NAT. CROAT. | VOL. 28 | No 2 | 441-454 | ZAGREB | December 31, 2019

original scientific paper / izvorni znanstveni rad
DOI 10.20302/NC.2019.28.29

NEW SPECIES FROM THE FAMILY HYDROPTILIDAE IN CROATIAN FAUNA COLLECTED IN THE KRKA NATIONAL PARK WITH PARTICULAR NOTICE TO BIODIVERSITY AND DNA BARCODING

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Kučinić, M., Ćukušić, A., Delić, A., Podnar, M., Gumhalter, D., Mičetić Stanković, V., Plantak, M., Čeple, G., Plavec, H. & Marguš, D.: New species from the family Hydroptilidae in Croatian fauna collected in the Krka National Park with particular notice to biodiversity and DNA barcoding. Nat. Croat., Vol. 28, No. 2., 441-454, Zagreb, 2019.

In this study we present: two species of caddisflies new for Croatian fauna from the family Hydroptilidae (*Hydroptila simulans* Mosley, *Orthotrichia costalis* Curtis), first DNA barcoding of caddisfly species in the Krka National Park and a discussion about recorded caddisfly fauna in the Krka NP from this study. From a faunistic point of view several species are interesting: *Hydropsysche mostarensis* Klapálek, *Hydroptila simulans* Mosely, *Hydroptila forfcipata* Eaton, *Orthotrichia costalis* Curtis and *Tinodes pallidulus* McLachlan. For the species *Oecetis notata* Rambur we have recorded interesting taxonomical remarks. Furthermore, within this study we used DNA barcoding which showed to be a very good and useful method for identification of very small and morphologically similar species from the family Hydroptilidae, or females from the family Psychomyiidae.

Key words: south Croatia, Dalmatia, the River Krka, *Hydroptila simulans*, *Orthotrichia costalis*, *Hydroptila forcipata*, *Hydropsyche mostarensis*, *Tinodes palidullus*, *Oecetis notata*, molecular analyses COI

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U ovome radu prikazujemo: dvije nove vrste zabilježene u fauni Hrvatske iz porodice Hydroptilidae (*Hydroptila simulans* Mosley, *Orthotrichia costalis* Curtis), prikaz DNA barkodiranja tulara u Nacionalnom parka Krka te osvrt na faunistički najzanimljivije vrste utvrđene tijekom ovih istraživanja. Faunistički najzanimljiviji su nalazi sljedećih vrsta: *Hydropsysche mostarensis* Klapálek, *Hydroptila simulans* Mosely, *Hdroptila forfcipata* Eaton, *Orthotrichia costalis* Curtis i *Tinodes pallidulus* McLachlan. Vrsta *Oecetis notata* Rambur nazanimljivija je s taksonomskog aspekta. U ovim istraživanjima metoda DNA barkodiranja pokazala se kao vrlo korisna u determinaciji vrlo malih i morfološki sličnih vrsta iz porodice Hydroptilidae te ženki iz porodice Psychomyiidae.

Ključne riječi: južna Hrvatska, Dalmacija, rijeka Krka, *Hydroptila simulans*, *Orthotrichia costalis*, *Hydroptila forcipata*, *Hydropsyche mostarensis*, *Tinodes palidullus*, *Oecetis notata*, molekularne analize COI

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INTRODUCTION

The family Hydroptilidae is the largest family within the order of Trichoptera. The family was established by Stephens (Holzenthal, 2007; Stephens, 1836). The family Hydroptilidae belongs to the suborder Integripalpia and according to Holzenthal *et al*. (2007) is divided into 2 subfamilies, Hydroptilinae Stephens 1836, and Ptilocolepinae Martynov 1913 (Martynov, 1913; Stephens, 1836). Adult stages are characterized with small body size, the length of some species forewings varies from 1.5 to 2.5 mm (Hickin, 1967; Malicky, 2004). However some species from the family Hydroptilidae are bigger and have a forewing length up to 4 or 5 mm (Hickin, 1967; Holzenthal *et al*., 2007; Malicky, 2004). Since their body size is so small they are called microtrichoptera. Adults of the family Hydroptilidae have very hairy wings (Hickin, 1967). Members of the family Hydroptilidae are of cosmopolitan distribution, therefore they are found worldwide on all continents (Holzenthal, *et al*., 2007) except in the Arctic. Currently, there are approximately 2000 species described worldwide (Holzenthal *et al*., 2007), out of which some hundred occur in Europe (Malicky, 2004).

