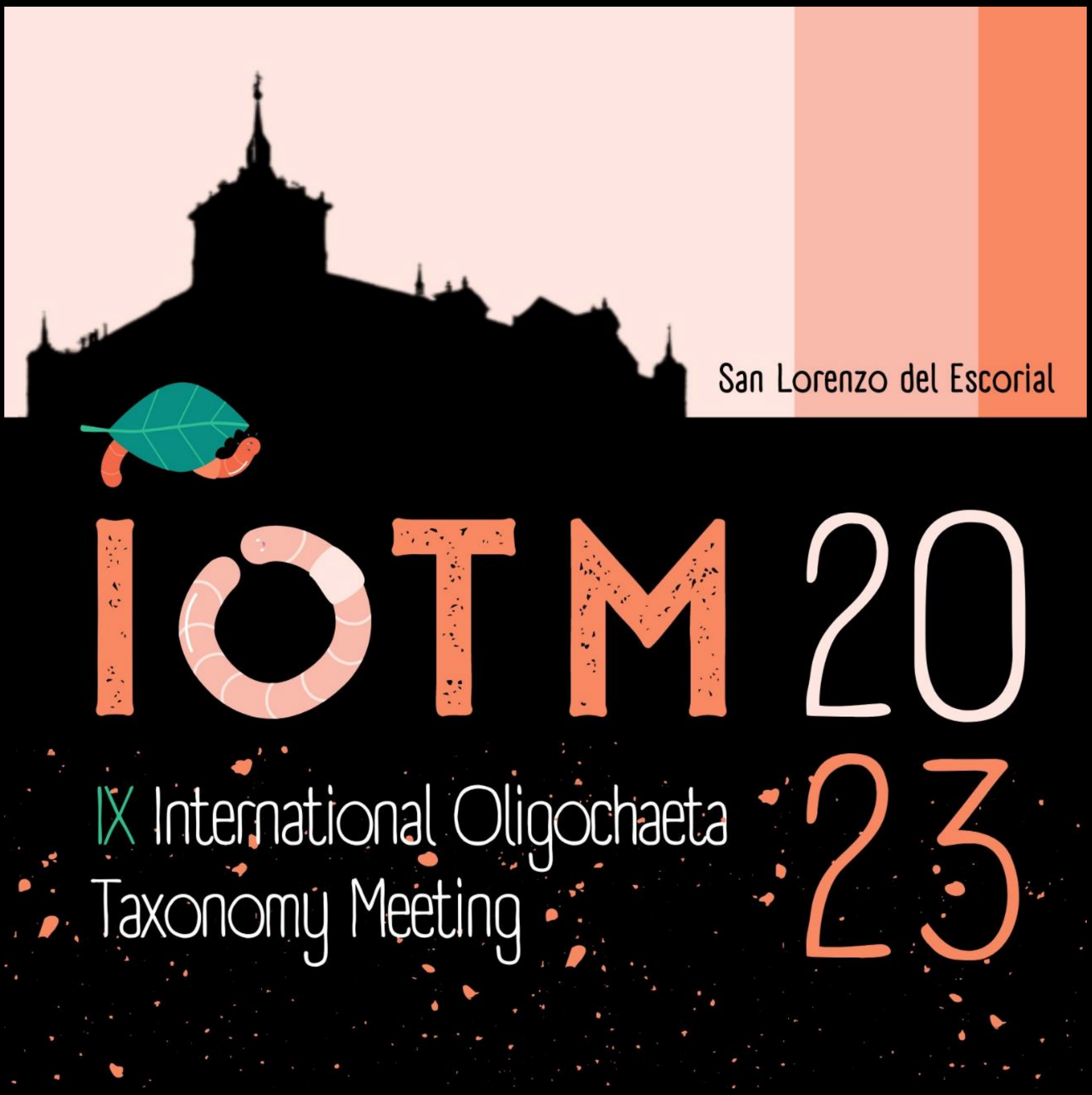
ABSTRACT BOOK



EARTHWORMS: OUR UNDERESTIMATED CREATURES!

December 11th to 15th 2023

IN HONOR OF VICTOR POP

EDITOR

Marie L. C. Bartz

COORDINATION

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LAYOUT

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INTERNATIONAL OLIGOCHAETA TAXONOMY MEETING 2023

IOTM 2023

The Oligochaeta is composed of the mostly aquatic microdriles and the mostly terrestrial megadriles, better known as earthworms. This diverse group (around 5000 described species) is one of the most ecologically important in their respective habitats.

