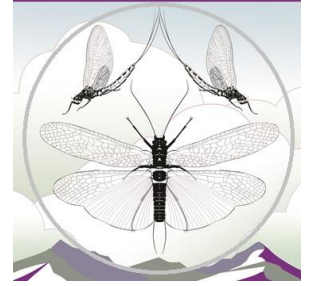




**2022 XVI<sup>TH</sup> INTERNATIONAL CONFERENCE ON  
EPHEMEROPTERA AND XX<sup>TH</sup> INTERNATIONAL  
SYMPOSIUM ON PLECOPTERA**



**PROGRAM, SCHEDULING, AND ABSTRACTS 2022 VIRTUAL MEETING**

**A Virtual Conference Hosted by the University of Illinois Urbana/Champaign, USA**

**ORGANIZERS**

**R. Edward DeWalt, University of Illinois, Illinois Natural History Survey, Champaign  
Boris C. Kondratieff, Fort Collins, Colorado**

**Held 26–29 July, 2022**

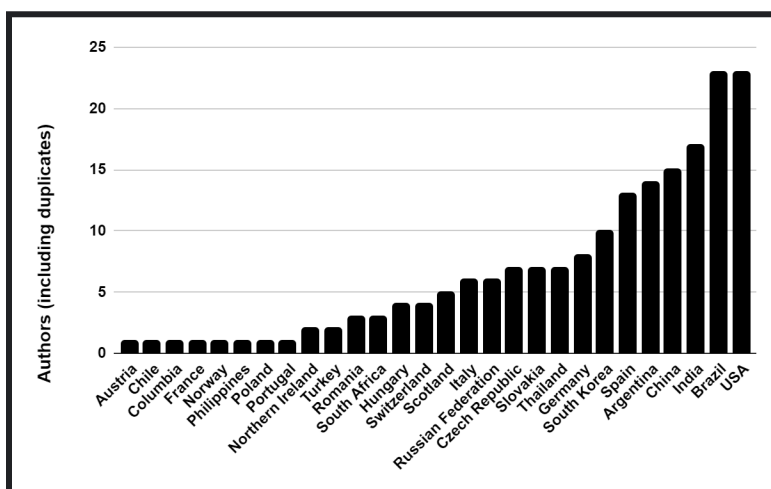
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## Introduction

Boris and I welcome you to the first virtual International Conference on Ephemeroptera and International Symposium on Plecoptera. This document will provide the who, what, and when of presentations and also how we will run sessions. We also provide some brief explanations for how the conference was organized in hope of heading off some inevitable questions and to provide a roadmap for those who wish to conduct a hybrid session in the future. We say this because the pandemic has broken our usual meeting paradigm, perhaps forever. Hybrid meetings will be necessary in the future to improve inclusivity, to be sensitive to the cost of travel, and to allow those who want to limit their carbon footprint to participate.

**Submitted presentations.** Presenters have submitted 36 video and 30 poster presentations. There are a few live presentations as well. The total number of presentations in Aracruz, Brazil in 2018 was about 75. A total of 133 authors appeared 187 times across all abstracts. Authors reported being from 28 countries with the USA and Brazil being the most frequent origin of authors. We expect the broader audience to be of greater geographical spread.



**Selection of time block for the conference.** Our meeting spans the dates of 26–29 July (Tuesday-Friday) and will meet for +/- three hours each day. We utilized four cities as anchor time zones for our conference (see table below): Berlin, Chicago, New Delhi, Beijing. [WorldClock](#) helped us visualize a time span that minimizes sleep deprivation for all involved. In Chicago the meeting runs approximately 06:00 to 09:00 hrs; in Berlin 13:00 to 16:00; Beijing 19:00 to 22:00, New Delhi 16:30 to 19:30. We are aware that these time zones do not account for all presenters or audience members. For those living outside of the time zones containing these cities, consult the WorldClock link and add a city in your time zone to obtain times to access the Zoom call.

UTC-time	Chicago	Berlin	Beijing	New Delhi
Tuesday, July 26, 2022 at 11:00:00	Tue 6:00 am *	Tue 1:00 pm *	Tue 7:00 pm	Tue 4:30 pm
Tuesday, July 26, 2022 at 12:00:00	Tue 7:00 am *	Tue 2:00 pm *	Tue 8:00 pm	Tue 5:30 pm
Tuesday, July 26, 2022 at 13:00:00	Tue 8:00 am *	Tue 3:00 pm *	Tue 9:00 pm	Tue 6:30 pm

**Presentation formats.** To limit potential errors inherent in live presentations, we required authors to record their talks as video. This was easily done using Zoom. Video presentations were limited to +/- 12 min. Three minutes are provided for questions and answers.

A modified poster format consisting of up to 5 slides, exported as a PDF document, was requested from each presenter. We chose this format to provide a less crowded way to view the posters. Some presenters chose the traditional format and we decided not to request changes. A single poster viewing time is scheduled for 06:00 Wednesday morning. Presenters are asked to attend this session to take live questions from the audience. Given the limited period for live questions, an open forum has been created that allows more interaction between presenters and the audience. Audience members may submit a question [here](#). Presenters, please check the [forum](#) on a regular basis to ensure questions on your presentations do not go unanswered.

The audience has access to all presentations by clicking on the title of each abstract. You may share this link with colleagues; the content is a duplicate of those used for the presentations.

**Recording of the conference.** We will be recording the presentations each day. After post-processing, these recordings, with questions and answers, will be made widely available. Because we are recording, we ask that you guard against the use of inappropriate language and loud or heated interactions.

**Audience.** We regret that we chose not to register attendees. We will have no record of non-presenters attending. Please help us to know who attended this conference by recording your name, affiliation, country, and email in this [spreadsheet](#).

**Etiquette during the meeting.** Show respect for colleagues by treating them as equals. Please do not display avatars that are distracting or demeaning. When attending the meetings, use the mute function to keep from competing with the video or live speaker. If you want to ask a question, use the “Reactions” button to choose the “hand-up” icon. When called upon, turn your button mute off and speak clearly. When your question has been answered. Lower your hand and mute your microphone. We will not have a lot of time for questions, so please do not monopolize the speaker.

Due to the large number of presentations, side meetings, and our desire to not go much beyond three hours in a day, group breaks are not routinely provided. Take breaks when you need to by turning off the video sharing and mute your microphone so you can leave quietly. Familiarize yourselves with the Zoom platform prior to the meeting if you are not already. This will reduce the number of distractions to presenters and other audience members.

**Side meetings.** Private invitations have been sent by email to all members of the Plecoptera and Ephemeroptera Standing Committee. Heath Ogden has asked for a meeting, “Ephemeroptera Consortium” to discuss logistics and arrange for collaborators on a genomic and morphological phylogeny of mayflies. You may enter this discussion through a breakout room called by the same name. See the schedule for this.

**Conference Proceedings.** We will hear from Boris C. Kondratieff about the conference proceedings. See schedule.

**Group picture.** Logistically, this is a little challenging. We will attempt to do this Tuesday at 09:05–09:10 Chicago time. Our moderator, Phillip Hogan, may also call for additional attempts to pick up more participants. Our group photo may turn out to be a compilation.

## Schedule of Meeting and Presentations

### Meeting Zoom Link

Moderator: Phillip Hogan, PhD student at the University of Illinois.

**Tuesday, 26 July**

**Live or video presentations. Times given as Chicago time (CST).**

1. 06:00-06:10. **Ed DeWalt**. Welcome.
2. 06:10-06:25. **John Brittain, Michel Sartori**. Introduction by Chairs of Standing Committees.

**Phylogeny, Systematics, Taxonomy**

3. 06:25-06:50. **Eric J. South**. Advances in Plecoptera Systematics.
4. 06:50-07:05. **Heath Ogden**. Higher-level relationships of Ephemeroptera.

**Ecology, Biomonitoring, Conservation**

5. 07:05-07:20. **Ji Hyoun Kang**. Population Genetic Structure of Massively Emerging *Ephemera orientalis* (Ephemeroptera: Ephemeridae) in South Korea Inferred from Novel Microsatellite Markers.
6. 07:20-07:35. **Andrés Peredo Arce**. Riparian Forest as Provider of Landscape Connectivity for Adult EPT.
7. 07:35-07:50. **Ed DeWalt**. Status Assessment of Illinois Watchlisted EPT in IL.
8. 07:50-08:05. **Maribet Gamboa**. Parallel Gene Expression Changes Reveal Genetic Plasticity Induced by the Temperature Gradient in Seven Stonefly Species.
9. 08:05-08:20. **Craig Macadam**. Ain't No Mountain High Enough – Studies on the Upland Summer Mayfly (*Ameletus inopinatus*).
10. 08:20-08:35. **Jack Stanford**. Ecology of Groundwater (Amphibitic) Stoneflies.
11. 08:35-08:50. **Gencer Türkmen**. Changes in the Community Structure of Ephemeroptera Species in a Biosphere reserve in Turkey, According to Stream Zonation, Stream Order, and Altitude.
12. 08:50-09:05. **Craig Macadam**. Current Knowledge of the Northern February Red (*Brachyptera putata*).

09:05-09:10. **Group picture**. Phillip will capture pictures. Turn on video sharing or use your avatar. Turn off video sharing if you do not want your picture taken.

## Wednesday, 27 July

Times given as Chicago time (CST).

1. 06:00-07:20. **Poster Presentations.**

### Video or Live Presentations Continued

#### Biogeography, Phylogeny

2. 07:20-07:35. **Luboš Hrivniak.** An Introduction to the Biogeography of New Caledonian Mayflies
3. 07:35-07:50. **Abigail P. Kirkaldy.** The Past Meets the Present: Examining the Biogeography and Evolution of the Plecoptera with Reference to their Ancient Forms
4. 07:50-08:05. **Alfredo Mayorga Villalobos.** Mandibular tusks as Weapons in the Aggressive Behavior of *Rhonanthus coreanus* (Ephemeroptera: Potamanthidae)
5. 08:05-08:20. **Eduardo Domínguez.** Paradigm shift in Mayfly Flight (Insecta: Ephemeroptera): Multi-methodological Approach Reveals True Function of Wing Bullae
6. 08:20-08:35. **Inês Ribeiro.** Neuroanatomy of the Central Nervous System of Adult Mayflies.

08:35-09:45. **Heath Ogden.** Ephemeroptera Consortium. Breakout room session.

#### Biology, Behavior, Reproduction

7. 08:35-08:50. **Annamaria Fausto.** Spermatological Models in European Plecoptera.
8. 08:50-09:05. **Maisa de C. Gonçalves.** Sperm as a Tool for Male and Female Association in Plecoptera.

## Thursday, 28 July

Live or video presentations. Times given as Chicago time (CST).

### Conference Proceedings

- 06:00-06:15. **Boris. C. Kondratieff**. Zoosymposium: cost, submission, formatting, editors, reviewers, etc.

### Proposal for the next Conference

- 06:15-06:30. **Stefano Fenoglio, Romolo Fochetti, Manu Rodríguez, Manuel Tierno de Figueroa**. 2024 Turin, Italy meeting proposal.

06:30-07:15. **Plecoptera Standing Committee**. Private invitation emails have been sent to committee members.

### Distribution, Faunistics

- 06:30-06:45. **Chonlakran Auychinda**. The Taxonomic Status and Species Richness of Some Ephemerelloid Mayflies (Ephemerelloidea: Ephemeroptera) in Thailand.
- 06:45-07:00. **Boonsatien Boonsoong**. Mayfly Larvae (Ephemeroptera) in Thailand.
- 07:00-07:15. **Zhenxing Ma**. A Contribution to *Epeorus (Iron)* of China (Ephemeroptera, Heptageniidae).
- 07:15-07:30. **Chanaporn Suttinun**. Baetidae of Thailand : the First Mass Survey.
- 07:30-07:45. **Anuntaya Wongyam**. Diversity of the Genus *Afronurus* (Ephemeroptera: Heptageniidae) in Thailand.
- 07:45-08:00. **John Sandberg**. Updated Drumming Signal Descriptions for Three *Soliperla* Species from California (Plecoptera: Peltoperlidae).
- 08:00-08:15. **Kumbakonam Govindarajaiyer Sivaramakrishnan**. Factors Structuring Patterns of Ephemeroptera (mayflies) Species Assemblages in Different Segments of the Western Ghats of Peninsular India – a Snapshot.
- 08:15-08:30. **M. Vasanth**. Mayflies (Insecta: Ephemeroptera) of Indian Himalaya and Future Challenges.
- 08:30-08:45. **Helen Margaret Barber-James**. New Insights into the Mayflies and Stoneflies of Angola, Africa.
- 08:45-09:00. **Phillip N. Hogan**. Using Distribution Models to Identify Range Shifts of Four *Acroneturia* Pictet, 1841 (Plecoptera: Perlidae) Species in the Midwest USA.
- 09:00-09:15. **Shigekazu Uchida**. *Flavoperla* (Plecoptera: Perlidae) Nymphs Inhabit Hyporheic Zones.

## Friday, 29 July

Live or video presentations. Times given as Chicago time (CST).

06:00-06:45. **Ephemeroptera Standing Committee**. Private invitation emails have been sent to committee members.