The biology of many species from the family Hydroptilidae is insufficiently known, since not all stages of their life cycle are described. According to Oliver Flint "The family Hydroptilidae is the *terra incognita* of Trichoptera" (Flint, 1992; Holzenthal *et al*., 2007). This applies especially to the immature stages of the larvae and the pupae, which are only in some species insufficiently known (Hickin, 1967). Nonetheless, the features of the majority of the family and the genera are well known, because the life cycle and the morphology of their life stages could be investigated through some well-known species from the genera (Hickin, 1967; Marshall, 1978). After copulation the female flies close to the water and lays the eggs attaching them below the water surface on rocks or submerged vegetation (Marshall, 1978). One of the main features of the family Hydroptilidae is that the first four instar stages of the larva last very short, they do not build cases and the morphology resembles those of some plankton crustaceans. Only the fifth instar builds cases out of sand and tiny stones. The cases are of a very specific and characteristic shape, the one of a bottle (Hickin, 1967; Waringer & Graf, 2011). Such a life cycle is called hypometamorphosis and is known only in this family of Trichoptera (Marshall, 1978).

Larvae feed on detritus or macrophytes, including filamentous green algae (Marshall, 1978). These feeding habits are expressed especially within the fifth larval stage of development when larvae are preparing to undergo to adult stage development. Many species have one or two generations in Europe, while a number of generations can be higher in subtropical and tropical area.

The family Hydroptilidae comprises many various genera that inhabit quite diverse habitats, from springs and mountain creeks, all the way to large rivers and rather less in lakes as well as in habitats made by men, e.g. canals (O'Connor, 2015). Adult forms tend to inhabit areas close to the aquatic habitats of larvae, like surrounding rocks, banks and bank vegetation (Marshall, 1978).

DNA barcoding is the newest identification method (Hebert *et al*., 2003a, b) that has significantly contributed (with other molecular analyses) to the knowledge of the diversity, taxonomy and discovery of cryptic species (for example Bilandžija *et al*., 2013; Brehm *et al*., 2019; Cárdenasi *et al*., 2013; Elías-Gutiérrez *et al*., 2008; Guo *et al*., 2016; Previšić *et al*., 2014; Smit *et al*., 2016; Tyagi *et al*., 2017; Vaglia *et al*., 2008; Vitecek *et al*., 2015), improvement of identification of species that are difficult to identify by morphological features, to biomonitoring, phylogenetic and phylogeography (for example Brehm *et al*., 2019; Kang *et al*., 2017; Santos *et al*., 2016; Vitecek *et al*., 2017). It is as almost no other method since the time of Linneaus (Linnaeus, 1758) has made such great influence in zoology like DNA barcoding. First papers on DNA barcoding and the BOLD System database (Ratnasingham & Hebert, 2007) published in Croatia are only several years old. Nonetheless, application of the method is intensive and has a growing trajectory in Croatia, as well as in the rest of Europe and the World.

The objectives of the present study were: 1.) to present two new records of caddisfly species in Croatia; 2.) to provide first DNA barcoding of caddisfly species in the Krka NP; 3.) to discuss recorded caddisfly fauna in the Krka National Park with previous studies. The results of this study are part of the project "DNA barcoding of biodiversity of Croatian fauna".

MATERIAL AND METHODS

Field work

Field investigations in the Krka National Park (Krka NP) (south Croatia, region Dalmatia) were conducted from 2017 until 2019 (leg. A. Delić, H. Plavec, G. Čeple, M. Kučinić). The material was collected on several localities in the NP.

