

Member News

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DNA barcoding for biodiversity assessment: Stoneflies of Croatia

The Dinaric Karst system represents one of the most diverse European freshwater habitats, including a variety of microhabitats, which resulted in speciation and endemism. As a part of Dora's PhD thesis within the project *DNA barcoding of Croatian faunal biodiversity* (IP-2016-06-9988), we conducted comprehensive field research on the stonefly fauna and applied DNA barcoding. Seventy-four morphospecies were confirmed for Croatia. Species delineation methods confirmed the existence of five divergent genetic lineages, which in addition to morphological differences from their congeners, represent distinct entities. Research has yielded the first molecular characterization of nine species that have mostly restricted distributions. Deep intraspecific genetic divergences within genera *Isoperla*, *Perla* and *Taeniopteryx* highlighted the need for taxonomic revision in several species-groups. The manuscript is in press in PeerJ.

***Isoperla popijaci*, a new stenoendemic stonefly species from Croatia**

Based on morphological (males and females adults, larval and eggs) and molecular data (sequencing of the DNA barcode region), we described a new *Isoperla* species. Based on the morphological characteristics, *Isoperla popijaci* belongs to the *I. tripartita* species-group and the lowest interspecific *p*-distance is 6.7%. *Isoperla popijaci* inhabits only two karstic sources of the intermittent rivulet Krasulja in Croatia, so the study puts emphasis on the conservation importance of the Dinaric Karst. The study was published in ZooKeys (<https://doi.org/10.3897/zookeys.1078.66382>).



Isoperla popijaci Hlebec & Sivec, 2021: Krasulja Rivulet, karstic source Ševerova Cave, 44°40.78'N, 15°37.87'E, 2 July 2021, I. Sivec. Photograph by Ignac Sivec.

***Taeniopteryx* n.sp. CRO-1: enigmatic status**

During comprehensive fieldwork we collected individuals which differ from all known species in the genus *Taeniopteryx* recorded in Croatia. Morphological differences are accompanied by genetic distinctiveness (interspecific *p*-distances ranged from 7.8–9.5%). As the genus *Taeniopteryx* is complex from a taxonomic point of view, we applied a multi-gene approach to resolve phylogenetic relationships among the species *T. schoenemundi* Mertens, 1923, *Taeniopteryx nebulosa* Linnaeus, 1758 and *T. hubaulti* Aubert, 1946. The manuscript is in progress.

Case of *Isoperla* cf. *lugens*

Specimens of *Isoperla* cf. *lugens* were found associated with the headwaters of several karstic rivers. These specimens differ morphologically from the typical *Isoperla lugens* and have exceptional genetic distinctiveness (the lowest interspecific *p*-distance is 6.7%). We applied a multi-gene approach to define the taxonomic status of *Isoperla* cf. *lugens* and we will try to resolve taxonomic relationships among other closely related *Isoperla* species. The manuscript is in progress.

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Together with A. Fausto (Tuscia University), I am studying the sperm patterns in European stoneflies. We studied the sperm structure of four species belonging to genera not analyzed in our previous studies, i.e., *Capnopsis schilleri* Rostock, *Amphinemura sulcicollis* Stephens, *Rhabdiopteryx neglecta* Albarda, *Tyrrhenoleuctra zavattarii* Consiglio, to whom it must be added the characterization of the spermatozoon of *Zwicknia gattolliati* (previously known as *Capnia bifrons*) and *Protonemura intricata* Ris, we discussed in a congress proceeding but never published so far. The results are being published.

The study of the sperm model of species belonging to families of the plecopterofauna of other continents (eg Notonemouridae, Scopuridae, Peltoperlidae etc.) is also envisaged, in order to obtain useful data to be used also for systematic purposes.

Besides, I have started the characterization of DNA barcodes of Italian Plecoptera, in collaboration with M. Oliverio (Sapienza University, Rome, Italy).

Also, together with friends and colleagues from Granada (Spain) I am continuing the study of molecular systematics and phylogeography of *Tyrrhenoleuctra* species.

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Transfer of Peter's collections to Staatliches Museum für Naturkunde, Stuttgart, Germany.

Although much of my work was on Plecoptera the stoneflies were not my first and not my only love. As a schoolboy I started with Coleoptera but shifted to Plecoptera in a thesis under J. Illies. During field work I then came across net-winged midges (Diptera: Blephariceridae) which fascinated me and became my third favourite. New species were named in all three orders, most beetles after 2012. Some time ago I decided that my collections of these groups should be safely stored for longer than I will probably be around. In November 2021 the collections, specimens

and related literature, were moved to the museum in Stuttgart where Dr. A. Staniczek is curator of aquatic insects.

