

# ELMIS RIETSCHELI STEFFAN, 1958 (INSECTA: COLEOPTERA: ELMIDAE) IN CROATIA: FIRST RECORD AND DNA BARCODING

VLATKA MIČETIĆ STANKOVIĆ<sup>1,\*</sup>, BRANKA BRUVO MAĐARIĆ<sup>2,\*</sup>,  
MANFRED A. JÄCH<sup>3</sup> & MLADEN KUČINIĆ<sup>4</sup>

<sup>1</sup>Croatian Natural History Museum, Demetrova 1, 10 000 Zagreb, Croatia

<sup>2</sup>Ruder Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia

<sup>3</sup>Natural History Museum Vienna, Burgring 7, 1010 Wien, Austria

<sup>4</sup>University of Zagreb, Faculty of Science, Department of Biology, Rooseveltov trg 6, 10 000 Zagreb, Croatia

Mičetić Stanković, V., Bruvo Mađarić, B., Jäch, M. A. & Kučinić, M.: *Elmis rietscheli* Steffan, 1958 (Insecta: Coleoptera: Elmidae) in Croatia: first record and DNA barcoding. *Nat. Croat.*, Vol. 27, No. 1, 185–194, Zagreb, 2018.

*Elmis rietscheli* Steffan, 1958 (Coleoptera: Elmidae) is a typical inhabitant of cold springs and small streams in the mountainous areas of Central Europe. Recently, three specimens of *E. rietscheli* were collected in the source area of the Šumi, a stream flowing from Mt. Ivanščica, northern Croatia. The morphological identification of the specimens was confirmed by DNA barcoding. This is the first record of *E. rietscheli* from Croatia, and it represents the southernmost record for this species known to date.

**Key words:** DNA sequencing, COI, faunistics, new record, species, crenal, riffle beetles

Mičetić Stanković, V., Bruvo Mađarić, B., Jäch, M. A. & Kučinić, M.: *Elmis rietscheli* Steffan, 1958 (Insecta: Coleoptera: Elmidae) u Hrvatskoj: prvi nalaz i DNA barkodiranje. *Nat. Croat.*, Vol. 27, No. 1, 185–194, Zagreb, 2018.

Tipična srednjoeuropska vrsta vodenog kornjaša iz porodice slapoljuba, Rietschelijev slapoljub, *Elmis rietscheli* Steffan, 1958 (Coleoptera: Elmidae), naseljava hladne izvore i malene vodotoke u središnjoj Europi. Tri primjerka ove vrste prikupljena su na području planine Ivanščice na izvoru potoka Šumi. DNA barkodiranje potvrdilo je morfološku identifikaciju jedinki. To je prvi nalaz vrste *E. rietscheli* za faunu Hrvatske te predstavlja do sada najjužniju zabilježenu točku rasprostranjenja te vrste.

**Gljučne riječi:** DNA sekvencioniranje, COI, fauna, novi nalaz, vrsta, izvor, slapoljubi

## INTRODUCTION

Although the conservation of biodiversity is nowadays recognised as a key priority, taxonomic and faunistic research, both providing basal information for conservation issues, are still undervalued. Therefore, many insect groups, such as Elmidae, are unfortunately still very poorly studied in Europe.

Elmidae, or riffle beetles, are a water beetle family typical of springs and mountain streams (BROWN, 1987; JÄCH, 1998; JÄCH *et al.*, 2005; ELLIOTT, 2008; KODADA *et al.*, 2016).

\* Vlatka.Micetic-Stankovic@hpm.hr / Tel: 0038514851700 / Fax: 0038514851644

\*\* Branka.Bruvo.Madjaric@irb.hr / Tel: 0038514571264 / Fax: 0038514561177

They are rather small-sized (adults are on average 2–3 mm long) with complex life cycles (CROWSON, 1981; ELLIOTT, 2008). They are frequently used as bioindicators for freshwater monitoring, *i.e.* assessments of ecological functionality and water quality (*e.g.* EYRE *et al.*, 1993; RICHOUX, 1994; ELLIOTT, 2008; BROJER *et al.*, 2017).

In an annotated checklist of the Elmidae of Croatia, MIČEĆIĆ STANKOVIĆ *et al.* (2015) listed 23 species; among these there are six species of the genus *Elmis* Latreille, 1802: *E. aenea* (Müller, 1806), *E. bosnica* (Zaitzev, 1908), *E. latreillei* Bedel, 1878, *E. maugetii* Latreille, 1802, *E. obscura* (Müller, 1806), and *E. rioloides* (Kuwert, 1890).