The main goal of the **International Oligochaeta Taxonomy Meeting (IOTM)** is to bring together experts in the field to exchange advances and to identify the current gaps in the knowledge of these important but poorly known animals, and to stimulate synergies, collaboration and the exchange of ideas.

The main focus of these meetings is oligochaete taxonomy and phylogeny, but other aspects of their biology are integrated as well such as ecology, faunistics, phylogeography and evolutionary biology.

The **9th International Oligochaete Taxonomy Meeting** was carried on the tradition of previous meetings and took place in **San Lorenzo del Escorial** in Spain, from **December 11th to 15th, 2023**.

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PROGRAM

	11/dez	12/dez	13/dez	14/dez	15/dez
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
9h00 -10h00		Plenary Talk	Plenary Talk	Plenary Talk	
10h00 - 10h20		Talk	Talk	Talk	
10h20 - 10h40		Talk	Talk	Talk	
10h40 - 11h00		Talk	Talk	Talk	Coffee break and poster presentation
11h00 - 11h30		Coffee break and poster presentation	Coffee break and poster presentation	Coffee break and poster presentation	Citizen science activities
11h30 - 11h50		Talk	Talk	Talk	Citizen science activities
11h50 - 12h10		Talk	Talk	Talk	Citizen science activities
12h10 - 12h30		Talk	Talk	Talk	Citizen science activities
12h30 - 12h50		Talk	Talk	Talk	Citizen science activities
13h00 - 14h30		Lunch	Lunch	Lunch	Lunch
14h30 - 16h30		Workshop	Workshop	Free time	
16h30 - 17h00		Coffee break and poster presentation	Coffee break and poster presentation	Monastery visit (from 15:30)	
17h00 - 19h30	Registration and reception coffee	Workshop	Workshop	Free time + monastery visit	
19h30 - 20h00	Opening talk	Free time	Free time	Free time + monastery visit	
20h00	Dinner	Dinner	Dinner	Dinner	

PLENARIES

THREE GENERATIONS IN EARTHWORM SYSTEMATICS: HOMAGE TO THE WORK OF THE POP FAMILY IN THE SYSTEMATICS OF LUMBRICIDAE

Csaba Csuzdi

Eszterházy Károly Catholic University, Eger, Hungary

The 9th International Oligochaeta Taxonomy Meeting is dedicated to the memory of Dr. Victor V. Pop who passed away in 2022 at the age of 86. Victor V. Pop cannot be authentically commemorated without remembering the role the Pop family has played in earthworm taxonomy and systematics since the early 20th century. Victor was the first son of senior Victor Pop (1903–1976) a professor of zoology at the Babes-Bolyai University, Cluj (Romania) and world renowned lumbricid systematist. Senior Victor Pop's role was instrumental in furthering the Central European earthworm research with continuing the work started by László Örley (1857-1887) and Andor Szüts (1884-). Victor Pop has published a relatively small number of scientific papers, but most of them have received wide international recognition. Of his work on earthworms, we must highlight two synthesizing works that have had the greatest international impact in laying the foundations for modern lumbricid taxonomy. One has published in 1941 „Zur Phylogenie und Systematik der Lumbriciden" where he put forward the Pop's earthworm classification, which later became widely accepted. The second was his monograph on the Romanian earthworm fauna published in 1949. The Pop's classification gradually replaced the Rosa-Michaelsen system, which gave too much taxonomic importance to the structure of the reproductive apparatus, which led to the lumping together of obviously unrelated species. Reassessment of the variability of morphological, anatomical and histological characters in species from different parts of Europe, led Victor Pop to develop a new hierarchical approach. Thus, Pop distinguished three characters with diagnostic value at genus level (body pigmentation, setal distances and histological structure of the body wall musculature) and several other characters with diagnostic value at species and subspecies level (e.g. position of the clitellar organs, prostomium type, calciferous glands, and others). On the basis of these characters, Pop managed to distinguish evolutionary lineages and placed all lumbricid species in 7 genera. This system was until the mid-1970s in use generally. It therefore seemed obvious that his son Victor V. Pop would continue his work. After a little detour in the study of soil science, Victor V. Pop followed in his father's footsteps and published his first paper on the earthworm fauna of the Retezat Nationnal Park in 1972. Shortly afterwards he published his first taxonomic work with the description of *Allolobophora zarandensis* V.V. Pop, 1978. He has since spent decades exploring the earthworm fauna of the Western Carpathians. This work has resulted in the description of numerous earthworm species such as the giant *Octodrilus permagnus* V.V. Pop, 1989. He was among the first earthworm scientists who recognized the importance of molecular methods and from the early 2000's his interest turned to molecular systematics. This field was taken up by his daughter Antonia Adriana Pop, who wrote her PhD thesis on this topic entitled "Molecular Taxonomy of the earthworm family Lumbricidae" at the University of Heidelberg in 2004. So, this is a special case where three successive generations of the same family have studied the animal group with world-renowned results.

