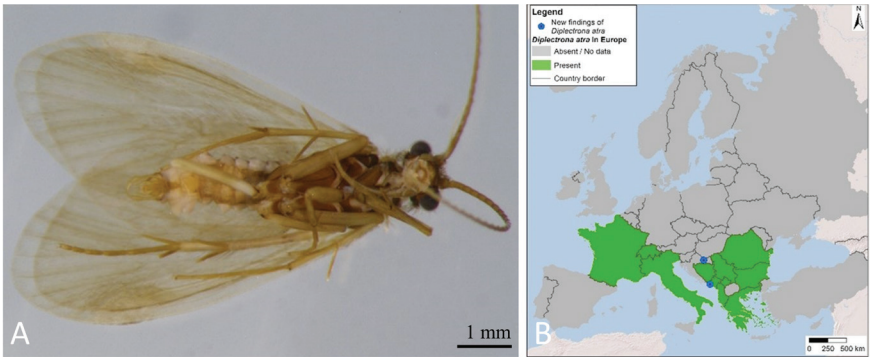

Fig. 5 A-B. Adults of *Diplectrona* cf. *atra* McLachlan, ventral view (A) (imago collected it the spring of the River Ljuta, 3.07.2007) with distribution according to Fauna Europea (B).

Diplectrona sp. & *Hydropsyche* sp.
spring of the River Ljuta, 24.09.2004, 1 ♀, 24.09.2004, 8 ♀♀, 28.06.2007, 1 ♀, 20.07.2015, 4 ♀♀, 20.07.2015, 6 ♀♀, 1.11.2015, 2 ♀♀, 17.08.2016 1 ♀, 20.06.2020, 1 ♀, the River Ljuta, upper part, 20.06.2020, 4 ♀♀, the River Ljuta, middle part, 1.11.2015, 1 ♀, spring in the village, Vodovađa, 21.07.2015, 1 ♀, 16.08.2016, 4 ♀♀, stream in the village of Palje Brdo, 21.07.2015, 4 ♀♀, stream in village of Lovorno, 4.10.2020., 1 ♀, stream in the village of Pridvorje, 17.08.2016, 2 ♀♀,

Family Goeridae

Silo nigricornis (Pictet, 1834)
spring of the River Ljuta, 24.09.2004, 4 ♀♀, 1 ♂,12.10.2008, 3 ♂♂, 25.04.2019, 1 ♀, 4.10.2020, 5 ♂♂, the River Ljuta, upper part, 12.10.2008, 2 ♀♀, 11 ♂♂, 9.05.2013, 1 ♀, 25.04.2019, 2 ♂♂, 20.06.2020, 1 ♀, 2 ♂♂, the River Ljuta, middle part, 4.10.2020, 1 ♀, spring in the village of Vodovađa, 25.04.2019, 1 ♂, the spring of Marija (village of Vodovađa), 5.10.2020, 1 ♂, stream in the village of Lovorno, 4.10.2020, 3 ♀♀, 1 ♂

Family Limnephilidae

Limnephilus lunatus Curtis, 1834
spring of the River Ljuta, 1.11.2015, 1 ♀, 1 ♂, spring in the village of Vodovađa, 31.10.2015, 1 ♂

Halesus digitatus (Rambur, 1842)
the River Ljuta, middle part, 31.10.2015, 1 ♀

Micropterna wageneri Malicky, 1971
spring of the River Ljuta, 1.11.2015, 1 ♀, 1 ♂, spring in the village of Vodovađa, 31.10.2015, 1 ♀, 4.10.2020, 1 ♀, 1 ♂, stream in the village of Palje Brdo, 31.10.2015, 1 ♂

Family Sericostomatidae

Sericostoma flavicorne Schneider, 1845
spring of the River Ljuta, 28.06.2007, 1 ♀, 21.06.2020, 1 ♀, spring in the village of Vodovađa, 16.08.2016, 1 ♀, 20.06.2020, 1 ♀

Tab. 1. The number of families and species recorded from the area of Konavle.