DNA barcoding (HEBERT *et al.*, 2003) is nowadays commonly used in taxonomic research as a means of species identification. This technique is based on sequencing of the standardized ~650 bp long fragment of the mitochondrial (mt) cytochrome oxidase gene subunit I (COI), which bears high interspecific and low intraspecific variability, thus enabling reliable species identifications (RATNASINGHAM & HEBERT, 2013). DNA barcodes were found to be especially useful in cases of morphologically ambiguous or sibling species (*see e.g.* FOSSEN *et al.*, 2016; BILTON & RIBERA, 2017; BILTON *et al.*, 2017).

In taxonomic studies on Elmidae, DNA barcoding has been used mostly for assigning immature developmental stages to adults, or in smaller phylogenetic studies, and, in a few cases, also in detecting undescribed species (ČIAMPOR & RIBERA, 2006; ČIAMPOROVÁ-ZATOVIČOVÁ *et al.*, 2007; ČIAMPOR & ČIAMPOROVÁ-ZATOVIČOVÁ, 2008; ČIAMPOR & KODADA, 2010; HAYASHI & SOTA, 2010; FREITAG & BALKE, 2011; CURIEL & MORRONE, 2012; FREITAG, 2013; LAŠŠOVÁ *et al.*, 2014; HAYASHI *et al.*, 2016; ČIAMPOR *et al.*, 2016, 2017; FREITAG & KODADA, 2017). Most of these studies deal with tropical species, while for European Elmidae only a few studies based on DNA data are available (*e.g.* HENDRICH *et al.*, 2015; JOVOVIĆ *et al.*, 2015; MÚRRIA *et al.*, 2017). For instance, in the Barcode of Life Database (*ref. webpage* BOLD System v4, accessed on March 25, 2018) there are currently data on 87 species (forming distinct BIN clusters) of Elmidae, but only 23 species sampled from seven European countries are included.

Here we present the first record of *Elmis rietscheli* Steffan, 1958 from Croatia, confirmed by DNA barcoding.

## MATERIAL AND METHODS

### Study area

Ivanščica is the highest mountain in the north of Croatia, with a maximum elevation of 1061 m a. s. l. The mountain stretches in a west-east direction; it is about 30 km long and 9 km wide. It forms a natural barrier between the Sava and Drava rivers. It is composed of sedimentary rocks, mineral limestones, sandstones, quartz and schist, and it is considered part of the Dinaric Alps (ČAPLAR, 2008; HERAK, 1960; ŠIMUNIĆ, 1983). The climate is moderate continental with some influence from the Adriatic Sea. The annual precipitation varies between 1000 and 1500 mm (ZANINOVIĆ *et al.*, 2008).

### Sampling and identification

The specimens were sampled with a hand net (mesh size: 500 µm), and stored in 96% ethyl alcohol. Specimens were examined with an Olympus ZX9 stereo microscope, and photographed with an Olympus Tough TG-5 camera. An inverted microscope Axio

Observer Z1 was used for genitalia photographs. All specimens are deposited in the Croatian Natural History Museum, Zagreb, Croatia.

### DNA extraction, PCR amplification, sequencing and sequence analyses

Genomic DNA was extracted from whole specimens by a non-destructive method, using GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, Germany) according to manufacturer's specifications, and eluted in 50  $\mu$ l of elution buffer.

The standard DNA barcode region (650 bp of 5' part of COI gene) was amplified with the use of universal LCO1490 / HCO2198 primers (FOLMER *et al.*, 1994). PCR was conducted in a 15  $\mu$ l reaction mixture, containing 1 x DreamTaq™ reaction buffer with 2 mM MgCl<sub>2</sub> (Thermo Scientific), 0.2 mM dNTP mix (Qiagen), 0.5  $\mu$ M of each primer, 0.75 units of DreamTaq polymerase (Thermo Scientific) and 3  $\mu$ l of eluted DNA. PCR cycling conditions were as follows: initial denaturation at 95°C for 2 minutes; 35 cycles of 95°C for 45 seconds, annealing at 52°C for 45 seconds, elongation at 72°C for 45 seconds; final elongation at 72°C for 7 minutes. PCR products were checked for quality and quantity on 1 % agarose gel stained with ethidium bromide and purified using Exonuclease I and FastAP™ Thermosensitive Alkaline Phosphatase enzymatic system (ThermoFischer Scientific), according to the manufacturer's specifications.

Bidirectional sequencing of PCR products was carried out at Macrogen Inc. (Amsterdam, Netherlands), using the amplification primers LCO1490 and HCO2198. Sequences were checked, edited and assembled from both directions in programs BioEdit v. 7.2.5 (HALL, 1999) and Geneious 8.1.4 (KEARSE *et al.*, 2012), and submitted to BOLD (RATNASINGHAM & HEBERT, 2007) and GenBank databases. BOLD ID and accession numbers are given in Tab. 1.