Keywords: earthworms, taxonomy, systematics, Carpathians, Europe.

SAMUEL JAMES TALK

MICRODRILES FIRST: THE BASAL BRANCHES OF THE CLITELLATE TREE

Rüdiger M. Schmelz

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The diversity of oligochaetes is much higher than the common conception of a typical earthworm may suggest. This talk gives an overview of the groups and families outside "Crassiclitellata" or “Megadrili” – the terms are not synonymous – and within the clitellate tree of life. These are the species-rich Enchytraeidae, Lumbriculidae, and Naididae (including the former Tubificidae), further the lesser-known Phreodrilidae, Haplotaxidae, Alluroidida, and a few other species-poor families mainly at the very base of the clitellate tree and hence especially interesting from a phylogenetic perspective. These groups have characters in common that make them different from the earthworms, for example small body size, large yolky eggs and a high variation of form and number of chaetae/setae. A taxon "Microdrili", based on these ancestral characters is no longer accepted, but the term remains useful to refer to the oligochaetes that are not earthworms. All microdriles except enchytraeids are exclusively aquatic. Some species are predatory, commensals, or they are gutless and feed on symbiotic bacteria, but most species feed by ingesting soil or sediment, which may explain the striking uniformity of body form and organization. Exuberant morphological differences are found in the male reproductive system; its basic pattern, however, is strikingly constant within groups and has given rise to phylogenetic hypotheses, some of which have been confirmed by molecular data. To know these groups, apart from being a valid objective in itself, helps understanding the peculiarities and the evolutionary history of earthworms.

Keywords: Clitellata; Oligochaeta; Microdrili; diversity; evolution.

NEW FUNCTIONAL GROUPS FOR EARTHWORMS BASED ON THEIR BEHAVIOR

Yvan Capowiez, Daniel Marchán, Thibaud Decaëns, Mickäel Hedde, Nicolas Bottinelli

INRAE Avignon; University of Madrid; University of Montpellier; INRAE Montpellier; IRD Hanoi

The seven ecological categories of earthworms were defined by Marcel Bouché in the 70s using only morphological and anatomical characteristics. They are however often used as proxies for functional groups, i.e. to describe how earthworms influence their environment. In the present study, we studied under standardized conditions (repacked soil cores) the feeding and burrowing behavior of 40 earthworm species from Europa with 5 replicates per species. After one month, the burrows system and the bioturbation intensity were analyzed using X-ray computed tomography. The quantity of litter consumed by earthworms was also recorded. We then defined 6 new functional groups using K-means classification. These 6 groups were only partially in agreement with the ecological categories of Marcel Bouché. We first provide quantitative basis for the splitting of « anecic » earthworms into 2 functional groups (epi-anecic and truly anecic earthworms). The main novelty is the splitting of endogeic earthworms into 2 different functional groups (hypo-endogenic and epi-endogeic earthworms). The two lasts groups were « intermediate » earthworms and « earthworms with no or low effects on the soil » which include mainly epigeic earthworms (but not only). We believe these new functional groups based on sound data will help all the soil biologist to better assess the effects of earthworm on their environment.

Keywords: ecological category; burrow system; tomography.

