Biodiversity of caddisflies (Insecta, Trichoptera) in Croatia with particular reference to DNA barcoding

Mladen Kučinić

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

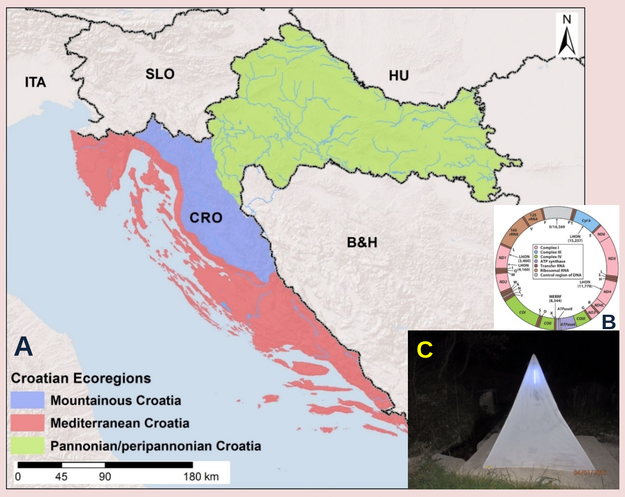

Figure 1. A – C. A - Republic of Croatia with three Ecoregions; B - mithochondrial DNA; C – UV lamp.

Introduction

Biodiversity is one of the most important biological features of an area. Systematic, mostly longtime, faunistic research into a specific area provides a list of species (a checklist) of certain animal groups that live in this area. Such checklists are very important in an evaluation of the diversity and faunistic specificity of certain areas and for the possibility of providing adequate protection for particular species. It is common for such lists to be compiled on a national level. In Europe there are caddisfly (Trichoptera) check lists for most countries (e.g. Krušnik & Urbanič et al. 2002, Živić et al. 2002.).

Geographically located in the Central and Mediterranean Europe (Fig. 1 A) (Bertić et al. 2001), Croatian territory has great habitat diversity and represent one of the European biodiversity hotspot.

Identification of various animal species is a starting point of research into the morphology, genetics, distribution, ecology and other biological characteristics of individual species. Morphological characteristics have for a long time been used as the basis for determination of organisms and this practice is being used until today.

A recent and one of the most frequently used methods in the analysis of biodiversity and the determination of organisms within a particular area is the DNA barcoding method of Paul Herbert. This method is used to identify species of different groups of organisms and is based on the sequencing of the standardized segment of the mitochondrial (mt) cytochrome c oxidase subunit 1 (COI) gene (Hebert et al. 2003). (Fig. 1 B).

The primary goal of the project „DNA barcoding of Croatian faunal biodiversity" (IP-2016-06-9988, supported by Croatian Science Foundation) is to investigate the genetic biodiversity and geographic distribution of fifteen groups of Croatian fauna (about 1500 species) by using DNA barcoding methodology.

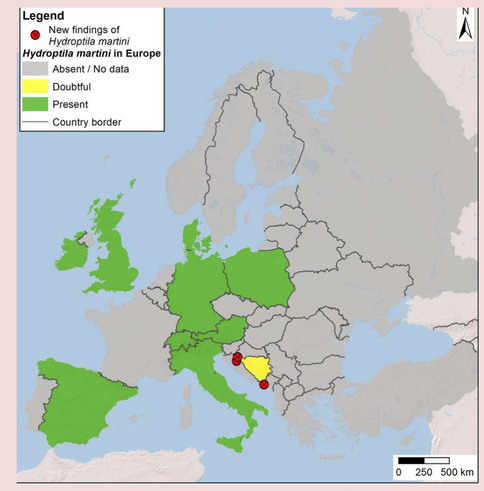

Figure 2. Distribution of species *Hydroptila martini* in Europe (green), red markings - localities in Croatia (according Fauna Europaea, https//fauna-eu.org).

Results and Discussion

Systematic studies of the biodiversity, distribution and taxonomy of caddisflies in the Republic of Croatia have started 20 years ago by researchers at the Croatian Natural History Museum in Zagreb and Faculty of Science, University of Zagreb. In these investigations, **209** Trichoptera species (Figs 2-5) from 17 families have been recorded so far (Tabs 1-2). Many of these species were found in Croatia for the first time: *Hydropsyche mostarensis*, *Tinodes antonioi*, *Hydroptila martini* (Fig. 2), *H. simulnas*, *Orthotrichia costalis*, *Chaetopetryx bucari* (e.g. Kučinić et al. 2013, unpublished data).