The African species of genus *Neoperla*. My son Andreas (Canberra; molecular studies) and myself (morphology) continue with our long-distance cooperation on the Ethiopian *Neoperla*. Only a single African *Neoperla* has been recognized since 1952 but in our almost completed work we will document the existence of close to 80 endemic species in Africa. They belong to several species groups, one also with a few Asian species.

In the context of the African study I also looked at *Neoperla* from elsewhere and now work on a synopsis of *Neoperla* at the level of major species groups, including historical genus group taxa.

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***Acroneuria* synonymy – Chris Verdone, B. Kondratieff & E. South.** Beginning in 2016, we conducted a conservation status survey of *Acroneuria kosztarabi* Kondratieff & Kirchner, 1993, for the US Fish & Wildlife Service. We made new collections and examined specimens from the Illinois Natural History Survey, The Virginia Museum of Natural History, C.P. Gillette Museum, and the National Museum of Natural History. We barcoded and analyzed data for 31 *Acroneuria* and concluded that *A. kirchneri* Stark & Kondratieff, 2004 is a junior synonym of *A. kosztarabi*. The paper was just published in Zootaxa (<https://doi.org/10.11646/zootaxa.5094.1.8>).

Review of Nearctic *Strophopteryx* – Chris Verdone, S. Beaty, V. Holland, E. South & B. Kondratieff. In 2021, we collected what appeared to be *Strophopteryx arkansae* Ricker & Ross, 1975 in North Carolina. Subsequent barcoding has since suggested this NC population is a distinct, albeit cryptic, species. We (CV, ES, BK) will be traveling to Arkansas in March 2022 to collect fresh material of *S. arkansae* and *S. cucullata* Frison, 1934 for a review of the Nearctic species, which will include DNA analyses and color photographs of the adults and larvae.

Review of Eastern Nearctic *Oemopteryx* – Chris Verdone, S. Grubbs, S. Beaty, V. Holland, Bronwyn Williams. In 2021, while looking through old NCDWR benthic samples, I found a larva of *Oemopteryx* from the North Carolina Sandhills ecoregion, a strange location for the genus. Subsequent rearing efforts yielded adults that are distinctive both morphologically and genetically. We will examine loan material from the Illinois Natural History Survey and C.P. Gillette Museum, review the two other eastern Nearctic species and hopefully describe this new species in the next year using DNA barcoding, Scanning Electron Microscopy and color photographs. We are considering reviewing all the Nearctic larvae as well.

New species of *Isoperla* – S. Beaty, V. Holland, Chris Verdone & Bronwyn Williams. In 2020 and 2021, Bronwyn Williams barcoded 308 specimens of *Isoperla* Banks, 1906. Preliminary results suggest that we have 10 undescribed species of *Isoperla* from North Carolina and Virginia. Victor Holland has made arrangements for us to use the SEM lab at NC State so we can finish descriptions for three of these, known by the temporary names *I.* “NOT dicala”, *I.* “Mayo River” and *I.* “species 10”. Among the other new species, we have three more in the *I.*

pseudosimilis/similis group and a couple similar to *I. transmarina* (Newman, 1838) that we hope to make progress on this year.

Possible new species of *Remenus* – Chris Verdone, S. Beaty, V. Holland & Bronwyn Williams. In 2019, while looking for *Isoperla*, we mistakenly reared several *Remenus* Ricker, 1952 that were morphologically distinct from the other described species. We currently have records of this taxon from the Catawba and Green River basins in North Carolina. Preliminary results from DNA barcoding were inconclusive. This year Bronwyn Williams will attempt a population genomics study to better elucidate this taxon’s relationship to the other congeners.

Stoneflies of the Qualla Boundary - Rainee Tetreault, Chris Verdone, S. Beaty & V. Holland. The Qualla boundary is a land trust governed by the Eastern Band of Cherokee Indians in western North Carolina. In 2021, I was contacted by Rainee Tetreault, biologist in charge of water quality monitoring for the Eastern Band of Cherokee. She was interested in learning about adult stoneflies and collection methods to survey for species petitioned for endangered species listing. What began as an instructional visit has evolved into a longer term survey to document the stoneflies of the Qualla Boundary, which includes many outstanding creeks and rivers like Raven Fork, Bunches Creek and the Oconaluftee River. Although we have only spent four days surveying thus far we have documented 42 species of stoneflies, including some stellar taxa like *Megaleuctra williamsae* Hanson, 1941, *Remenus duffieldi* Nelson & Kondratieff, 1995, *Allocapnia stannardi* Ross, 1964, and *A. fumosa* Ross, 1964. We hope to carve out a few days every other month over the next few years to develop a comprehensive checklist for the area.