Families	Number of species	Families	Number of species
Rhyacophilidae	1	Goeridae	1
Glossosomatidae	1	Limnephilidae	3
Hydroptilidae	4	Sericostomatidae	1
Polycentropodidae	1	Odontoceridae	1
Psychomyiidae	3	Leptoceridae	1
Hydropsychidae	4		

Family Odontoceridae

Odontocerum albicorne (Scopoli, 1763)
the River Ljuta, upper part, 20.06.2020, 1 ♀, 5.10.2020, 1 ♂, spring in the village of Vodovađa, 20.06.2020, 1 ♀, the spring of Marija (the village of Vodovađa), 16.08.2016, 1 ♂

Family Leptoceridae

Adicella filicornis (Pictet, 1834)
spring of the River Ljuta, 21.05.2000, 1 ♀, 21.06.2020, 2 ♂♂, the spring of Marija (the village of Vodovađa), 20.06.2020, 1 ♂

DISCUSSION

Previous research recorded four species of Trichoptera in the Konavle region: *Rhyacophila tristis* Pictet, *Tinodes andrasi* Oláh, *Silo nigricornis* Pictet and *Micropterna wageneri* Malicky (Kučinić *et al*., 2017a, 2020b; Oláh, 2010). With the results of this research, these four species have been reconfirmed and 17 further previously unrecorded species were reported (Tab. 2). The number of 21 recorded species represents 10% of the Croatian Trichoptera fauna, which counts approximately to 210 species in total (Kučinić unpublished data).

The species number recorded in the Konavle region is not large if compared with the number of Trichoptera species recorded in other areas of Croatia. For example, 89 caddisfly species were recorded from Plitvice Lakes National Park (Kučinić *et al*., 2017b), 77 species in the area of the River Dobra, River Kamačnik and Lake Sabljaci (Cerjanec, 2020), 77 species in the area of the River Cetina and its affluents (Graf *et al*., 2008a; Malicky 2014; Vučković, 2011; Vučković *et al*., 2021; Waringer *et al*., 2009) or 56 species of the River Krka and its affluents (Kučinić *et al*., 2011, 2019a; Ridl *et al*., 2017). Nonetheless, the peculiarity of the Konavle region is represented by its interesting composition of Trichoptera fauna (Tabs 2–4).

In the Konavle region a smaller number of species is conditioned primarily by the hydrological features, because the only liquid surface freshwater that does not dry up in this area is the River Ljuta with some smaller brooks. Other watercourses, like the river Konavočica, dry out. This surely impacts the structure and composition of the fauna on these habitats.

The results of this research include surveys from eight localities (Tab. 2). In the future, the number of localities will increase, and it can be expected that the number

Tab. 2. Distribution of caddisflies at the research localities in the area of Konavle: 1 – spring of the River Ljuta, 2 –the River Ljuta, upper part, 3 – the River Ljuta, middle part, 4 – spring in the village of Vodovađa, 5 – the spring of Marija, 6 – stream in the village of Palje Brdo, 7 – stream in the village of Lovorno, 8 – stream in the village of Pridvorje, with literature data: Oláh (2010) and Kučinić *et al*. (2017a, 2020b).

Systematics	LOCALITIES							
	1	2	3	4	5	6	7	8
Family Rhyacophilidae								
Rhyacophila tristis Pictet	•			•				
Family Glossosomatidae								
Agapetus cf. *kampos* Oláh	•	•						
Family Hydroptilidae								
Hydroptila martini Marshall								•
Hydroptila sparsa Curtis								•
Hydroptila vectis Curtis								•
Oxyethira falcata Morton								•
Family Polycentropodidae								
Polycentropus excisus Klapálek	•							
Family Psychomyiidae								
Tinodes andrasi Oláh	•	•						
Tinodes braueri McLachlan	•	•	•					
Tinodes pallidulus McLachlan	•							
Family Hydropsychidae								
Diplectrona cf. *atra* McLachlan	•	•	•	•		•		
Hydropsyche angustipennis Curtis							•	
Hydropsyche fulvipes Curtis						•		
Hydropsyche instabilis Curtis	•		•	•		•	•	•
Family Goeridae								
Silo nigricornis Pictet	•	•	•	•	•		•	
Family Limnephilidae								
Limnephilus lunatus Curtis	•			•		•		
Halesus digitatus Rambur			•					
Micropterna wageneri Malicky	•			•		•		
Family Sericostomatidae								
Sericostoma flavicorne Schneider	•			•				
Family Odontoceridae								
Odontocerum albicorne Scopoli		•		•	•			
Family Leptoceridae								
Adicella filicornis Pictet	•				•			
TOTAL NUMBER OF TAXA	13	6	5	8	3	5	3	5