**Tab. 1.** Specimens and sequences used in the analyses. Newly sequenced samples are marked in bold.

Species name	Country	Sample ID	BOLD sequence ID	GenBank Acc. Nr.
<i>Elmis rietscheli</i>	Croatia	<b>ELRIO-B24/CROBB1</b>	<b>CROBF001-18</b>	<b>MH368658</b>
		<b>ELRIO-B25/CROBB2</b>	<b>CROBF002-18</b>	<b>MH368659</b>
	Germany	BC ZSM AQU 00290	FBAQU290-09	HM422035
<i>Elmis aenea</i>	Croatia	KJ381177	GBCL23072-15	KJ381177
		KJ381175	GBCL23073-15	KJ381175
		KJ381172	GBCL23074-15	KJ381172
		KJ381167	GBCL23078-15	KJ381167
	Austria	KJ381159	GBCL23028-15	KJ381159
		KJ381158	GBCL23029-15	KJ381158
		Germany	ZFMK-TIS-2522902	GCOL10027-16
	BC ZSM AQU 00495		FBAQU400-10	HM401301
		ZFMK-TIS-19747	GCOL3640-16	KU910942
Finland	ZMUO.006128	COLFF808-13	KJ965229	
<i>Elmis rioloides</i>	Croatia	KJ381192	GBCL23067-15	KJ381192
		KJ381193	GBCL23066-15	KJ381193
		KJ381189	GBCL23068-15	KJ381189
	Austria	KJ381179	GBCL23027-15	KJ381179
		KJ381180	GBCL23026-15	KJ381180
		KJ381181	GBCL23025-15	KJ381181

Species name	Country	Sample ID	BOLD sequence ID	GenBank Acc. Nr.
	Germany	BC ZSM AQU 00154	FBAQU154-09	HM376183
		BC ZSM AQU 00500	FBAQU405-10	HM401304
	Italy	ZFMK-TIS-7959	GCOL1005-16	KU911071
<i>Elmis obscura</i>	Germany	BC ZSM AQU 00499	FBAQU404-10	HM401303
		BCZSMAQU00968	FBCOG778-12	KM446204
<i>Elmis latreillei</i>	Germany	BC ZSM AQU 00289	FBAQU289-09	HM401281
<i>Elmis maugetii</i>	Austria	KJ381196	GBCL23023-15	KJ381196
		KJ381194	GBCL23024-15	KJ381194
	Germany	ZFMK-TIS-2522897	GCOL10025-16	KU907029
		BC ZSM AQU 00498	FBAQU403-10	HM401302
		BC ZSM AQU 00055	FBAQU055-09	HM376142
		BC ZSM AQU 00228	FBAQU228-09	HM422008
<i>Riolus subviolaceus</i>	Germany	BCZSMAQU01044	FBCOG854-12	KM451547
<i>Limnius opacus</i>	Germany	BC ZSM AQU 00538	FBAQU443-10	HM401335

BOLD Identification Engine (accessed March 2018) was used for comparison of DNA barcodes amplified from *Elmis rietscheli* with the barcode data available in BOLD. The single sequence of *E. rietscheli* in BOLD was provided by the Barcoding Fauna Bavaria Project in Germany (HENDRICH *et al.*, 2015). Sequences of *E. rietscheli* were aligned with all barcode sequences of *Elmis* species retrieved from BOLD (Tab. 1), with the addition of two outgroup sequences (Elmidae: *Limnius opacus* Müller, 1806 and *Riolus subviolaceus* (Müller, 1817)). Sequences were collapsed to haplotypes in FaBox online toolbox (VILLESEN, 2007); uncorrected p-distances and Kimura 2-parameter distances (K2P) between haplotypes were calculated using MEGA 7.0.25 (KUMAR *et al.*, 2016). Neighbour-joining (NJ) tree based on the K2P distance model was calculated in MEGA 7.0.25 (KUMAR *et al.*, 2016), and the robustness of the clades was assessed through 1000 bootstrap replicates. The maximum likelihood (ML) tree was constructed on PhyML 3.0 web-server (GUINDON *et al.*, 2010), with automatic model selection by SMS (determined through AIC selection criterion) (LEFORT *et al.*, 2017) and aLRT SH-like support.

## RESULTS AND DISCUSSION

*Elmis rietscheli* was described comparatively recently. Despite its rather distinctive male genitalia, this species was overlooked for a long time, mainly because of its remarkable external variability (JÄCH, 1992). For instance, BERTHÉLEMY (1979: 34) noted that *E. rietscheli* resembles *E. maugetii*, while SCHULTE (1989) found the specimens which he had examined more similar to *E. latreillei*; specimens recorded as *E. rietscheli* from Romania by IENIȘTEA (1974) turned out to belong to *E. latreillei* (BERTHÉLEMY, 1979: 26). This remarkable external changeability, known also from several other elmids species, may depend, at least to some extent, on the water temperature (see *e.g.* KNIE, 1978).

Three specimens of *Elmis rietscheli* were sampled by the first author on Mt. Ivanščica (northern Croatia), in the spring area of the Šumi, a mountain stream with gravel and sand benthos (21.VI.2016, 46°11'21.7"N 16°9'16.6"E, ca. 410 m a.s.l.) (Figs. 1, 2).

