



CADDISFLY FAUNA CHARACTERISTICS (INSECTA, TRICHOPTERA) OF FOUR ADRIATIC ISLANDS WITH A NOTE ON DNA BARCODING

MLADEN KUČINIĆ^{1*}, ANĐELA ČUKUŠIĆ², HRVOJE PLAVEC³, MIRO LANDEKA⁴,
MLADEN PLANTAK⁵, MARIJANA VUKOVIĆ⁶, VIŠNJA BUKVIĆ⁷,
MILIVOJ FRANJEVIĆ⁸, SANJA ŽALAC⁹ & GORDAN LUKAČ¹⁰

¹Department of Biology (*Laboratory for Entomology*), Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia

²Ministry of environment and energy, Radnička cesta 80/7, 10000 Zagreb, Croatia

³Grožnjanska 18, 10 000 Zagreb, Croatia

⁴Marina Tartaglie 2, 10 000 Zagreb, Croatia

⁵Elektroprojekt d.d., Civil and Architectural Engineering Department, Water Resources, Nature and Environmental protection, Alexandera von Humboldta 4, 10000 Zagreb, Croatia

⁶Croatian Natural History Museum, Demetrova 1, 10000 Zagreb, Croatia

⁷University Hercegovina, Blajburških žrtava 100, 88000 Mostar, Bosnia and Herzegovina

⁸Department for forestry and wildlife management, Faculty of Forestry, Svetošimunska 25, University of Zagreb, 10000 Zagreb

⁹ZSC „Dr. Ivo Pevalak“, National park Plitvice Lakes, Josipa Jovića 19, 53231 Plitvička jezera, Croatia

¹⁰National park Paklenica, Dr. Franje Tuđmana 14a, 23244 Starigrad-Paklenica, Croatia

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To date, 13 species of Trichoptera from 11 genera and 8 families have been reported for the Adriatic islands of Cres, Krk, Pag and Hvar. Relatively recent research has been conducted on the islands of Cres, Krk and Pag, where 329 adults of Trichoptera belonging to 10 species from 8 genera and 6 families were collected. Most dominant was *Ecnomus tenellus* with 235 specimens collected (islands of Krk and Cres), followed by *Leptocerus tineiformis* (Krk) with 51 specimens collected. Only one specimen of the each species *Stenophylax mitis* (Cres) and *Mystacides azurea* (Krk) was recorded. Most of the species were recorded on the island of Krk (7), and the lowest on the island of Pag (2). Faunistically most interesting was the first finding of species *Stenophylax mitis* McL. for the fauna of Croatia.

Key words: Adriatic, aquatic insects, distribution, *Stenophylax mitis* McL., DNA barcoding

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Na području jadranskih otoka (Cres, Krk, Pag, Hvar) utvrđeno je do sada 13 vrsta Trichoptera iz 11 rodova i 8 porodica. Novija istraživanja provedena su na otocima Cresu, Krku i Pagu u kojima je prikupljeno 329 adultnih oblika Trichoptera s 10 vrsta iz 8 rodova i 6 porodica. Najdominantnije vrste bile su *Ecnomus tenellus* s 235 prikupljenih primjeraka (Krk i Cres) i *Leptocerus tineiformis* (Krk) s 51 prikupljenim primjerkom, a samo s jednim primjerkom zabilježene su vrste *Stenophylax mitis* (Cres) i *Mystacides azurea* (Krk). Najveći broj vrsta utvrđen je na otoku Krku (7), a najmanji na otoku Pagu (2). Faunistički najzanimljiviji je nalaz vrste *Stenophylax mitis* McL. koja je ovim istraživanjima utvrđena po prvi puta u fauni Hrvatske.

Ključne riječi: Jadran, vodeni kukci, rasprostranjenost, *Stenophylax mitis* McL., DNA barkodiranje

INTRODUCTION

The Republic of Croatia is situated in the central and southern part of Europe (Figs. 1, 3), belongs among the Central European and Mediterranean countries and has a surface area of 56 000 km². According its climatic, geological, vegetational and geographical characteristics, three main parts can be distinguished: the Pannonian-Peripannonian part, the central mountains, and the Mediterranean part (BERTIĆ *et al.*, 2001). The Mediterranean part of Croatia is the longest and, apart from the area of Istria in the north, does not have many watercourses, only a few isolated rivers. It is characterized by a Mediterranean climate with warm/hot summers and mild winters. Some of the streams in this area dry up during the summer period. Besides the inland part, Croatia has a coastal area with a surface of 28 000 km² and about 1100 islands.

