15th ISAO 2022

15th International Symposium on

Aquatic Oligochaeta

19-24 September 2022

Book of abstracts

Royal Belgian Institute of Natural Sciences, Brussels, Belgium

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15th International Symposium on Aquatic Oligochaeta

19-24 Sep 2022
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Welcome to the Symposium

On behalf of the Organising and Scientific Committees of the 15th International Symposium on Aquatic Oligochaeta (ISAO), I would like to welcome you all to the Symposium.

Since the first ISAO meeting was hosted by Ralph Brinkhurst in Sidney, British Columbia, Canada in 1979, our meetings have been held without interruption every three years. Just as the years seemed to be passing peacefully and our 15th ISAO was slowly coming together, the SARS-Cov-2 epidemic came suddenly into our lives, causing a complete disruption in our way of life.

But now, after going through this ordeal, we are finally here, four long years after our last symposium in Hirosaki, Japan, which was a great moment for all those who attended.

Although the virus is losing the battle, it still retains some resources that have prevented some of our colleagues from joining us, I am thinking in particular of our Chinese colleagues. Likewise, our Russian colleagues were looking forward to joining us, but the international tensions decided otherwise.

This 15th edition of the ISAO will finally take place with a smaller attendance than we had hoped for but, and this is probably the most important thing, it will allow us to maintain the spirit of fraternity that reigns among us, which has favoured the exchange of ideas between us and has always been the fertile ground on which our collaborations have started and grown.

The quality of the abstracts contained in this volume augurs well for our conference. I would particularly like to thank our colleague Rüdiger Schmelz for agreeing to moderate a debate on the taxonomy of oligochaetes in the 21st century, and to organise a workshop dedicated to the identification of a group that has put off many by its difficulty: the Enchytraeidae.

I wish you an excellent symposium.

Patrick Martin

The Symposium Host and Convener

15th International Symposium on Aquatic Oligochaeta

General information

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Mark J. Wetzel, Illinois Natural History Survey, Champaign, Illinois, U.S.A.

Venue

Welcoming Party

Date and Time: Monday, 19 September 2022, 18:30 – 20:00

Site: Brasserie "Volle Gas", Place Fernand Cocq 21, 1050 Ixelles – www.vollegas.be

Opening Ceremony and Main Symposium Sessions

Date and time: Tuesday, 20 - 24 September 2022, 09:00 – 17:00

Site: VIP Room, Royal Belgian Institute of Natural Sciences

Closing banquet

Date and time: Friday, 23 September 2022, 19:00 – 22:00

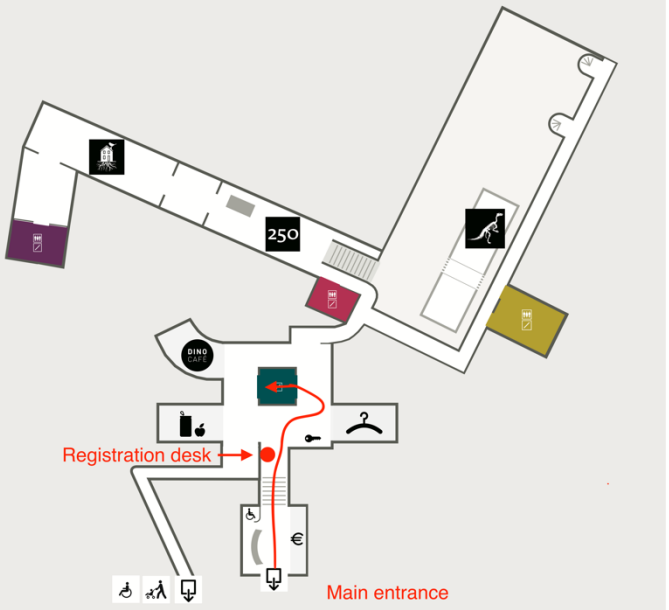
Site: Restaurant "Origine", Rue Général Leman 36, 1040 Etterbeek – origine-restaurant.be

Lunch

Site: Restaurant « Schieve Lavabo », 344 chaussée de Wavre, 1040 Etterbeek - schievelavabo.com/restaurants/jourdan

0

- Eten en drinken — Boire et manger — Essen und Trinken — Food and drinks
- Picknickplaats — Espace pique-nique — Picknickraum — Picnic room
- Biodivercity
- Vestiaire — Vestiaire — Garderobe — Cloakroom
- 250 jaar Natuurwetenschappen — 250 ans de Sciences naturelles — 250 Jahre Naturwissenschaften — 250 years of Natural Sciences
- Uitgang — Sortie — Ausgang — Exit
- Dinosauriërs — Dinosaures — Dinosaurier — Dinosaurs
- Museumbalie — Info et tickets — Museumskassen — Front desk



-1

- Galerij van de Mens — Galerie de l'Homme — Galerie des Menschen — Gallery of Humankind
- Toiletten — Toilettes — Toiletten — Toilets
- Dinosauriërs — Dinosaures — Dinosaurier — Dinosaurs
- Babyroom — Baby room — Wickelraum — Baby care room
- Doe en ontdek — Espace découverte — Entdeckungsraum — Discovery room
- Museumwinkel — Boutique — Museumsladen — Museum Shop
- Tijdelijke tentoonstelling — Exposition temporaire — Wechselausstellung — Temporary exhibition
- Auditoria — Auditoriums — Auditorien — Auditoria
- Mineralen — Minéraux — Mineralien — Minerals
- EHBO — Premiers secours — Erste Hilfe — First aid