### Phylogeny, Systematics, Taxonomy

1. 06:00-06:15. **Rao Rao Mo**. Systematic Classification of Plecoptera from Southern China.
2. 06:15-06:30. **Arthien Lovell Pelingen**. Updates on the *Neoperla* Needham, 1905 (Plecoptera, Perlidae) from the Philippines.
3. 06:30-06:45. **Scott A. Grubbs**. New Synonymy and Updated Range Alter the Conservation Conversation About the Louisiana Needlefly, *Leuctra szczytkoi* Stark & Stewart, 1981 (Plecoptera: Leuctridae).
4. 06:45-07:00. **Dávid Murányi**. Contribution to the *Protonemura* Kempny, 1898 (Plecoptera: Nemouridae) of the Caucasus.
5. 07:00-07:15. **T. Sivaruban**. A New *Baetis* (Ephemeroptera: Baetidae) from Tamil Nadu, India.
6. 07:15-07:30. **Chang-Fa Zhou**. A Review of Asian *Habrophlebiodes* Species (Ephemeroptera: Leptophlebiidae).
7. 07:30-07:45. **T. Kubendran**. Phylogeny of Indian Baetidae (Insecta: Ephemeroptera) Using Morphological Characters.
8. 07:45-08:00. **Rogério Campos**. Estimating Phylogeny and Tempo of the Antillean Colonization of Neotropical *Hagenulina* (Ephemeroptera: Leptophlebiidae): did the GAARlandia Landspan Drive a Central Role?
9. 08:00-08:15. **Thales Yann da Silva Orlando**. A New Species of *Leentvaaria* Demoulin (Ephemeroptera: Leptophlebiidae): A long Awaited Addition to a Previously Monotypic Genus.
10. 08:15-08:30. **Avery Larsen**. Phylogenomics of Baetidae: A Comparison in Classification.
11. 08:30-08:45. **Jhon Faber Marulanda Lopez**. Cladistic Analysis of Terpidinae Kluge (Ephemeroptera: Leptophlebiidae).
12. 08:45-09:00. **Trevor D. Millar**. Bioinformatics Pipeline for Targeted Phylogenomic Data: A case study in Mayflies (Ephemeroptera).
13. 09:00-09:15. **Pavel Sroka**. Evolution of Filter-feeding in Aquatic Insects Dates Back to the Middle Triassic: New Evidence from Stemgroup Mayflies (Insecta: Ephemera) of Grès à Voltzia, Vosges, France.



## **Side Meetings in Breakout Rooms.**

### **Ephemeroptera Consortium: Classification Effort and Grant to Sequence 300 more Taxa.**

Wednesday, 27 July. 08:35-09:45.

Join the Ephemeroptera Consortium breakout room to attend.

T. Heath Ogden

Utah Valley University, Orem, UT, USA. [heath.ogden@uvu.edu](mailto:heath.ogden@uvu.edu)

I am proposing to schedule a time during the meeting to invite mayfly colleagues to be a part of what I am calling the “Ephemeroptera Consortium”. This working group will discuss and plan how to accomplish two main goals 1) Initiate the conversation and plan how to create a new Mayfly Classification (based on all the evidence, molecular and morphological). 2) Establish a group of investigators to be part of a NSF proposal (Ogden will submit this year) to acquire and sequence around 300 selected species to carry out targeted capture/Genomic/transcriptomic sampling and data generation. Essentially I am looking for potential Co-PIs and Senior Collaborators and collaborators.

Keywords: Phylogenetics, Taxonomy, Systematics, Evolution, Ephemeroptera

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### **Plecoptera Standing Committee**

Thursday 28 July. 06:30-07:15.

Breakout room session by invitation only.

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### **Ephemeroptera Standing Committee**

Friday 29 July. 06:00-06:45.

Breakout room session by invitation only.

## Video Presentation Abstracts

### *Biogeography, Phylogeny*

#### [An Introduction to the Biogeography and Speciation of New Caledonian Mayflies \(Ephemeroptera\)](#)

Luboš Hrivniak <sup>1</sup>, Jindřiška Bojková <sup>2</sup>, Michel Sartori <sup>3</sup>

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New Caledonia (NC) is a Melanesian archipelago located 1,700 km northwest of New Zealand. It is well-known for exceptional regional endemism and local microendemism in terrestrial and aquatic ecosystems. The mayflies of NC belong to a single family Leptophlebiidae; other widespread families such as Baetidae and Caenidae are absent there. The patterns of diversity and radiation in New Caledonian mayflies are unique worldwide. They have diversified to 19 endemic genera with 46 species known to date, which is exceptional within mayflies and aquatic insects in general. New Caledonian mayflies are also unusual in terms of morphological convergences with unrelated Northern Hemisphere families. Ecological diversification of larvae to occupy different niches in freshwaters resulted in strikingly similar morphological forms. Although the diversity of mayflies in NC has been extensively studied by Peters et al. (1978–2016), the origin and mechanisms driving their diversification are poorly understood. Based on our field studies in 2015 and 2022, we aim to investigate their phylogenetic relationships with other Leptophlebiidae and clarify their evolutionary history using molecular data. Our preliminary results show that New Caledonian mayflies are closely related to genera distributed in Australia, New Zealand, and South America that belong to Atalophlebiinae. The relationships can be explained by the tectonic history of NC. Despite its recent location in Melanesia, its history is linked to the breakup of Gondwana and the geologic history of Zealandia. Long-term isolation, development of ultramafic bedrocks, and intense orogeny may have played an important role in the radiation of New Caledonian species.

Keywords: Diversity, Speciation, Melanesia, Islands, Biogeography

## The Past Meets the Present: Examining the Biogeography and Evolution of the Plecoptera with Reference to their Ancient Forms

Abigail P. Kirkaldy<sup>1,2,3</sup>, Rosemary Prevec<sup>3,4</sup>, Helen M. Barber-James<sup>1,5,6</sup>, Arnold Staniczek<sup>7</sup>

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The Permian Period (299—252Ma) was a critical time in the evolution and dispersal of many insect groups, including the Plecoptera. However, insects from this period remain poorly understood, particularly in the Southern Hemisphere where invertebrate fossils are rare. The Onder Karoo locality in South Africa has recently yielded an unprecedented diversity of middle Permian insects (~266Ma) preserved in deposits of an aquatic lake margin system in the lowermost Abrahamskraal Formation. Amongst the exceptionally well preserved insects found at the site, the nymphs of three new species of stem-Plecoptera were collected, all of which are new to science. These are the earliest stem-Plecoptera collected from Gondwanan landmasses to date. Since only 17 fossil species have been recorded previously from Gondwana, this discovery represents a significant contribution to our understanding of ancient stoneflies. A combination of morphological and phylogenetic evidence was used to examine these specimens in the context of Plecoptera evolution, to better understand the origin, dispersal and biogeography of this group.

Keywords: Biogeography, Permian, Stem Plecoptera, Lagerstätte

*Biology, Behavior, Reproduction***Paradigm Shift in Mayfly Flight (Insecta: Ephemeroptera): Multi-Methodological Approach Reveals True Function of Wing Bullae**

Eduardo Domínguez <sup>1</sup>, Thomas van de Kamp <sup>2,3</sup>, Istvan Miko <sup>4</sup>, María Gabriela Cuezco <sup>1</sup>, Arnold H. Staniczek <sup>5</sup>

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Unlike wings in other insect orders, mayfly forewings are equipped with so-called bullae. These oval, blister-like spots are found in some of the main negative veins. Traditionally, it has been suggested that bullae might play a central role in flight (Edmunds & Traver 1954). According to these authors, bullae would allow the distal part of the wing membrane to bend in the upstroke, thus diminishing pressure and reducing resistance. However, our own observations by high speed videography did not confirm this generally adopted theory. Instead of bending during upstroke, mayfly wings remain straight for the whole wing cycle, only in their basal wing half its membrane cambers to produce uplift. This observed wing movement contradicts a function of bullae in wing bending during flight, which led to further investigations by SEM,  $\mu$ CT, and fluorescence microscopy. In both winged stages, bullae are present at half length in negative wing veins Sc, R2, R4+5, and MP1 at most. Unlike the entire wing membrane, bullae do not contain resilin and have no elastic properties. They resemble well-defined, desclerotized areas in otherwise heavily sclerotized veins. This still suggests a role of bullae in wing bending, but this does not happen in flight. Instead, video recordings reveal a crucial function of bullae in subimaginal molting: Halfway through the molt, wings fold over at bullae position, thus facilitating extraction of the imaginal wings from the subimaginal skin. This theory is also supported by the lack of bullae in species, which remain in their subimaginal stage.

Keywords: Bulla, Flight, Molting, Subimago, Wing

## Spermatological Models in European Plecoptera

Annamaria Fausto, Annarita Taddei, Gabriella Gambellini, Massimo Ceci, Romolo Fochetti

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The sperm of Plecoptera is of a typical polyneopteran pattern, filiform and flagellate, with an elongated and compact nucleus topped by an acrosomal complex and a “9+9+2” axoneme flanked by two mitochondrial derivatives. The structure shows a certain heterogeneity, in particular as regards to morphology of the acrosomal complex (mono-bi-three layered), shape of nucleus, presence or absence of one or two accessory bodies flanking the axoneme and degree of crystallization of mitochondrial derivatives.

Many systematic issues regarding Plecoptera are still debated, and it seems that the use of molecular data is unable to definitively clarify the systematic relationships within the order. We believe that spermatology characters can be useful also in the phylogenomic molecular era to help solve some of the unanswered systematic questions. We studied in the past the spermatozoa structure of ten Plecoptera species belonging to all the seven European stoneflies families (Fausto et al., 2001; 2002a; 2002b). Our comparative analysis of sperm characters allowed us to suggest the presence of a “sperm model” valid at family level; a certain degree of heterogeneity was, however, found in sperm structure within the seven European families analyzed. We describe in the present communication the sperm structure of four more species belonging to European stoneflies genera not analyzed in our previous studies, i.e. *Capnopsis schilleri* Rostock, *Amphinemura sulcicollis* Stephens, *Rhabdiopteryx neglecta* Albarda, *Tyrrhenoleuctra zavattarii* Consiglio. Based on the data obtained, it is possible to infer affinity between all the taxa examined so far and discuss aspects of the systematic arrangement within the order.

Key words: Sperm Structure, Plecoptera Systematics, European Stoneflies

## Sperm as a Tool for Male and Female Association in Plecoptera

Maisa de C. Gonçalves<sup>1,2</sup>, Paulo H. Rezende<sup>3</sup>, Frederico F. Salles<sup>2</sup>, José Lino-Neto<sup>3</sup>

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The sperm morphology is species-specific, so the analysis of these cells in different animal groups, including insects, can determine the species of the male in which these were produced. The identification of many species can be problematic because several descriptions are based only on adult males, which often are the most morphologically conspicuous semaphoront. In this context, we studied the sperm morphometry of two Perlidae species, *Kempnyia obtusa* Klapálek, 1916 and *Kempnyia neotropica* (Jacobson & Bianchi, 1905). Male and female specimens were collected by light trap at the Serra do Brigadeiro, Araponga, Minas Gerais, Brazil. We dissected the adults, and prepared slides of sperm cells from both sexes (in females, sperm cells were dissected from the spermatheca). The material was stained with Giemsa and DAPI and photodocumented. Measures were made using Sperm Sizer 1.6.6 software. The sperm of both species were long, filiform, composed of a head, with acrosome and nucleus, and the flagellum; and organized in bundles in the seminal vesicles. The sperm morphometry was unique for each species, allowing the determination of the females. The sperm from males of *K. obtusa* measured  $593,3 \pm 21,7 \mu\text{m}$ , and nucleus were  $18,6 \pm 1,2 \mu\text{m}$ , and females  $573,6 \pm 20,5 \mu\text{m}$ , nucleus:  $18,1 \pm 1,1 \mu\text{m}$ . And for males of *K. neotropica*, measured  $901,6 \pm 20,9 \mu\text{m}$ , and nucleus were  $22,0 \pm 1,0 \mu\text{m}$ , and females  $854,4 \pm 13,4 \mu\text{m}$ , nucleus:  $20,5 \pm 1,1 \mu\text{m}$ . Our study shows that comparative spermatological studies are useful to associate males and females of Plecoptera and in solving or at least clarifying systematic questions.

Keywords: Stoneflies, Anacroneuriini, Sperm morphology, Identification, Taxonomy, Systematics

**Mandibular Tusks as Weapons in the Aggressive Behavior of *Rhoenanthus coreanus***  
**(Ephemeroptera: Potamanthidae)**

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Structures like tusks or horns are well-known in regard to their function as weapons in most animals. However, in the case of the mandibular tusks in Ephemeroidea, these structures are known to be associated with burrowing and feeding. In this study, a total of 232 larvae of *Rhoenanthus coreanus* (Yoon & Bae, 1985) were observed (54 males and 178 females) to corroborate previous hypotheses on the functions of the mandibular tusks. As a result, in addition to burrowing and feeding behaviors, we reveal for the first time the use of mandibular tusks as weapons in the aggressive behavior among larvae of this species. Larvae of this species use their tusks directly as weapons in combat. Females were more aggressive than males. Approximately 21% of all aggressive observations corresponded to male vs male, 75% female vs female, and 4% mixed (male vs female). Aggressive behavior was observed specially in the larvae with larger body size in each sex (pronotum length = male 2.48—3.42 mm, female 3.63—5.10 mm). We concluded that larger mandibular tusks might increase the multifunctionality of tusks, and the aggressive behavior in this species occurs mainly due to territory conflict as a mechanism to keep or find shelters for feeding and hiding. This study is the first of its kind and unveils a new perspective on functions of the mandibular tusks, and aggressive behavior within Ephemeroptera, which has potential in future studies.

Key words: Ephemeroptera, Ephemeroidea, *Rhoenanthus*, Aggressive Behavior, Mandibular Tusks

## Neuroanatomy of the Central Nervous System of Adult Mayflies

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Mayflies dedicate their entire adult life to reproducing. Upon transitioning from aquatic larvae to the terrestrial adults, mayflies shed all the mouth parts and most antennal segments, along with the associated chemo- and mechanosensory sensilla. In contrast, the compound eyes grow progressively over numerous larval molts. Moreover, adult mayflies do not feed or search for food. The short, terrestrial adult life of mayflies is thus solely dedicated to reproduction, which includes searching for a mate and swarming, mating and laying eggs. The central nervous system of adult mayflies might have specific adaptations at the structural and/or functional levels that support this peculiar strategy of reducing the behavioral and sensory feature spaces in order to reproduce. The central nervous system of mayflies is distributed along the anterior-posterior axis of larvae or adults, with the brain lodged in the head, along with three thoracic and seven abdominal ganglia along the anterior-posterior length of mayflies. The central nervous system of adults of the mayflies species *Ephemera danica*, *E. vulgata* and *E. lineata*, was analyzed with immunostaining with anti-Dlg (synapses) and phalloidin (F-actin). The central brain, lying between two large optic lobes, mainly consists of neuropiles belonging to the central complex. Visual information enters the central brain via several pathways, including a very prominent anterior optic tract. Comparisons with nymphal stages are ongoing. The anatomy of the adult brain suggests that it is mainly dedicated to processing visual cues, providing further incentive to probe the span of sensory cues adult mayflies might perceive.