However, the DNA barcoding method was used on specimens collected only on the following four localities (Figs 1, 2 A-D): Brljan Lake (Fig. 2A), hydroelectric power plant Miljacka (HPP Miljacka) (Fig. 2B), waterfall Roški slap (Fig. 2C) and Lake Visovac (a dock near the island Visovac) (Fig. 2D). The first locality is located at the very end of the Brljan Lake (Fig. 2A), behind the dam on the bridge, close to the left side of the River Krka. The locality is situated on a paved road, which leads over the bridge. On both sides, it is predominantly covered by forest vegetation, including some fig trees and cherry trees. The locality hydroelectric power plant Miljacka is located inside the hydroelectric power plant close to the Brljan Lake (Fig. 1), on the upper course of the River Krka. The aquatic insects were collected on the left side of the river, next to the main building of the hydroelectric power plant. The River Krka forms at this place canyons and the water current is rather strong with expressed saturation (Fig. 2B). Mosses cover flagstones and the river resembles at this place an upper stream of a mountain river. Hydrological specifications of this part of the River Krka are underground springs on the right side of the river, which connect the River Krka and river Zrmanja (Bonacci & Roje-Bonacci, 2015). The waterfall Roški slap is one of the most recognizable part of the River Krka. Characteristic are tufa and a cascade of smaller waterfalls which contribute to a stronger saturation (Fig. 2C). In this part the River Krka is wide,

with a lot of bushy and forest vegetation on its riverbanks. Material was collected on the bridge, close to the right side of the River Krka. Aquatic insects, including Trichoptera, were collected approximately 50 meters away from the right side of the river. The last locality, Visovac Lake is located on the lower section of the River Krka (Fig. 1), near the island Visovac. It is situated on a small boat dock on the right side of river Krka. The river is in this place very wide and resembles a lake. Therefore, this part is called Visovac Lake (Fig. 2D). The riverbanks are covered by marsh vegetation of reed and water plants.

Laboratory work

In order to use DNA based method of specimen identification along with morphological features all collected material was preserved with absolute ethanol. The DNA vouchers of the barcoded samples were stored in the Croatian Natural History Museum in Zagreb and in the Trichoptera collection „Kučinić".

Macrophotographing of Trichoptera adults was carried out using a Leica Wild MZ8 stereomicroscope and Olympus SP-500 UZ digital camera, processed with computer program Olympus Quick Photo Camera 2.2 at the Laboratory for patology of trees, Department of Forest Protection and Wildlife Management at the Faculty of Forestry, University of Zagreb.

Determination of species was done according to Malicky (2004) and Kumanski (1985, 1988). Systematic followed Morse (2019).

DNA extraction and PCR amplification

Genomic DNA was extracted from legs of 21 specimens listed in Tab. 1 with specimen ID marked with bold letters. In Tab. 1. there are data of determination according to morphological features (first column), specimens ID, Locality/ Family, BOLD Sequence ID and species identification (%) after DNA barcoding analyses (last column).

Genomic DNA was extracted using GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, Germany) according to the manufacturer's specifications and eluted in 50 μl of elution buffer. For the amplification of the COI-5P barcode region primers: LCO1490 and HCO2198 (C 1994) ware used. The volume of mixture for polymerase chain reactions (PCR) was 50 μl. The PCR mixture contained 1 x Go Taq®Reaction Buffer (containing 1.5 mM MgCl2, Promega), 0.2 mM of each dNTP, 0.4 μM of each primer, 1.25 units of Go Taq®DNA Polymerase (Promega) and 5 μl of DNA eluate. PCR cycling conditions comprised an initial denaturation step (94°C for 2 min) followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 90 s and a final extension step of 72°C for 7 min. Product purification and bidirectional sequencing was performed by Macrogen Inc. sequencing service (Seoul, South Korea) and Macrogen Europe (Amsterdam, the Netherlands) using the amplification primers. Sequences were edited manually and aligned using the program BioEdit (Hall, 1999).