Tab. 3. Seasonal dynamics of caddisflies in the area of Konavle (1–12 months), with literature data: Oláh (2010) and Kučinić *et al*. (2017a, 2020b).

Systematics	MONTHS											
	1	2	3	4	5	6	7	8	9	10	11	12
Family Rhyacophilidae												
Rhyacophila tristis Pictet						•				•		
Family Glossosomatidae												
Agapetus cf. *kampos* Oláh				•		•	•	•	•			
Family Hydroptilidae												
Hydroptila martini Marshall								•				
Hydroptila sparsa Curtis								•				
Hydroptila vectis Curtis								•				
Oxyethira falcata Morton								•				
Family Polycentropodidae												
Polycentropus excisus Klapálek									•			
Family Psychomyiidae												
Tinodes andrasi Oláh						•	•			•		
Tinodes braueri McLachlan						•			•	•		
Tinodes pallidulus McLachlan								•				
Family Hydropsychidae												
Diplectrona cf. *atra* McLachlan				•	•	•	•			•		
Hydropsyche angustipennis Curtis								•				
Hydropsyche fulvipes Curtis							•					
Hydropsyche instabilis Curtis						•	•	•	•	•	•	
Family Goeridae												
Silo nigricornis Pictet				•		•			•	•		
Family Limnephilidae							•					
Limnephilus lunatus Curtis										•	•	
Halesus tesselatus Rambur										•		
Micropterna wageneri Malicky										•	•	
Family Sericostomatidae												
Sericostoma flavicorne Schneider						•		•				
Family Odontoceridae												
Odontocerum albicorne Scopoli						•		•		•		
Family Leptoceridae												
Adicella filicornis Pictet					•	•						
TOTAL NUMBER OF TAXA	–	–	–	3	2	10	6	10	5	10	3	-

Tab. 4. Collection details of DNA barcoded specimens from the Konavle region: Specimen ID marks in the collection "Pogledi Nova – Trichoptera DNA barcoding collection"; Locality; BOLD Sequence ID.