Keywords: Central Nervous System, Adult Mayflies, Reproductive Behavior, Visual Sensory Cues



*Distribution, Faunistics***The Taxonomic Status and Species Richness of Some Ephemerelloid Mayflies (Ephemerelloidea: Ephemeroptera) in Thailand**Chonlakran Auychinda <sup>1</sup>, Michel Sartori <sup>2,3</sup>, Boonsatien Boonsoong <sup>4</sup><sup>1</sup> Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom Province, Thailand. [c.auychinda@gmail.com](mailto:c.auychinda@gmail.com)<sup>2</sup> Museum of Zoology, Palais de Rumine, Place Riponne 6, CH-1005 Lausanne, Switzerland.<sup>3</sup> Department of Ecology and Evolution, Lausanne University, CH-1015 Lausanne, Switzerland.<sup>4</sup> Animal Systematics and Ecology Specialty Research Unit (ASESRU), Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

In Thailand, five families belonging to the superfamily Ephemerelloidea were previously reported. These ephemerelloid mayflies were classified into 11 genera and 21 species. However, the study of the superfamily Ephemerelloidea in Thailand is still deficient and also there are some taxonomic confusions. In this report, ephemerelloid mayflies were collected from 88 localities of Thailand from 2015 to 2022 to examine the species richness and revision of taxonomic status using morphological and molecular analysis. Our specimens were classified into the same five families as the previous data. We found only eight genera, but more than 21 species:

Ephemerellidae: *Notacanthella commodema*, *N. quadrata*, *Cincticostella femorata*, *C. gosei*, *C. insolta*, *Teloganopsis* spp., *T. brocha*, *T. oriens*, *Torleya* spp., *T. cf. grandipennis*, *T. naga*, *T. coheri* and *T. lacuna*;

Teloganellidae: *Teloganella umbrata*;

Teloganodidae: *Dudgeodes* spp.;

Tricorythidae: *Sparsorythus* sp. and *S. multilabeculatus*;

Vietnamellidae: *Vietnamella* sp. B, *V. thani*, *V. maculosa* and *V. nanensis*.

A *COI* phylogeny was reconstructed by Bayesian inferences. The result reveals that there are many cryptic species in the genus *Teloganopsis*. Furthermore, the genus *Torleya*, which is morphologically problematic, shows three separated clades with unique characters in each of them. From our results, we suggest that the genus *Torleya* should be completely revised. However, our data are not complete for both morphology and *COI* sequences. Although our investigations help fulfill the taxonomic gap of Thai ephemerelloid mayflies, their integrative taxonomy requires further study.

Keyword: *COI* Phylogeny, Cryptic Species, Ephemerellidae, Integrative Taxonomy, *Torleya*

## [New Insights into the Mayflies and Stoneflies of Angola, Africa](#)

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Several surveys of the unique river systems of the Angolan highlands have been carried out under the auspices of the National Geographic Okavango wilderness project, managed by the Wild Bird Trust, South Africa, starting in 2014. The most recent survey in November 2019 aimed to see what species might be present during summer, as earlier surveys had been conducted in autumn and spring. The surveys included headwater tributaries of both the Zambezi and Okavango systems, and produced 40 morphologically distinct mayfly species, some known, many unknown. This added several additional species to the previously reported (2019) list of 71 species for Angola, nine of which have since been described. The Plecoptera fauna of Africa is still very poorly known, and while only three species were found, two of these are undescribed species of *Neoperla*. This work highlights the importance of ongoing survey work in Africa, where many species remain unknown.

Keywords: Africa, Angola, Okavango, Zambezi, New Species

## Mayfly Larvae (Ephemeroptera) in Thailand

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Mayflies (Ephemeroptera) are one of the most common components of aquatic assemblages in freshwater environments and contribute to ecosystem services. They have been widely used as indicators of water quality and are frequently an important component of biomonitoring protocols. They exhibit a decrease in taxa richness with increased stream or river pollution and degradation. In a series of the taxonomic studies of Thai mayflies, nineteen families, 73 genera, and approximately 165 species of mayfly larvae were reported from Thailand. The families Baetidae and Heptageniidae are the most diverse and widespread groups of Thai mayflies. Knowledge about the diversity of Thai mayflies has steadily increased in the present decade. New genera (*Cymbalocleon*, *Elatosara*, *Mekongellina*, *Sangpradubina* and *Thainis*), new species and new record of mayflies were continuously discovered in Thailand. Nevertheless, the number of genera and species seems to be lower than the species diversity estimation. There are still several undescribed genera and species. Studies on systematics and ecology of mayflies are increasing in recent years. DNA barcoding can provide a powerful supplement to the traditional morphological approach to species delimitation. The book entitled “Mayfly Larvae in Thailand” (Thai version) and the boardgame “Thai Mayflies” were developed for environmental science communication. Further efforts to assess the conservation status of mayfly species are required. Thus, mayfly diversity and ecological requirements data could be used as tools to evaluate environmental impacts on water resources and drive future research for biodiversity conservation management strategies.

Keywords: Biodiversity, Bioindicator, DNA Barcode, Systematics

**Using distribution models to identify range shifts of four *Acroneuria* Pictet, 1841 (Plecoptera: Perlidae) species in the Midwest USA**

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Regional faunal assessments of stoneflies in the United States Midwest (herein defined as Illinois, Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin) indicate increasing imperilment resulting from human disturbance and climate change. Large-bodied Perlid stoneflies with multivoltine life cycles are among the most at risk for regional extirpation, with losses reported in several midwestern states. Species distribution modeling was undertaken to describe distribution shifts for four widespread riverine species: *Acroneuria abnormis* (Newman, 1838), *A. frisoni* Stark & Brown, 1991, *A. internata* (Walker, 1852) and *A. lycorias* (Newman, 1839). The distribution modeling algorithm MaxEnt was selected to predict both the historical (i.e., pre-1960) and contemporaneous distributions for each species using separate occurrence datasets. These models permit the identification of suitable habitat loss through range contractions associated with human disturbance. Predictions of suitable habitat losses were recorded for multiple species, but were greatest for *A. abnormis* and *A. internata*. These models serve to guide future collection efforts and to further describe patterns of regional biodiversity loss.

Keywords: Biogeography, Conservation, Distribution Modeling

## *Flavoperla* (Plecoptera, Perlidae) Nymphs Inhabit Hyporheic Zone

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*Flavoperla* (Plecoptera, Perlidae) nymphs were suggested to be inhabitants of the hyporheic zone, but their habitat is not sufficiently revealed yet. We reexamined numerous specimens of nymphs, exuviae and adults of the genera *Flavoperla* and *Paragnetina* (Plecoptera, Perlidae; both emerge in summer and have more than 2 years of nymphal period) collected from 2000 to 2022, mainly in the Yahagi River system, central Honshu, Japan. The number of *Flavoperla* nymphs was far fewer than that of *Paragnetina* nymphs. However, for exuviae and adults, the number of *Flavoperla* was similar (exuviae) to, or fewer (adults) than that of *Paragnetina*. We excavated dry riverbeds just off the water at seven sites in the Yahagi River system from 2019 to 2021 to collect hyporheos. At six of the seven sites, we collected animals that could be considered hyporheos (nymphs of *Flavoperla* and Leuctridae, Plecoptera, larvae of *Stenelmis*, Elmidae, Coleoptera and Ceratopogonidae, Diptera) or groundwater animals (Bathynellacea, Pseudocrangonyctidae, both Crustacea). These results suggest that the low numbers of *Flavoperla* nymphs collected by the usual methods for benthos may be due to their habitat in the hyporheic zone. Other candidates for hyporheos include the nymphs of Leuctridae and the larvae of *Stenelmis* and Ceratopogonidae. Most of these animals showed a preference for the waters with high oxygen saturation. Strong disturbance of the riverbed is thought to increase oxygen saturation in the hyporheic zone, and therefore, *Flavoperla* nymphs etc. could be used as indicators for strong riverbed disturbance.

Keywords: *Flavoperla*, hyporheic zone, disturbance, oxygen saturation, Yahagi River

## A Contribution to *Epeorus (Iron)* of China (Ephemeroptera, Heptageniidae)

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*Epeorus* Eaton, 1881 is a genus with high species diversity in Holarctic and Oriental regions, nine subgenera have been proposed to date. However, the delimitation and monophyly of some subgenera remain controversial and without molecular support. Here, an investigation combining molecular and morphological evidence is adopted to resolve the subgeneric positions of several Chinese *Epeorus* species which are intermediate between *Epeorus (Proepeorus)* and *Epeorus (Iron)*. As a result, three new *Epeorus* species, which form a new *E. gibbus*-group, are recognized herein from both the nymphal and imaginal stages. The molecular topology supports strongly that this new species group is the sister group of *E. longimanus*-group, which belongs to *E. (Iron)*. Thus, these three new species are also grouped into subgenus *Iron*. Morphologically, the unfolded gills VII of *E. gibbus*-group are dissimilar to all other *Iron* species while their mouthparts are closely alike to those of *E. longimanus*-group. It seems that the suction discs of the different subgenera of the genus *Epeorus* are homoplasies but their mouthparts need more consideration in phylogenetic reconstructions.

Keywords: Mayfly, Integrative Taxonomy, COI, Species Delimitation, Phylogenetics

Updated Drumming Signal Descriptions for Three *Soliperla* Species from California  
(Plecoptera: Peltoperlidae)

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Previously reported drumming signal descriptions of three *Soliperla* Ricker, 1952 species in the family Peltoperlidae are updated. The description of monophasic call signal patterns for *Soliperla quadrispinula* (Jewett) and *S. thyra* (Needham & Smith) are revised. The male call and response of *S. sierra* Stark and the response signal of *S. quadrispinula* are described for the first time. The male call signals of the three California species are distinct, having a repeated monophasic call pattern containing one to six monophasic signals, with inconsistent inter-call intervals. Sequenced monophasic female answers did not follow all repeated male calls. Male monophasic responses occasionally followed female answers in sequenced 3-way exchanges. The repeated monophasic call appeared similar to the grouped signal pattern, but inter-call intervals were not consistent.

Keywords: Plecoptera, Peltoperlidae, *Soliperla*, Drumming, Repeated Monophasic Signal Pattern

## Baetidae of Thailand : the First Mass Survey

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Baetidae are one of the most diverse and widespread families. However, the members often remain neglected and unidentified in most ecological studies and environmental surveys especially in tropical areas due to their small body size and its difficult identification. Baetidae were first reported from Thailand in 1969; fifty years later, sixteen species belonging to nine genera were reported. The aims of this study are to provide a species checklist based on morphological and molecular evidence as well as to investigate the ecological niches and distribution of the different taxa. 1,500 specimens were collected between May 2017 to May 2019 from 70 localities along the southern and western parts of Thailand. Thirty-nine species belonging to 16 genera were identified. Eleven genera are reported from Thailand for the first time. One genus, *Cymbalcloeon* Suttinun, Gattolliat, & Boonsoong, 2020 as well as four new species *C. sartorii*, *Indocloeon (H.) continentale* Kluge & Suttinun, 2020, *Procerobaetis totuspinosus* Suttinun, Kaltenbach, & Boonsoong, 2021 and *Tenuibaetis panhai* Suttinun, Gattolliat, & Boonsoong, 2022 were described. The description of two new genera and several new species are still pending. *Labiobaetis* with twelve potential species is the most diversified genus but is still in need of study. A new species is provisionally assigned to *Securiops*; but the report of this Afrotropical genus in Southeast Asia is very surprising and needs to be confirmed. This study shed a new light on the diversity of the Baetidae in Thailand and extend their use beyond simple bioindicators and reveal their importance to document habitat changes in this developing country.

Keywords: Mayfly, Southeast Asia, Oriental Region, Species Diversity, Distribution



## Mayflies (Insecta: Ephemeroptera) of Indian Himalaya and Future Challenges

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A concise overview of biology, diversity and distribution of the Ephemeroptera of Indian Himalaya, one of the global biodiversity hotspots, is presented. The Ephemeroptera of India is composed of at least 210 species in 59 genera and 15 families. The current study summarizes the diversity and distribution of Ephemeroptera of five Himalayan states of India *viz.*, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Sikkim and Arunachal Pradesh. The study documents 91 species and 32 genera belong to the 10 families and 54 species are endemic. The richest diversity of mayfly species of Indian Himalaya, especially Himachal Pradesh and Arunachal Pradesh and the maximum diversity is seen in three families and three genera in this region. Updated information on Ephemeroptera of Himalayan part of Pakistan, Nepal, Bhutan and China is required to comprehensively understand the diversity in the hotspot. Further, to advance our knowledge of Ephemeroptera of the region, a synthesis of the taxonomy of adults and larvae with description of all life history stages within a molecular phylogenetic framework is urgently required.

Keywords: Biodiversity, Systematics, Ecosystems, Cryptic Species

**Factors Structuring Patterns of Ephemeroptera (mayflies) Species Assemblages in Different Segments of the Western Ghats of Peninsular India – a Snapshot**

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Understanding the importance of interacting factors structuring biodiversity of the communities of freshwater biota in tropical biodiversity hotspots has become a challenging task in the midst of sixth megaextinction and rapid climate change. Alarming imperilment and extinction of biodiversity elements especially in fragile inland waters along with fragmentation of their habitats have crippled them jeopardizing the goods and services.

The Western Ghats of peninsular India have a long geological tectonic, biogeographic and eco-climatic history. Its inland water fauna is subjected to unique species assemblage patterns as a result of continued interaction between evolutionary and ecological processes primarily impacted by topographic barriers (Goa gap, Palghat gap and Shencottah gap) in the geological past followed by creation of vertically arranged sequences of stable ecological belts along elevational and bioclimatic gradients ("sky islands"). Since the turn of the twentieth century, multiple human induced stressors on freshwater ecosystems of the Western Ghats' depauperate fauna created a 'biodiversity crisis'. Identification of remaining near pristine habitats and developing long term conservation strategies are urgently required. In this context, trait-based species assemblage patterns of an ancient aquatic insect order, the Ephemeroptera, an ideal taxon for biogeographic and phylogenetic analyses with sensitive biomonitoring potential are presented with suggestions for future work to help in conservation of this precious biological heritage.