DNA sequences obtained in this study were submitted to Barcode of Life Data Systems (BOLD, Ratnasingham & Hebert 2007, Tab. 1). For 21 DNA barcode sequences obtained in this study, similarity search was performed using the BOLD Identification Engine (available on http://boldsystems.org/) which uses

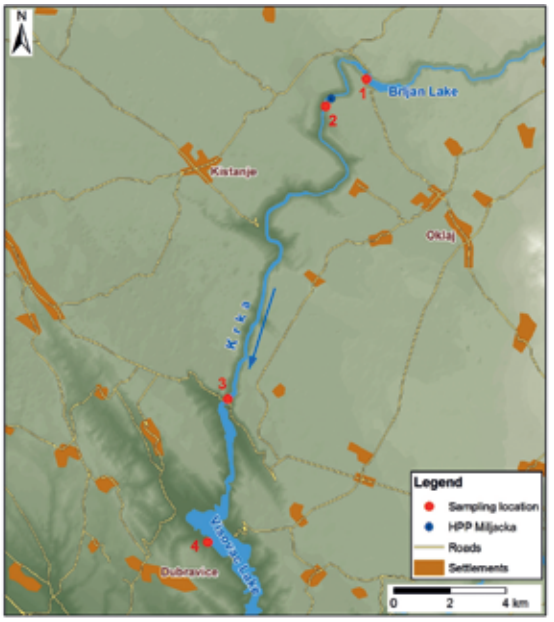

Fig. 1. Simpling sites in the Krka NP: 1 – Brljan Lake, 2 – HPP Miljacka, 3 – Roški slap, 4 – Visovac Lake.

Fig. 2 A–D. Photographs of the sampling sites in the Krka NP: Brljan Lake (A), HPP Miljacka (B), Roški slap (C) and Visovac Lake (D) (photo A. Delić).

all sequences uploaded to BOLD from public and private projects to locate the closest match.

RESULTS AND DISCUSSION

Throughout recent investigation on Trichoptera of Krka NP (Kučinić *et al*., 2011; Ridl *et al*., 2015), conducted between 2017 and 2019, some interesting faunistic findings have been recorded and confirmed by using DNA barcoding method (Tab. 1). Altogether 21 Trichoptera specimens have been DNA barcoded. The specimens belong to 8 families, 11 genera and 16 different species (Tab. 1), which makes approximately 30% of all established Trichoptera fauna in the Krka NP (Kučinić *et al*., 2011; Ridl *et al*., 2015).

From a faunistic point of view several species are very interesting: *Hydroptila simulans* Mosely, 1920, *Orthotrichia costalis* (Curtis, 1834), *Hydroptila forfcipata* Eaton, 1873, *Hydropsysche mostarensis* Klapálek, 1898 and *Tinodes pallidulus* McLachlan, 1878. The species *Oecetis notata* (Rambur, 1842) (Tab. 1) is interesting from a taxonomical point of view.

Only one species from the family Hydroptilidae has been previously known from Krka NP: *Hydroptila sparsa* Curtis, 1834 (Kučinić *et al*., 2011; Ridl *et al*., 2015). This species has also been reconfirmed during this investigation. Another three new species have been discovered in Krka NP: *Hydroptila simulans, H. forfcipata* and *Orthotrichia costalis*. The findings of the species *H. simulans* and *O. costalis* are the first findings of these species in Croatia. Determination of all three species has been undertaken by applying DNA barcoding, and their COI sequence has been entered in the BOLD System Database (Tab. 1). Since the family Hydroptilidae has adults of very small dimensions, DNA-barcoding has proven to be a very important tool for accurate determination of species (Tab. 1). This example, as well as the whole family Hydroptilidae, shows the importance of DNA barcoding,