An undescribed *Isoperla* (*pseudosimilis* group) species awaiting description. Virginia, Scott County, Devils Fork, 8 May 2017, C. Verdone, B. Kondratieff. Photograph by C. Verdone.

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Eastern Nearctic Nemourinae

The treatment of eastern Nearctic Nemourinae with Richard Baumann is slowly approaching completion. We have assembled plates for all species consisting of line drawings and scanning electron micrographs. Digital light microscopy imaging is the last task before this monograph can be submitted for publication (journal TBD). This treatment will complement previous monographs on eastern Nearctic Chloroperlidae (Surdick 2004), Peltoperlidae (Stark 2000), Perlidae (Stark 2004), Perlodidae – Isoperlinae (Szczytko and Kondratieff 2015), Perlodidae – Perlodinae (Kondratieff 2004), Pteronarcyidae (Nelson 2000), and Taeniopterygidae (Stewart 2000).

Systematics and taxonomy eastern Nearctic *Leuctra*

This is a collaborative project with several individuals, namely Ed DeWalt (INHS), Ruthie Eastham (current WKU undergraduate student, Madison Layer (current WKU M.S. student), and Madeline Metzger (former WKU M.S. student). Ed and I are collaborating on a conservation and taxonomic assessment of the Louisiana Needlefly *L. szczytkoi* presently funded by the U.S. Fish and Wildlife Service. The related species *L. paleo* is presumed to be a junior synonym (Harrison & Stark 2010, *Illiesia* 6(03):16-33). Ed and I collected fresh *L. paleo* and *L. szczytko* specimens in October 2020 from both type localities and other localities and DNA barcoded them, demonstrating approximately 1% sequence divergence. In October 2021, we successfully collected more populations of *L. szczytkoi* in central and western Louisiana and found *Leuctra* (larvae only) for the first time in Texas (Sabine and Jasper counties). A second plate of tissue is now being barcoded to identify the larvae. We are currently working on both a systematics treatment and a broader conservation ecology paper. I had successful trips in October-December 2021 to collect fresh *L. cottaquilla* in Alabama and both *L. colemanorum* and *L. hicksi* in Mississippi. All of the species mentioned are part of Madison's project on the systematics and phylogeography of the *L. ferruginea* Group. Ruthie is performing a parallel treatment of the *L. tenuis* group, and I am working on the *L. biloba* and *L. grandis* groups. Spring-summer 2022 collecting will target fresh material of *L. usdi* (*L. tenuis* group), *L. crossi* (unplaced), *L. monticola* and *L. pinhoti* (*L. biloba* group), late May-emerging populations determined as *L. tenuis*, a potentially undescribed species similar to *L. ferruginea*, and a June-emergent population of *L. variabilis* (*L. tenuis* group).

New North American *Perlesta* (Perlidae)

Ed DeWalt and I have at least two undescribed species from eastern USA that we hope to describe by the end of 2022. Fieldwork in Indiana, Michigan, Minnesota, and Wisconsin is planned to collect fresh material of both species for morphological description and to expand upon the partial barcode-based phylogenetic treatment of the genus presented in South et al. (2019).

Update - Maryland Plecoptera

Phillip Hogan (former WKU M.S. student) completed his two-part thesis on Maryland stoneflies. The emphasis was on the Appalachian portion of the state in its western panhandle. Part 1 was a

distributional modeling assessment of several uncommon/rare Appalachian species, most have been listed as Species of Greatest Conservation Need on at least one USA State Wildlife Action Plan. Part 2 was a first attempt at a distributional atlas of the Appalachian Maryland Plecoptera. Manuscripts of both parts are in progress.