It is the first record of *Elmis rietscheli* in Croatia. This species was so far recorded from France, Germany, Switzerland, Austria, Italy, Slovenia and Hungary (JÄCH *et al.*, 2016); specimens from Hungary were collected in the Kőszeg Mountains, the eastern-

most region of the Alps, about 700 m from the Austrian border (Lókkös, 2010). This species is typical of mountainous areas, where it prefers the eucrenal, the hypocreanal



Fig. 1. Position of Mt. Ivanščica in Croatia (beetle symbol).

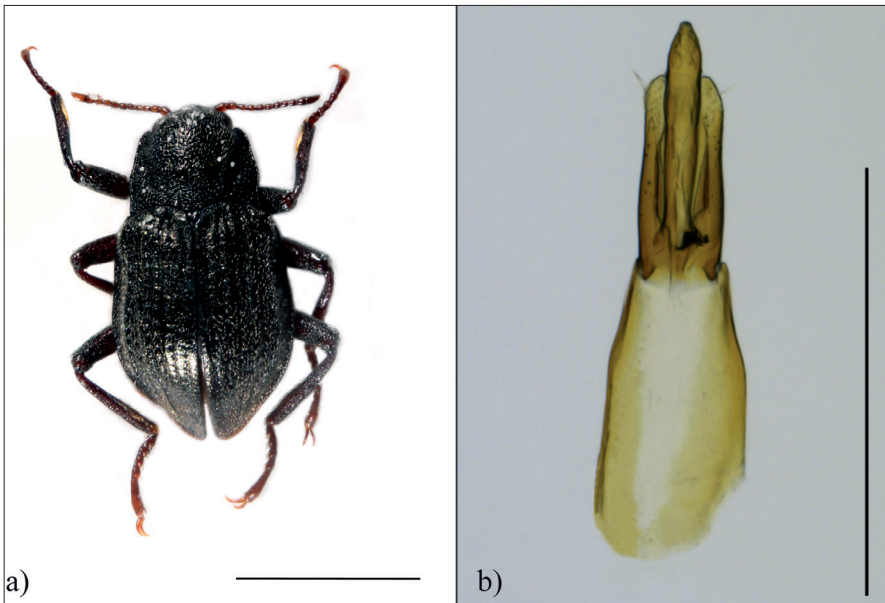
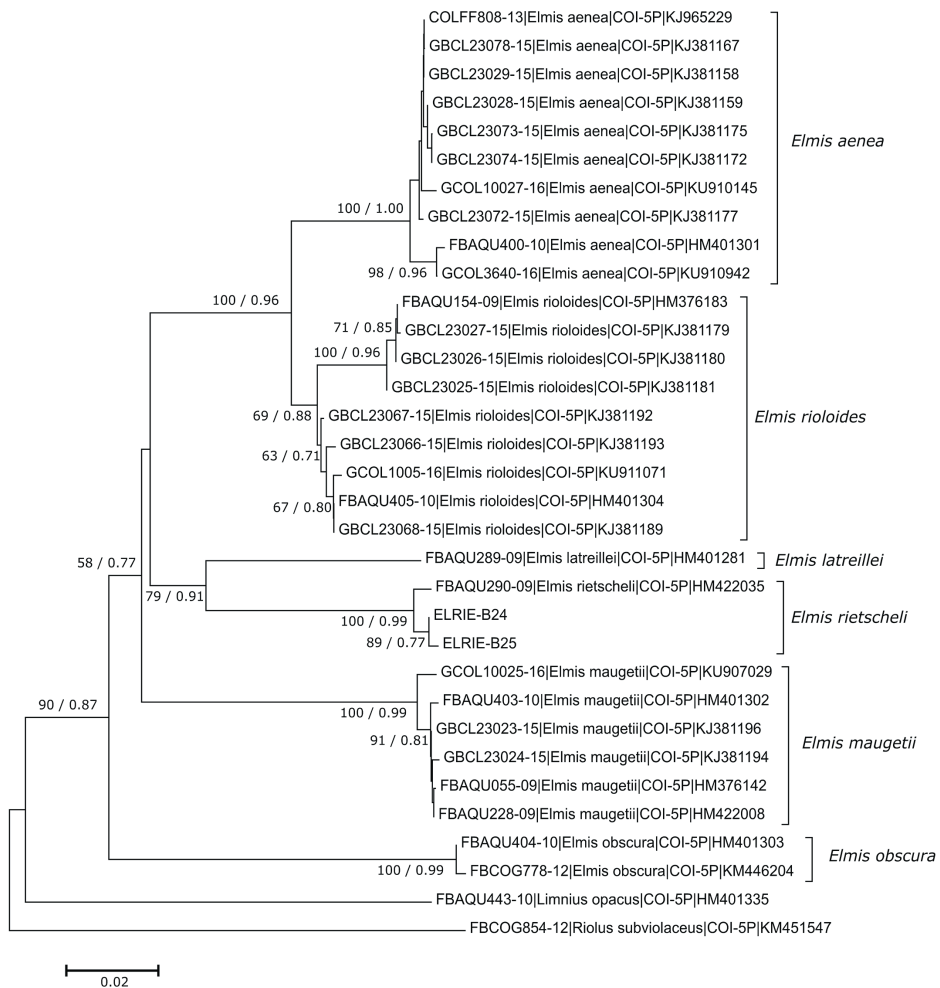