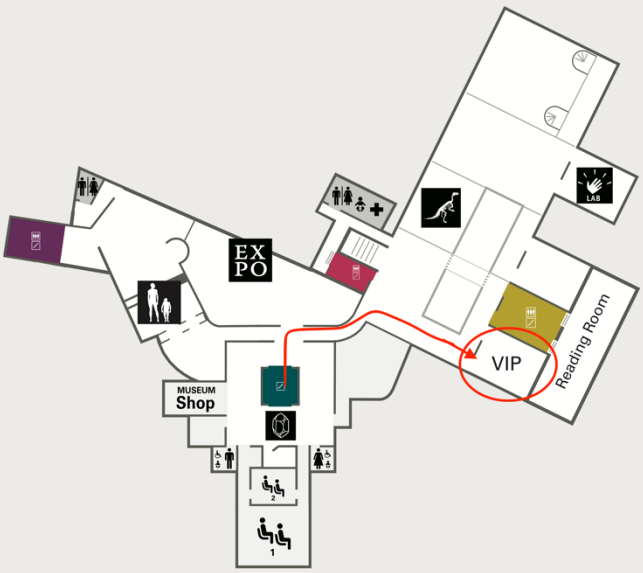


Table of contents

Biogeography

The discrepancy between host crayfish phylogeny and ectosymbiotic branchiobdellidan distribution in Japan; insights into historical co-dispersal from a molecular phylogeny perspective

Tomoaki Konno *† [1], Akifumi Ohtaka [2], Itsuro Koizumi [1], Stuart Gelder [3]

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[2] Faculty of Education, Hirosaki University – Hirosaki, Japan
[3] Department of Science, University of Maine at Presque Isle – Maine, United States

Genetically the Japanese crayfish, *Cambaroides japonicus*, show clear differentiations between populations of the western and eastern Hokkaido, while those in southern Hokkaido and Honshu Islands sometimes share the same haplotype. In contrast, the species composition of the ectosymbiotic branchiobdellidans found on Hokkaido and Honshu is different. Generally, the phylogeny and distribution patterns of a host and its symbionts are expected to be congruent, however, we found discrepancies between the distribution of branchiobdellidan species and that based on crayfish phylogeny. We hypothesize two possible reasons for this discrepancy: 1) branchiobdellidans may have unknown long-distance dispersal mechanism, and 2) random species extinction of branchiobdellidans may have occurred at each site after the co-dispersal with its host, while maintaining high species diversity.

We conducted a comprehensive sampling for branchiobdellidans on Hokkaido and northern Honshu Islands. Specimens were sequenced for mitochondrial COI and 16S rRNA and the results compared with host phylogenies and distribution. Phylogenetic analysis of the branchiobdellidans revealed the presence of independent clusters at the species level and suggest that the divergence age among species estimated from the COI was earlier than Japanese crayfish immigration to Japan. The phylogenetic relationship, within the *Cirrodrilus cirratus* complex sampled from Hokkaido, indicated significant localization in either eastern or western Hokkaido, and is consistent with the phylogenetic relationship and distribution of the host crayfish linage. In addition, the estimated divergence age of the two major branchiobdellidan groups was consistent with the divergence age of the host. These results were the same for *C. sapporensis*, having been collected in various regions along with the *C. cirratus* complex.

Results support hypothesis 2 and suggest that Japanese branchiobdellidan species had already speciated to the current level before Japanese crayfish immigrated to Japan and co-dispersion with the host is a major factor in defining the current distribution of Japanese branchiobdellidans.

Keywords: Branchiobdellida, Crayfish, Biogeography, Phylogeography, Japan

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Reassessment of the geographical distribution of indigenous branchiobdellidans (Annelida, Clitellata) ectosymbiotic on Japanese crayfish

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The Japanese crayfish, *Cambaroides japonicus*, is found on the islands of Hokkaido and northern Honshu in association with 12 species of *Cirrodrilus*, a genus of ectosymbiotic branchiobdellidans. To clarify the distributional ranges of these species, we examined museum-deposited specimens and collected branchiobdellidan assemblages from 68 locations on Hokkaido and 60 on northern Honshu. Molecular analyses (mt COI) were performed on a part of the specimens examined.

The present investigation found 10 morphologically distinct species; however, *Cirrodrilus cirratus* and *C. uchidai* were combined into a single taxon, *Cirrodrilus cirratus* complex, following molecular analyses in which the two taxa nested together in a phylogenetic tree. The following 6 species: *Cirrodrilus digitatus, C. sapporensis, C. nipponicus*,*C. ezoensis*, *C. homodontus* and *C. cirratus* complex were recorded from Hokkaido, while *C. aomorensis*, *C. tsugarensis* and *C. iwakiensis* were found on Honshu. The presence of *Cirrodrilus digitatus* (= *C. inukaii*) and *C. cirratus* complex (*C. uchidai*) on Honshu resulted from the translocation of crayfish from Hokkaido, but finding *C. tsugarensis* and *C. iwakiensis* on Hokkaido is currently inexplicable. No significant difference in the species composition was found between eastern and western regions on Hokkaido, where divergent lineages have been detected in the Japanese crayfish host. Morphological and molecular studies suggest that six species with dissimilar dorsal and ventral jaws form a monophyly, in which four species were found on Hokkaido and two on Honshu. The present reassessment supports the hypothesis that ectosymbiotic branchiobdellidans had undergone diversification into multiple species prior to the host's divergence on Hokkaido and northern Honshu.

Two species previously reported from Hokkaido, *Cirrodrilus megalodentatus* and *C. makinoi* were not found in the present study. It is possible that the two species are rare, or that they are intraspecific variants of the respective, similarly appearing *C. ezoensis* and *C. homodontus*.

Keywords: branchiobdellidans, distribution, Japan

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Extremophile phreodrilids? Revealing new phreodrilid diversity on granite outcrops in south-western Australia

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Phreodrilids mostly occur in cooler wetter parts of the southern hemisphere. Where they occur in warmer climes they are mostly found in mesic habitats such as at high altitude, deep lakes, groundwater or springs. It is thus anomalous that numerous new species are being discovered on isolated granite outcrops in the semi-arid to arid inland areas of southern Western Australia. These outcrops have uneven surfaces and shallow depressions are occupied by temporary rain-filled pools known as ngammas and moss dominated meadows growing on shallow soil, often in combination. These habitats support diverse invertebrate communities including species that occur only in this habitat. Phreodrilids occur in the gnammas and the meadows. After rains, primarily in winter, the pools fill and the meadows become saturated, but in summer both habitats dry completely with little moisture even in the meadow soils. This drying combines with summer temperatures that can reach 40°C below the meadow soils to create an extreme environment for oligochaetes. The granite surface is impervious and there are no obvious refuges from xeric conditions on or near the outcrops. This raises questions about how the worms survive such harsh conditions. The only described species, *Astacopsidrilus edwardi* Pinder 2003, was named for the late Prof. Donald Edward (The University of Western Australia) who collected four species while searching for chironomids in the same habitat in 1995. Two additional species have been collected since 2019. Each species is known from only 1 to 3 outcrops and only 22 out of many thousands of outcrops have been surveyed for worms.