For DNA barcoding in the last 7 years, about 185 species of Trichoptera have been collected, and successful DNA barcode analyses were done for **153** Trichoptera species (Tabs 1-2) (74% of Croatian fauna).

DNA barcoding data has shown some very interesting results. In some cases a large genetic diversity among populations of some species from Croatia and other parts of Europe was established e.g. genus *Diplectrona* (Fig. 3 A-B), *Rhyacop. cabrakanensis* (Fig. 4), *Tinodes antonioi, Triaenodes lefkas* – **stat. nova**, *Potamophylax cingulatus*, *Setodes virids bulgaricus* – **stat. nova**.

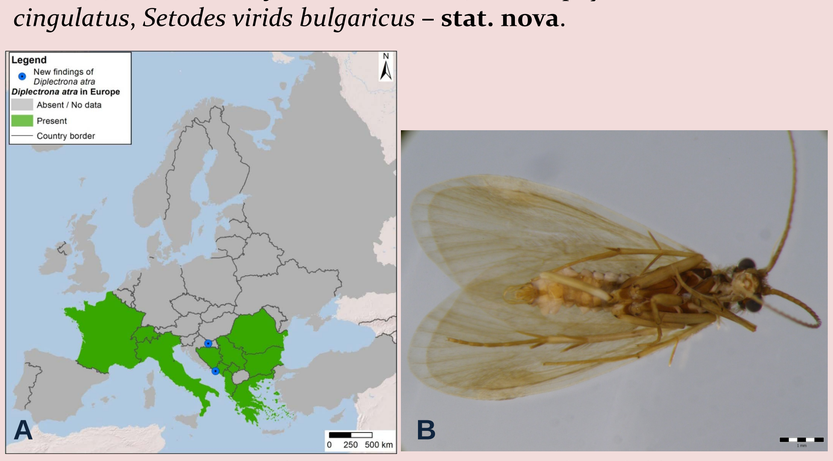

Figure 3. A – B. A – Areal od *Diplectrona atra* in Europe (green), blue - *Diplectrona* **sp. n. 1** and *Diplectrona* **sp. n. 2** in Croatia; B – *Diplectrona* **sp. n. 1**, adult, male (Konavle region) (according Fauna Europaea, https//fauna-eu.org).

Table 2. Families with number of species (1, 2) and number of of DNA barcoding species (*).

Family	1 (*)	Family	2
Rhyacophilidae	**14** (14*)	Brachycentridae	**5** (2*)
Glossostomatidae	**8** (6*)	Goeridae	**5** (3*)
Hydroptilidae	**20** (11*)	Lepidostomatidae	**3** (3*)
Philopotamidae	**6** (5*)	Limnephilidae	**63** (49*)
Ecnomidae	**1** (1*)	Sericostomatidae	**2** (1*)
Polycentropodidae	**11** (8*)	Odontoceridae	**1** (1*)
Psychomyiidae	**12** (9*)	Beraeidae	**7** (3*)
Hydropsychidae	**17** (13*)	Leptoceridae	**28** (20*)
Phryganeidae	**6** (4*)	-	

Material and Methods

Samples of adult caddisflies were collected using entomological net and UV light traps (Fig. 1 C) in different aquatic habitats in Croatia – about 200 localities (Fig. 6). The samples were stored in containers with 96% EtOH, for morphological and molecular analysis, respectively. The collected material is deposited in caddisflies' collections in the Croatian Natural History Museum in Zagreb and in the University of Zagreb. DNA sequences were submitted to Barcode of Life Data Systems (BOLD, Ratnasingham & Hebert 2007) and GenBank. Systematic presentaion was done accordinig Malicky (2004) and Morse (2019).

Table 1. Systematic presentation of caddisflies species from Croatia with notice of DNA barcoding (*DNA barcoding spec.).

Family Rhyacophilidae
Rhyacophila aurata Brauer, 1857*
Rhyacophila balcanica Radovanović, 1953*
Rhyacophila cabrankensis Mal., Prev. & Kuč., 2007*
Rhyacophila dorsalis McLachlan, 1789*
Rhyacophila fasciata Hagen, 1859*
Rhyacophila hirticornis McLachlan, 1879*
Rhyacophila laevis Pictet, 1834*
Rhyacophila loxias Schmid, 1970*
Rhyacophila palmeni McLachlan, 1879*
Rhyacophila praemorsa McLachlan, 1879*
Rhyacophila schmididinarica Urb, Kr. & Mal., 2000*
Rhyacophila torrentium Pictet, 1834*
Rhyacophila tristis Pictet, 1834*
Rhyacophila vulgaris Pictet, 1834*