**Keywords:** Trait-Based Species Assemblages, Ephemeroptera, Western Ghats, Topographic Barriers, Sky Islands

## Diversity of the Genus *Afronurus* (Ephemeroptera: Heptageniidae) in Thailand

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Nymphs of the genus *Afronurus* Lestage, 1924 are usually the dominant heptageniid mayflies in Thai streams. The lack of revision of the genus *Afronurus* in Thailand led to this study of *Afronurus* in Thailand. The *Afronurus* nymphs were collected from different localities in each region of Thailand using hand-picking and D-frame net methods. The specimens were preserved in absolute ethanol and identified based on morphology. The eggs of mature nymphs were investigated using a scanning electron microscope (SEM). A total of 494 specimens were identified into four species (*A. rainulfiana*, *A. rubromaculata*, *A. gilliesiana* and *A. cervina*). The *A. rainulfiana* is widely distributed in Thailand. The most diverse region is in the western region including the Mae Klong watershed, the Salavin watershed and the Phetchaburi watershed which indicated the suitable habitat. The optimal habitat for *Afronurus* nymphs is the fast flowing area at the middle of the stream and the nymphs were usually found in cobble substrate that was covered by algae. The egg chorionic structure of *Afronurus* was useful for a comparison between these species.

Keywords: Mayfly, Heptageniidae, *Afronurus*, Diversity, Thailand

**Riparian Forest as Provider of Landscape Connectivity for Adult EPTs: An Overview  
Across European Regions**

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Most adult EPTs (Ephemeroptera, Plecoptera and Trichoptera) disperse by flying upstream from their emergency site, following the water stream. Although it is generally assumed that riparian vegetation can facilitate the dispersion of many species, this relationship has not been properly characterized yet. The effects of riparian vegetation on landscape connectivity for EPTs can be assessed by analyzing differences in the EPT community structure: highly connected sites will host communities with a large proportion of weak dispersers and *vice versa*. Using the community structure to assess the relationship between riparian vegetation and landscape connectivity, allows for large scale analyses. In this research project, we first selected the most reliable method to assess the dispersion capacity of EPT communities. To do so, we compared the consistency and precision of several functional indices using experimental data. Then, we determined which environmental parameters, including the riparian forest, can predict the dispersion capacity of the EPT community. We confirmed a direct positive effect of riparian vegetation on EPT dispersion under certain conditions, and we also determined the most suitable spatial scale to apply our methodology. In the next step, we extended the analysis to different European biogeographical regions applying the conclusions from the previous study, aiming to find differences among distant geographical areas. Finally, we will try to predict changes in EPT phenology, as emergency periods, triggered by the increase or river water temperatures across the European continent, and how those changes could be ameliorated by riparian forest.

Keywords: Aerial Dispersion, Riparian Vegetation, Functional index, Community Composition, Europe

Status Assessment of Illinois Watchlisted Ephemeroptera, Plecoptera, and Trichoptera  
Species in Support of State Wildlife Action Plan Objectives

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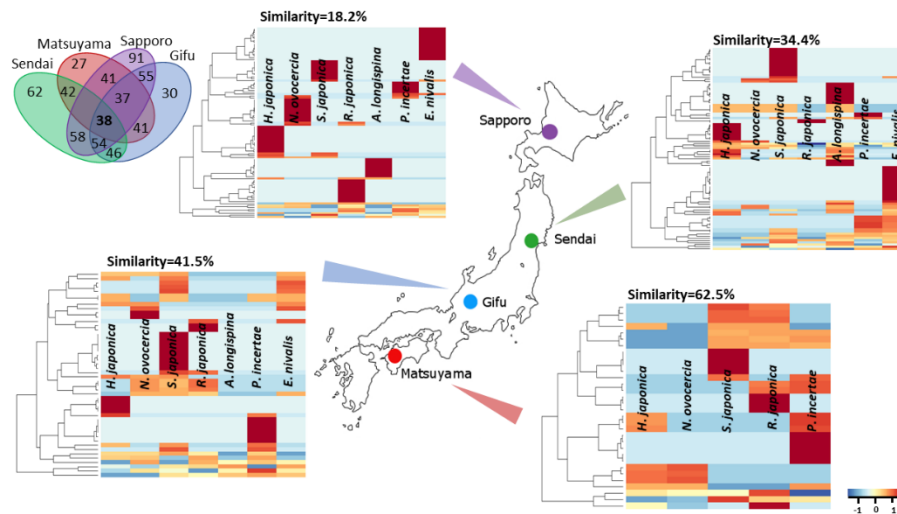
Illinois Ephemeroptera, Plecoptera, and Trichoptera (EPT species) are relatively well known due to state monographs, recent extensive collections, and vast, digitized research collections at the Illinois Natural History Survey (INHS). The Illinois Wildlife Action Plan (IWAP) seeks to protect a “Watchlist” of 72 EPT species in its IWAP: E: 29, P: 24, T:19. Objectives are to sample historical and new locations, define habitat requirements, upgrade status to no concern or NatureServe state “S” rankings. Sampling focuses on adults, larvae where necessary, in multiple seasons keeping all EPT bycatch. Museum specimens were examined to find more locations and verify identifications. Limited DNA barcoding was used to help identify species, associate unidentifiable immatures, and build the barcode library. Sampling through May 2022 yielded , completing 407 collecting events at 258 unique locations. Numerically, 421 EPT are known from Illinois, we have collected 288 species, including nearly 20 new state records. To date, we confirm the presence of 44 of 72 Watchlist EPT species in Illinois, refute the suggested extirpations of the stoneflies *Attaneuria ruralis* (Perlidae) and *Isogenoides varians* (Perlodidae), report the extirpation of several large river species of *Hydropsyche*, and suspect that six of 9 Watchlisted Ephemerellidae species to have been lost from the state. Our efforts identify many more EPT worthy of SGCN status, including new state records. Establishment of state level conservation ranks (SCGN and S ratings) is planned for Watchlist and several other EPT species during summer 2022.

Keywords: Aquatic Insects, Conservation Status Assessment, EPT Species

## Parallel Gene Expression Changes Reveal Genetic Plasticity Induced by the Temperature Gradient in Seven Stonefly Species

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Latitudinal variation plays an important role in creating strong selection pressure for genomic variation that enables the survival and adaptation of organisms. By altering their patterns of gene expression, organisms can modify their adaptive potential to heterogeneous environmental conditions along a latitudinal gradient. Here, we investigated how seven stream stonefly species sampled from four geographical regions in Japan would differ in their responses to latitudinal variation by measuring gene expression (RNA-seq) differences within species and gene co-expression among species. Based on 622 differentially expressed genes, we observed a high species-specific gene expression at higher latitudes suggesting high adaptive potential in colder regions, whereas similar expression patterns among species at lower latitudes suggested that stronger environmental stress is occurring in warmer regions. Weighted gene co-expression network analysis (WGCNA) identified 22 genes with similar expression patterns among species, where respiration-related, metabolism, and developmental co-expressed genes acted in different environmental contexts along spatial scales leading to divergent adaptive responses to latitude. This study highlights the importance of considering multiple species when evaluating the consequences of environmental change on aquatic insect communities and possible mechanisms to cope with environmental changes.

Keywords: Gene Expression, RNA, Environmental Change, Adaptation

**Population Genetic Structure of Massively Emerging *Ephemera orientalis* (Ephemeroptera: Ephemeridae) in South Korea Inferred from Novel Microsatellite Markers**

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Mayflies are one of the key members constituting aquatic ecosystems with their diverse roles in freshwater communities as a consumer and also as a prey. A burrowing mayfly, *Ephemera orientalis* McLachlan, widely distributed across the Korean streams, has recently become nuisance aquatic insects due to repeated local mass emergences in urban areas throughout spring to late summer. Despite its ecological and environmental importance in the aquatic ecosystems, the level of genetic diversity and genetic structures of *E. orientalis* populations remains unknown in South Korea. Here, we developed novel polymorphic microsatellite markers using a next generation sequencing (NGS) technique. A total of 18 populations across South Korea including massively emerging populations were then analyzed by using the developed microsatellites. Information on the level of genetic diversity and genetic structure of the Korean *E. orientalis* populations will facilitate understanding the level of interpopulation differentiation, effective population size, migrations between populations and origin of mass emerging populations of *E. orientalis*. Seven newly developed microsatellite markers in the present study would assist to reveal the spatial and temporal patterns of population dynamics of *E. orientalis*.

Keywords: Burrowing Mayfly, Mass Emergence, Microsatellite, Population Genetics

**Ain't No Mountain High Enough – Studies on the Upland Summer Mayfly (*Ameletus inopinatus*)**

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The Upland Summer Mayfly (*Ameletus inopinatus*) is the only arctic-alpine mayfly species found in Great Britain. Previous research in Europe using climate models have shown that the geographical range of this species is likely to contract as water temperatures increase. As such, the Upland Summer Mayfly is particularly vulnerable to the impacts of climate warming. This study investigated populations of this species to establish its current distribution and to investigate the impact of temperature on its development. Surveys were undertaken at sites with historical records to determine whether populations persist there. The altitudinal limits of the species were also established at five sites, with one site visited a decade later to determine whether there was any change in the altitudinal range over the period. The impact of water temperature on the development of the Upland Summer Mayfly was also investigated. The species has apparently been lost from four out of five sites with historical populations. At sites with extant populations the altitudinal limit of the species ranged between 323 and 447 meters above sea level. The species has retreated to higher altitudes by 16 meters over a 10-year period. Development is closely linked to water temperature, with snow cover playing an important role in ensuring emergence occurs before the watercourse ceases to flow in the early summer. The results of this study confirm the vulnerability of the Upland Summer Mayfly to rising water temperatures and provides new information to help target mitigation measures to prevent further losses of this species.

Keywords: Climate Change, Water Temperature, Development, Conservation



## Current Knowledge of the Northern February Red (*Brachyptera putata*)

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The Northern February Red Stonefly (*Brachyptera putata*) is a British endemic species, now only found in rivers in the Highlands and north of Scotland. Previously there have been few studies focusing on this species, despite its conservation importance. This study reports on recent investigations and observations on the taxonomy, distribution, life cycle, ecology, and habitat of the species. A novel citizen science survey provides new information on the occurrence and flight period of the adults; the drumming call of the male is documented; and the phylogenetic position of the species is confirmed through molecular sequencing. Further knowledge gaps are identified, and recommendations made for future research on this species.

Keywords: Endemic, Drumming, Flight Period, DNA Sequencing

## Ecology of Groundwater (Amphibitic) Stoneflies

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Over 50 years ago *Paraperla frontalis* larvae were shown to exist in a shallow floodplain aquifer of a gravel-bed river in western Montana and later they were documented throughout the main stems of the Flathead River system. Larvae are almost never found in surface waters until they emerge on the river shorelines. As teneral adults, they mate and subsequently deposit fertilized eggs into the river in the usual stonefly fashion. This novel life cycle is termed “amphibitic.” Over the years we and others have found *P. wilsoni*, *Kathroperla perdita*, and 5 species of *Isocapnia*, including long-winged and brachypterous adults and wingless dwarfs (male and female), occupying amphibitic niches in the alluvial aquifers of rivers in Washington, Idaho, Colorado and British Columbia, all of which have glacial history. Recently, *P. frontalis* and multiple species of *Isocapnia* were discovered emerging from aquifers underlying afeis (landscape-scale ice cover) on the north slope of Alaska. Groundwater stoneflies are remarkably tolerant of hypoxia which allows them to exist as top consumers in groundwater food webs subsidized by ancient methane. Details of life cycles, trophic relationships, distribution and abundance have been documented by a suite of studies on the Nyack Flood Plain of the Middle Flathead River, Montana. In this paper we describe local and regional population structure and synthesize the ecophysiology and ecology of these novel stoneflies in the context of their functional role in gravel-bed river ecosystems.

Keywords: Groundwater Stoneflies, Ecology, Rivers, Plecoptera, Amphibitic Life Cycle

## Changes in the Community Structure of Ephemeroptera Species in a Biosphere Reserve in Turkey, According to Stream Zonation, Stream Order, and Altitude

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The study aims to reveal the changes in the species composition of Ephemeroptera species and to determine the species richness from upstream to downstream from various streams in Camili Biosphere Reserve in Turkey. Ephemeroptera species were collected from 23 sites in the Camili Valley in July 2019. As a result, 17 species belonging to 8 genera and 4 families were identified. The highest species richness was observed in epirhithron. The most dominant species were *Epeorus (Caucasiron) znojkoii*, *Baetis (Rhodobaetis) vadimi*, and *Rhithrogena decolorata*. The highest species richness according to altitudes was observed at 2,000m–2,500m a.s.l. The most dominant species in this altitude were *B. (R.) vadimi*, *E. (C.) turcicus*, and *E. (C.) znojkoii*. In addition, the changes in the relative abundance of each species in each stream zone and altitudes were also provided. The most abundant species in eucrenon and hypocrenon (2,000–2,500m a.s.l.) was *B. (R.) vadimi*. This species was replaced by *E. (C.) znojkoii* in epirhithron (1,000–2,000m a.s.l.). *Rhithrogena decolorata* was found to be the most abundant in metarhithron (below 1,000m a.s.l.). Our study brings the first information on the habitat preferences and species diversity changes along the altitudinal gradient in Camili Biosphere Reserve. Since the area is located in an important area in terms of biodiversity, our results create the basis for future hydrobiological studies in the region.

Keywords: Biodiversity, Habitat, Mayfly, Protected Area

*Phylogeny, Systematics, Taxonomy*

**Phylogeny of the Plecoptera: Past Challenges, Recent Developments, and Future Direction**

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Phylogenetic study of the Plecoptera has been a challenge for stonefly systematists for over a century. Numerous competing hypotheses supported by morphological and/or molecular evidence have shown incongruent or unresolved topologies. However, the advent of genomic and transcriptomic molecular phylogenetics has facilitated the generation of well-supported and fully-resolved phylogenies. Within the superfamily Perloidea, recent study has recovered a separate family level lineage for *Kathroperla* Banks, 1920, a genus historically included in the subfamily Paraperlinae within the family Chloroperlidae. Combined molecular and morphological evidence support elevation of *Kathroperla* to Kathroperlidae, the seventeenth family of extant Plecoptera. Other recent study has provided new phylogenetic insights into the superfamily Nemouroidea and the biogeographic history of all extant families. Additionally, mitochondrial genomes have been used to construct numerous well-supported family level phylogenies. Although recent studies provide new phylogenetic perspectives, incongruence between hypotheses still exists. A future global collaborative effort aimed at analysis of genomic and/or transcriptomic datasets generated from previous recent studies combined with newly generated molecular data from key taxa could provide a robust phylogeny of the world fauna.