In Croatia two basic hydrological basins can be distinguished: the Black Sea basin, which includes all the watercourses in the Pannonian-Peripannonian part and much of the mountain part (the Danube river flows into the Black Sea), and the Adriatic basin, which covers isolated watercourses (e.g. the rivers Rječina, Cetina, Krka, Zrmanja, Jadro, Ljuta) that flow into the Adriatic Sea. Adriatic islands are very poor regarding inland waters, and only a few of them have lakes (e.g. the islands of Pag and Cres) or streams; many more of them have pools (e.g. the island of Rab), many of which dry up.

In the last 20 years there have been quite a few faunistic papers (ĆUKUŠIĆ *et al.*, 2017; KUČINIĆ *et al.*, 2011a, 2017), taxonomic papers (GRAF *et al.*, 2008; MALICKY *et al.*, 2007; KUČINIĆ *et al.*, 2008, 2013; PREVIŠIĆ *et al.*, 2014; VUČKOVIĆ *et al.*, 2011; WARINGER *et al.*, 2009), ecological papers (IVKOVIĆ *et al.*, 2013; PREVIŠIĆ *et al.*, 2007; ŠEMNIČKI *et al.*, 2012) and phylogenetic papers (PREVIŠIĆ *et al.*, 2014; SZIVÁK *et al.*, 2017) dealing with Trichoptera in Croatia, but in them Trichoptera and island fauna were never represented. In this paper, therefore, we highlight that particular part of the Croatian fauna, focusing on the diversity and distribution of Trichoptera on four Croatian islands with their peculiar hydrological features that enable the occurrence of Trichoptera. Besides recent research in this paper we included an overview of literature data for adult forms of Trichoptera on Croatian islands (MALICKY, 1979). In macrozoobenthos research on the island of Krk (lake Jezero) Trichoptera are recorded but without identification of larvae to the species level (MIHALJEVIĆ *et al.*, 2004).

The DNA barcoding and BOLD System database (HEBERT *et al.*, 2003a, 2003b; RATNASINGHAM & HEBERT 2007) has been one of the newest methods used in biodiversity, taxonomy, ecology, conservation biology, phylogeny and phylogeography (e.g. VITECEK *et al.*, 2017; YÁNEZ-MUÑOZ *et al.*, 2018), and it is the most significant one since the time of Carl von Linné (LINNAEUS, 1758). DNA barcoding method based on 658-bp region of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene leading to larger and faster species identification services and better biodiversity information. One of the success of the DNA barcoding method lays in a library of DNA barcodes of morphologically identified specimens from a wide and diverse area. Therefore, in this paper we present data about the first DNA barcoded specimens of Trichoptera collected on the Croatian islands.

MATERIAL AND METHODS

Study area

Croatia has over 1100 islands and islets. In this paper we have included three Croatian islands (Krk, Cres and Pag; Figs 1-2), and literature data for the island of Hvar. Krk and Cres are located in the Kvarner Bay and are the biggest Croatian islands; two other big islands, Pag and Hvar, are on the border between the Croatian Littoral (Hrvatsko primorje) and Dalmatia, and in Dalmatia, respectively (Figs 1-2). Biogeographically, the Croatian Littoral and Dalmatia belong to the Mediterranean part of Croatia (BERTIĆ *et al.*, 2001).

Field work

The collecting of material was done during 2015 and in August 2018. Islands Krk, Cres and Pag were visited in the spring, summer and autumn of 2015 for a total of 15 field days. The island of Krk was visited also in August 2018. On the island of Cres the material was collected at Vransko Lake (Fig. 2C) and in the pool near the village of Beli; on Krk it was collected at the lakes Njivice and Ponikve (Fig. 2B); and on Pag at Lake Veliko Blato (Fig. 2A) near the town of Pag. During the field work we collected mainly adults, which can be identified to species level, and only on Pag were a few larvae collected (leg. G. Lukač, M. Kučinić). Adults were collected during the day with entomological nets during 30 minute catch per unit effort (CPUE) periods, and during the night with a small portable light traps equipped with UV lamps of 12 V voltage power (lighting time = 60 minutes). All collected specimens were preserved in 96% ethanol in order to be available for DNA barcoding of selected species. The majority of collected Trichoptera specimens are now a part of the Collection NIP – Trichoptera in the Croatian Natural History Museum in Zagreb.



Fig. 1. Map of Croatia with the investigated islands in blue color (Cres, Krk, Pag and Hvar).



Fig. 2A-C. Researched localities: A) Lake Veliko Blato, island of Pag (photo M. Kučinić); B) Lake Ponikve, island of Krk (photo D. Cerjanec); C) Vransko Lake, island of Cres, daytime caddisfly collecting (photo H. Plavec).