Keywords: Diversity, Phreodrilidae, Australia

*Speaker

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A database on aquatic Oligochaeta

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An electronic database "World Distribution of Aquatic Oligochaeta" is currently in preparation. It will be completed in 2023 but is already available in Internet. It is presenting all published finding cases of microdrile oligochaetes (up to 150,000) together with their literature sources, geographical coordinates, and distribution maps. An updated systematic list of taxa is also given. For the latter, the order level of Oligochaeta system was recently improved by Schmelz et al. (2021).

Schmelz, R. M., Erséus, C., Martin, P., van Haaren, T. & Timm, T. (2021). A proposed order-level classification in Oligochaeta (Annelida, Clitellata). *Zootaxa*, 5040: 589-591.

Keywords: Biogeography, database, systematics, Oligochaeta

[*]Speaker
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Community Ecology

Aquatic oligochaetes species from River Tigris Sediments / Iraq

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Samples of aquatic oligochaetes were collected from sediments of 10 sites along the middle sector of River Tigris / Iraq for a period from November 2018 to December 2019. Species of tubificid worms (subfamily Tubificinae) were morphologically identified from a total collection of 7556 specimens, with *Limnodrilus* spp as the most abundant species (74% of the total individuals), especially *L. hoffmeisteri* Claparède,1862, and *L claparedianus*, Ratzel,1868, in addition to individuals of *L. cervix* Brinkhurst, 1963 , *and L. udekemianus* Claparède 1862. Other tubificid worms, with hair chaetae were identified as *Aulodrilus pigueti* Kowalewski,1914; *Embolocephalus velutinus* (Grube, 1879) ; *Potamothrix bavaricus* (Őschmann, 1913) and *P. hammoniensis* Michaelsen 1910.; *Psammoryctides albicola* (Michaelsen, 1901)*; P. barbatus* (Grube,1891)*; P. moravicus* (Hrabě, 1934)*; and Tubifex tubifex* (Müller, 1774).Two species of the subfamily Rhyacodrillinae, were also identified as *Branchiura sowerbyi* Beddard, 1892, and *Rhyacodrilus coccineus* (Vejddovský, 1875), and two species of the subfamily Naidinae, *Amphichaeta leydigii* Tauber, 1879, and *Slavina appendiculata* (d' Udekem**,** 1855), in addition to *Pristina sima* (Marcus, 1944) (subfamily Pristininae). *Hemifridericia* sp. (Nielsen & Christensen, 1959**)** of the family Enchytraeidae was recorded from one site only during November, and identified by conserved 18s rRNA encoding gene sequence. The query sequence was 96.13% identical to *Hemifridericia parva* Nielsen & Christensen, 1959.

Keywords: Aquatic Oligochaeta, tubificid worms, River Tigris, Iraq

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Composition and abundance of oligochaete in large Scandinavian lakes in a morpho-edaphic index framework - in the 1970s and 50+ years later. An indicator system that holds

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[2] Water and Environment, Swedish University of Agriculture Sciences – Sweden

We know that bottom-living oligochaetes and chironomid larvae thanks to their supposedly integrative power could provide more robust measures of the water quality than separate chemical data. In Milbrink (2020) it was shown that precision increases greatly if the relationship between oligochaete species in stead could be demonstrated within a morpho-edaphic index framework (Ryder et al. 1974). The percentage composition and total abundance of oligochaetes per sample in a number of large lakes or selected basins of large lakes in southern Sweden and Norway were thus tested against this index in the 1970s (Milbrink, 1978). In logarithmic scales abundance values turned out to be more or less linear. Since the late 1970s we today know much more about the ecological preferences of oligochaete species and their characteristic species associations. The oligochaete fauna of the large lakes of southern Scandinavia has been studied in detail over many years - in Lakes Vättern, Vänern and Mälaren for more than 100 years and in Lake Hjälmaren for over 50 years (Milbrink, 2020). The trophic change which had taken place in Lake Vättern was illustrated graphically and discussed in this work. A much similar trophic change could also be seen in Lake Vänern (the basin of Norra Värmlandssjön), as well as in the more eutrophic Lakes Hjälmaren and Mälaren. We simply have a situation of oligotrophication thanks to advanced sewage treatment since the 1960s. With our new knowledge it comes natural today to study changes in species composition after trophic change and to investigate if the above close relationships in abundance are still largely linear. It was so for Lake Vättern. Here we could demonstrate that the same is true also for Lakes Vänern, Hjälmaren and Mälaren. In a time when efforts to save the environment often fail, this is a real success story.

Keywords: oligochaete communities, morphology, edaphic frame, work, indicator system

*Speaker
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Impact of drying on small streams in the Czech Republic: are aquatic clitellates more affected by the duration or periodicity of the dry phase?

Jana Schenková *† [1], Michal Horsák [1], Marek Polášek [1], Petr Pařil [1]

[1] Masaryk university, Faculty of Science, Department of Botany and Zoology – Kotlářská 2, 61137 Brno, Czech Republic