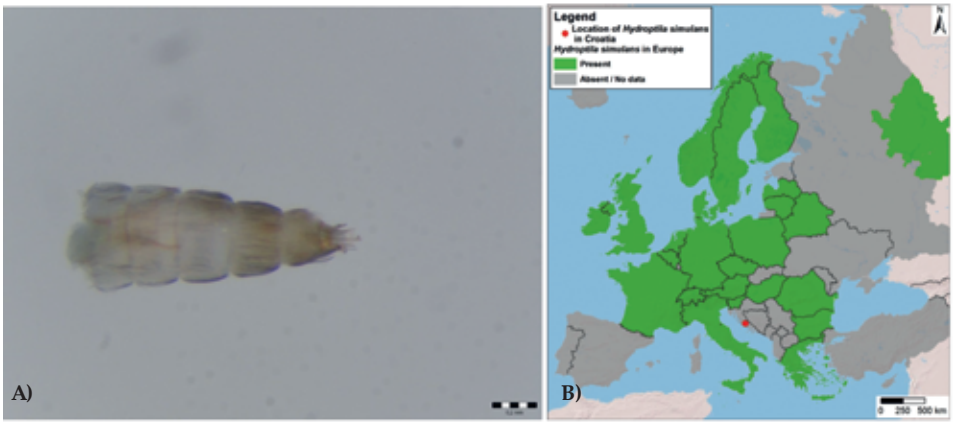

Fig. 3A-B. Abdomen and genitalia of *Hydroptila simulans* Mosely, 1920 (A), ventral view (photo M. Kučinić). Distribution in Europe according to Fauna Europaea (B). Red circle: record in the Krka NP.

which serves as an addition to taxonomical, phylogenetic and phylogeographical investigation, as well as investigation of different aspects of biomonitoring, conservation biology and protection of specific species and their habitats.

Hydroptila simulans (Fig. 3A) is widely distributed species in Europe, from Scandinavia, Ireland (O'Connor 2015) and Great Britain (Hickin, 1967; Barnard & Ross, 2012) in the north all the way to Greece in the south (Malicky, 2005) (Fig. 3B). The forewing length is 3 mm (Malicky, 2004). The fifth larval stage of this species builds cases from surrounding mud (O'Connor, 2015). Adults are active from May to August (O'Connor, 2015) which is consistent with our study and data from Krka NP. In the National Park we found this species in August 5, 2019. So far, *H. simulans* was recorded in the lower part of the Krka River at the locality in Visovac Lake (Fig. 2D). That is the only record of the species in Croatia so far.

Orthotrichia costalis is one of five European species of the genus *Orthotrichia* (Malicky, 2004). They are small sized caddisflies with the length of their forewings of only 3 mm (Malicky, 2004). They mainly inhabit slow running waterbodies such as large rivers, lakes or fishponds (O'Connor, 2015). So far, this species was recorded only at two localities in Croatia, both in Krka NP: Roški slap (Fig. 2C) and Visovac Lake (Fig. 2D). At the locality near the island of Visovac Krka River reaches its widest shape with a very slow current which hydrologically resembles a large lake. On the contrary, locality Roški slap has different hydrological characteristics, but the Krka River is very wide there with areas where very slow current occurs. In the contrast to majority of European species of family Hydroptilidae, larval stages of *O. costalis* are well known (O'Connor, 2015; Waringer & Graf, 2011). Larvae build protective cases from silk threads, which are rounded, elongated and narrowed in front and hind end, but not pointed (Waringer & Graf, 2011). The species *O. costalis* is widely distributed species in Europe, from Scandinavia and Great Britain in the north to Greece in the south