Laboratory work

Literature data for the island of Hvar originate from the Collection of Pater Gabriel Strobl deposited in the Museum of Natural History at Admont in Austria (MALICKY, 1979). The material was collected by entomologist and teacher Ivan Krstitelj Novak on the island of Hvar. Collected specimens are labelled Lesina (MALICKY, 1979), which is the old name of this island. Localities on the labels are not given any more precisely.

Identification was done according to MALICKY (2004) and KUMANSKI (1988), and with respect to taxonomy we followed MORSE (2019). In this paper only adults identifiable to species level are considered.

For DNA extraction and PCR, genomic DNA was extracted from legs of four specimens of four species: *E. tenellus*, *H. pellucidula*, *L. affinis* and *S. mitis*. All specimens used in this study are kept as vouchers in the Trichoptera DNA Barcode collection in the Croatian Natural History Museum in Zagreb. 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) were used. The volume for polymerase chain reactions (PCR) was 50 µl. The PCR mixture contained 1 × 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) using the amplification primers. Sequences were edited manually and aligned using the program BioEdit (HALL, 1999).

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

RESULTS AND DISCUSSION

In the present study, 329 adult specimens identified to species level were collected on the islands of Cres, Krk and Pag. In total, 10 species of Trichoptera from 8 genera and 6 families were recorded (Tab. 1). Malicky studied the Collection of Pater Gabriel Strobl and mentions five species for the island of Hvar (MALICKY, 1979); three of them (*Rhyacophila hirticornis* McLachlan, 1879, *Mesophylax aspersus* (Rambur, 1842) and *Sericostoma flavicorne* Schneider, 1845) were not recorded during our study. Therefore, the number of species recorded for the Adriatic islands amounts to 13 from 11 genera and 8 families (Tab. 1). This number is not

Tab. 1. Distribution of caddisflies on four Adriatic islands

Species	Islands			
	Cres	Krk	Pag	Hvar
Family Rhyacophilidae <i>Rhyacophila cf. hirticornis</i> McL.				•
Family Ecnomidae <i>Ecnomus tenellus</i> Ram.	•	•	•	
Family Psychomyiidae <i>Timodes waeneri</i> L.	•			
Family Hydropsychidae <i>Hydropsyche pellucidula</i>		•		
Family Phryganeidae <i>Agrypnia varia</i> Fab.		•		
Family Limnephilidae <i>Limnephilus affinis</i> Curt. <i>Limnephilus marmoratus</i> Cur. <i>Mesophylax aspersus</i> Ram.		• •	•	• • •
<i>Stenophylax mitis</i> McLachlan <i>Stenophylax permistus</i> McL.	• •			
Family Sericostomatidae <i>Sericostoma flavicorne</i> Schneider				•
Family Leptoceridae <i>Leptocerus tineiformis</i> Cur. <i>Mystacides azurea</i> L.		• •		
TOTAL	4	7	2	5

Tab. 2. List of caddisfly species with DNA barcode in the BOLD database.

Species	Specimen ID	BOLD Sequence ID	Location
<i>Ecnomus tenellus</i>	TETEN_1	CROAA085-18	Lake Vransko jezero - island of Cres
<i>Hydropsyche pellucidula</i>	THPEL_2	CROTR155-19	Lake Ponikve - island of Krk
<i>Limnephilus affinis</i>	TLAFI_2	CROTR032-19	Lake Ponikve - island of Krk
<i>Stenophylax mitis</i>	TSMIT_2	CROTR013-19	pool near Beli - island of Cres

high and constitutes approximately 5% of the Croatian caddisfly fauna, which has more than 200 species (KUČINIĆ, 2019). The majority of species were recorded on the islands of Krk and Hvar (7 and 5 species, respectively); the lowest number of species was collected on Cres and Pag (4 and 2 species, respectively) (Tab. 1).

In the present study *Ecnomus tenellus* (Rambur, 1842) dominated with 235 collected specimens (Krk and Cres), followed by *Leptocerus tineiformis* Curtis, 1834 (Krk) with 51 collected specimens. Other species were recorded in lower numbers; only one specimen was recorded in *Stenophylax mitis* McLachlan, 1875 (Cres) and *Mystacides azurea* (Linnaeus, 1761) (Krk). These data show that some species at certain localities can be found in large numbers, but on the other hand, for the finding of other species, a significantly greater sampling effort is needed and more freshwater island habitats should be checked.