USA Northeast Regional Species of Greatest Conservation Need (RSGCN)

The U.S. Fish & Wildlife Service has earmarked \$300,000 to fund conservation status assessments of 33 RSGCN species in 13 northeastern USA states. The species were proposed as RSGCNs by Ed DeWalt, Boris Kondratieff, Luke Myers, and me during a collaboration in 2020. Funding will start in 2023 for a four-year project. We are in early planning phases. Field work prioritizes 13 RSGCNs “Priority 1” species for fieldwork: *Acroneuria arida*, *Acroneuria flinti*, *Allocapnia frumi*, *Alloperla vostoki*, *Diura washingtoniana*, *Isoperla major*, *Isoperla myersi*, *Leuctra laura*, *Leuctra monticola*, *Neoperla mainensis*, *Soyedina merritti*, *Sweltsa holstenensis*, and *Taeniopteryx nelsoni*. Other objectives include compilation of museum and literature specimen data for all 33 species, the development of standard operating protocols for field and museum work, and training of graduate students, state and regional conservation staff, and select volunteers to help with data collection.

Mount Washington and Presidential Range Plecoptera

The Presidential Range is part of the White Mountains of the USA state of New Hampshire. Its peaks are named for several 19th century USA presidents. Mount Washington, the highest peak in the range, towers at 1,917 m (= 6,288 ft). Because of its elevation, latitude, and brutally cold, unpredictable weather, the mountain is home to several populations of species known nowhere else in the world or are present as disjunct populations with Holarctic distributions. The USA Forest Service and state of New Hampshire provided research permits to Ed DeWalt, Luke Myers, and myself to collect Mount Washington during summer 2021 principally to find *Diura washingtoniana* (endemic), *Arcynopteryx dichroa* (disjunct), *Leuctra laura* (endemic), and *Zapada katahdin* (disjunct or continuous to Mount Katahdin, Maine, USA). We were successful in collecting larvae of *A. dichroa* (Cutler River at Huntington Ravine Trail) and *D. washingtoniana* larvae and one adult male (Upper Lakes of the Clouds, a glacial tarn). The record of *A. dichroa* is remarkable in that the closest known populations are in ca. 1,430 km westward (= ca. 890 mi) in western Lake Superior. Sampling in and near Mount Washington produced 17 new state records. DNA barcoding of *D. washingtoniana* and *A. dichroa* is planned to compare to congeners and other disjunct populations, respectively. Future trips are planned to continue work on Mount Washington and expand to other peaks in the Presidential range to find other locations for *D. washingtoniana* and other rare species.

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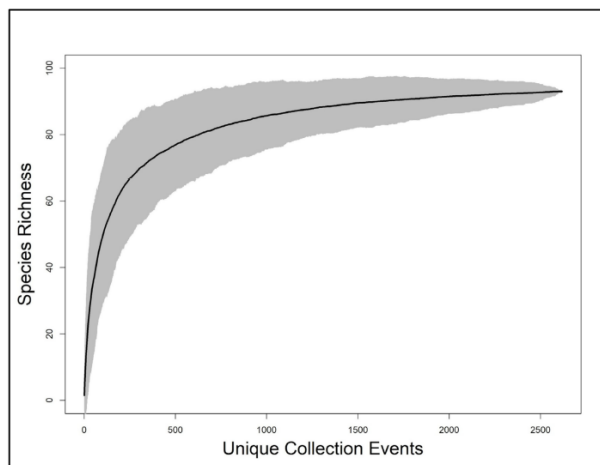
Currently we are sampling macroinvertebrates, particularly Plecoptera and Trichoptera, in Sierra Nevada National Park (Southern Spain) within the European project LIFE Watch-ERIC (N/REF LifeWatch-2019-10-UGR-01). We will analyze some stonefly populations from the demographical point of view in three reaches at different altitudes within the same stream course. We are also conducting monthly sampling in seasonal streams of the Guadiana Basin (Spain) to

study the metacommunity structure and the biology and demography of particular species of stoneflies, in order to discuss if they may be used as “sentinel species” of climate change. This is part of a project recently funded by the European Commission through the regional government (*Junta de Andalucía*, Spain) and the University of Granada, in which we are going to analyze the genetic structure of several populations of Plecoptera too in order to detect if they behave as a metapopulation and their conservation status. On the other hand, we are finalizing (together with Drs. Julio Luzón-Ortega, Patrizia Vannucchi and Romolo Fochetti) a work on the drumming behavior and the molecular characterization of several populations of the genus *Tyrrhenoleuctra* from Spain and (together with Drs. Cristina Trenzado-Romero and Ramón Carmona) a study on the histology of the digestive tract of several species of Plecoptera aimed at determining the possible existence of differences between species and within a same species with different development stages and with different diets.