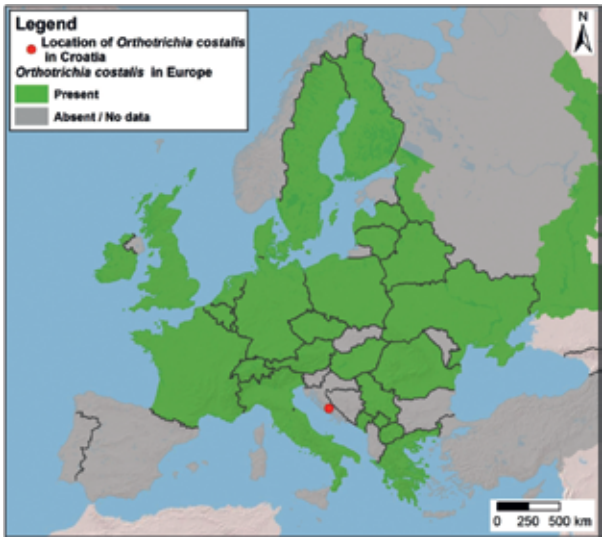

Fig. 4. Distribution of *Orthrotrichia costalis* (Curtis, 1834) in Europe according to Fauna Europaea. Red circle: records in the Krka NP.

(Malicky, 2005). The species is absent from Iberian Peninsula, Corsica, Sardinia, Sicily and Malta (Fauna Europaea) (Fig. 4). DNA barcoding showed to be a very good and useful method in identification of *O. costalis* (Tab. 1).

Hydroptila forcipata (Fig. 5, Tab. 1) is the third newly recorded species from the family Hydroptilidae in Krka NP. With three new records presented in this article, and previous two records of *Hydroptila sparsa* and *Allotrichia pallicornis* Eaton (1873) (Kučinić *et al*., 2011; Ridl *et al*., 2015), in Krka NP altogether 5 species of Hydroptilidae have been found so far. It is the first finding of *H. forcipata* in the Mediterranean part of Croatia. Adults appear in Greece from April to October, and in central Europe from May to October (Malicky, 2005). In the Krka National Park we found this species in September. The species *H. forcipata* is widespread in Europe except Iceland in the north, and Portugal, Corsica, Sardinia and Malta in the south (Fauna Europaea, 2019; Hickin, 1967; Ibrahimi *et al*., 2012; Malicky, 2005).

During this study the species *Hydropsyche mostarensis* is reconfirmed for the area

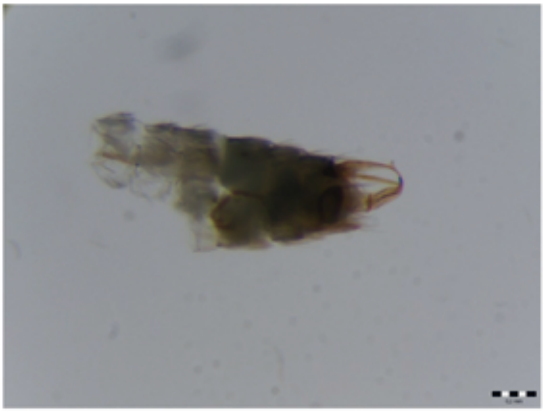

Fig. 5. Abdomen and genitalia of *Hydroptila forcipata* Eaton, 1873, lateral view (photo M. Kučinić).

of the NP Krka in the same locality, HPP Miljacka (Kučinić *et al*., 2011) (Fig. 2B). Only one specimen was collected on August 5, 2018. DNA-barcoding was applied to this specimen and the results entered in the BOLD platform. This represents the second record of *H. mostarensis* in the Barcode of Life Data System (BOLD). This finding from the Krka NP is very interesting for the Croatian Trichoptera fauna, since it represents the only site for Croatia (Kučinić *et al*., 2011). Krka NP is the westernmost point of the species distribution, which biogeographically belongs to southern European species (Fig. 6). The closest finding originates from the spring of river Lištica in Bosnia and Herzegovina (Stanić-Koštroman *et al*., 2012, 2015). The larva of *H. mostarensis* has been described from Greece (Karaouzas, 2018) and is different from well described larvae of the genus *Hydropsyche* from this area. This species belongs to *Hydropsyche gutata* Group (Karaouzas, 2018). *Hydropsyche mostarensis* prefers spring areas (Stanić-Koštroman et al., 2012) and prefers lower habitat temperatures (Karaouzas, 2018). In this specific case the side springs of river Krka, close to the HPP Miljacka, cause low temperatures from 10.5 up to 15.2 °C with a very low maximum water during summer (Kučinić *et al*., 2011). This probably suits the species *H. mostarensis*. Since this is the only finding of