Species	Specimen ID	Locality	BOLD Sequence ID
Rhyacophila tristis	TRTRI_4	spring in the village of Vodovađa	CROAA098-18
Rhyacophila tristis	TRTRI_5	spring in the village of Vodovađa	CROTR011-19
Rhyacophila tristis	TRTRI_7	spring in the village of Vodovađa	CROTR031-19
Agapetus cf. *kampos*	TAFUS_1	spring of the River Ljuta	NIP011-17
Agapetus cf. *kampos*	TAFUS_2	spring oh the River Ljuta	CROTR225-19
Agapetus cf. *kampos*	TAFUS_3	spring of the River Ljuta	CROTR230-19
Agapetus cf. *kampos*	TAFUS_4	spring of the River Ljuta	CROTR216-19
Agapetus cf. *kampos* (larva)	MK_1	spring of the River Ljuta	CROTR347-21
Agapetus cf. *kampos* (larva)	MK_2	spring of the River Ljuta	CROTR348-21
Tinodes andrasi	MK7	spring of the River Ljuta	CROTR352-21
Tinodes andrasi	MK14	spring of the River Ljuta	CROTR353-21
Tinodes braueri	MK70	spring of the River Ljuta	CROTR354-21
Tinodes pallidulus	MK6	spring of the River Ljuta	CROTR350-21
Hydroptila sparsa	THYP_6	stream in the village of Pridvorje	CROTR196-19
Hydroptila vectis	THVEC_2	stream in the village of Pridvorje	CROTR168-19
Oxyethira falcata	THYP_4	stream in the village of Pridvorje	CROTR166-19
Oxyethira falcata	THYP_ 5	stream in the village of Pridvorje	CROTR167-19
Oxyethira falcata	THYP_7	stream in the village of Pridvorje	CROTR169-19
Diplectrona cf. *atra*	TDATR_1	strema in the village of Palje Brdo	CROTR291-19
Diplectrona cf. *atra*	TDATR_2	spring in the village of Vodovađa	CROTR292-19
Diplectrona cf. *atra*	TDATR_4	spring of the River Ljuta	CROTR247-19
Diplectrona cf. *atra*	DIPL_1	spring of the River Ljuta	CROTR293-19
Diplectrona cf. *atra*	DSPH2	spring of the River Ljuta	CROTR294-19
Diplectrona cf. *atra*	DSPH4	spring of the River Ljuta	CROTR295-19
Hydropsyche fulvipes	THINS_8	stream in the village of Palje Brdo	CROTR163-19
Silo nigricornis	CROBK28	the River Ljuta, upper part	CROTR363-21
Silo nigricornis	CROBK29	spring in the village of Vodovađa	CROTR364-21
Silo nigricornis	CROBK30	the River Ljuta, upper part	CROTR365-21
Silo nigricornis	CROBK31	the River Ljuta, upper part	CROTR366-21
Silo nigricornis	CROBK32	the River Ljuta, upper part	CROTR367-21
Silo nigricornis	CROBK33	stream in village of Lovorno	CROTR368-21
Silo nigricornis	CROBK34	stream in village of Lovorno	CROTR369-21
Silo nigricornis	CROBK35	the spring of Marija	CROTR370-21
Silo nigricornis	CROBK36	spring of the River Ljuta	CROTR371-21
Micropterna wageneri	TMWAG_1	stream in the village of Palje Brdo	NIPM001-17
Micropterna wageneri	TPWAG_1	stream in the village of Palje Brdo	NIPM002-17
Sericostoma flavicorne	CROBK38	spring in the village of Vodovađa	CROTR372-21
Odontocerum albicorne	CROBK39	the River Ljuta, upper part	CROTR373-21
Odontocerum albicorne	CROBK40	spring in the village of Vodovađa	CROTR374-21
Adicella filicornis	MK4	spring of the River Ljuta	CROTR349-21
Adicella filicornis	MK6	spring of the River Ljuta	CROTR351-21

of Trichoptera species will also increase. The Konavle field is located in the southernmost part of Croatia, which is close to the border to Bosnia and Herzegovina, and Montenegro. Since these countries have a very specific caddisfly fauna (e.g. Krušnik, 1987; Marinković-Gospodnetić, 1976, 1978, 1979, 1988; Stanić-Koštroman *et al*., 2015) it is possible that more new species will be reported.

The relatively small number of the species recorded does not diminish the faunistic value of the Konavle region, on the contrary. If compared to other Croatian regions (e.g. Cerjanec *et al*., 2020; Kučinić *et al*., 2011, 2017b; Previšić *et al*., 2013; Vučković *et al*., 2021), the Trichoptera fauna of Konavle is a very interesting segment of Croatian caddisfly fauna, e.g. *locus typicus* for *Tinodes andrasi* Oláh, new species for fauna of Croatia, *Agapetus* cf. *kampos* Oláh, for its Mediterranean part, e.g. *Hydroptila martini* Marshall or record of *Diplectrona* cf. *atra* McLachlan. These faunistic and taxonomic results show that the area of Konavle, as an isolated valley in the south of Croatia (Bertić *et al*., 2001; http://www.enciklopedija.hr), went through geological processes that led to its isolation and resulted in some segments in the specific structure of the Trichoptera fauna in this part of Croatia.