Key Words: Plecoptera Phylogenetics, Stonefly Transcriptomes, Kathroperlidae

## **Higher-level Relationships of Ephemeroptera: Evolution, Phylogenomics, and Classification**

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Higher-level relationships among major mayfly lineages remains controversial and previous molecular and morphological data are not very well supported (especially along the back bone and nodes deep within the topology) and tend to contradict each other in certain parts of the mayfly tree of life. This has led to classification systems that may not reflect the evolutionary history of the order. This project represents the largest phylogenetic analysis for mayflies to date. Over 450 targeted genomic protein coding regions (exons) were generated using the novel hybrid enrichment probe set (Ogden et al., 2019) for nearly 150 genera representing the main ephemeropteran clades. The dataset was analyzed as DNA position one and two in the codons and as amino acid sequences. The results allowed us to test the monophyly of many of the proposed higher-level groupings and to initiate a conversation about a new classification scheme for the order. This study supports the conclusion that more taxa and data, especially phylogenomic data, result in more robust trees and represent a good option for future studies in mayfly phylogenetics and systematics.

Keywords: Phylogenetics, Taxonomy, Systematics, Evolution, Ephemeroptera

**Estimating Phylogeny and Tempo of the Antillean Colonization of Neotropical Hagenulina (Ephemeroptera: Leptophlebiidae): Did the GAARlandia Landspan Drive a Central Role?**

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Hagenulina is a diversified group encompassing 49 known species arranged in 11 genera, of which seven are Antillean restricted, two are South American restricted, and the remainder are recorded in both areas. Here, we integrated data from phylogeny, morphological clocks, living and fossil species, and biogeography to test the Hagenulina monophyly, and the relationship among the genera. In addition, we test if the Antillean colonization occurred at once through the time and if the GAARlandia landspan drove a central role in Antillean colonization. Our analyses indicate that Hagenulina is monophyletic and has a South American origin around the Cretaceous, starting its diversification around the Cretaceous-Paleogene transition when *Ecuaphlebia* diverged from the other Hagenulina. Considering the biogeography, our results indicate that Hagenulina dispersed from South America to the Antilles through several independent events. The role of a possible land bridge in this colonization process was not clearly evidenced, but, the temporal proximity of 5 million years between the estimated period for the GAARlandia and the age of the lineages that colonized the Greater Antilles does not allow us to fully refute this hypothesis.

Keywords: Mayflies, West Indies, Colonization, Morphological Clocks, Dispersal

[New Synonymy and Updated Range Alter the Conservation Conversation About the Louisiana Needlefly, \*Leuctra szczytkoi\* Stark & Stewart, 1981 \(Plecoptera: Leuctridae\)](#)

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The Louisiana Needlefly, *Leuctra szczytkoi* Stark & Stewart, 1981 (Plecoptera: Leuctridae) is a rarely collected stonefly known from four small, spring fed streams of the South-Central Plains Ecoregion (SCPE) of Louisiana. In 2010, the Center for Biological Diversity petitioned the US Fish and Wildlife Service for inclusion and protection of this species under the U.S. Endangered Species Act. In 2020, the authors were tasked with assessing the conservation status of the species. A possible synonymy with *L. paleo* Poulton & Stewart, 1991 and incomplete sampling of similar habitat in the SCPE of Arkansas, Louisiana, and Texas broadened our objectives to include sampling of adjacent states and conducting DNA barcoding (mtCOI) of all found populations to assess the taxonomic validity of *L. paleo* and that all specimens were *L. szczytkoi*. Maximum Likelihood analysis confirms that *L. paleo* is indeed a junior synonym of *L. szczytkoi*. Additionally, males, females and nymphs sampled across 17 locations in southern Arkansas, Louisiana, and eastern Texas all form a single species with geographic subdivision such that Arkansas and eastern Louisiana populations form one well supported clade and western Louisiana and eastern Texas populations another. Intraspecific variation ranged from 0.0 to 4.8% without obvious morphological differences. The SCPE is an old landscape with rolling hills and intervening lowlands consistent with infrequent dispersal events and population subdivision. Comparison to other *Leuctra* supports the monophyly of *L. szczytkoi*. This work points out the usefulness of integrated systematic assessment of species petitioned for protection under the Endangered Species Act so that taxonomic errors and incomplete distributional information are not encoded into legal structures that are difficult to change and that may impugn the process of protecting imperiled species.

Keywords: Distribution, Conservation, Natural History, Plecoptera, *Leuctra*

## Resolving Phylogeny of Indian Baetidae (Insecta: Ephemeroptera) using morphological characters

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Thorough taxonomic and phylogenetic study of mayflies in India is lacking. In India, Baetidae is the most speciose family. The purpose of this study is to investigate the phylogenetic relationships of family Baetidae using morphological evidence. A morphological dataset was generated using 42 larval species and 14 imago species of external characters. Using these data, a phylogenetic tree was reconstructed through the Neighbour Joining (NJ) method accompanied by bootstrapping. Additionally, Principal Component Analysis was implemented on the dataset to check how species cluster together in morphological space. The resulting tree inferred two distinct clades of *Labiobaetis* each of which were retrieved as two different clusters in the PCA. Furthermore, one clade of *Baetiella* species was also retrieved which clustered distinctly in the PCA. Although representatives from other genera were considered in the study, morphological data could not resolve the relationships among and within them. These results indicate that more resolution is required in the phylogeny and incorporating molecular data can possibly resolve them better.

Keywords: Morphology, Phylogeny, India, Baetidae, PCA



## Phylogenomics of Baetidae: A Comparison in Classification

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This study focused on the evolutionary relationships of the family Baetidae, one of the three largest families in Ephemeroptera. Baetidae is of phylogenetic interest because of its somewhat basal position in the order and its diverse and varied life histories and morphological characteristics. This analysis was carried out using Anchored Hybrid Enrichment from over 450 protein coding loci from 37 baetid ingroup taxa and five outgroup taxa. The data was analyzed as DNA (1<sup>st</sup> and 2<sup>nd</sup> positions in the codons) and as translated amino acid sequences in both maximum likelihood and Bayesian methodological frameworks. The results are compared to the recognized clades and classifications seen in previous phylogenetic proposals.

Keywords: Ephemeroptera, Phylogenetics, Anchored hybrid enrichment, Baetidae

## Cladistic Analysis of Terpidinae Kluge (Ephemeroptera: Leptophlebiidae)

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Terpidinae is restricted to the Neotropics and was originally proposed as a lineage of Atalophlebiinae including the genera *Terpides* and *Fittkaulus* (later, *Tikuna* was included). Currently, the subfamily contains 17 species, nine of *Terpides*, four of *Tikuna* and four of *Fittkaulus*. Terpidinae or the *Terpides* lineage were not proposed using a quantitative phylogenetic analysis and the internal relationships of the genera and species were never deeply analyzed. Our aim is to test the current classification of Terpidinae using a morphology-based cladistic analysis. To evaluate the monophyly and its internal relationships, 16 species of the subfamily were selected as in-groups, as well as six species of other subfamilies of Leptophlebiidae. A total of 80 characters were analyzed with the parsimony method and using implied weighting. The monophyly of Terpidinae was recovered and Castanophlebiinae formed its sister group, whereas *Tikuna* was recovered as a sister group of *Fittkaulus*. One polytomy was recovered with the species *Te. jessiae*, *Te. contamanensis* and *Te. sooretamae*. We found no autapomorphies for each of these species as well as for *F. cururuensis* and *F. amazonicus*. The diagnoses of all species were revised, illustrated, and interactive taxonomic keys for nymphs and male imagoes were proposed. The evolution of some selected morphological characteristics used a priori in the classification of the group were discussed.

Key words: Systematics, *Fittkaulus*, *Terpides* Lineage, Neotropic, *Tikuna*

**Bioinformatics Pipeline for Targeted Phylogenomic Data: A Case Study in Mayflies  
(Ephemeroptera)**

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Next Generation Sequencing (NGS) technologies are regarded as a highly accurate and cost effective method of DNA sequencing. Anchored Hybrid Enrichment (AHE) has been used across different taxonomic groups for phylogenomic estimation, including select mayflies (Miller et al. 2018; Ogden et al. 2019). The aim of this work was to develop a novel pipeline for analyzing anchored hybrid enrichment datasets that avoids problems with the readability and software availability that plague other modern pipelines. A test of the pipeline was given using around 250 mayfly taxa from raw Illumina NGS reads to a complete phylogenetic analysis.

Keywords: Phylogenetics, Bioinformatics, Phylogenomics, Ephemeroptera

## Systematic Classification of Plecoptera from Southern China

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Southern China is one of the regions where stoneflies are widely distributed. Before this study (2019), a total of 132 species, belonging to 29 genera and 5 families of 3 superfamilies of Plecoptera were recorded from Southern China, and the research scope was concentrated in Guangxi. Furthermore, the important identification characteristics of some species in this area, such as aedeagus and aedeagal sac, remained unknown so far, resulting in a serious problem in the identification of poorly understood and closely related species. Some type specimens have been lost, and the species were not recollected. In order to improve the taxonomy of Plecoptera in Southern China, traditional morphological classification was used for systematic classification during the present study. Now a total of three superfamilies in two infraorders, including 31 genera of 6 families, representing 173 species for the suborder Arctoperlaria of Plecoptera have been reported from Southern China. These included 38 recently described species and three undescribed species; Chloroperlidae and Peltoperlidae were recorded from Guangxi and Guangdong for the first time, respectively; three genera and one genus were recorded from Guangxi and Guangdong for the first time, respectively; five new Chinese records and twenty-one new provincial records were also reported. This research described the relevant morphological characteristics in detail and provided illustrations for them, contributed improved taxonomic information for the region, and enriched our knowledge of the Chinese fauna of Plecoptera.

Keywords: Morphology, New Species, Plecoptera, Southern China, Taxonomy

## Contribution to the *Protonemura* Kempny, 1898 (Plecoptera: Nemouridae) of the Caucasus

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The genus *Protonemura* is one of the most diverse stonefly taxon in the Caucasian Region. All of the Caucasian species are endemic or subendemic, occurring only in the Anatolian and/or Alborzian ranges of the Caucasus. During five trips in 2015, 2018 and 2019, we collected Plecoptera in Armenia, Azerbaijan and Georgia. Yet only the materials from Azerbaijan were published, herein we detail the *Protonemura* species collected. Three new species are proposed on the basis of males, females and larvae: *Protonemura* sp.1 from Azerbaijan and Georgia, *Protonemura* sp.2 from Georgia and *Protonemura* sp.3 from Armenia and Georgia. Comments are given on the variability of the adults of *P. spinulata* Martynov, 1928 and *P. viridis* Balinsky, 1950 on the basis of Georgian specimens. The hitherto unknown larva of *P. aculeata* Theischinger, 1975, *P. bacuriana bacuriana* Zhiltzova, 1957, *P. oreas* Martynov, 1928, *P. spinulata* and *P. viridis* are described on the basis of association by pharate adults.

Keywords: Armenia, Azerbaijan, Georgia, New Species, Larval Description

**A New Species of *Leentvaaria* Demoulin (Ephemeroptera: Leptophlebiidae): A Long Awaited Addition to a Previously Monotypic Genus**

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*Leentvaaria* was established by Demoulin in 1966 as a monotypic genus based on nymphs from Suriname and then redescribed by Domínguez et al. (2001). The adult was described several years later by Salles et al. (2016). Supported by mouthparts characters associated with the filtering habitus, *Leentvaaria palpalis* was placed as a member of the *Hermanella* generic complex. The relationships in *Hermanella* complex were approached for the first time by Flowers & Domínguez (1991) and posteriorly by Sartori (2005), who suggested that *Hydrosmilodon* is paraphyletic because of the close relationship between *L. palpalis* and *H. gilliesae* [a view supported by Salles et al. (2016) while describing the adults of both species]. Approximately 56 years since the genus proposition, the imago stage of a new species of *Leentvaaria* is described based on male and female imagoes collected using ultraviolet light traps from São João stream, district of Taquaruçu, Palmas (Tocantins state), northern Brazil (10° 23' 35" S, 48° 7' 47" W). *Leentvaaria* sp. nov. is diagnosed by characteristics shared with *L. palpalis* and *H. gilliesae*, such as fore wing venation, distance between male compound eyes and the presence of wide projections on the styliger plate; and by unique ones, such as small body size (fore wings: 3.3—3.6 mm), body color pattern, and the morphology of the penis lobe and styliger plate projection. The description of this new species will help in understanding the relationship between the genera from the *Hermanella* complex and the generic limits of this clade within the group.

Keywords: Taxonomy, Neotropical region, *Hydrosmilodon*, Brazil, *Hermanella* Complex

## Updates on the *Neoperla* Needham, 1905 (Plecoptera, Perlidae) from the Philippines

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One of the Sustainable Development Goals of the United Nations is to conserve and protect freshwater-related ecosystems such as rivers, lakes, aquifers, and wetlands while at the same time improving their water quality (SDG 6). Essentially, part of this initiative encompasses biodiversity discovery of aquatic life in these habitats. Plecoptera (stoneflies) are a group of aquatic insects known for its intolerance to organic pollution and their presence and abundance are important in rapid assessments of water quality in rivers and streams. However, stonefly research in the Philippines dealing with their taxonomy and ecology has been scarce over the past decades. In this study, we present the updated checklist of *Neoperla* species including the recently described *N. mindoroensis* (Mindoro) and *N. pangantihoni* (Palawan), putative new species *Neoperla* sp. (Mindanao), and newly recorded *N. atripennis* (Camiguin). Four other species, namely *N. recta* (Mindanao), *N. oculata* (Mindanao), *N. sabang* (Palawan), and *N. salakot* (Palawan), were also recorded from additional localities in Mindanao and Palawan. Morphological examination of diagnostic characters such as aedeagi and eggs were done using compound and scanning electron microscopes (SEM), respectively. Additionally, a TCS haplotype network and a Kimura-2-parameter genetic divergence analysis were performed to distinguish each species and to associate female and male adults is recommended that additional efforts are done in the collection and identification of Philippine stoneflies using integrative taxonomy to further advance the knowledge of their systematics, zoogeography, and ecology.