this species from Croatia special attention should be paid to its conservation. In fact, the species has never been before or after collected on any of approximately 200 other Trichoptera sampling sites, which have been visited during the last 20 years. Although this area is protected as a national park, negative effects are possible since this is also the location of the hydroelectric power plant. Until now, no negative effects could be traced. Therefore it is suggested to continue with the same management of the hydroelectric power plant and the nearby area.

Fig. 6. Distribution of *Hydropsyche mostarensis* (Klapálek, 1898) in Europe according to Fauna Europaea. Red circle: record in the Krka NP.

Tinodes pallidulus is the fourth species recorded for the first time in the Krka NP during this this study. This species is fourth from the genus *Tinodes* recorded in the NP and along with the genera *Hydropsyche* and *Limnephilus* it is the most numerous genus in the Krka NP (Kučinić *et al*., 2011; Ridl *et al*., 2015). *Tinodes pallidulus* is widely distributed in Europe from Scandinavia and Great Britain in the north, and Greece and France in the south, except Island, Iceland, Apennine Peninsula, Corsica, Sardinia and Sicily (Fauna Europaea, 2019; Hickin, 1967; Malicky, 2005; Živić *et al*., 2006). In Croatia this species was recorded in the continental (according to Bertić *et al*., 2001 - Panonnian-Peripanonian) and Mediterranean parts (Kučinić *et al*., 2016). DNA barcoding proved to be a useful method in identification of *T. pallidulus* females sampled in the NP.

DNA barcoding of caddisfly fauna in the Krka NP revealed some taxonomically interesting results. Species *Oecetis notata* (Tab. 1) is recorded in the lower reach of Krka River as a very common species, especially at the locality Skradinski

Buk (Ridl *et al*., 2015). In our recent studies, we have additionally found one specimen of *O. notata* at Roški slap, but also in other rivers in Croatia: Mrežnica River, Gacka River, Dobra River and Cetina River. Besides this specimen from the Roški slap, we have also DNA barcoded two specimens from Gacka River and Dobra River (Ćukušić, 2019). The sequences obtained from specimens collected in rivers Krka, Dobra and Gacka were submitted to the BOLD Identification Engine which showed a match of 99.84% (Gacka River), 100% (Dobra River) and 94.2% (Krka River) (Tab. 1). Latter result from Krka River clearly indicates intraspecific variability within caddisflies (variability of 8%), with the possibility that the specimens from Krka River represent a subspecies (Ćukušić, 2019). Nonetheless, future studies with comprehensive morphological and molecular analyses with inclusion of nuclear genes are required.

Tab. 1. List of species used in this study: first column - identification according to morphological features, last column - DNA species identification with similarity percentage to existing DNA sequences in BOLD database (identification according to the BOLD Identification Engine).

Species	Specimen ID	Locality	BOLD Sequence ID	Species identification (%)
Family Hydroptilidae				
Hydroptila forcipata	THFOR_5	Roški slap	CROTR-131-19	*Hydroptila forcipata* (100%)
Hydroptila cf. *simulans*	THSIM_1	Roški slap	CROTR129-19	*Hydroptila simulans* (99.53%)
Hydroptila sparsa	THSPA_4	Roški slap	CROTR156-19	*Hydroptila sparsa* (99.52)
Hydroptilidae	THYD_10	Visoavac Lake, right bank near island Visovac	CROTR224-19	*Orthotrichia costalis* (100%)
Hydroptila sparsa	THSPA_5	Roški slap	CROTR184-19	*Orthotrichia costalis* (100%)
Hydroptilidae	THYD_11	Visovac Lake, right bank near island Visovac	CROTR184-19	*Orthotrichia costalis* (99.84%)
Family Polycentropodida				
Cyrnus trimaculatus (Curtis, 1834)	TCTRI_5	Visovac Lake, right bank near island Visovac	CROTR161-19	*Cyrnus trimaculatus* (99.68%)
Family Ecnomidae				
Ecnomus tenellus (Rambur, 1842)	TETEN_5	Visovac Lake, right bank near island Visovac	CROTR234-19	*Ecnomus tenellus* (99.69%)