Fig. 2. *Elmis rietscheli* Steffan, 1958: a) habitus (scale = 1 mm), b) male genitalia (scale = 100 µm).

and the epirhithral (JÄCH *et al.*, 2005; BROJER *et al.*, 2017); in some parts of the eastern Alps it is very common. Ivanščica Mountain was sometimes considered to be an extension of the Alps (HERAK, 1960; ŠIMUNIĆ, 1983).

A molecular analysis confirmed the identification. Two COI haplotypes of the specimens sampled on Mt. Ivanščica have very low sequence divergence with respect to the single *E. rietscheli* sequence available from BOLD (0.0058 uncorrected p-distance, Tab. 2), which is within the standard intraspecific sequence divergence for COI in beetles (PENTINSAARI *et al.*, 2014). On the other hand, average sequence divergence between haplotypes of different species of *Elmis* is 11 % (0.043 – 0.134 uncorrected p-distance), which is also within the range of the COI interspecific divergences observed in other groups of beetles.



**Fig. 3.** Neighbour-joining phylogenetic tree constructed by COI sequences of *Elmis rietscheli* from Croatia and haplotypes of *Elmis* species from BOLD, based on Kimura-2-parameter distance model. Numbers on branches are NJ bootstrap support values calculated from 1000 bootstrap replicates (above 50 %) / aLRT-SH like ML support (above 0.7); two sequences of *E. rietscheli* from Croatia: ELRIE-B24 and ELRIE-B25.

**Tab. 2.** Interspecific and intraspecific (bold) uncorrected p-distances calculated for COI sequences of *Elmis* species.

Species name	<i>E. aenea</i>	<i>E. rioloides</i>	<i>E. latreillei</i>	<i>E. rietscheli</i>	<i>E. maugetii</i>	<i>E. obscura</i>
<i>E. aenea</i>	<b>0.0049</b>					
<i>E. rioloides</i>	0.0432	<b>0.0117</b>				
<i>E. latreillei</i>	0.1086	0.0964	–			
<i>E. rietscheli</i>	0.1103	0.0982	0.0888	<b>0.0058</b>		
<i>E. maugetii</i>	0.1148	0.1010	0.1076	0.1194	<b>0.0035</b>	
<i>E. obscura</i>	0.1326	0.1182	0.1254	0.1312	0.1339	<b>0.0017</b>

This result is further supported by the phylogenetic analysis. The topology of NJ and ML trees was mostly congruent, with only a few exceptions regarding the moderately or weakly supported nodes (Fig. 3). Three *E. rietscheli* haplotypes group together in a 100 % BS (0.99 aLRT-SH like) supported clade, with *E. rietscheli* being recovered as sister to *E. latreillei*.

The record of *Elmis rietscheli* in Croatia represents the southernmost record for this species. In Croatia it was found in a habitat similar to many of its habitats in Central Europe, confirming its cretal affinities. Further studies should include sampling in other parts of Croatia, with special focus on springs and mountain streams.

## ACKNOWLEDGEMENT

The authors would like to thank Dr. I. Mihoci and Dr. M. Šašić Kljajo for their assistance during the field work. We are grateful to Dr. I. Stanković for the photographs, and for the map (Figs. 1, 2). Special thanks are due to Hrvatske Vode for allowing us to use their equipment for taking the photographs of the habitus and male genitalia. We thank both referees for their careful reading of the MS. The study was supported by Croatian Science Foundation grants IP-2016-06-9988 “DNA barcoding of biodiversity of Croatian fauna” (Project leader: Prof. M. Kučinić) and IP-2014-09-3733 (Project leader: Prof. Đ. Ugarković), and by MZOŠ-HR / MBWFW grant HR 09/2016 (Project leaders: B. Bruvo Mađarić and M.A. Jäch).