Keywords: COI, Integrative Taxonomy, New Species, SEM, Stonefly

### [A new \*Baetis\* \(Ephemeroptera: Baetidae\) from Tamil Nadu, India](#)

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As part of the ongoing research in the southern Western Ghats, a new species of *Baetis* Leach is described based on nymphs collected from the Western Ghats, Southern India. This new species does not belong to any of the *Baetis* species groups proposed so far. It is most closely related to *B. collinus* Müller-Liebenau & Hubbard. However, the new species is distinguished from *B. collinus* by the presence of tergalius I, the presence of postero-tergal spines on tergum IX, and by the size of labial palp segment III. The new species is compared to other Oriental species.

Keywords: *Baetis collinus*, New Species, Tamil Nadu, Western Ghats



**Evolution of Filter-Feeding in Aquatic Insects Dates Back to the Middle Triassic: New Evidence from Stemgroup Mayflies (Insecta: Ephemeroptera) of Grès à Voltzia, Vosges, France**

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Within aquatic insects, mayfly nymphs have developed a remarkable diversity of life strategies and feeding habits. However, early evolution of these strategies and the role of mayfly nymphs in freshwater palaeoecosystems remain largely unknown. We investigated fossil mayfly nymphs from the middle Triassic of Grès à Voltzia formation, France, which represents the oldest known outcrop including a diverse mayfly fauna. We focused on three species, namely *Mesopteropteron longipes*, *Vogesonympa ludovici* and *Triassoephemera punctata*, and investigated their original type material along with numerous previously unstudied specimens. As a result, we provide a new interpretation of their morphology, life history, and systematics: The nymphs of *M. longipes*, *V. ludovici*, and *T. punctata* were aquatic and at least the former two species undoubtedly lived in flowing water as passive filter-feeders. These findings represent the earliest occurrence of this feeding strategy in aquatic insects. Most obvious morphological adaptations to this lifestyle are long filtering setae arranged in rows on forelegs, analogous to some modern mayfly nymphs. We place the three investigated species *M. longipes*, *V. ludovici* and *T. punctata* within a newly established clade, representing the sister group to Heptabanchia (Coxopteroptera + Ephemeroptera). *M. longipes*, *V. ludovici* and *T. punctata* share a single-segmented tarsus synapomorphic with Heptabanchia, but also retain the plesiomorphic condition of double tarsal claws. They are also characterized by their autapomorphic lack of tracheal gills, probably connected to their life in flowing, well-oxygenated water.

Key words: Fossil, Life History, Mesozoic, Nymphs, Systematics

## **A Review of Asian *Habrophlebiodes* Species (Ephemeroptera: Leptophlebiidae)**

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The genus *Habrophlebiodes* Ulmer, 1920 is found in North America and Asia. In the latter area, four species have been described, three from China, but their exact morphologies have never been photographed. Upon types and specimens, all four Asian species are compared in this study morphologically and molecularly. The combined results show males of four *Habrophlebiodes* species can be separated easily upon their genitalia but their nymphs are very similar, which can be identified only by the tiny structures, like abdominal color pattern, labrum and prostheca. The Chinese Taiwan species *H. tenella* Kang & Yang, 1994, which is known from nymphs, is close to the mainland species *H. zijinensis* You & Gui, 1995 although their genetic distance is higher than that of different populations of the latter. The descriptions and photos of this study provide excellent information for further mayfly taxonomy of Asian Leptophlebiidae.

Keywords: Morphology, Genetic Distance, Mayfly, China, Taxonomy

## Poster Presentation Abstracts

### *Biogeography, Phylogeny*

#### 16s Variability and Distribution Modeling of *Andesiops peruvianus*

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*Andesiops peruvianus* (Baetidae) is widely distributed in the Andean region. A previous work with specimens from Colombia found out, based on molecular and morphology, that *A. peruvianus* is a complex of pseudocryptic species. We show the preliminary result of our study which aimed to reveal the phylogeographic pattern of *A. peruvianus* in Argentina Northwest (NW). We analyzed 31 16S' sequences to explore genetic diversity and its geographic pattern. Additionally, we compared these sequences with sequences from Ecuador (4 seq.) and Bolivia (1 seq.) to explore genetic divergence. Finally, we performed a niche modeling to describe the habitat preference of this species. Our results showed a great genetic divergence among haplotype from Ecuador, Bolivia and Argentina. Within Argentina, we found a low genetic diversity (7 haplotypes), however, an unexpected high genetic divergence was found between localities belonging to the same ecoregion (Puna). The niche modeling revealed a clear difference in habitat preference between Ecuador specimens to those from Bolivia and Argentina. These preliminary results suggest that *A. peruvianus* may be a larger species complex than expected. In addition, *A. peruvianus*' populations may exhibit a highly genetic structure in Argentina NW.

Keywords: Ephemeroptera, *Andesiops*, Phylogeography, Ecological Niche Modeling

**Diversification in Caucasian *Epeorus* (*Caucasiron*) Mayflies (Ephemeroptera: Heptageniidae) Follows Topographic Deformation Along the Greater Caucasus Range**

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The evolution and distribution of mountain biota are closely linked with mountain formation and topographic complexity. Here we explore the diversification of cold-tolerant mayflies of the subgenus *Epeorus* (*Caucasiron*) in the south-eastern Greater Caucasus, an area of dynamic changes in topography since the Miocene, driven by the convergence of the Greater and Lesser Caucasus. We hypothesized that orogenic processes promoted the diversification of *E. (Caucasiron)* and that the resultant diversification pattern followed the timing and progress of mountain formation along the range. A new evolutionary lineage of *E. (Caucasiron)* distributed in the central-eastern Greater Caucasus was found and described as *Epeorus (Caucasiron) tripertitus* sp. n. It consists of three clades clearly differentiated based on mitochondrial sequence data, but indistinguishable by morphological traits. Based on a time-calibrated phylogeny using mitochondrial (COI, 16S) and nuclear (EF, wg, 28S) markers, we found that a gradual allopatric diversification of the ancestral population of *E. (C.) tripertitus* sp. n. dated to a period lasting from the late Miocene to the Pleistocene spread eastward along the range. This pattern corresponded with the process of topographic deformation which started in the central part of the range in the Miocene and progressed to the east during the Pliocene/Pleistocene. The results implied the dominant role of mountain building on the biotic diversification of this region and continuing recent speciation in the south-eastern part of the mountains.

Key words: Caucasus, Phylogeny, Speciation, Orogeny, Aquatic Insects

## Biogeographical History of Baetidae Family: Events and Speciations

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Baetidae Leach was one of the first families of mayflies established. It is now the most taxon-rich family in the order, with approximately 110 genera and 1,100 species constituting a quarter of the world's mayfly diversity. Baetids inhabit diverse freshwater habitats around the world with the exception of Antarctica, New Zealand, and some oceanic islands. The worldwide distribution of Baetidae is reflected in its long history of biogeographic processes difficult to observe and evaluate. We examine spatial diversification patterns in the family taking into account their phylogenetic relationships and their explicit distribution. We used a phylogeny of the family Baetidae with 158 species distributed in 96 genera of the family and 245 morphology characters. We analyzed with Geographically explicit Event Model (GEM) the ancestral habitats and distributional areas. The results show that the founded event is the main driver in the diversification of the taxon followed by vicariance and sympatry. The main event in the origin of the family was the vicariance between Siphlaenigma and Baetidae. The vicariance is frequent in the origins of higher lineages (Baetidae clade, Baetinae clade, 'Cloeninae' clades). The present ancestral distribution of the Baetinae is now known as South American-African continents (Gondwanan). The founder events are higher frequency in small lineages of Baetinae, and many clades with close relationships between South American/African lineages and fauna from all other biogeographical regions. While most 'Cloeninae' clades present East African and Southeast Asia as ancestral distribution, and close relationship between African-Eurasian fauna, with many founder events; except Cloeon-complex, which present vicariance (between Africa, Europe, Asia, and Nearctic) and sympatry as the most significant events.

Keywords: Biogeography, Speciation, Baetidae, GEM, Distribution

*Biology, Behavior, Reproduction***Drumming Signals of Nearctic Stoneflies (Plecoptera): 1977-2015**Luke William Myers <sup>1</sup>, John Burton Sandberg <sup>2</sup>

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The earliest Nearctic stonefly drumming descriptions are over 40 years old, yet a comprehensive summary remains unfinished. This report on 279 drumming descriptions and experiments from 38 studies provides comparative summaries of signal characters and descriptions for 64 genera and 154 species. Two hundred eighteen drumming descriptions from 128 species are unfinished and include descriptions of only the male call, descriptions of only the female answer, or lack a complete interval pattern description. One hundred ninety-five interval pattern descriptions for 107 species are 1) incomplete: reporting a range of means and standard deviations or 2) lacking: reporting only a single mean and standard deviation. We provide provisional descriptions for “incomplete” and “lacking” interval patterns estimated from the reported overall mean interval, standard deviation and range. Currently there are 41 distinct signal descriptions which are based upon the combination of signal pattern and interval pattern or rub count and rub duration characters. A comparative framework of drumming characters is proposed and includes eleven signal categories that group similar signals together. The framework ranks character states based upon assumptions of relative complexity and frequency of occurrence. A provisional family summary ranks Perlodidae (68 species and 24 distinct calls) and Perlidae (23 species and 18 distinct calls) with the highest signal diversity and complexity. However, the number of drumming descriptions between nine stonefly families varies considerably.

Keywords: Nearctic, Plecoptera, Vibrational Communication, Mate-Finding Behavior, Percussive And Non-Percussive Signals

**An Investigation of the Effects of Individual Differences and Temperature on the Drumming Signal Traits of the British Endemic Stonefly, *Perlodes mortoni***

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Stoneflies produce complex vibratory calls, generated via percussive abdomen movements, which are an essential part of mate recognition and detection. These vibratory calls can be short monophasic signals or long duets, initiated by a complex male signal, followed by an unmated female response and a second male signal, mimicking the female. In this study the calls of *Perlodes mortoni* were recorded and analyzed against the ambient temperature and the individual's morphology. To achieve that *P. mortoni* nymphs were collected and reared until maturation and vibrational calls were then recorded and analyzed. From each call the signal duration, number of knocks, frequency and inter-knock duration were collected along with the morphological measures: body weight, and the body length, abdomen length and width and head width were also measured. The results demonstrated significant temperature-dependence. Furthermore, there was distinct variation between the morphological features and call characteristics, with heavier individuals having shorter inter-knock intervals. The female responded faster and was significantly more likely to respond to calls of higher frequency and shorter inter-knock intervals, which might be indicative of sexual selection for heavier males. However, further research is needed to confirm these findings.

Keywords: Vibrational Communication, Plecoptera, *Perlodes mortoni* Calls, Duets, Temperature-Dependence

## Histological Evaluation of the Digestive Tract of Two Species of Plecoptera with Different Feeding Habits During Their Life Cycle

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It is known that feeding habits of species of Plecoptera change throughout their life cycle. In the present study, a histological evaluation of the digestive tract was carried out in *Dinocras cephalotes* (Curtis, 1827) and *Isoperla grammatica* (Poda, 1761). In both species, nymphs are mainly predators, although they also ingest vegetal resources in the younger instars. In the adult stage, *D. cephalotes* almost does not ingest food, while *I. grammatica* feeds on pollen and other vegetal matter. For the analyses, individuals were fixed by immersion in Bouin's fluid for 48 hours and then embedded in paraffin, and cut into serials sections of 10 µm thickness. Sections were stained with hematoxylin and eosin. The histological evaluation showed that, in comparison with the nymphal stage, adults of *D. cephalotes* lack chitinous teeth in the stomodeum and have a reduced mesodeum volume with epithelial changes that could be related to fasting. Furthermore, a noteworthy higher proportion of adipose tissue around the digestive tract in the nymphal stage could cover energy demands during the subsequent adult fasting period. On the other hand, *I. grammatica* did not reveal histological differences between the nymph and the adult, possibly due to active feeding during both stages. In light of the results, it can be concluded that those differences in the digestive tract may be related with their different feeding habits during the adult stage. Further studies including more species with different feeding behaviors could increase our knowledge on this topic.

Keywords: *Dinocras cephalotes*, *Isoperla grammatica*, Digestive Tract, Feeding, Histology



*Distribution, Faunistics*

The Species Diversity of Genus *Baetiella* from the Chinese Mainland (Ephemeroptera: Baetidae)

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Seven *Baetiella* species were reported from China previously. In our collection, eight species were identified upon nymphal materials, two of them (*Baetiella ausobskyi*, *B. imanishii*) were first reported from this country. Their mouthparts are similar but thoracic and abdominal protuberances and terminal filaments are different. They show that China has high species diversity of the genus.

Keywords: China, *Baetiella*

## Korean Stoneflies (Plecoptera) with Five New Species and Three New Korean Records

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The stonefly diversity has been investigated more intensively from remote areas for the last several decades and the recorded number is increasing with approximately 4,000 species in the world. In the Korean peninsula, a total of 96 species, 38 genera, and 11 families of Plecoptera were cataloged by Kim et al. (1998), Ham (2009), and Hwang and Murányi (2015; 2020) including the following three species recorded only from North Korea: *Pictetiella asiatica* Zwick & Levanidova, *Claassenia radiata* (Klapálek), and *Xanthoneuria unimaculata* (Zhiltzova). In this study, five new species are described from South Korea with an additional three species are new to the Korean peninsula: *Kogotus* n. sp., *Skwala* n. sp., *Pictetiella* n. sp., *Pomoleuctra* n. sp., *Xanthoneuria* n. sp., *Pictetiella zwicki* Zhiltzova, 1976, *Haploperla maritima* Zhiltzova & Levanidova, 1978, and *Alloperla tiunovae* Teslenko, 2009. Material and distributional data, descriptions for new species, and diagnoses for newly recorded species are provided.