ORAL PRESENTATIONS

50 YEARS AFTER BOUCHÉ'S EARTHWORM SAMPLING IN FRANCE: WHO WINS? WHO LOSES?

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Little is known about the long-term response of earthworm species to global change. Earthworm species conservation status have been poorly assessed and only 221 species are cited in the IUCN red list in a total of 5406 described yet and 113 with insufficient information for a proper assessment, and none of these species being French. Bouché's sampling of earthworm communities on more than 1300 localities in France in the 1960s represents a unique opportunity to assess how French species have responded to global change. During the #Vers2022 program, more than 400 of these localities have been resampled, fifty years after Bouché's work. In this study, we assessed what species "won" and what species "lost" from the 1960s to the 2020s. We first compared sample completeness between the two sampling to verify they can be compared, using estimators developed by Chao et al., 2020. We then measured changes in species occurrences between the 1960s and the 2020s using a multilevel pattern analysis. We showed that winner species are very common and peregrine species, like *Aporrectodea rosea, Allolobophora chlorotica* or *Aporrectodea longa*, and loser species are quite rare ones, like *Lumbricus bouchei*. We also discussed the status of very rare species, especially those known from one locality only, because the lack of data makes them hard to assess. Finally, we built a functional trait space to test whether winner and loser species share common or rare combinations of functional traits.

Keywords: global change; winner species; loser species; France; functional traits.

EARTHWORM DIVERSITY HOTSPOT IN FRENCH GUIANA

Thibaud Decaëns[a], Arnaud Goulpeau[a], Mickaël Hedde[b], Emmanuel Lapied[c], Marie-Eugénie Maggia[d]

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French Guiana covers an area of 86,504 km^2, most of it forested. The diversity of earthworms in this region had hardly been studied before the early 2010s, and the few works on the subject mentioned only around thirty species (Brown & Fragoso 2007), a figure far below what might be expected for a tropical territory of this size. Since 2010, we have carried out field work at 16 locations throughout French Guiana. Nearly 5,000 specimens have been collected, and their analysis using DNA barcoding (COI gene) has revealed the existence of nearly 300 operational taxonomic units (OTUs) (Maggia et al. 2021, Goulpeau et al. 2022). In localities where the samples were also studied morphologically, a perfect congruence between molecular data and morpho-anatomical characters was found, suggesting that these OTUs correspond to true biological species, most of which are new to science (Decaëns et al. 2016). We have undertaken the formal description of these new species, notably describing 18 species and one genus new for science from the Mitaraka range alone (Decaëns et al. in press). The distribution of this extraordinary diversity was analysed using a combination of hierarchical diversity partitioning, demonstrating that regional diversity is essentially explained by large-scale beta diversity (between localities). Generalised models (GLM and GDM) also allowed us to rank the relative importance of biotic interactions, environmental filters and historical factors in explaining patterns of community diversity and composition (Goulpeau et al. in prep). Finally, on the basis of rarefaction curves and asymptotic indices of diversity, we estimate that more than 2,000 species could exist in French Guiana, which challenges commonly accepted estimates of the number of earthworm species on a global scale.

Keywords: DNA barcoding, diversity partitioning, taxonomy.

EARTHWORM TAXONOMY, BIODIVERSITY AND ECOLOGY IN BRAZIL: AN UPDATE SINCE IOTM7 (2016)

George Brown[a,g], Samuel James[b], Marie Bartz[c,d], Alexander Feijoo[e], Luis Hernández[f], Talita Ferreira[g], Wilian Demetrio[h], Herlon Nadolny[g], Guillaume Rousseau[f], Rafaela Dudas[g]

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Brazil is currently home to 317 described earthworm species, though a very large number of new species have been found (>100) that still must be described. This has been hindered mainly by the lack of trained taxonomists active in the country, although recent capacity building efforts have slightly diminished this limiting factor. Since 2016, one new family, nine new genera and 56 new species have been described, including those currently ready to be sent for peer-review. Most belong to the three most speciose families in Brazil: Rhinodrilidae, Glossoscolecidae and Ocnerodrilidae. Extensive collection and identification efforts have been made since 2016 including the Atlantic Forest, Amazonia, Cerrados and Pampa biomes, and have revealed a wealth of new species, particularly in the genera *Fimoscolex* and *Glossoscolex* (Glossoscolecidae), *Urobenus*, *Rhinodrilus* and several new genera (Rhinodrilidae), *Kerriona* and *Belladrilus* (Ocnerodrilidae). Over 150 species were subjected to IUCN endangered species classification efforts, leading to the red-listing of seven species in a higher danger of extinction (1 CR, 4 EN and 2 VU). However, most species assessed were categorized as either DD (88 spp.) due to lack of data, or LC because of their relative resistance to disturbance and ocurrence in anthropogenic ecosystems (45 spp.). The annual taxonomy training courses continue to attract many students and new materials. Updated checklists have been prepared for all states, and all quantitative sampling information has been synthesized, leading to several important publications currently underway. São Paulo (100 spp.) and Paraná (93 spp.) are the most speciose states, followed by Santa Catarina (74 spp.), Rio Grande do Sul and Amazonas (68 spp. each), highlighting the very geographical skewed sampling and taxonomic efforts in Brazil. In the