Family Glossosomatidae
Synagapetus krawanayi Ulmer, 1939
Glossosoma bifidum McLachlan, 1879*
Glossosoma conformis Neboiss, 1963*
Glossosoma discophorum Klapálek, 1902*
Agapetus delicatulus Mclachlan, 1884
Agapetus fuscipes Curtis, 1834*
Agapetus laniger Pictet, 1834*
Agapetus ochripes Curtis, 1834*

Family Hydroptilidae
Hydroptila cognata Mosley, 1930
Hydroptila forcipata Eaton, 1873*
Hydroptila phaon Malicky, 1976
Hydroptila lotensis Mosely, 1930*
Hydroptila martini Marshall, 1977*
Hydroptila rheni Ris, 1896
Hydroptila simulans Mosely, 1920*
Hydroptila sparsa Curtis, 1834
Hydroptila tigurina Ris, 1894
Hydroptila vectis Curtis, 1834*
Hydroptila vichtaspa Schmid, 1959
Hydroptila tineoides Dalman, 1819
Ithytrichia lamellaris Eaton, 1873*
Orthotrichia angustella McLachlan, 1865*
Orthrotrichia costalis (Curtis, 1834)
Orthotrichia tragetti Mosely, 1930*
Oxyethirta falcata Morton, 1893*
Oxyethira flavicornis Pictet
Agraylea sexmaculata Curtis, 1834 *
Allotrichia pallicornis , 1834*

Family Philopotamidae
Wormaldia copiosa (Mclachlan, 1868)
Wormaldia subterranea Radovanović, 1932*
Wormaldia pulla Mclachlan, 1878*
Wormaldia subnigra McLachaln, 1865*
Philopotamus montanus (Donovan, 1813)*
Philopotamus variegatus (Scopoli, 1763)*

FamilyEcnomidae
Ecnomus tenellus (Rambur, 1842)*

Family Polycentropodidae
Cyrnus trimaculatus (Curtis, 1834)*
Neureclipis bimaculata (Linnaeus, 1758)*
Plectrocnemia brevis McLachlan, 1878*
Plectrocnemia conspersa (Curtis, 1834)*
Plectrocnemia geniculata McLachlan, 1871
Polycentropus excisus Klapálek, 1894 *
Polycentropus flavomaculatus (Pictet, 1834)*
Polycentropus ieraptera Malicky, 1972
Polycentropus irroratus (Curtis, 1835)*
Polycentropus schmidi Novak & Botosaneanu, 1965*
Holocentropus stagnalis (Albarda, 1874)

Family Psychomyiidae
Psychomia klapaleki Malicky, 1995*
Psychomia pusilla (Fabricius, 1781)*
Lype phaeopa (Stephens, 1836)
Lype reducta (Hagen, 1868)*
Tindes andrasi Oláh, 2010
Tinodes antonioi Botosaneanu & Tat.-Vigano, 1974*
Tinodes braueri McLachlan, 1878*
Tinodes dives Pictet, 1834*
Tinodes pallidulus McLachlan, 1878*
Tinodes rostocki McLachlan, 1878
Tinodes unicolor (Pictet, 1834)*
Tinodes waeneri (Linnaeus, 1758)*

Family Hydropsychidae
Cheumatopsyche lepida (Pictet, 1834)*
Diplectrona **sp. n. 1** (Konavle region, south Cro.)*
Diplectrona **sp. n. 2** (Papuk Mt, central Croatia)*
Hydropsyche angutipennis (Curtis, 1834)*
Hydropsyche bulbifera McLachlan, 1878*
Hydropsyche bulgaromanorum Malicky, 1977*
Hydropsyche contubernalis McLachlan, 1875 *
Hydropsyche guttata Pictet, 1834
Hydropsyche dinarica Marinković-Gospdn., 1979
Hydropsyche fulvipes Curtis, 1834*
Hydropsyche incognita Pitsch, 1993*
Hydropsyche instabilis (Curtis, 1834)*
Hydropsyche modesta Navas, 1925*
Hydropsyche mostarensis Klapálek, 1898*
Hydropsyche ornatula McLachlan, 1878
Hydropsyche pellucidula (Curtis, 1834)*
Hydropsyche saxonica McLachlan, 1884

Family Phryganeidae
Agrypina varia (Fabricius, 1793)*
Phryganea bipunctata Retzius, 1783*
Phryganea grandis Linnaeus, 1758*
Trichostegia minor Curtis, 1834*
Hagenella clathrata (Kolenati, 1848)
Oligostomis reticulata (Linnaeus, 1761)