In this research altogether 11 families of Trichoptera were recorded, with the highest number of four species recorded per family from the families Hydroptilidae, and Hydropsychidae (Tabs 1–3) and only one species per family for seven families (Tabs 1–3). New species can most probably be expected as well as from the family Limnephilidae, which is the most numerous European family, and from the families Rhyacophilidae, Polycentropodidae and Leptoceridae. Nonetheless, records from other families are also possible. Until now, for example no species from the family Philopotamidae or Lepidostomatidae have been recorded, but future findings cannot be ruled out. According to the data in this study and other caddisflies studies in Croatia (e.g. Cerjanec *et al*., 2020; Kučinić *et al*., 2011, 2017b; Vučković *et al*., 2021), including the hydrological features of Konavle and other areas (the rivers Cetina, Dobra, Krka and their tributaries, the Plitvice Lakes National Park), we can conclude that we have registered about 70% of the potential fauna of caddisflies of Konavle region, which had about 30 potential species.

The highest number of species was recorded on the locality spring of the River Ljuta (Tab. 2). According to its hydrological features and different types of aquatic microhabitats, this is no surprise (Fig. 2 A). Interesting fauna was also recorded on the small stream in the village of Pridvorje. At this locality alone, four species from the family Hydroptilidae were recorded (Tab. 2). In future studies, smaller streams and watercourses will be given more attention and significance, for example the small stream in the village of Lovorno (Fig. 2 G-H).

The highest number of Trichoptera species was recorded during the warm months, which was also expected (Tab. 3) and is consistent with similar surveys in other parts of Croatia (Cerjanec *et al*., 2020; Kučinić *et al*., 2017b; Vučković *et al*., 2021).

Because of the need to supplement data on seasonal dynamics, a part of the future fieldwork will be carried out in winter months (November and December), and in spring (March and May). Throughout the present research, the most interesting months were October, with ten recorded species and November, with three recorded species. This is consistent with the Mediterranean features of the climate in Konavle region, and the Trichoptera biodiversity of this period.

A faunistic preview of some interesting Trichoptera species (*Agapetus* cf. *kampos* Oláh, *Hydroptila martini* Marshall, *Oxyethira falcata* Morton, *Tinodes andrasi* Oláh,

Diplectrona cf. *atra* McLachlan and *Micropterna wageneri* Malicky) that have been recorded during this research will be given.

The species *Agapetus* cf. *kampos* Oláh (Fig. 3 A-C) belongs to one of the most common Trichoptera species in Konavle region. A great number of collected female and male imagines, and larvae, all originate from the localities spring of the River Ljuta and upper stream of the Ljuta. The examination of genitalia of a large number of males showed specific morphological varieties of *Agapetus* cf. *kampos* Oláh from Konavle (Oláh & Kovács, 2013). The River Ljuta is the second locality of this species after its type locality at Basa spring (Mt Rumija) in Montenegro (Oláh & Kovács, 2013). Since *Agapetus* cf. *kampos* was described based on two male specimens, in future studies more specimens of both sexes have to be collected from Montenegro, to make a detailed morphological analysis, to perform DNA barcoding, and define the exact taxonomic status of its population in Konavle region. Future studies should also aim at describing undescribed female specimens and undescribed larvae from the populations in Konavle region.

According to Fauna Europea (https://fauna-eu.org), *Hydroptila martini* Marshall, is distributed in Great Britain, Central Europe, Italy and Spain (https://fauna-eu.org; Barnard & Ross, 2012). The finding from Konavle region is the third finding of this species in Croatia, the first finding being from Lika and the second finding being from the Panonian-peripanonian part of Croatia (Kučinić *et al*., 2020b). It also represents the first finding of this species from Mediterranean parts and southeastern Europe. Morphological determination was confirmed by DNA barcoding (Tab. 4). This species belongs to the smallest caddisfly species in Croatia (microtrichoptera), with an anterior wingspan from 2.5 to 3.5 mm (Malicky, 2004). DNA barcoding is a very useful method for identifying taxonomically difficult species (Bączkiewicz *et al*., 2017), in present study that was the case for *Hydroptila martini* Marshall, but also other species from family Hydroptilidae (Tab. 4).