Pampa, Atlantic Forest and Cerrado biomes of Southern, Southeastern and Central Brazil, a larger proportion of the species encountered are exotic, while in the Amazonian biome in the North, most species collected are still native. However, this is highly skewed by a greater sampling effort in disturbed vs. preserved native vegetation outside of Amazonia. Most native species are found in native vegetation, but throughout Brazil, native species can also survive in several anthropogenic ecosystems. Local species richness is generally low using quantitative samples (1.9 to 2.6 spp. per site), and qualitative sampling tends to greatly increase richness estimates, though rarely >10 spp. are found at a particular site. Genetic work has highlighted the cryptic diversity of some species, and the need for integrated taxonomic studies, as well as the use of other markers to help elucidate taxonomic complexities. More taxonomists and collaboration are needed in order to further advance earthworm taxonomy and ecology in Brazil, and to increase the understanding of the biodiversity and potential benefits of these animals in Brazilian ecosystems.

Keywords: biodiversity; native species; exotic species; distributions.

EVOLUTION AND DIVERSITY OF TRIGASTERID EARTHWORMS OF PUERTO RICO AND THE VIRGIN ISLANDS ARCHIPELAGO

Roberto Carrera-Martínez[a], Nicolás Gómez-Andújar[b], Mercedes Hernandez-Natera[c], Kenneth Banks[d], Edwin Cruz-Rivera[e], David A. Hensley[f], Grizelle González[g], Sean D. Schoville[h]

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The Caribbean Islands are home to a diverse earthworm fauna that has been incompletely documented. Among the groups in this region, the trigasterids are remarkable for having a high level of endemism and a set of unique synapomorphies on their digestive system, which includes two or three discrete gizzards. While a single member of the “core trigasterids” (those with three gizzards: *Trigaster*, *Neotrigaster*, and *Borgesia*) is known from Cuba, Puerto Rico and the Virgin Islands archipelagoes contain numerous taxa. For Puerto Rico and Virgin Islands, trigasterid earthworms are known from relatively restricted regions and habitats within the islands. This has potentially resulted in the evolution of isolated species or lineages for each genus. Therefore, understanding the evolutionary history of the trigasterids will help us to understand how each group diversified over time and adapted to a such wide range of habitats. Nonetheless, the status of some of these species, including the type species of *Trigaster*, has not been revised since the original description in the late 1890s. Therefore, the objective of this study is to validate the known species in this region based on morphology and molecular data, and to reconstruct their evolutionary history with mitochondrial markers. Our preliminary results indicate the presence of eight lineages of *Trigaster*, six of *Borgesia*, and another five for *Neotrigaster*. Of the *Trigaster* lineages, two correspond to *T. longissima*, one to *T. lakesteri* (type species of the genus), while the remaining lineages represent four undescribed species. This includes a first record for Culebra Island in Puerto Rico. Furthermore, *Neotrigaster rufa* is composed of three divergent lineages associated with each major mountain range in Puerto Rico. Lastly, *Borgesia* is suggested to be paraphyletic, with the taxon *B. wegei* represented by two strongly divergent lineages. Based on species delimitation analyses, several new species may be nested within *N. rufa*. Lastly, based on the preliminary morphological description of these specimens, *T. lankesteri* is a variable species, which might include the diagnostic features of *T. calwoodi* and *T. intermedia*.

Therefore, the latter two species might represent variations of *T. lankesteri*. While this study represents the first attempt to reconstruct and validate species of earthworms in the Greater Antilles, we also recognized that more data is needed. Future efforts will include phylogenomic and integrative approaches to further understand trigasterid evolution and diversification.

Keywords: gigantism; island biogeography; Acanthodrilidae; mountains; tropical biodiversity.