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Phylogenomics of North American Plecoptera. Eric J. South finished his PhD dissertation in December, 2020, publishing his main chapter using transcriptomes and 95% of the genera present in North America (<https://doi.org/10.1111/syen.12462>). He followed this up with a phylogenomic analysis of most members of the Paraperlinae and several outgroups and morphological description of the first new family since 1987, Kathroperlidae Banks, 1948 (not South & DeWalt 2021, see <https://doi.org/10.1093/isd/ixab014>). Banks described the subfamily Kathroperlinae under the Perlidae, an act previously unrecorded in Plecoptera literature. Eric now has an assistant professor position at Lyon College, Batesville, Arkansas, USA.

Plecoptera of Indiana. Evan A. Newman graduated with a master’s degree in December, 2021. One chapter is published on a watershed based analysis of the diversity of stoneflies in Indiana, a Midwest, USA state (<https://doi.org/10.3390/d13120672>). From 5330 specimen records he found 93 species (including one undescribed *Perlesta*). Species richness was near saturated with terminal prediction of 95 ± 5 species. The total is intermediate between Ohio (103 species) to the east and Illinois (80) to the west. US Geological Survey HUC8 scale watersheds with the highest



richness were those in southern unglaciated and once-glaciated (Illinoisan glacial event) drainages. Species richness patterns were supported by relationships with several hydrological variables and watershed drainage area. A distribution atlas of the Plecoptera of Indiana is in construction in Biodiversity Data Journal and a third paper is planned to formally rank conservation status of species using the NatureServe rank calculator for USA states. Scott A. Grubbs is a valued advisor and coauthor on these papers.

Search for Illinois 72 Watchlisted Plecoptera, Ephemeroptera, and Trichoptera. This four year project, funded by the state of Illinois, examines all parts of the state for 72 Watchlisted EPT species as defined in the Illinois Wildlife Action Plan of 2015. Species that entered the list were rare contemporarily or had been known historically but not found recently. Stoneflies once thought extirpated, *Attaneuria ruralis* (Perlidae) and *Isogenoides varians* (Perlodidae), have been found with concerted sampling effort. Light trap collections at two locations on the Mississippi River produces males of *A. ruralis* and April investigations along large rivers found exuvia of *I. varians* on the Mississippi and Wabash rivers on opposite borders of the state--the first occurrence records for five decades. The rare *Allocapnia smithi* (Capniidae), *Leuctra alta*, *Zealeuctra narfi* (Leuctridae), and *Prostoia hallasi* (Nemouridae) have been found in several intermittent streams in the Shawnee Hills of southern Illinois, an east-west ridge vaulted 100-200 m above the surrounding landscape. Finding stoneflies new to Illinois is rare. Boris Kondratieff, collecting at a 2019 North American Plecoptera Symposium function found *Alloperla hamata* (Chloroperlidae) in a small stream of the Shawnee Hills. Extensive collecting since then has not produced other locations. This work has also yielded many new mayfly and caddisfly records for Illinois. Unfortunately, we have only found 43 of the 72 Watchlisted species with late winter and spring collections to go before analysis and report writing this summer.

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Distribution modeling study of the midwestern USA (Illinois, Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin) stonefly assemblage. Using a dataset of 38,000 records, distribution modeling is being employed to describe range dynamics following European settlement of the Midwest, identify habitat for uncommon species, and aid in the development of conservation assessments for each species modeled.

Phylogeographic analysis of the eastern Nearctic genus *Allocapnia* Claassen, 1924. This research addresses the post-glacial dispersal hypotheses summarized by Ross & Ricker (1971) using haplotype networks and distribution modeling. Additional predicted outcomes of this research include an updated review and a complete phylogeny of the *Allocapnia*.

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Observations of crumpled wings in stoneflies (Plecoptera). Further to our paper (as titled above; pdf available via ResearchGate [here](#)), my colleague Craig Macadam and I wish to draw your attention to our increased observations of wing ‘crumpling’ in Irish and British stonefly populations. We have now observed the phenomenon in all families found in our respective islands. We are wondering if this is a case of Baader-Meinhof phenomenon or if it is in fact an increasingly common phenomenon in our stonefly populations? Several theories are proposed (see paper) but potential climate effects and related degree-days are also a consideration. Should anyone have noticed a similar increase in observations of this phenomenon in their respective

territory please get in touch, or if you have a theory as to its cause we would be very interested to hear from you.



Photo of wing crumpling in *Capnia atra* (Capniidae) taken in Ireland (Hugh Feeley).

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