Species	Specimen ID	Locality/Family	BOLD Sequence ID	Species identification (%)
Family Psychomyiidae				
Tinodes braueri McLachlan, 1878	TTBRA_2	Roški slap	CROTR125-19 CROTR125-19	*Tinodes braueri* (99.84)
Tinodes waeneri (Linnaeus, 1758)	TTWAE_3	Visovac Lake, right bank near island Visovac	CROTR034-19	*Tinodes waeneri* (98.71%)
Tinodes waeneri	TTWAE-4	Visovac Lake, right bank near island Visovac	CROTR159-19	*Tinodes waeneri* (98.71%)
Tinodes sp.	TRIC_5	HPP Miljacka	CROTR158-19	*Tinodes pallidulus* (99.52%)
Family Hydropsychidae				
Hydropsyche instabilis	THINS_10	Brljan Lake	CROTR242-19	*Hydropsyche inatabils 100%*
Hydropsyche mostarensis	THMOS_1	HPP Miljacka	CROTR086-19	*Hydropsyche mostarensis* (99.34%)
Family Lepidostomatidae				
Lepidostoma hirtum (Fabricius, 1775)	TLHIT_4	HPP Miljacka	CROTR235-19	*Lpeidostoma hirtum* (99.29%)
Lepidostoma hirtum	TLHIT_3	Roški slap	CROTR132-19	*Lepidostoma hirtum* (100%)
Family Limnephilidaee				
Limnephilus marmoratus	TLMAR_1	Visovac Lake, right bank near island Visovac	CROTR096-19	*Limnephilus marmoratus* (99.84%)
Limnephilus marmoratus Curtis, 1834	TLMAR_2	Visovac Lake, right bank near island Visovac	CROTR097-19	*Limnephilus marmoratus* (99.84%)
Family Leptoceridae				
Athripsodes albifrons	TAALB_2	Roški slap	CROTR123-19	*Athripsodes albifrons* (99.22%)
Ceraclea dissimilis (Stephens, 1836)	TCDIS_3	Lake Brljan	CROTR254-19	*Ceraclea dissimiliss* (99.69%)
Oecetis cf. *notata*	TONOT_5	Roški slap	CROTR130-19	*Oecetis notata* **(94.2%)**

CONCLUSIONS

It this study DNA barcoding method has proved to be a very good and useful method in identification of very small and morphologically similar species from the family Hydroptilidae, as well as female specimens from family Psychomyiidae. We collected 21 specimens for DNA barcoding from four localities in the Krka NP, and identified 16 species among them. All specimens are entered into the BOLD base. Four species are new for the caddisfly fauna of the Krka NP and two species are new for the fauna of Croatia. From taxonomic standpoint the most interesting species found in the NP is *Oecetis notata* Rambur. DNA barcoding analyses revealed high molecular divergence of specimens collected in the Krka

NP compared to the specimens collected at other sites, River Gacka and River Dobra.

Future studies on Trichoptera in the Krka NP will be focused on further sampling and DNA barcoding with aim to contribute to detailed knowledge on distribution and biodiversity of caddisflies.

ACKNOWLEDGMENTS

We are very grateful to the Department of Forest Protection and Wildlife Management) at the Faculty of Forestry, University of Zagreb, two anonymous referees for their useful suggestions and also to Graham McMaster for his assistance with the English language This research is a part of scientific project "DNA barcoding of Croatian faunal biodiversity" (IP-06-2016-9988) funded by the Croatian Science Foundation.

Received December 1, 2019

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