Climate change in Europe predicts an increasing frequency of droughts, even for areas with a temperate continental climate. The Czech Republic is geographically located in the center of Europe on the watersheds of the Elbe, Danube, and Oder Rivers, where precipitation is almost the only source of water. In this study, we examined 25 small streams evenly distributed along the gradient of the flow intermittence in the Czech Republic. Aquatic clitellates were sampled between 2013 and 2016 in spring (March-April) before the expected dry phase and in autumn (September-November) after the dry phase in the case of intermittent streams. We observed that the community exposed to both irregular and periodic drying, following extreme summer conditions, could maintain local species diversity. However, significant differences in clitellate species composition were observed between perennial streams (indicated by the rheobionts *Nais alpina* and *Propappus volki*), irregularly drying streams (indicated by the interstitial species *Rhyacodrilus coccineus* and predator *Erpobdella vilnensis*), and intermittent streams, having representatives of Lumbricidae and Enchytraeidae as indicators. The effect of drying was even stronger in intermittent streams, for which significantly lower beta diversity was observed compared to perennial streams. We found that the most important changes in clitellate composition were controlled by water temperature and geographic variables in perennial streams, while streams affected by drying were controlled by climate, with high mean July air temperature and low annual precipitation sums amplifying the effect. In the intermittent streams, communities shifted significantly towards semiaquatic clitellate species. The abundance of semiaquatic species was also affected by the periodicity of the dry phase, with their proportion predicted in the regression tree analysis to be 11% in perennial and irregularly drying streams, and 40% in periodically drying streams. These results may help define streams potentially vulnerable to drought caused by future climate change. Supported by P505/20-17305S.

Keywords: drying, beta diversity, semiaquatic species, climate change

*Speaker

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Performance of morphological and DNA-based methods to assess diversity of earthworms and enchytraeids at 25 permanent soil monitoring sites in Germany

Rüdiger Schmelz *† [1], Stephan Jänsch [2], Paul Henning Krogh [3], Daniela Alves [4], Tiago Natal-Da-Luz [4,5], Verónica Rojo [6], Adam Scheffczyk [2], José Paulo Sousa [5], Joaquín Vierna [6], Antón Vizcaíno [6], Jörg Römbke [2]

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[4] CloverStrategy Lda – Coimbra, Portugal
[5] Centre for Functional ecology – Department of Life SciencesUniversity of Coimbra, Portugal
[6] AllGenetics Biology SL – A Coruña, Spain

Communities of earthworms, enchytraeids and collembolans were investigated at twenty-five Permanent Soil Monitoring Sites in Germany, with the objective to compare the performance of three different methods to assess soil invertebrate diversity, (1) classical identification of specimens to species using morphological characters, (2) DNA-metabarcoding of invertebrate communities (comDNA), and (3) metabarcoding of environmental DNA (eDNA) extracted from soil. Background of this project, named MetaSol and launched by the Federal Environmental Agency of Germany (UBA, Umweltbundesamt), is the need to develop a standardized and cost-effective method for the assessment of soil invertebrates at the Permanent Soil Monitoring Sites of the German Länder. We present sampling design and workflow and we discuss the strengths and weaknesses of each method in light of the results obtained.
Advantages are as follows: Morphology: detection of very small, rare, or new species, inclusion of abundance and biomass data. comDNA: detection of cryptic species, identification of juvenile specimens. eDNA: cryptic species, detection of cryptic species and species not sampled but present at a site; identification of juvenile specimens. Methodological constraints and uncertainty are lowest in "morphology" and highest in "eDNA", the reverse is true for costs and workload.
16S eDNA is very efficient to assess earthworm diversity. Congruence of results using the 3 methods is especially good in enchytraeids. The main challenges for metabarcoding are: to minimize selective DNA amplification (primer bias), to enhance the reference libraries, and to integrate quantitative data. The main challenges for morphology is to describe and name the new species, and to obtain funds to do so.
For a full picture of the diversity, a combination of methods is recommended. The biological state of soil annelid communities (e.g. community types, degree of disturbance) can be assessed with each of the 3 methods.

Keywords: metabarcoding, annelid diversity, method comparison, species assemblages, environmental assessment

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The rise and fall of Oligochaeta (mass densities) in the Zeeschelde estuary

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The river Schelde is 355 km long, originating on the plateau of Saint-Quentin in France and ending in the North Sea in the Netherlands near Vlissingen. The Schelde estuary is approximately 160 km long and has a complete salinity gradient from polyhaline to a tidal freshwater zone, including extensive freshwater, brackish and saline tidal mudflats and marshes. It is a well-mixed estuary characterized by strong currents, high turbidity and large tidal amplitude (up to 6 m). The river has gone through dramatic and abrupt changes since the beginning of the millennium: especially since 2007 when the purification of the Zenne tributary - which receives the wastewater of Brussels - set in motion a chain of events:

- a reduced input of organic pollution into the Zeeschelde
- an anoxic zone in the river was resolved allowing marine fish, shrimp and mysid shrimp to reach the freshwater zone. These organisms often feed on Oligochaetes. due to the reduced food supply and the increasing predation of marine migratory organisms, the numbers of aquatic Oligochaeta which reached up to 5.000.000 worms m^{-2} plummeted
- high numbers of wintering waterfowl declined only months after the purification
- surprisingly, so far the overall species richness of aquatic Oligochaeta did not change much: in general, the species richness stayed the highest in the more upstream parts of the estuary and subtidal samples seem to have some extra species, especially in upstream river parts.

Keywords: Estuaries, biodiversity, waste water treatment, feeding ecology, Belgium

*Speaker
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The Worm Buoy Collective Behavior and its Seasonality

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California blackworms (*Lumbriculus variegatus*) are detritivores that typically live in anoxic environments (< 0.5 mg L^{-1} dissolved oxygen). They measure 20-40 mm in length and spend most of their time with their anterior segments burrowed in granular material to forage while waving their posterior regions above them. While blackworms breathe from their mucosal body wall, they supplement respiration using their ciliated hindgut. In shallow water, blackworms obtain oxygen directly in air by placing their tails parallel along the air-water interface. Portions of their tail subsequently break surface tension due to its material property which we hypothesize to contain a series of hydrophobic segments and hydrophilic intersegments. These hydrophobic surfaces provide latching force, allowing worms to hang freely using just the interface. Outside of granular substrates, blackworms form collective "blobs" due to their thigmotactic behavior. After about 50% of the population has latched onto the interface, worm blobs can lift off from the ground. In this talk, we introduce the "worm buoy", which is a floating collective of blackworms. We estimate that a single worm weighing 7 mg, in air, can support itself by exposing only one segment on the interface. Worms can adjust latching force by dynamically exposing more or less of its tail surface. We hypothesize that the worm buoy promotes collective survival in anoxic environments by allowing smaller worms climb up the structure and obtain oxygen directly from the interface. Furthermore, through year-long timelapse imaging, we present that the worm buoy behavior is time-dependent and occurs seasonally in warmer weather.