Received April 20, 2018

## REFERENCES

- BERTHÉLEMY, C., 1979: Elmidae de la région paléarctique occidentale: systématique et répartition (Coleoptera Dryopoidea). *Annales de Limnologie* **15**(1), 1–102.
- BILTON, D.T. & RIBERA, I., 2017: A revision of *Meladema* diving beetles (Coleoptera, Dytiscidae), with the description of a new species from the central Mediterranean based on molecules and morphology. *ZooKeys* **702**, 45–112. doi:10.3897/zookeys.702.14787
- BILTON, D.T., TURNER, L. & FOSTER, G.N., 2017: Frequent discordance between morphology and mitochondrial DNA in a species group of European water beetles (Coleoptera: Dytiscidae). *PeerJ* **5**, e3076. doi:10.7717/peerj.3076
- BROJER, M., JÄCH, M.A. KODADA, J. & MOOG, O., 2017: COLEOPTERA: Water Beetles s.l. In: Moog, O. & HARTMANN, A. (eds.), *Fauna Aquatica Austriaca*, 3. Edition 2017. Wien: Federal Ministry of Agriculture, Forestry, Environment and Water Management, 49 pp. Available at: <http://www.ecoprof.at/index.php/faunaaquaticaaustriaca.html>

- BROWN, H.P., 1987: Biology of riffle beetles. *Annual Review of Entomology* **32**, 253–273. doi:10.1146/annurev.en.32.010187.001345
- CROWSON, R.A., 1981: *The biology of the Coleoptera*. Academic Press. London, 802 pp.
- CURIEL, J. & MORRONE, J.J., 2012: Association of larvae and adults of Mexican species of *Macrelmis* (Coleoptera: Elmidae): A preliminary analysis using DNA sequences. *Zootaxa* **3361**, 56–62. doi:10.5281/zenodo.182342
- ČAPLAR, A., 2008: Planinarski vodič po Hrvatskoj. *Bibliotheca geographia Croatica*, pp. 186–187. (in Croatian).
- ČIAMPOR, F. & ČIAMPOROVÁ-ZATOVIČOVÁ, Z., 2008: A new species of *Hedyselmis* Hinton and notes on the phylogeny of the genus (Coleoptera: Elmidae). *Zootaxa* **1781**, 55–62. doi:10.5281/zenodo.182342
- ČIAMPOR, F., ČIAMPOROVÁ-ZATOVIČOVÁ, Z. & KODADA, J., 2017: A new species of *Neblinagena* Spangler from Kukenán tepui and DNA barcoding of *Neblinagena* and related genera (Coleoptera: Elmidae). *Zootaxa* **4286**(2), 176–186. doi:10.11646/zootaxa.4286.2.2
- ČIAMPOR, F. & KODADA, J., 2010: Taxonomy of the *Oulimnius tuberculatus* species group (Coleoptera: Elmidae) based on molecular and morphological data. *Zootaxa* **2670**, 59–68.
- ČIAMPOR, F., LAŠŠOVÁ, K., MAIER, C.A., ČIAMPOROVÁ-ZATOVIČOVÁ, Z. & KODADA, J., 2016: *Phanoceroidea* Hinton, 1939: Description of new species, morphology of larvae, and revised taxonomic position of the genus (Coleoptera: Elmidae) based on molecular evidence. *Zootaxa* **4117**(2), 277–288. doi:10.11646/zootaxa.4117.2.9
- ČIAMPOR, F. & RIBERA, I., 2006: *Hedyselmis opis*: Description of the larva and its phylogenetic relation to *Graphelmis* (Coleoptera: Elmidae: Elminae). *European Journal of Entomology* **103**, 627–636. doi:10.14411/eje.2006.084
- ČIAMPOROVÁ-ZATOVIČOVÁ, Z., ČIAMPOR, F. & KODADA, J., 2007: *Stenelmis puberula* Reitter (Coleoptera: Elmidae) — description of larva and its association with adults, using DNA sequences. *Zootaxa* **1661**, 17–28. doi:10.11646/zootaxa.1661.1.2
- ELLIOTT, J.M., 2008: The ecology of riffle beetles (Coleoptera: Elmidae). *Freshwater Biology* **1**, 189–203. doi:10.1608/FRJ-1.2.4
- EYRE, M.D., PILKINGTON, J.G., CARR, R., McBLANE, R.P., RUSHTON, S.P. & FOSTER, G.N., 1993: The running-water beetles (Coleoptera) of a river catchment in northern England. *Hydrobiologia* **264**, 33–45.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R., 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.
- FOSSEN, E.I., EKREM, T., NILSSON, A.N. & BERGSTEN, J., 2016: Species delimitation in northern European water scavenger beetles of the genus *Hydrobius* (Coleoptera, Hydrophilidae). *ZooKeys* **564**, 71–120. doi:10.3897/zookeys.564.6558
- FREITAG, H., 2013: *Ancyronyx* Erichson, 1847 (Coleoptera, Elmidae) from Mindoro, Philippines, with description of the larvae and two new species using DNA sequences for the assignment of the developmental stages. *ZooKeys* **321**, 35–64. doi:10.3897/zookeys.321.5395
- FREITAG, H. & BALKE, M., 2011: Larvae and a new species of *Ancyronyx* Erichson, 1847 (Insecta, Coleoptera, Elmidae) from Palawan, Philippines, using DNA sequences for the assignment of the developmental stages. *ZooKeys* **136**, 47–82. doi:10.3897/zookeys.321.5395
- FREITAG, H. & KODADA, J., 2017: Larvae of *Ancyronyx* Erichson, 1847 (Insecta: Coleoptera: Elmidae) from Sulawesi, using DNA sequences for the assignment of the larval stages. *Zootaxa* **4299**(1), 121–130. doi:10.11646/zootaxa.4299.1.6
- GUINDON, S., DUFAYARD, J.F., LEFORT, V., ANISIMOVA, M., HORDIJK, W. & GASCUEL, O., 2010: New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**(3), 307–321. doi:10.1093/sysbio/siq010
- HALL, T.A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.
- HAYASHI, M., KAMITE, Y., KOBAYASHI, T. & SOTA, T., 2016: Description of larvae of genera *Stenelmis*, *Ordobrevia* and *Nomuraelmis* (Coleoptera: Elmidae: Elminae) of Japan with their molecular taxonomy. *Zootaxa* **4178**(4), 451–480. doi:10.11646/zootaxa.4178.4.1
- HAYASHI, M. & SOTA, T., 2010: Identification of elmid larvae (Coleoptera: Elmidae) from Sanin District of Honshu, Japan, based on mitochondrial DNA sequences. *Entomological Science* **13**, 417–424. doi:10.1111/j.1479-8298.2010.00404.x