Keywords: Plecoptera, Stoneflies, New Species, New Korean Records, Korean Peninsula

## Ephemeroptera of Northern North America

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An updated checklist of mayfly species for northern North America was compiled as a byproduct of a periodical review of the conservation status of mayfly species in Canada, facilitated by the Canadian Wildlife Service for the General status of species in Canada program. The geographic coverage of the list includes Alaska, Greenland, Saint Pierre & Miquelon, and all of Canada. The list was created based on critical review of scientific literature and evaluation of databases maintained by institutional and private collections, the Canadian Centre for DNA Barcoding, and various Canadian government entities. A total of 348 nominal species were tallied for the region, and the jurisdictional distributions of each noted. Additional species are treated in terms of being historic misidentifications or as species likely to occur in the region based on their known geographic ranges globally. The species richness of the study region represents a little more than half of the North American species known north of Guatemala and Belize. The list for this large area will contribute to better understanding of biodiversity trends at the regional and planetary levels.

Keywords: Mayflies, Nearctic, Checklist, Biodiversity

***Anacroneuria* (Plecoptera, Perlidae) from Southern Yungas Cloud Forests (Argentina and Bolivia)**

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The genus *Anacroneuria* is the dominant group of stoneflies in the Yungas cloud forest (southern Bolivia and northwestern Argentina) a region known for its diversity and low accessibility. In Argentina and in this ecoregion in particular, *Anacroneuria* was little studied, therefore we aim here to know its species diversity (based on adult characters). We also explore useful larval characters for species recognition. We have collected adults using light traps and larvae were reared from different localities. Adults and associated larval exuviae were studied, photographed and drawn. Ten species were identified by the aedeagus of the males: *A. arrazayalensis* Orce, *A. handlirschi* Klapálek, *A. pakitza* Stark and Sivec, *A. tucuman* Stark, *A. saltensis* Froehlich and five other species, probably new to science. The larval and adult stages of five of these species were also successfully associated. Photographs of the holotype of *A. reedi* Navás (type locality: Tucumán) were also studied. Based on our results, new records and new stage descriptions are given. We propose a key to distinguish all the species from Yungas ecoregion.

Keywords: Taxonomy, Life Stages, Southern Yungas, Aquatic Insects, Stonefly

## Diversity of Ephemeroptera in South America

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The study of Ephemeroptera has increased enormously in the last few decades in South America. Many researchers in South America have been describing many new species and genera for this group in this region. We used spatial data to evaluate how much diversity is on a global scale. We identified endemic species for each country and studied how many species have been described in each South American ecoregion. We analyzed all the geographic records available for the taxon in South America (7,921 records); previously, we checked records and taxonomy identifications. The results indicate that 720 species are distributed in South America. The highest species richness is in Brazil (with 422 species), followed by Argentina (165) and Colombia (139). Endemism wise, the tendency is similar for Brazil, with 422 endemic species, followed by Colombia with 46 and Peru with 31. The Cerrado ecoregion is the most diverse one (132 spp), followed by Bahia Coastal Forests (104 spp) and Alto Paraná Atlantic Forests (100 spp). On the other hand, some regions are still not reflecting their expected diversity, such as Peru, Paraguay and Suriname. Based on the current data, we hypothesize that there is still a high mayfly diversity to discover.

Keywords: Endemism, Ecoregions, Ephemeroptera, Areas of Distribution, Records

**Peculiarities of Ecology and Phenology of Stoneflies (Plecoptera)  
on the Northern Slopes of the Central Caucasus**

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Species diversity of the amphibiotic fauna inhabiting watercourses of the northern slopes of the Central Caucasus (Terek River basin) is directly related to the diversity of the environmental factors that play a decisive role in the formation of the landscape-climatic zones here. The amphibious insects of the Caucasus evolved during the process of long-term formation of a hydrographic network associated with complex mountain-building processes; therefore, aquatic insects of different genesis meet in the region. The most interesting zone in terms of faunal diversity is the mountainous region. The aim of the work is to study the features of ecology and phenology of early spring species of stoneflies within the mountain and foothill zones of the studied region. Traditional methods of hydrobiological research were used: field observations with sampling of preimaginal and imaginal material. In the course of joint research a total of 46 stonefly species were confirmed for the region. As a result of phenological observations, the timing of adult emergence was specified. At the end of winter and early spring first species molt into adults: *Taeniopteryx caucasica* Zhiltzova, 1981, *Capnia arensi* Zhiltzova, 1964, *Leuctra fusca* Linnaeus, 1758. Other adult plecopterans appear in March-May: *Leuctra hippopus* Kempny, 1899; *L. uncinata* Martynov, 1928; *L. furcatella* Martynov, 1928; *L. tarnogradskii* Martynov, 1928; *Capnia nigra* Pictet, 1833; *C. arensi* Zhiltzova, 1964; *Capnopsis schilleri archaica* Zwick, 1984; *Zwicknia bifrons* (Newman, 1838); *Nemoura martynovia* Claassen, 1936; *N. cinerea* (Retzius, 1783); *Protonemura capitata* Martynov, 1928; *P. alticola* Zhiltzova, 1958; *P. aculeata* Theischinger, 1976; *P. vernalis* Zhiltzova, 1958; *P. brachystyla* Zhiltzova, 1988; *P. microstyla* Martynov, 1928; *Amphinemura trialetica* Zhiltzova, 1957; *A. mirabilis* (Martynov, 1928); *Brachyptera transcaucasica* Zhiltzova, 1956; *Taeniopteryx caucasica* Zhiltzova, 1981; *Pontoperla teberdinica* (Balinsky, 1950); *P. katherinae* (Balinsky, 1950); *Chloroperla zhiltzovae* Zwick, 1967; *Filchneria balcarica* Balinsky, 1950; *Isoperla bithynica* (Kempny, 1908); *Perlodes microcephala* (Pictet, 1833). Stoneflies are dominant litoreophilic group of hydrobiocenoses in freshwater streams of the Central Caucasus. Unlike the larvae of caddisflies and mayflies, they have an extremely low zero point for nymphal growth that lies slightly above the freezing point. The development of larvae continues at very low water temperatures, especially in species living in mountain streams and springs. Mature larvae of *Protonemura alticola* were found in the sources of the river Tseydon, at the point of exit from under the glacier, where the water temperature approached 0°C. Slightly downstream (temperature 2°C), imagoes of this species were recorded (Zhiltzova, 1955). The most widely represented spring species are stoneflies of the families Nemouridae, Leuctridae, Chloroperlidae. Common species for the studied watercourses are Taeniopterygidae, Perlidae, Perlodidae. Rare species are *Filchneria balkarica* Balin., *Capnopsis schilleri archaica* Zwick, *Capnia arensi* Zhiltz.

Keywords: Stoneflies, Plecoptera, Central Caucasus, Ecology, Phenology

**Unraveling the Global Habitat Availability Over Time and South American Freshwater  
Invasion Routes of the Mayfly *Cloeon dipterum***

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The *Cloeon dipterum* is the most widely-distributed and studied mayfly species and recently proposed as a new model to investigate insect evolution. The species is one of the rare cases of exotic aquatic insects due to its wide environmental tolerance and potential to spread beyond its native range. Its habitat suitability, invasion pathways, now and in the future, are aspects not yet studied. Ecological Niche Models were produced to current condition and two future scenarios (mitigation-SSP2-4.5 or business-as-usual-SSP5-8.5), with six algorithms, 40,562 occurrences, using 19 climatic variables, up to 2080. The results reveal no significant changes in potential areas of introduction or invasion pathways over time on a global scale. The perennial introduction areas are in Greenland, Iceland, Brazil, Argentina, South Africa, Australia, New Zealand, China, far east of Russia. The Argentinean introduced population has not yet occupied most of the suitable areas. Two routes of expansion were identified, Coastal from Argentinean and Uruguayan small streams on the coast, and Paraná-Paraguay comprising the area of influence of Rivers Paraná and Paraguay. Both routes contain areas of global importance for conserving freshwater biodiversity that covers 14 RAMSAR regions, Paraná-Paraguay watershed, and Chacoan and Pantanal biomes, the two of the most important wetland complexes of the world. Field surveys to detect its presence are imperative in areas around Montevideo in Uruguay, Bahía Blanca, and Rosário in Argentina, and Concepción in Paraguay. *Cloeon dipterum* has the time and conditions to be the most successful alien mayfly, representing a worldwide potential risk for native ecosystems.

Keywords: Alien, Exotic, Global, Colonization, Dispersal

**Inter-Annual and Habitat Variation of the Mayfly Assemblage in Grassland and Pine Afforested Headwater Streams (Córdoba, Central Argentina)**

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Mayflies are widely used as indicators of stream conditions since their presence and abundance are closely associated with changes in environmental characteristics. We aim to analyze the richness and abundance patterns of mayflies in headwater streams of the Ctlamochita River basin in central Argentina, where some sub-basins have been fully afforested with exotic pines. We sampled 3 grassland streams and 3 pine afforested streams in 2008, 2012, 2014, and 2015. We took Surber samples in riffles, and in 2012 we also sampled 2 additional habitats: pools and macrophyte patches. We found 9 mayfly genera. *Americabaetis* spp. was the most frequent and abundant while *Cloeodes* spp., *Farrodes* spp. and *Tricorythodes* spp. were the least ones, with a relative frequency < 3%. Richness and abundance were higher in grassland streams (ANOVA,  $p < 0.05$ ), but the abundance pattern of different mayfly genera varied. In 2012 mayfly abundance was higher in pools and macrophytes in the afforested streams (ANOVA,  $p < 0.05$ ), but it was similar among habitats in grassland streams. *Americabaetis* spp. dominated in macrophyte patches while *Baetodes* spp. and *Nanomis* spp. in riffles. *Caenis* spp. dominated in pools but it was also abundant in macrophytes in the afforested streams. *Leptohyphes* spp. dominates in riffles but it was also abundant in macrophytes in the grassland streams. *Callibaetis* spp. was only found in grassland streams in pools and macrophyte patches. Distribution patterns are influenced by microhabitat characteristics which may be conditioned by changes in riparian land uses.

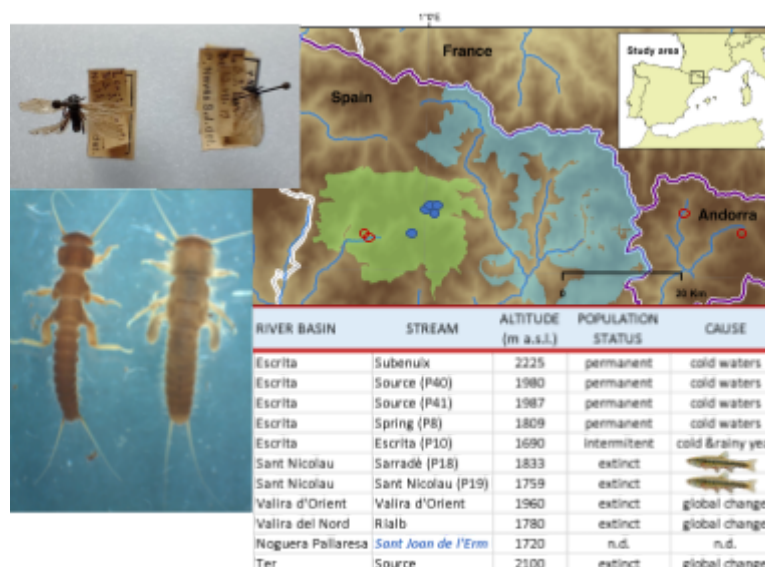
Keywords: Ephemeroptera, Habitat, Richness, Land Use, Exotic Pines



## Can *Pachyleuctra benllochi* (Navás, 1917) Survive in the South Face of the Pyrenees?

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*Pachyleuctra benllochi* Navás (1917) was described in the Catalan Pyrenees in an area of medium altitude (1,710—1,720 m A.S.L.), later two populations were located in brooks of Andorra and one near the source of the river Ter. Surprisingly, in an extensive study of the river basins of the Aigüestortes and Estany de Sant Maurici National Park (PNAESM), no populations of *P. benllochi* were found (Ventura, 1997). In order to understand the distribution of this species in the Pyrenees of Lleida, a long-term study was started in 2008 in different river stretches, including brooks and sources in the High Pyrenees. Some populations have been found only in the PNAESM, two in the Sant Nicolau River basin and four in the Escrita River basin. The two populations present in the main riverbeds are receiving their populations from sources located upstream, and these populations remain only during cold and rainy years. Since 2014, no nymphs have been captured in the stretches of the Sant Nicolau River basin in which they inhabited, due to the introduction of invasive predatory minnows populations by poachers. Currently there are 4 populations of *P. benllochi* on the southern slopes of the Pyrenees, of which only one has acceptable densities. If we take into account the problem of increased temperature and reduced rainfall predicted as a result of climate change for this area, we can conclude that the survival of this species is clearly in danger on the southern slopes of the Pyrenees.

Keywords: *Pachyleuctra benllochi*, Endemic, Spanish Pyrenees, Global Change, Invasive Minnows

## The Influence of the Geological Bedrock on the Mayfly Diversity in the Western Carpathians

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The distribution of aquatic insects is strongly influenced by the physical and chemical properties of water. Several papers have so far been published that focus on the effects of pH, alkalinity, conductivity, and other chemical substances in streams on the distribution of hydrobionts. However, stream chemistry, including the hydrological and temperature regime of running waters, is significantly affected by the geological bedrock. Crystalline streams, which are medium permeable, have a higher concentration of silicates. In contrast, limestone streams have higher calcium concentrations, alkalinity, and high rock permeability. However, there are very few publications that address this issue. In our work, we focused on determining the influence of the geological bedrock on diversity and distribution of mayflies. We selected a model area with three different geological bedrocks (crystalline, neovolcanic, and carbonate) situated within the same river basin in the Western Carpathians (Slovakia). Ordination analysis, non-metric multidimensional scaling, has shown formation of the three different mayfly taxocenoses associated with each type of geological bedrock. This is likely caused by differing alkalinity, silicate and calcium content, and altitude of bedrocks which influence the distribution of species. Neovolcanic bedrock, with *Baetis rhodani* (Pictet, 1843) and *Habroleptoides confusa* (Sartori & Jacob, 1986) characteristic for these streams, showed the lowest species diversity. The highest diversity was found in carbonate streams with characteristic species, *Baetis alpinus* (Pictet, 1843) and *Rhithrogena iridina* (Kolenati, 1859). The taxocenoses of crystalline streams were more similar to limestone streams with significantly different species richness.