Keywords: Worm buoy, physically entangled collective behavior, blackworms, *Lumbriculus variegatus*, interfacial mechanics

*Speaker

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Environmental Studies

Wastewater effects from a pulp and paper mill on the oligochaetes community structure

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Studies receiving pulp and paper mill effluents (Mondi Syktyvkar JSC) on the Vychegda River (European North) were initiated in 2018/2020 to address questions about effects of wastewater effluents on biodiversity aquatic communities. The investigated section is 55 km long, located in the zone of wastewater influence and control section is 72 km upstream. Sediment assays and water concluded that no significant toxicity effects were observed from pulp mill discharges of river. But there is an increased temperature regime, excess of biogenic elements and the predominance of uncharacteristic substrates (artificial pebbles, gravel, silt).

In the zone of influence 40 species and forms of oligochaetes have been identified (Baturina et al., 2021a, b). We found species having been earlier identified only in tributaries, in floodplain water bodies and in lakes of the Vychegda River basin (Lastochkin, 1955).

According to the calculations of the diversity index (Shannon index (HN) and Simpson index (DS)), evenness index (Pielow index (E)), and Whittaker (βw) indices, the studied section of the river exceeds the control section by the species diversity, there is also a significant increase abundance and biomass of oligochaetes. The correlation analysis between the quantitative development indicators of oligochaetes with the environmental parameters (type of substrate, presence of algal and macrophyte, depth, chemical composition), the assessment of biotopic confinement of species (Fj) and conjugate between species (rA) showded that the distribution of oligochaetes in the study area largely depends on the substrate type. This study indicated that effluent-loadings to the river greatly increase benthic riverine productivity and associated with severe eutrophication. Nutrients (phosphorus and nitrogenin) the effluent have enrichment effect on the river food web immediately below pulp mill discharges and on technogenic substrates algae rapidly accumulated. This increases the availability of food for secondary producers, changes the structure of zoobenthos, including oligochaetes community structure.

Keywords: Oligochaeta, biodiversity, pulp and paper mill treated wastewater, European North

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Dero superterrenus (Michaelsen, 1912) (Annelida, Clitellata, Naididae, Naidinae) associated with phytotelmata and artificial containers in Monroe County, Florida USA

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The island chain known as the Florida Keys surrounds the southernmost tip of the state of Florida, USA. We present the results of a multi-year inventory of phytotelmata in the Florida Keys, focusing on the aquatic oligochaetes collected and identified from these unique and usually temporary natural and artificial habitats. For many years (mid 1990s–present), the Florida Keys Mosquito Control District has been conducting larval mosquito surveillance in natural areas and in domestic situations. Domestic surveillance has included examination of artificial and natural containers near houses, inspection of sewage treatment plants, and monitoring mosquito larval development in storm water catch basins. Several publications discussed the results of this long-term monitoring program – focusing specifically on the mosquitoes and other arthropods in samples; the oligochaetes in samples remained unidentified. Recently, we identified the oligochaetes present in samples collected from 86 natural and artificial phytotelmata, including 1,114 specimens of *Dero superterrenus* (Michaelsen, 1912), several *Dero* sp. (incomplete, likely *D. superterrenus*), and five unidentifiable Tubificinae. The relationships of *D. superterrenus* with the four mosquitos encountered during the monitoring program – *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, *Culex nigripalpus* Theobald, and an unidentified *Wyeomyia* species – are summarized. Other recently documented records for *D. superterrenus* elsewhere in the southern U.S. states are highlighted.

Keywords: phytotelmata, water bodies, aquatic oligochaetes, Annelida, Clitellata, Naididae, *Dero superterrenus*, distribution, new state records, USA, Florida

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Microscopy

A comparison of macrobdellid leech morphology using microCT

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Micro-computed tomography (MicroCT) is a powerful tool for studying the internal morphology of soft-bodied invertebrates, such as leeches, and has advantages over destructive techniques like dissection and serial sectioning. We used microCT to compare the morphology of the internal reproductive organs of two morphologically similar, yet molecularly distinct, species of North American medicinal leeches, *Macrobdella mimicus* and *Macrobdella decora*. *Macrobdella mimicus* is morphologically similar, yet molecularly distinct, from the type species of the genus, *Macrobdella decora*. Our goals were to compare the internal reproductive morphology of these two species, to assess the influence of breeding condition (i.e., seasonality) on the reproductive organs, as well as to assess scan quality of specimens recently collected (< five years) versus historical specimens. Six specimens each of *M. mimicus* and *M. decora* were stained with 0.3% phosphotungstic acid and 3% DMSO in 70% ethanol for 2 weeks and scanned using a GE Phoenix v|tome|x micro-CT scanner with the 180 kV Nanofocus tube configuration. The best scan of each species was segmented for 3D reconstruction using the software package Amira v. 2019.1. We found subtle morphological differences between the two species, although size differences of the male reproductive systems and the accessory organs was likely influenced by the breeding condition of the leech. The oviduct of *M. decora* was more convoluted and longer than that of *M. mimicus*, and the oviducts join inside the vagina of *M. mimicus* rather than outside as in *M. decora*. The epididymes of *M. decora* had more coils and the internal vas deferens were much longer than observed in *M. mimicus*. Historical specimens seemed to absorb stain better, but all specimens resulted in high quality scans. MicroCT is a promising technology for documenting fine scale internal morphology of soft-bodied invertebrates, but is not yet scalable for collection-wide digitization efforts.

Keywords: imaging, Hirudinea, 3D reconstruction

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Morphology

Recent progress in ovary analyses in clitellate annelids

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Ovary micromorphology and oogenesis in clitellate annelids have been intensively studied for several years. Our main goal is to follow the ways of ovary evolution in Clitellata. To date, special attention has been paid to leeches (Hirudinida) and their allies (Branchiobdellida, Acanthobdellida) and microdrile clitellates (Capilloventridae, Phreodrilidae, Enchytraeidae, Propappidae, Lumbriculidae, Haplotaxidae, Naididae). Among naidids, representatives of four subfamilies, Naidinae, Tubificinae, Limnodriloidinae, and Rhyacodrilinae, were studied.