All the species we recorded on islands are faunistically interesting, because although the Trichoptera inhabiting islands are not very diverse they do represent an additional, interesting part of the faunistic biodiversity of our islands. Beside species which are quite common in our fauna, e.g. *E. tenellus*, *Tinodes waeneri* (Linnaeus, 1758) (Fig. 4A-B), *Agrypnia varia* (Fabricius, 1793) (Fig. 4), *Limnephilus affinis* Curtis, 1834, *Stenophylax permistus* McLachlan, 1895, *Sericostoma flavicorne* Schneider, 1845 and *Mystacides azurea* (Fig.5 A-B) (e.g. CERJANEC, 2012; KUČINIĆ et al., 2011a), faunistically the most interesting are finds of *Limnephilus marmoratus*, *S. mitis*, *Mesophylax aspersus* and *Leptocerus tineiformis*. According to FAUNA EUROPAEA (2019), *M. aspersus* is distributed in Great Britain, France, Switzerland, the Iberian and Apennine peninsulas and Croatia (e.g. MALICKY, 1998; BONADA et al., 2004). The species has been found recently in Kosovo as well (IBRAHIMI et al., 2015). Records for the island of Hvar (MALICKY, 1979) are the first for Croatia, and more recently it was recorded for Istria, too (unpublished data). In the Collection of the Croatian Natural History Museum there are data on *M. aspersus* in the area of Virovitica, Trnovec (continental part of Croatia) and Mt Biokovo (Mediterranean part of Croatia). More recently, it was recorded in the Plitvička jezera NP and Krka NP. *L. tineiformis* is widely distributed in Europe, and for Croatia Malicky mentions it for the lower course of the river Cetina, situated in the Mediterranean part (MALICKY, 2014). As well as on the island of Krk it was also recorded in the area of the river Bednja in the continental part of Croatia (unpublished data). The most interesting finding in the present study was *Stenophylax mitis* which, according to FAUNA EUROPAEA (2019), is not recorded for Croatia (Fig. 3). It is distributed in parts of western, central and southeastern Europe (Fig. 3). It is also recorded on two localities in the Mediterranean part of Croatia: the source of the River Zrmanja and Paklenica National park (the mountain hut Borisov dom). The only collected male specimen from Cres was DNA barcoded (Tab. 2) which confirmed our identification. The record of *R. hirticornis* from the island of Hvar from the Collection of Gabriel Strobl (MALICKY, 1979) will have to be checked and confirmed by new research because there is a certain possibility that this species is not distributed in the Mediterranean part of Croatia.

Specimens of four species (*E. tenellus*, *H. pellucidula*, *L. affinis* and *S. mitis*) are listed in Tab. 2 with specimen ID and BOLD Sequence ID (Tab. 2; ČUKUŠIĆ, 2019). These are the first DNA barcoded specimens of Trichoptera from the Croatian



Fig. 3. Distribution of *Stenophylax mitis* McLachlan, 1875 in Europe according to FAUNA EUROPAEA (2019) with records in Croatia.

islands, listed in the Barcode of Life Data System (BOLD) (RATNASINGHAM & HEBERT, 2007). DNA barcoding method confirmed morphological identification of caddisflies species from Croatian islands based on sequences deposited in the BOLD database. Sequences analysed the using BOLD identification engine, were matched for their maximum identity with those available in the BOLD database (maximum similarity 99%).

Family Rhyacophilidae

Rhyacophila cf. *hirticornis* McLachlan, 1879

island Hvar – „*Lesina, Dalmatien*, 1♀“ (MALICKY, 1979)

Family Ecnomidae

Ecnomus tenellus (Rambur, 1842)

Lake Njivice, island of Krk, 1.07.2015, 28 males; Lake Ponikve, island of Krk, 18.05.2015, 1 female, 34 males; Lake Ponikve, island of of Krk, 13.07.2015, 26 females, 32 males; Vransko Lake, island of of Cres, 29.05.2015, 10 females, 24 males; Vransko Lake, island of of Cres, 8.08.2015, 9 females, 34 males; Vransko Lake, island of Cres, 3.10.2015, 2 males; Lake Veliko Blato, island of Pag, 3.09.2015, 18 females, 27 males