Keywords: Carbonates, Silicates Streams, Biodiversity, Geological Bedrock, Distribution

*Phylogeny, Systematics, Taxonomy*

**Description of Adults of *Acentrella nadineae* (Baetidae:Ephemeroptera) with Notes on Rearing and Madicolous Behavior of Nymphs**

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*Acentrella nadineae* McCafferty, Waltz, & Webb, 2009 is broadly distributed in cool, clean, swift rocky streams across parts of the southeast, northeast, and midwestern United States (McCafferty et al. 2009; Webb and Burian 2017), but currently its adult stages are unknown. The difficulty associated with successfully rearing nymphs of *Acentrella* species is mostly responsible for our lack of knowledge of these life stages. New field observations of *A. nadineae* nymphs from the Hubbard River, CT/MA (State-line), USA were used to modify an already successful rearing system for baetid nymphs to obtain a reared series of male and female imagos for study. Both life stages were photographed alive and are described herein for the first time. In addition, details of the modified rearing system and notes on the unusual madicolous behavior of near final instar nymphs are presented.

Keywords: *Acentrella*, Taxonomy, Imagos, Rearing, Madicolous Nymphs

**The Nymph and Imago of *Nigrobaetis purpurata* from China (Ephemeroptera: Baetidae)**

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The species *Pseudocloeon purpurata* Gui, 1999 was described only from the male imago. We collected both imaginal and nymphal material from streams of Zijin Mountain of eastern China. Our examination confirms that this species belongs in the genus *Nigrobaetis* Novikova & Kluge, 1987. We provide a description of the nymph and a complementary description of the male and female imago.

Keywords: China, Baetidae, *Nigrobaetis*

## Association of Nymphs and Adults of *Massartella* (Ephemeroptera: Leptophlebiidae) Using Oological Characters

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The genus *Massartella* Lestage, 1930 (Leptophlebiidae: Atalophlebiinae) was erected to include the species *Atalophlebia brieni* Lestage, 1924. Currently, the genus contains five species, two known from southeastern Brazil, while the other are known from the Pantepuis. The nymphal and adult stages of the Brazilian species were not described and associated based on the same locality. Additionally, some morphological features show overlap between species, which may compromise their diagnosis, and the description of new species. This constitutes an obstacle to the taxonomy of the genus, requiring the discovery of additional characters. The objective of our work was to characterize the egg morphology of some species of *Massartella* and to test whether it can be useful to associate life stages and for interspecific discrimination. Eggs from female nymphs and adults of five species (*M. brieni* and four new species) were analyzed using Scanning Electron Microscopy and described. The eggs of *Massarella* are characterized by presenting a hemispherical shape, sculpted chorion with ridges or hemispherical protuberances, and attachment structures uniformly distributed in the chorion. We also found variation at a specific level in the chorionic crests, being regular or irregular; and the hemispherical projections varied in number and location. Given this interspecific variation, we were able to associate nymphs and female adults based on these characteristics. Other studies have highlighted the usefulness of oological characters in the identification and association of species. These oological characters may complement the characters conventionally used in the taxonomy of the genus.

Keywords: Atalophlebiinae, Taxonomy, Oology, Ultrastructural Characters

**A New Species, *Zwicknia* sp. n. (Plecoptera, Capniidae) from the Carpathians (Romania),  
Detected by DNA Barcode Data and Divergent Morphology**

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The DNA barcode region (658 base pair fragment of the 5' terminus of the mitochondrial cytochrome c oxidase gene) is now widely used in integrative taxonomy to support divergent morphologies in close related species, both for identification (DNA barcoding) and for description (DNA taxonomy). Capniidae is a small family of winter-active stoneflies (Plecoptera) of about eight species in the Carpathian area (Europe) with several endemic and overlooked cryptic diversity, weakly supported DNA taxonomy. Here we present morphological variability and DNA barcode data for a newly discovered *Zwicknia* Murányi, 2014 from the Bucegi Mountains, the Carpathians. The barcode sequence of this overlooked species is well differentiated from the available DNA sequences found in the international databases, based on the topology of the generated NJ tree and the *p*-distances. It is closely related to *Zwicknia kovacsi* Murányi & Gamboa, 2014, with a distance of  $p=3.72$ , and with visible morphological differentiation based on the structure of the main epiproct sclerite and the bicornuate process of tergite 9. As a result, the number of Capniidae species in the Carpathian area is raised to nine. Our findings support the Carpathians as one of the most important hotspots of diversity of aquatic insects in Europe, and further highlights that the faunistic and taxonomy data of the region is still incomplete.

Keywords: DNA Barcoding, Molecular Taxonomy, Cryptic Diversity, Carpathians, Aquatic Insects

**The Exact Morphology of *Serratella brevicauda* Jacobus, Zhou & McCafferty, 2009**  
**(Ephemeroptera: Ephemerellidae)**

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The species *Serratella brevicauda* Jacobus et al., 2009 is based on nymphs only. Detailed morphology and images of *S. brevicauda* show a stout, strong body, with remarkably short caudal filaments, maxillary palp, and the apical segment of the labial palp. In addition, the body of this species is very stout and strong, their caudal filaments have lateral setae on the apical half. It may be a species in another genus.

Keywords: nymph, mayfly, *Serratella*, generic position

**The First Description of the Nymph of *Potamanthodes macrophthalmus* and Reinstatements of the Genera *Potamanthodes* and *Stygifloris* (Ephemeroptera: Potamanthidae)**

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The Chinese endemic mayfly *Potamanthodes macrophthalmus* You, 1984 is the single species relating the taxon *Potamanthodes* Ulmer, 1920 to other generic taxa of the family Potamanthidae because it has larger compound eyes and longer penes than congeners. However, the nymphs remained unknown and its detailed imaginal morphology was incomplete until now. Upon newly collected material and type specimens, the main diagnostic characters of its nymph, which is described for the first time here, are revealed. This includes relatively large eyes, tiny mandibular tusks and remarkable body color pattern. In the imaginal stage, besides longer penes and larger eyes, the blunt apex of penis and shape of bent R<sub>1</sub> vein of hindwing are additional recognizable characters. Taxonomically, *Potamanthodes* presents unique characters, such as MP<sub>2</sub> originating at base of CuA in forewing, partially pigmented forewings, acute costal projection on hindwings of imagoes and a bristle-like setae row on nymphal fore femora. Therefore, *Potamanthodes* and *Stygifloris* Bae, McCafferty, and Edmunds, 1990 stat. rev. are reinstated as genera in this study.

Keywords: China, *Potamanthodes*, *Stygifloris*, revised status, nymphal description



## The Genus *Tyrrhenoleuctra* Consiglio, 1957 in the Iberian Peninsula: Behavioural and Molecular Approaches

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In this communication we present the results of a study of different populations of the morphologically cryptic genus *Tyrrhenoleuctra* in the Iberian Peninsula. A total of seven populations were analysed from a molecular and behavioral point of view. Two phylogenetic trees, maximum likelihood (ML) and neighbor joining tree (NJ), were constructed using both new COI sequences and COI sequences already available in GenBank. Furthermore, intra- and interspecific p-distances were calculated within and between sequences of the different populations. Regarding behavioral analyses, the male call of individuals from all studied populations were recorded, analyzed and described. Both phylogenetic molecular approaches used, ML and NJ, were congruent in discriminating five well-defined clusters, representing the four known Iberian *Tyrrhenoleuctra* species and a previously unknown taxon. The sequences from the seven studied populations produced in this study fell within two clades: *T. lusohispanica* (five populations) and the new taxon (two populations). These results were supported also by the behavioral study, in which the drumming calls of individuals belonging to the two clades differed in some important parameters such as the number of sequences, intersequence interval and interbeat interval. The obtained results confirm the wide distribution of *T. lusohispanica* and show the existence of a new species (under description) within this genus from a more restricted area.

Key words: Plecoptera, *Tyrrhenoleuctra*, DNA Analysis, Vibrational Signals, Spain

Notes on the Eggs of *Gripopteryx* Pictet, 1841 and *Tupiperla* Froehlich, 1969  
(Gripopteryginae: Gripopterygidae)

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The current state of the diagnostic morphological characters associated with females of Gripopteryginae is fragmentary and in some cases insufficient. Characteristics of the eggs of many taxa among Gripopterygidae had led to great contributions for their taxonomy. However, only a few works have studied and described the eggs of Gripopteryginae, mainly using optical microscopy observation. Thus, the search for differentiating morphological characters is necessary in this scenario. The present study aims at deepening the understanding about the ultrastructure of the eggs of Gripopteryginae by firstly examining the eggs of two species of *Tupiperla* [*T. robusta* Froehlich, 1998 and *T. tessellata* (Brauer, 1868)] and *Gripopteryx* [*G. garbei* Navás, 1936 and *G. cancellata* (Pictet, 1841)]. The eggs were removed from adult females, mounted on metal stubs, dehydrated, covered with palladium/gold and observed with a scanning electron microscope. We observed interspecific and intergeneric variation in shape and ultrastructure of the chorion, which presents hexagonal forms in *Tupiperla* and it is reticulated in *Gripopteryx*. The variation of shape and size agrees with the description of Froehlich (1969; 1990) for the genera. This study provides preliminary results about the taxonomy of this subfamily, as well as the first SEM images of the eggs of *Gripopteryx* and *Tupiperla*. The eggs of the *Tupiperla* species are described for the first time.

Keywords: Taxonomy, Plecoptera, SEM, Internal Morphology

## Redescription of Five *Filchneria* Klapálek, 1908 (Plecoptera, Perlodidae) Species Based on the Type Material

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As part of the revision of the paraphyletic genus *Filchneria*, five species known since the last century (*F. balcarica* Balinsky, *F. mesasiatica* Zhiltzova, *F. irani* (Aubert), *F. kuenluensis* (Šámal), and *F. olgae* (McLachlan)) were redescribed based on types stored in museums in the Netherlands, Switzerland, Russia and newly acquired material. Redescription was illustrated with color photographs of the habitus, wings, head and pronotal patterns, eversible male paraproct lobes (as possible), male paraproctal sclerite, aedeagus, details of chaetotaxy, female subgenital plate, and egg morphology using digital cameras and SEM. The characteristic features of each species are revealed. The main differences were established in the male paraproctal sclerite shape, in the posterior margin of tergum 10, female subgenital plate, and the collar of the eggs. *F. kuenluensis* is distinguished from other *Filchneria* species in the shape of the paraproctal sclerite, which bears a small triangular protrusion along the inner edge closer to the apex, indicating a close relationship to *Perlodinella* Klapálek, 1912 and *Zhiltzovaia* Özdikmen, 2008. At the very least, evidence of an unknown egg is necessary to confirm the assignation of *F. kuenluensis* to one of these genera.

Key words: *Filchneria*, Redescription, Male, Female, Egg

Systematic Studies of Perlinae (Plecoptera: Perlidae), with Focus on Neoperlini and Genus *Neoperla*

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In *Neoperla*, the slender structure in front of the hemitergal callus is not a hermitergal process, but the front part of the hemitergite itself. It is caudally much wider, the discrepancy in width is an apomorphy of the genus *Neoperla*. Morphological data suggest a revised infrastructure of the genus: the polyphyletic *montivaga*-group is replaced by reinstated synonyms of *Neoperla* with the new status of subgenera, and one new subgenus. *Neoperla (Neoperla)* Claassen, 1905 (type: *Perla occipitalis* Pictet, 1841) includes all species with a spermathecal stalk. The nominal subgenus occurs in Africa, North America, and Asia. Species without a spermathecal stalk occur only in Asia, they are assigned to: *Neoperla (Ochthopetina)* Enderlein, 1909 (type: *Ochthopetina aeripennis* Enderlein, 1909, = *Tropidogynoplax* Enderlein, 1910: 141; type: *T. fuscipes* Enderlein, 1909 = *N. aeripennis* (Enderlein), fide Klapálek 1923: 1779); *Neoperla (Formosita)* Klapálek, 1914 (type: *Formosina costalis* Klapálek, 1913, preocc. = *Formosita costalis* Klapálek, 1914); *Neoperla (Oodeia)* Klapálek, 1921 (type: *Oodeia dolichocephala* Klapálek, 1921); *Neoperla (Burmeisterina)*, subgen. nov. (type: *Perla luteola* Burmeister, 1839). The new subgenera are briefly characterized by apomorphies. Confirmed associations of genders of the Asian species are urgently needed for the necessary comprehensive revision of *Neoperla*.

Keywords: Morphology of Hemitergite, Infrastructure of Genus *Neoperla*, Reinstated Synonyms, *montivaga*-Group Replaced by Subgenera

## **Proposal for the 2024 International Joint Meeting on Ephemeroptera and Plecoptera**

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This group of Plecoptera/aquatic ecology scientists propose to host the next meeting in 2024. They propose an in-person meeting, with possibly a hybrid format allowing the opportunity for digital contributions. View the link from the title to see a short video presentation that proposes to host the meeting in late July to early August, 2023 in Turin, Italy.

**Proceedings of the 2022 XVI<sup>th</sup> International Conference on Ephemeroptera & XX<sup>th</sup> International Symposium on Plecoptera**

Boris C. Kondratieff and R. Edward DeWalt

Zoosymposium has again been chosen as the journal for our proceedings. Requirements for submission and publication of conference proceedings are outlined. Submission deadline, requests for peer-reviewers, open access page costs, and details of the selected journal are included. Information for authors can be found at [Zoosymposia](https://zoosymposia.org). Follow this guide carefully. Please send manuscripts to R. Edward DeWalt ([dewalt@illinois.edu](mailto:dewalt@illinois.edu)) and Boris C. Kondratieff ([boris.kondratieff@gmail.com](mailto:boris.kondratieff@gmail.com)) by **15 October 2022**. For review purposes we require a word document with any tables plus images plated as you envision them in pdf. Final images (in format and resolution requested by Zoosymposia) will be requested after acceptance. We will call on mayfly and stonefly scientists with expertise in the area study review manuscripts. Please contribute to this effort by accepting review requests and quickly returning constructive reviews. If we all follow instructions carefully, the proceedings can be in publication in January 2023.