In the most recent study, we concentrated on the representatives of four species, i.e., *Peristodrilus montanus* (Rhyacodrilinae), *Achaeta affinis* (Enchytraeidae), *Batracobdella algira* (Glossiphoniidae), and *Haemadipsa japonica* (Haemadipsidae).
Our analyses revealed that ovaries show flexibility in their morphology and organization, but they are conservative at the family/subfamily level. Microdrile ovaries are paired, inconspicuous structures enveloped only by thin somatic cells and connected via ligament to the septum. In leeches, they form variable ovarian units (egg follicles, ovary cords) enclosed within the ovarian sacs.

In all species studied, ovaries are composed of syncytial groups of germ cells (germ-line cysts) associated with somatic cells. Each germ cell has one intercellular bridge connecting it to a central and anuclear cytoplasmic mass (cytophore). Within cysts, oogenesis progresses, and germ cells in the consecutive stages can be found, i.e., oogonia, meiotic cells, nurse cells, and growing oocytes (future eggs). This pattern of cysts organization is typical to all clitellates studied to date, with only the one exception – *Capilloventer australis*, a representative of the basal family Capilloventridae, in which no germ-line cysts have been found.

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Besides this unity, we have found many differences and distinct characters, and several types of ovaries have been distinguished to date. We believe these characters may help disentangle the complex phylogenetic relationships within Clitellata. Our next aim is to find and compare the ovary and oogenesis across Crassiclitellata and allied taxa.

Keywords: ovaries, germline cysts, oocytes, nurse cells

Phylogeny

Acanthobdellida (hook-faced fish worms) – molecular analyses and external morphology shed light on the history of the ancient leech group

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Acanthobdellids (hook-faced fish worms) are regarded as the sister group to true leeches (Hirudinida). Acanthobdellida comprises two known species: *Acanthobdella peledina* Grube, 1851 and *Paracanthobdella livanowi* (Epstein, 1966), each placed in a separate family (Acanthobdellidae and Paracanthobdellidae). These leech-like annelids are blood-feeding fish parasites; like their closest relatives, they are equipped with suckers, and they also bear chaetae which are absent in true leeches. They live in the Northern Hemisphere, from Scandinavia to Alaska, however, *P. livanowi* was found in the Russian Far East only. We present new molecular data (selected sequences of nuclear and mitochondrial genes) for two populations of *A. peledina* (Scandinavian and Alaskan) and, for the first time, sequences for *P. livanowi* (COI, 12S, 18S and 28S). We also analysed the external morphology of both species using light microscopy and SEM, mainly concentrating on the anterior suckers with hooked chaetae and gonopores which are the main features distinguishing the species. Our analyses showed species-level differences in the anterior sucker and facial hooks; molecular phylogenetics mirrors this divergence between species (*P. livanowi* is sister to *A. peledina*). Alaskan and Scandinavian *A. peledina* populations are morphologically similar but appear distinct genetically. Molecular data suggest that hook-faced fish worms are an ancient lineage; however, both species and populations have diverged

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relatively recently. Due to not very distinct differences between the two species, we propose to abandon the family Paracanthobdellidae. To match better leech taxonomy, we propose to erect a new suborder Acanthobdelliformes. We also propose the common name 'hook-faced fish worms' for acanthobdellids to underline the presence of characteristic bent, hook-like chaetae.

Keywords: Leeches, phylogenetics, scanning electron microscopy

Taxonomy

Establishing DNA-barcode libraries of Scandinavian clitellates

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Species of Clitellata (oligochaetes and leeches) are common in aquatic and terrestrial ecosystems, and they will be of great importance when large-scale barcoding becomes an everyday approach in biodiversity assessment. During 2019-2022 and with funding from the Swedish Environment Protection Agency, we have compiled a large dataset of DNA-barcode sequences obtained in our lab over the last two decades. The objective is to establish taxonomically verified barcode reference data of as many as possible of species primarily occurring in Norway and Sweden. We have material also from Denmark and other countries in North Europe. Important is to account for haplotype variation of the genes within the species, and the latter are in many cases species hypothesis rather than formally described, binomial taxa. Four libraries, one for each of Cytochrome C Oxidase subunit I (COI), 16S rDNA (both mitochondrial), and two loci in the nuclear genome, the Internal Transcribed Spacer Region 2 (ITS2), and Histone 3 (H3) are assembled. We successfully PCR-amplified and sequenced DNA (of at least one gene marker) for totally ca. 21,000 worms (9,800 from Sweden; 9,800 Norway; 500 Denmark; 900 other countries), and we thus obtained at least Cytochrome C Oxidase subunit I (COI) mtDNA, or (if COI is missing) 16S mtDNA, sequences for all individuals. The intra-specific variation is generally lower in ITS2 and H3, and they were more selectively sampled for sequencing. We are in the process of uploading our libraries on Genbank, initially using tools in the Barcode of Life Data (BOLD) Systems. BOLD normally publishes only COI data for animals, but ultimately, all four markers will be publicly available on GenBank. At the end of 2022, we will have uploaded 12,700 sequences (ca. 4,000 COI, 3,550 16S, 2,700 ITS2, 2450 H3) from 4,000 specimens, representing ca. 530 species.

Keywords: DNA libraries, Scandinavia, Clitellata

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Aquatic oligochaete worms (Annelida: Clitellata) in Texas spring, hyporheic and phreatic groundwater habitats: preliminary findings.