The species *Oxyethira falcata* Morton is not rare as it is distributed throughout most parts of Europe (Fauna Europea, https://fauna-eu.org; O'Connor, 2015; Hickin, 1967). It was previously not reported from Norway, Sweden, Poland and Baltic countries (Fauna Europea; O'Connor, 2015). Nonetheless, the finding from Konavle region is the first one from Croatia. Morphological determination was confirmed by DNA barcoding (Tab. 4). Larvae have the typical morphology of the family Hydroptilidae. The first four larval stages do not build cases, only the fifth stages do, and is very different from the earlier instars. They are herbivores that eat aquatic algae. Larvae can be found in streams, rivers and lakes and adults were also found at stagnant pools in a marsh (https://trichopteraireland.wordpress.com; O'Connor, 2015). The size of the imago is 3.5 mm (Malicky, 2004) and it flies from May until October (O'Connor, 2015).

Tinodes andrasi Oláh (Fig. 4) is an endemic species of the Dinarides. It was described in the year 2010 (Oláh, 2010) with its *locus typicus* being the upper stream of the River Ljuta. *Tinodes andrasi* Oláh has only been reported from Konavle polje (Oláh, 2010) and one locality (Črnojevica) in Montenegro (Malicky, 2018). Our DNA barcoding results show that the population from Konavle region has a difference of 1.5% from the population of *Tinodes rostocki* McLachlan in Europe. Based on the morphological features, the population from Konavle could have a subspecies status, which will be covered in another study.

Diplectrona cf. *atra* McLachlan (Fig. 5 A) was reported for the second time in Croatia. The first record originates from Mt Papuk in Pannonian/peripannonian Croatia

(Previšić *et al*., 2013). Besides *Agapetus* cf. *kampos* Oláh and *Silo nigricornis* Pictet it is one of the most numerous species in the River Ljuta and Konavle region. The results of DNA barcoding show certain specifics of the populations from Konavle, which will be analyzed in more detail in the results of the study of Croatian Trichoptera DNA barcode library (under preparation). *Diplectrona* cf. *atra* McLachlan has a disjunct areal with a partial distribution in West Europe (France, Spain), and a partial distribution in southeastern Europe (Bosna and Herzegovina, Greece, Bulgaria) (Fig. 5 B, Fauna Europea; Kumanski, 1985; Malicky, 2005).

The species *Micropterna wageneri* Malicky was described based on specimens that were collected in Italy (Malicky, 1971). In Croatia, *M. wageneri* Malicky was reported not only from the Konavle region but on Mt. Biokovo as well (Kučinić *et al*., 2017a). It belongs to the group of Mediterranean species and was also reported from Montenegro and Serbia (Kučinić *et al*., 2017a, Fauna Europea). According to Graf *et al*. (2008b), it is a montane species, which was confirmed by our findings from Biokovo (Kučinić *et al*., 2017a), but not from Konavle region where it was reported only from localities at lower altitudes of 150 m (brook in the village of Palje Brdo). Like other species from the genus, *Micropterna* Stein, this species can be found in caves as part of the troglophile fauna (Moretti, 1988).

Due to the faunistic characteristics of the Trichoptera fauna from the Konavle region future activities should be aimed at establishing a special attitude towards this area, as well as the protection of this hotspot of Croatian Trichoptera biodiversity.

CONCLUSION

The area of Konavle, the southernmost area of Croatia, represents one of the most interesting regions for Trichoptera fauna. Due to specific hydrological features and the sensitivity of such biotopes (springs, brooks, channels), it is necessary to install high standards for their protection, without major anthropogenic influence and with agricultural activities that should be aimed at the highest level of ecological production. In relation to the Croatian fauna, the area of Konavle represents a hotspot (not according to number of species, but because of the finding of very interesting species) for caddisflies with an interesting and special Trichoptera fauna. Legal protection only makes sense if it is aimed at the protection of habitats. Education of the local community represents one of the most important factors for the active protection of the biodiversity of a region such as Konavle. Nonetheless, the hydrological regime that was detected during the last 20 years must be maintained with the lowest possible negative effect from humans.

The DNA barcoding method proved to be very important and in fact essential in confirming the results of morphological determination (e.g. Geraci *et al*., 2011; Hebert *et al*., 2004; Kumara *et al*., 2019; Yang *et al*., 2015; Zhou *et al*., 2016).

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