EXPLORING EDAPHOBASE 2.0: A ROBUST SOIL BIODIVERSITY DATA WAREHOUSE AND ITS BENEFITS

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Edaphobase (www.edaphobase.org) is a data warehouse and information system that focuses on soil zoology and soil ecology. It is used for the collection, management, and dissemination of data related to soil-dwelling organisms, particularly soil invertebrates like earthworms, nematodes, mites, and other micro- and macrofauna. Edaphobase is a valuable resource for researchers and scientists involved in soil science and ecology, as it provides access to data on the distribution, taxonomy, and ecology of soil-dwelling organisms, which is essential for understanding soil ecosystems and their role in various ecological processes. This database contributes to the advancement of soil biodiversity research and aids in the development of sustainable land management practices. Within the EU COST Action "EUdaphobase" (https://www.eudaphobase.eu/), Edaphobase has been further developed and adjusted for enhanced aid to researchers, conservationists and decision makers to further understand and protect soil biota. The structure of Edaphobase is complex in order to enable not just input of biodiversity data, but also a large number of associated metadata. The strengths of Edaphobase are data transparency policy, quality control and a possibility of providing DOIs to uploaded datasets. Data is uploaded to Edaphobase via specific software ("Upload Wizard"). Even though only 17-22 datafields are mandatory during the upload, te existence of more than 650 available data fields can be overwhelming. In this presentation, we will offer a concise introduction to the upload software and provide guidance on utilizing the available resources effectively.

Keywords: soil biodiversity.

HISTORY, ECOLOGICAL IMPACTS, AND ADAPTATION OF AN INTRODUCED SOIL DWELLER IN THE CHANGING CLIMATE OF IRELAND

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Prosellodrilus amplisetosus (Lumbricidae) is a small endogeic species with a very limited distribution in SW France and NW Spain. Given its low dispersal ability, it is surprising that *P. amplisetosus* has been reported at three locations in southeastern Ireland, ~ 1000 km north of its endemic range. A single adult of *P. amplisetosus* was found at a rural site in Dunlear, Co. Louth, as part of an extensive survey of soil biota across Ireland in 2007; up to ~100 worms/m^2 were found in 2012 at Airfield Estate, a Victorian-era farm in suburban Dublin. In October 2022, we confirmed the continued presence of the species at Airfield and also found it in high numbers in several habitats south of Dunlear in Co. Meath, an area that likely represents some of the oldest farmed land in Ireland. All of the records are within 70 km of each other, near the east coast of Ireland; ongoing survey work at multiple locations across Ireland has not otherwise revealed the occurrence of the species beyond these sites, but the species was recently reported at a single location in the UK. To understand the history and consequences of these occurrences of the species we are sampling across the native and introduced ranges of the species and integrating isotope analyses and phylogeographic analyses as well as physiological experiments and genomic analyses of gene expression patterns under different environmental conditions. We will report on preliminary results from our field surveys and DNA barcode analyses. Ultimately, we will interpret our results in the context of the well-documented human history of the introduction sites.

Keywords: invasive species; barcoding; phylogeography; isotope analyses; experiments.

INFERENCE OF MOLECULAR MARKERS IN EARTHWORM SYSTEMATICS: AN INDIAN PERSPECTIVE

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India hosts four biodiversity hotspots, out of which the North-East Indo-Burma global biodiversity hotspot and the Western Ghats are important regions to explore fauna and flora respectively. Although, the Indian earthworms have been studied under a wide spectrum that includes checklists, biodiversity assessments, land use systems, population dynamics, invasive species, new reports, gut microbiome, barcoding, phylogenetic relatedness, and range extension. Nonetheless, less attention is given to barcoding, genetic variability, phylogenetic relatedness, and population structure studies. Also, in major categories of these studies, classical morpho-anatomical approaches were the foundation to delimit earthworm taxa. As a result, several challenges have emerged in assessing biodiversity of earthworms owing to homoplasy and symplesiomorphic morphological characters that are generally subjective and taxonomic impediments. These impediments have led to the existence of many morphotypes species that are lumped together into single species, thus being uncertain in the taxonomic category. For the first time in India, the molecular characterization of earthworms was given access in 2008. Recently, various molecular markers Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Simple Sequence Repeat (SSR),18S rRNA are mitochondrial COI gene are being used by various workers. Recently, our team studied the phylogenetic relationships of Indian terrestrial clitellates utilizing the COI approach and reviewed gene catenation corresponding to the observation of Fernández et al. (2012), integrating multigene or multilocus studies to recover closely related species within monophyletic clades. The study presents genetic variability in *Amynthas c. carinensis*, *Metaphire anomala*, *Perionyx rufulus*, *P. depressus*, *Pontoscolex corethrurus, Drawida bullata*, *D. victoriana*, *D. vulgaris*, *D. papillifer papillifer*, *D. nagana*, *D. periodosa*, and *D. assamensis* using mitochondrial markers and presents sets of novel species/ records from the study area. Further, in India, the use of molecular markers in the study of clitellates systematics is still in its initial stage. There is a need for the systematic application of molecular phylogenetic approaches to differentiate clitellates groups, which are very diverse and endemic to the country.