Family Brachycentridae
Brachycentrus montanus Klapálek, 1892*
Brachycentrus subnubilus Curtis, 1834
Micrasema minimum McLachlan, 1876
Micrasema sericeum Klapálek, 1902
Micrasema setiferum (Pictet, 1834)*

Family Goeridae
Goera pilosa (Fabricius, 1775)*
Litax niger (Hagen, 1859)
Silo nigricornis (Pictet, 1834)
Silo pallipes (Fabricius, 1781)*
Silo piceus Brauer, 1857*

Family Lepidostomatidae (3,
Crunoecia kempnyi Morton, 1901*
Lasiocephala basalis (Kolenati, 1848)*
Lepidosotoma hirtum (Fabricius, 1775)*

Family Limnephilidae
Apatania muliebris McLachlan, 1866
Ironoquia dubia (Stephens 1837)*
Drusus chrysotus (Rambur, 1842)*
Drusu croaticus Marinković-Gospodnetić, 1971*
Drusus discolor (Rambur, 1842)*
Drusus schmidi Botosaneanui, 1953*
Drusus vespertinus Marinković-Gospodnetić, 1971*
Ecclisopteryx ivkae Previšić, Graf & Vitecek, 2014*
Ecclisopteryx keroveci Previšić, Graf & Vitecek, 2014*
Grammotaulius nigropunctatus (Retzius, 1783)*
Glyphotaelius pellucidus (Retzius, 1783)*
Anabolia furcata Brauer 1857*
Rhadicoleptus alpestris Kolenati, 1848*
Limnephilus affinis Curtis, 1834*
Limnephilus auricula Curtis, 1834*
Limnephilus bipunctatus Curtis, 1834
Limnephilus decipiens Kolenati , 1848
Limnephilus extricatus McLachlan, 1865*
Limnephilus flavicornis (Fabricius, 1787)*
Limnephilus graecus Schmid, 1965*
Limnephilus griseus (Linneaus, 1758)
Limnephilus hirsutus (Pictet, 1834)*
Limnephilus ignavus McLachlan, 1865*
Limnephilus incisus Curtis, 1834
Limnephilus lunatus Curtis, 1834*
Limnephilus marmoratus Curtis, 1834
Limephilus rhombicus (Linnaeus, 1758)*
Limnephilus sparsus Curtis, 1834*
Limnephilus stigma Curtis, 1834
Limnephilus vittatus (Fabricius, 1798)*
Chaetopteryx bosniaca Marinković-Gospod., 1955*
Chaetopteryx bucari Kučinić, Szivák & Delić, 2013*
Chaetopteryx gonospina Marinković, 1966*
Chaetopteryx fusca Brauer, 1857
Chaetopteryx major McLachalan, 1876*
Chaetopteryx marinkovicae Mal. & Kru., 1988*
Chaetopteryx rugulosa Kolenati, 1848*
Chaetopteryx schmidi Botosaneanui, 1957*
Chaetopteryx uherkovichi Oláh, 2011
Annitella apfelbecki Klapálek, 1899*
Micoptera fissa (McLachan, 1875)*
Micropterna lateralis Stephens, 1837*
Micropterna nycterobia McLachlan, 1875*
Micropterna sequax McLachlan, 1875*
Micropterna testacea (Gmelin, 1789)*
Micropterna wageneri Malicky, 1971*
Stenophylax meridiorientalis Malicky, 1980*S*
Stenophylax mitis McLachlan, 1875*
Stenophylax permistus McLachlan, 1895*
Stenophylax vibex Curtis, 1834*
Potamophylax cingulatus (Stephens, 1837) *
Potamophylax latipennis (Curtis, 1834)
Potamophylax luctuosus Piller & Mitte., 1783*
Potamophylax nigricornis (Pictet, 1834)*
Potamophylax pallidus (Klapálek, 1899)*
Potamophylax rotundipennis (Brauer, 1857)*
Allogamus auricollis (Pictet, 1834)*
Allogamus uncatus (Brauer, 1857)
Halesus digitatus (Schrank, 1781)*
Halesusu tessellatus (Rambur, 1842)*
Hydatophylax infumatus (McLachlan, 1865)
Platyphylax frauenfeldi (Brauer, 1857)
Mesophylax aspersus (Rambur, 1842)*

Family Sericostomatidae
Notidobia ciliaris (Linnaeus, 1761)
Sericostoma flaviorne Schneider, 1845

Family Odontoceridae
Odontocerum albicorne (Scopoli, 1763)*

Family Beraeidae
Beraea dira McLachlan, 1875
Beraea maurus (Curtis, 1834)
Beraea pullata (Curtis, 1834)
Beraeamyia schmidi Botosaneanu, 1960*
Ernodes articularis (Pictet, 1834)*
Ernodes articularis (Pictet, 1834)*
Ernodes vicinus (McLachlan, 1879)