- HEBERT, P.D.N., CYWINSKA, A., BALL, S.L. & DEWAARD, J.R., 2003: Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* **270**, 313–322. doi:10.1098/rspb.2002.2218
- HENDRICH, L., MORINIÈRE, J., HASZPRUNAR, G., HEBERT, P.D.N., HAUSMANN, A., KÖHLER, F. & BALKE, M., 2015: A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. *Molecular Ecology Resources* **15**(4), 795–818. doi:10.1111/1755-0998.12354
- HERAK, M., 1960: Kreda s ofiolitima u Ivanščici (sjeverozapadna Hrvatska) s geološkom kartom. (Kreide mit Ophiolithen in der Ivanščica, NW Kroatien). *Acta geologica* **2**(29), 111–120.
- INIȘTEA, M.A., 1974: Die Elmiden Rumäniens. *Travaux du Muséum d'Histoire naturelle „Grigore Antipa“ XIV*, 209–238.
- JÄCH, M.A., 1992: 42. Familie: Dryopidae, 42.a Familie: Elmidae, 44.a Familie: Psephenidae, pp. 67–82. In: LOHSE, G.A. & LUCHT, W. (eds.), *Die Käfer Mitteleuropas XIII* (2nd suppl.), Krefeld: Goecke & Evers.
- JÄCH, M.A., 1998: Annotated check list of aquatic and riparian/littoral beetle families of the world (Coleoptera), pp. 25–42. In: JÄCH, M.A. & JI, L. (eds.), *Water beetles of China*. Wien: Zoologisch-Botanische Gesellschaft in Österreich and Wiener Coleopterologenverein. 371 pp.
- JÄCH, M.A., DIETRICH, F. & RAUNIG, B., 2005: Rote Liste der Zwergwasserkäfer (Hydraenidae) und Krallenkäfer (Elmidae) Österreichs (Insecta: Coleoptera), pp. 211–284. In: ZULKA, K.P. (ed.), *Rote Listen gefährdeter Tiere Österreichs. Checklisten, Gefährdungsanalyse, Handlungsbedarf. Part 1: Säugetiere, Vögel, Heuschrecken, Wasserkäfer, Netzflügler, Schnabelfliegen, Tagfalter* (Grüne Reihe des Lebensministeriums, Vol. 14/1). Wien: Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wirtschaft, 407 pp.
- JÄCH, M.A., KODADA, J., BROJER, M., SHEPARD, W.D. & ČIAMPOR, F., 2016: Coleoptera: Elmidae and Protemnidae. *World Catalogue of Insects*, Vol. **14**. Leiden: Brill, XXI + 318 pp.
- JOVOVIĆ, L., BRUVO MAĐARIĆ, B., MIČETIĆ STANKOVIĆ, V., JÄCH, M.A. & KUČINIĆ, M., 2015: Phylogeny and phylogeography of genus *Elmis* (Coleoptera, Elmidae) in karstic running waters in southeastern Europe, pp. 173–174. In: KLOBUČAR, G., KOPJAR, N., GLIGORA UDOVIČ, M., LUKŠA, Ž. & JELIĆ, D. (eds.), *12th Croatian Biological Congress with International Participation*. Sveti Martin na Muri, Croatia: Croatian Biological Society 1885.
- KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THIERER, T., ASHTON, B., MENTJES, P. & DRUMMOND, A., 2012: Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**(12), 1647–1649. doi:10.1093/bioinformatics/bts199
- KNIE, J., 1978: Untersuchungen an *Elmis maugetii* var. *hungarica* var. n. und ihre Abgrenzung gegenüber *Elmis maugetii* Latreille, 1798 und *Elmis maugetii* var. *megerlei* (Duftschmid, 1805) (Coleoptera: Dryopoidea). *Folia Entomologica Hungarica* (Rovartani Közlemények) (Series Nova) **31**(1), 61–67.
- KODADA, J., JÄCH, M.A. & ČIAMPOR, F., 2016: 19.2. Elmidae Curtis, 1830, pp. 561–589. In: BEUTEL, R.G. & LESCHEN, R.A.B. (eds.), *Handbook of Zoology, Volume IV (Part 38), Coleoptera, Beetles, Volume 1: Morphology and Systematics* (Archostemata, Adephaga, Myxophaga, Polyphaga partim), 2nd ed. Berlin, New York: Walter de Gruyter, XVII + 684 pp.
- KUMAR, S., STECHER, G. & TAMURA, K., 2016: MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**(7), 1870–4. doi:10.1093/molbev/msw054
- LAŠŠOVÁ, K., ČIAMPOR, F. & ČIAMPOROVÁ-ZATOVIČOVÁ, Z., 2014: Two new Larinae species from Guayana region, Venezuela (Coleoptera: Elmidae). *Zootaxa* **3753**(2), 187–195. doi:10.11646/zootaxa.3753.2.8
- LEFORT, V., LONGUEVILLE, J.E. & GASCUEL, O., 2017: SMS: smart model selection in PhyML. *Molecular Biology and Evolution*, **34**(9), 2422–2424. doi:10.1093/molbev/msx149
- LÖKKÖS, A., 2010: First record of *Elmis rietscheli* Steffan, 1958 from Hungary (Coleoptera: Elmidae). *Folia entomologica hungarica* **71**, 31–33.
- MIČETIĆ STANKOVIĆ, V., JÄCH, M.A. & KUČINIĆ, M., 2015: Annotated checklist of Croatian riffle beetles (Insecta: Coleoptera: Elmidae). *Natura Croatica* **24**(1), 93–109. doi:10.20302/NC.2015.24.5
- MÚRRIA, C., BONADA, N., VELLEND, M., ZAMORA-MUÑOZ, C., ALBA-TERCEDOR, J., ELISA SAINZ-CANTERO, C., GARRIDO, J., ACOSTA, R., EL ALAMI, M., BARQUÍN, J., DERKA, T., ÁLVAREZ-CABRIA, M., SÁINZ-BARIAIN, M., FILIPE, A.F. & VOGLER, A.P., 2017: Local environment rather than past climate determines community composition of mountain stream macroinvertebrates across Europe. *Molecular Ecology* **26**(21), 6085–6099. doi:10.1111/mec.14346
- PENTINSAARI, M., HEBERT, P.D.N. & MUTANEN, M., 2014: Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLoS ONE* **9**(9), e108651. doi:10.1371/journal.pone.0108651