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In contrast to the situation in Europe, stygobitic and hyporheic annelids have received little attention in North America. Recent spring, hyporheic and phreatic groundwater collections suggest a rich oligochaete fauna endemic to the region of the Edwards Aquifer in central Texas. Some species in springs and spring runs, including several Naidinae (Naididae), are widespread in surface waters (Worsham & Huffman 2016). *Eclipidrilus palustris* (Lumbriculidae) was common in springs and associated habitats, and is widespread in cleaner, cooler waters of the southeastern USA. *Varichaetadrilus angustipenis* (Naididae, Tubificinae) was also common in these habitats; this species has been associated with groundwater in Illinois by Wetzel & Taylor (2001). However, several stygophilic or hyporheic species of Haplotaxidae and Lumbriculidae appear to be new and endemic. In the Haplotaxidae, 2 basic morphotypes, each with more than one apparent species, are distinguished by morphology of the gizzard: typical for the family (in IV-V, with a median sphincter) in one type, vs. more elongate (III or IV-VII), with uniform musculature in the other. The few mature specimens also differ in gonadal arrangement and presence of genital chaetae. Lumbriculids are diverse, with over 10 novel morphotypes distinguished so far. Several morphotypes can be assigned to *Eremidrilus* based on semiprosoporous male ducts with male pores in X, spermathecae in XI, and a filiform proboscis. Two hyporheic species (in genera *Eremidrilus* and *Pararhynchelmis*) have spermathecae that appear to join the gut, and also have unusual mid-dorsal glands in posterior segments. Other Texas lumbriculid species have unusual arrangements of reproductive organs, and are difficult to classify. An unknown species of Phallodrilinae was represented by a single specimen; these small oligochaetes may not be efficiently captured by the relatively large mesh sizes used in standard collection methods.

Keywords: Texas hyporheic groundwater

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A new approach to the delineation of taxa at the species and higher levels

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The study of ecological interactions within biological communities requires a thorough understanding of biodiversity which is inseparable from taxonomy. Despite centuries of dispute and controversy, the guidelines for assigning organisms to certain taxonomic levels have not been established. Admittedly, all human attempts to systematize living organisms are artificial, and there are no strict cut-off boundaries between taxa in nature. However, accounting for inaccurate taxonomic variables may lead to misguided estimates of ecological effects. Unfortunately, there are no clear guidelines for taxonomic assignment at levels above species. Here, we present a novel approach to the use of genetic divergence to evaluate the taxonomic position of certain samples with the simultaneous estimation the correctness of current systematics. This approach includes measuring raw and model-adjusted distances between samples' genetic sequences and attributing them to the lowest taxonomic levels that are common in sample pair to reveal distance distributions matching different taxonomic levels (species, genus, family etc.). This approach facilitates the reassessment of the taxonomic position of the samples, whose genetic distances relative to other samples in the dataset did not match their taxonomic divergence. A set of molecular data of segmented worms was chosen to test this approach. As a result, numerous inconsistencies in the systematics of Clitellata were pointed out. These inconsistencies included both oversplitting and overlumping of specimens into the taxa of different levels and clear cases of misidentification. Our approach sparks re-evaluation of current systematics where traditional methods fail to provide sufficient resolution. Getting insights into a real degree of taxonomic divergence between inferred samples provides better opportunity to account for taxonomic variables in ecological research by comparing the same level taxa.

Keywords: genetic divergence, high level taxa delimitation, molecular based systematics, oligochaetes, leeches, leechlike parasites

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On *Haplotaxis* Hoffmeister, 1843 (Annelida, Clitellata)

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Within the oligochaetes, the genus *Haplotaxis* Hoffmeister, 1843 is particular in several respects. Its constitutive species are generally considered as predators, a rare feeding behaviour within the oligochaetes, essentially detritivores. The genus has a cosmopolitan distribution but remains poorly known because of its rarity related to its presence mainly in groundwater and the fact that several species are only known from immature specimens. The type species *Haplotaxis gordioides* (Hartmann in Oken, 1819) alone concentrates the problems linked to a fundamental lack of knowledge of this group: supposedly present on most continents, it remains poorly defined because of the absence of reference types and the lack of information on the type locality. At present, we can consider this species as a "waste basket taxon".
The last decade has seen a renewed of interest in the genus *Haplotaxis,* because of the development of research programmes dedicated to the study of groundwater biodiversity, and issues related to the existence of cryptic species that genetic data brought back into focus.
This presentation will address various aspects and thoughts related to *Haplotaxis*, its morphology, diversity and ecology: (1) identification of the type locality of *H. gordioides* and identification of morphological material that can be used for neotypification of the species, (2) presence of the group in Africa, description of novel morphological structures and discussion of their potential role, arguments favouring the creation of a new genus to accommodate Afrotropical species, (3) reflections on real feeding mode, and (4) discussion on ecological categorization of the species within the subterranean aquatic environment.
Finally, recently obtained genetic results on the diversity of *Haplotaxis* in Switzerland and France will allow us to reflect on the real diversity of this group and the probable need to revalidate many species that have been synonymized with *H. gordioides* in the past.

Keywords: *Haplotaxis*, taxonomy, phylogeny, distribution, diversity

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Reflections on species-level taxonomy of oligochaetes in the 21st century

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Nowadays, oligochaete taxonomy is increasingly using DNA sequence data, additional to morphological characters. Both methods are dependant on each other, but in different ways. Considering the amount of data, the richness of analytical tools, and the depth of resolution, DNA sequences outdo morphological characters by orders of magnitude. In cases of conflict, it is therefore the DNA sequence data that take the lead. On the other hand, the species identity of DNA sequences is still largely based on morphological characters of the underlying specimens. DNA data have, in general, confirmed, the outer limits of species taxa, but questioned the inner limits of taxa through the detection and delimitation of cryptic species. It is important to name these cryptic species, but the former morpho-species should not remain unnamed, especially when the cluster of cryptic species is a monophyletic group.

DNA sequencing technology is developing at high speed, and new data are becoming increasingly available. Yet it is paramount to agree on a specific type of data. New data do not help when they are unknown in similar species. Therefore extension should be favoured over intension when it comes to research programmes. DNA sequences of currently established markers should be generated for as many known species as possible, and for specimens of a morpho-species that cover its distribution range.

Morphological identification will probably be replaced increasingly by molecular identification. Such identification can be carried out by non-experts, which will increase greatly the body of knowledge about the species. Paramount here is the quality of the reference databases.

For those who take delight in observing oligochaete worms, their liberation from the taxonomic tunnel vision may open new ways to study these worms, towards lifestyle, body functioning, or any other aspect of their biology.