Family Leptoceridae
Erotesis baltica McLachlan, 1877
Adicella balcanica Botosaneanu & Novak, 1965*
Adicella cremisa Malicky, 1972 *
Adicella filicornis Pictet, 1834
Adicella reducta (McLachlan, 1865)
Adicella syriaca Ulmer, 1907
Mystacides azurea (Linnaeus 1761)*
Mystacides longicornis (Linnaeus, 1758)*
Mystacides nigra (Linnaeus, 1758)
Triaenodes lefkas, Malicky 1974* - **stat. nova**
Athripsodes albifrons (Linnaeus, 1758)*
Athripsodes aterrimus (Stephens, 1836)*
Athripsodes bilineatus (Linnaeus, 1758)*
Athripsodes cinereus (Curtis, 1834)*
Athripsodes dalmatinus Malicky, 1980*
Leptocerus tineiformis Curtis, 1834*
Leptocerus tineiformis Curtis, 1834*
Ylodes kawraiskii Martinov, 1909
Ceraclea albimacula Rambur, 1842
Ceraclea annulicornis Stephens, 1836
Ceraclea dissimilis (Stephens, 1836)*
Ceraclea riparia (Albarda, 1874)*
Oecetis furva (Rambur, 1842)*
Oecetis lacustris (Pictet, 1834)*
Oecetis notata Rambur, 1842*
Oecetis ochracea (Curtis, 1825)
Oecetis testacea (Curtis, 1834)*
Setodes viridis bulgaricus Kum., 1967* - **stat. nova**
Setodes punctatus Fabricius, 1793*

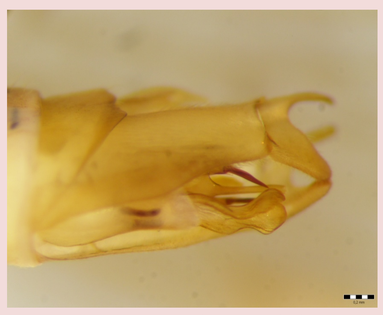

Figure 4. *Rhyacophila cabrankensis*, endemic species in fauna of Croatia, male genitalia, lateral view, *locus typicus*, spring of the River Čabranka (mountain Croatia).

Figure 5. *Tinodes andrasi, locus typicus* - the River Ljuta. Founded in two localities: Konavle (Croatia) and one locality in Monte Negro.

Figure 6. Spring of the River Ljuta - Konavle region, south-east Croatia, *locus typicus* of species *Rhyacophila andrasi* and *Diplectrona* sp. n. 1.

Literature

Bertić I., Lampek Pavčnik, I. & Radovinović, R., 2001. Satelitski atlas Hrvatske. Naklada Ljevak i Gisdata,Zagreb, 360 pp.; according Fauna Europaea, https//fauna-eu.org Hebert, P.D.N., Cywinska, A., Ball S.L. & deWaard J.R., 2003a: Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B 270, 313–322; Malicky, H. 2004. Atlas of European. Trichoptera. Springer, Dordrecht.; Krušnik, C., & Urbanič, G., 2002: Preliminary List of Slovenian Trichoptera. Proceedings of the 10th International Symposium on Trichoptera (Ed. W. Mey). Nova Supplementa Entomologica 15, 359-364. Kučinić, M., Szivák, I., Pauls, S.U., Bálint, M., Delić A. & Vučković I. 2013: *Chaetopteryx bucari* sp. n. a new species from the *Chaetopteryx rugulosa* group from Croatia (Insecta, Trichoptera) with some molecular, taxonomical and ecological notes on the group. Zookeys 320 1-28; Malicky, H., Previšić, A. & Kučinić, M., 2007: *Rhyacophila cabrankensis* nov. spec. from Croatia. Braueria, 34: 14; Morse, J.C. (Ed.), 2019: Trichoptera World Checklist. Available from: http://entweb.clemson.edu/database /trichopt/index.htm (accessed 13 Sepmember 2018); Ratnasingham, S. & Hebert, P.D.N., 2007: BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7, 355–364. DOI: 10.1111/j.1471-8286.2006.01678.x ; Živić, I., Marković, Z. & Brajković, M., 2002: First check list of Serbian Trichoptera. Folia Historico-Naturalia Musei Matraensis 26: 269-277.

Acknowledgments

This work has been supported in part by the Croatian Science Foundation under the Project: IP-2016-06-9988, DNA Barcoding of Diversity of Croatian Fauna.