- RATNASINGHAM, S. & HEBERT, P.D.N., 2007: BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* **7**, 355–364. doi:10.1111/j.1471-8286.2007.01678.x
- RATNASINGHAM, S. & HEBERT, P.D.N., 2013: A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE* **8**(8), e66213. doi:10.1371/journal.pone.0066213
- RICHOUX, P., 1994: Theoretical habitat templates, species traits, and species richness: aquatic Coleoptera in the upper Rhône River and its floodplain. *Freshwater Biology* **31**, 377–395. doi:10.1111/j.1365-2427.1994.tb01747.x
- SCHULTE, H., 1989: Beiträge zur Ökologie und Taxonomie der Gattung *Elmis* Latreille (Insecta: Coleoptera, Elmidae) unter besonderer Berücksichtigung niederbayerischer Vorkommen. *Lauterbornia* **1**, 23–37.
- ŠIMUNIĆ, A., 1983: Pregled geološke građe sjeverozapadne Hrvatske, pp. 41–50. In: MOHORVIČIĆ, A. (ed): Varaždinski zbornik. Zbornik radova sa znanstvenog skupa povodom obilježavanja 800. godišnjice grada. Varaždin, Zagreb: JAZU (in Croatian).
- VILLESEN, P., 2007: FaBox: an online toolbox for FASTA sequences. *Molecular Ecology Notes* **7**(6), 965–968. doi:10.1111/j.1471-8286.2007.01821.x
- ZANINOVIĆ, K., GAJIĆ-ČAPKA, M., PERČEC TADIĆ, M. et al., 2008: Klimatski atlas Hrvatske / Climate atlas of Croatia 1961–1990, 1971–2000. Croatian Meteorological and Hydrological Service, Zagreb, 200 pp. (in Croatian).