Keywords: morphology, DNA sequences, integrative taxonomy, microdrile oligochaetes

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Other topics on Aquatic Oligochaeta

Update: Profiling the Illinois Natural History Survey Annelida Collection – a case study in assessing the health of stored biological specimens

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Natural history collections holding one or more groups in the Phylum Annelida are deposited in museums and other public as well as private research collections. Natural history collections document biological diversity, highlight historical changes, monitor current trends of species, model population changes, and provide the basis for development of protocols and legislation to protect endangered and threatened species and the uniqueness of the habitat in which they occur. Critical issues facing natural history collections include governmental regulations, permits, and repatriation of critical knowledge; loss of taxonomists, systematists, curators, and collections managers; and long-term funding to support the physical and digital maintenance and security of specimen holdings. Our current profiling protocol quantitatively assesses the "health" of the oligochaete specimens in the Illinois Natural History Survey Annelida Collection. Assessment categories for both dry (slide-mounted) and wet (fluid storage) specimens include conservation status, processing state, storage container, condition of labels, identification, arrangement, data quality, and computerization. The status of this profiling exercise was presented during ISAO14 in Hirosaki, Japan, in September 2018. This presentation updates the current status of this multi-year project – including the discovery of widespread stopper failure throughout the ethanol-preserved specimens in the Annelida Collection and in several other INHS biological collections. We outline our mitigation plan recently implemented to rescue specimens that otherwise would have soon been 'lost' through desiccation.

Keywords: natural history collections, curation, collection management, profiling, archiving, digitization, storage container failures, Annelida, oligochaetes, leeches, branchiobdellidans

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Nomenclatura Oligochaetologica Editio Secunda – A catalogue of names, descriptions and type specimens of the Oligochaeta: update on this web-based catalogue

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The history of the first edition of this nomenclator is summarized, followed by its transition to a web-based format in 2014. This website was recently restructured and migrated to a WordPress content management system in December 2021. A general layout and guide to navigation throughout this updated website is highlighted.

Keywords: Annelida, Clitellata, oligochaetes, species accounts, original descriptions, nomenclator, distribution, literature, searchable website

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Poster

Placobdella n. sp. (Hirudinea: Glossiphoniidae), a new glossiphoniiform leech from North Africa

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Currently, there are 24 recognized species belonging to the genus *Placobdella*. The most remarkable diversity is observed in North and Central America. The Palearctic region remains very poorly studied, with only one described species; the type species of the genus: *Placobdella costata* Moore, 1953. However, the Palearctic species diversity is likely to increase soon as the classification of the genus continues to be refined, and new taxa are still being discovered and described. It was found recently that *P. costata* complex consists of at least five independent lineages, including the Tunisian-Algerian *Placobdella* clade, which potentially deserves to be considered as separate species based on COI locus. Here we present preliminary data about morphology and provide even more molecular markers (COI, 12S, 28S, and histone H3) of the newly discovered Tunisian-Algerian *Placobdella.* This species is diagnosed as belonging to the genus *Placobdella* based on the following synapomorphies: **(1)** placobdellid ocular morphology (one prominent pair of ocelli plus another smaller coalesced pair), **(2)** two pairs of compact salivary glands and **(3)** possessing of one pair of sac-like bacteriomes. It is worth noting that in North Africa, *Placobdella costata* were recorded previously by several authors; however, these records may refer to the new species presented herein. In fact, *P. costata* sensu lato was thought to be widely distributed in Tunisia and Algeria, and it was initially assigned to this taxon based on morphological criteria. The new species was compared with Ukrainian *Placobdella costata* sensu stricto and Polish *P. costata* specimens and can be distinguished morphologically and anatomically from its congeners by possessing both compact and diffuse salivary glands and also by the morphology of the atrium. Results of the Automatic Barcode Gap Discovery (ABGD) and the Bayesian implementation of the PTP model (bPTP) delimitation methods support the species rank of the Tunisian-Algerian Placobdella.

Keywords: Leeches, species diversity, North Africa

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Newly recorded species of marine oligochaetes (Annelida: Clitellata) from the East Sea in Korea

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The three seas that surround the Korean Peninsula (East Sea of Korea, West Sea, and South Sea) vary considerably in their temperature, currents, and salinity, which in turn affect their environment. Of these three, the East Sea of Korea is the deepest. Its water temperature is lower, and it has a different sea bottom composition. Marine oligochaetes can be found in most coastal or deep-sea sediments and the diversity of some species is incredibly rich and cosmopolitan. In Korea, however, it has rarely been taxonomically studied. This study describes six species of marine oligochaete collected from the East Sea of Korea.

Keywords: Marine Oligochaeta, Tubificida, Naididae, Phallodrilinae, *Limnodriloides*, *Marinonina*, *Heterodrilus*, *Tubificoides*, *Uniporodrilus*, East Sea, Dokdo Island, Korea.

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Oligochaeta (Annelida) from some temporal water bodies of East Estonian forests

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Oligochaeta of forest vernal pools and small draining ditches were sampled together with other aquatic macroinvertebrates yearly in spring 2013-2018 at six sites in the eastern part of Tartu County, Estonia. In 298 samples, a limited number of oligochaete taxa (23) was found. The most common species were *Lumbriculus variegatus* (2/3 of all individuals sampled), *Cognettia glandulosa, Tubifex tubifex* and *Aulodrilus limnobius*, all able to reproduce rapidly in asexual way, either by architomy or parthenogenesis. The phytophilous Naididae were rare or lacking, as were most of the other Tubificidae, common in permanent water bodies, and the majority of the soil-inhabiting Enchytraeidae and Lumbricidae. Some in general rheophilous Lumbriculidae (*Rhynchelmis tetratheca, Stylodrilus heringianus, S. brachystylus*) apparently live permanently in the humid soil under several temporary pools. Vernal temporary waters seem to be a specific habitat for the naidid *Bratislavia palmeni*. The oligochaete fauna was almost similar in the natural pools of drained and undrained forests, slightly more diverse in draining ditches, and the poorest in recently dug mitigation pools.

Keywords: Key words: Oligochaeta, forest pools, forest ditches, Estonia

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Author Index