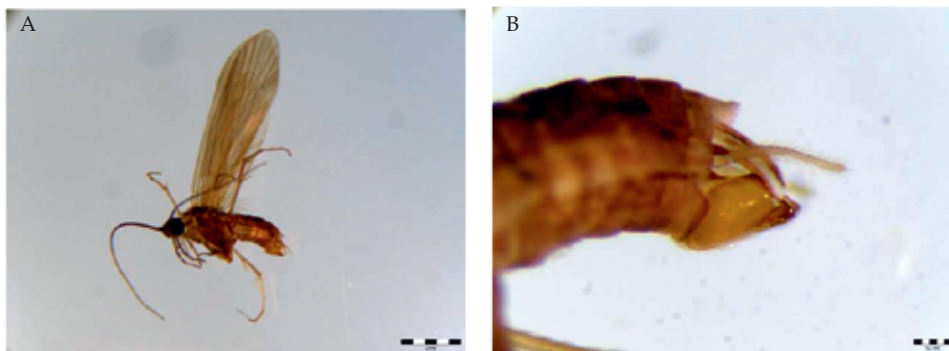


Fig. 4. A-B. *Tinodes waeneri* (Linnaeus, 1758); male (A) and zoomed male genitalia (B), from Vransko jezero Lake, island Cres. (scale bar = 2mm).

Family Psychomyiidae

Tinodes waeneri (Linnaeus, 1758) (Fig. 4 A-B)

Vransko Lake, island of Cres, 29.05.2015, 1 male; Vransko Lake, island of Cres, 8.08.2015, 1 male; Vransko Lake, island of Cres, 3.10.2015, 5 females, 12 males

Family Hydropsychidae

Hydropsyche pellucidula (Curtis, 1834)

Lake Ponikve, island of Krk, 06.08.2018



Fig. 5. *Agrypnia varia* (Fabricius, 1793) male, from Lake Njivice, island Krk. (scale bar = 2mm)

Family Phryganeidae

Agrypnia varia (Fabricius, 1793) (Fig. 5)

Lake Njivice, island of Krk, 16.10.2015, 3 females, 7 males, Lake Ponikve, island of Krk, 21.10.2015, 1 male

Family Limnephilidae

Limnephilus affinis Curtis, 1834

Lake Veliko Blato, island of Pag, 30.03.2015, 1 female, 1 male; Lake Ponikve, island of Krk, 21.10.2015, 1 male; island of Hvar, „Dalmatien, Lesina: 2 males, 2 females (Lesina, Novak)“ (MALICKY, 1979)

Limnephilus marmoratus Curtis, 1834

Lake Njivice, island of Krk, 16.10.2015, 3 females, 7 males; island of Hvar - „Dalmatien Lesina: 1♂, 2♀♀“ (MALICKY, 1979)

Mesophylax aspersus (Rambur, 1842)

island of Hvar, „Dalmatien, 1♂, 1♀, auf der Bodenetikette: Lesina (Novak)“ (MALICKY, 1979) – Dalmatia, 1 female, 1 male, on the ground label: Hvar (Novak)

Stenophylax mitis McLachlan, 1875

pool near Beli village, island of Cres, 3.10.2015, 1 male

Stenophylax permistus McLachlan, 1895

pool near Beli village, island of Cres, 30.05.2015, 2 females

Family Sericostomatidae

Sericostoma flavicorne Schneider, 1845

island of Hvar, 1 female, 1 male (MALICKY, 1979)

Family Leptoceridae

Leptocerus tineiformis Curtis, 1834

Lake Ponikve, island of Krk, 13.07.2015, 25 females, 26 males

Mystacides azurea (Linnaeus, 1761) (Fig. 6 A-B)

Lake Njivice, island of Krk, 1.07.2015, 1 male



Fig. 6A-B. *Mystacides azurea* (Linnaeus, 1761); male (A), zoomed male genitalia (B) from Lake Njivice, island Krk. (scale bar = 2mm)

CONCLUSION

During our investigation of four Croatian Adriatic islands we found a very interesting, but not numerous Trichoptera fauna. In the future, caddisfly research of Adriatic islands other than Cres, Krk and Pag should also include islands with particular types of freshwater habitats, i.e. Rab, Lastovo, Korčula, Brač, Mljet and so on. For example, research of butterflies on the island of Mljet, conducted in the first decade of the 21st century (Kučinčić *et al.*, 2011b) at localities near aquatic habitats (pools) was done by collecting nocturnal insects (moths, caddisflies and so on), but Trichoptera were not registered during that study. Since only a smaller

part of aquatic habitats of the island of Mljet was included, in the future many more aquatic biotopes should be systematically investigated in order to detect other aquatic insect groups, including Trichoptera, as shown by FRANKOVIĆ & BOGDANOVIĆ (2011) for dragonflies. In the future research we can expect more interesting faunistic results on Croatian Adriatic islands. Their biodiversity is not as high as on Greek islands (MALICKY, 2005) but they are a very important segment of Croatian fauna.

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