Keywords: molecular markers, earthworms of India, Octochaetidae, Megascloescidae, Moniligastridae, Pheretima-complex.

MITOCHONDRIAL DNA EVIDENCES REFLECT POPULATION STRUCTURE AND PHYLOGENETIC GEOGRAPHY IN AMYNTHAS ASPERGILLUM (OLIGOCHAETA: MEGASCOLECIDAE) IN SOUTHERN CHINA

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Amynthas aspergillum (Perrier, 1872), a natural resource of traditional Chinese medicine (Guangdilong) with high economic value, is widely distributed in southern China. To investigate the extent of genetic differentiation and diversity of *A. aspergillum*, a population genetic structure study was performed on 157 samples from 75 locations in southern China using the mitochondrial genes COI, COII, 12S rRNA, 16S rRNA, and NDI. The results showed *A. aspergillum* had a high level of genetic diversity and the variation within populations was the main source of the total variation. Five deeply divergent mitochondrial clades (clade I - V) were detected via both phylogenetic tree and haplotype network analyses, and this finding was supported by the high K2P genetic distance and *Fst* value. And there was no significant phylogeographic structure. Clade II, IV, V, widely distributed geographically, was suggested a recent demographic expansion by multiple analysis results, such as high level of haplotype and low nucleotide diversity, star-shaped haplotype network structures, significantly negative neutrality test values and unimodal mismatch distribution pattern. The divergence time estimates and reconstruction of ancestral-area revealed that *A. aspergillum* originated in Guangxi Province and underwent the initial intraspecific diversification in the early Pliocene to generate clade I, then gradually dispersed eastward and rapid differentiated into clade II-V during the Pleistocene.

Keywords: earthworms; Megascolecidae; *Amynthas aspergillum*; population genetic structure; genetic differentiation.

NEW EARTHWORMS FROM A RURAL VILLAGE IN KWAZULU NATAL

Thembeka Nxele

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Communal areas as well as agroecosystems have received less attention compared to protected areas. Rural villages have natural habitats like grasslands that people use for livestock grazing, and natural forests that they use for firewood and traditional medicine. These spaces have a potential of providing valuable information on earthworm species richness and distribution. However, the natural spaces in villages are threatened by humans building houses in the grazing land. A survey in 2021–2022 took place at kwa-Swayimane rural village, where sugarcane fields and grassland were sampled. New species were discovered from the grassland belonging to *Tritogenia* and *Geogenia* and are illustrated in this paper. The discovery of these species shows how species can be lost even before they are described, because of land transformation.

Keywords: grassland; communal areas; land transformation; Swayimane.

NOTHING IS AS IT SEEMS: PHYLOGENETIC AND MORPHOLOGICAL STUDY OF THE HYPERVARIABLE EARTHWORM SPECIES COMPLEX ALLOLOBOPHORA MOLLERI-MOEBII

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Life in soils has led to a morphological stasis in key edaphic organisms such as earthworms, giving rise to cryptic species with great genetic diversity. However, the opposite phenomenon may be occurring in earthworms such as the *Allolobophora molleri-moebii* species complex, resulting in the description of different species from an alleged single genetic lineage. To solve this taxonomic problem, 35 populations of this complex from Portugal, Spain and Morocco were collected, representing a wide range of variation in one morphological trait (clitellum position). Clitellate individuals were identified and their internal and external characters were studied. The phylogenetic relationships of the sampled populations and other representatives of Lumbricidae, Criodrilidae and Hormogastridae were recovered by Bayesian inference and Maximum Likelihood analyses for a set of seven molecular markers (COI, ND1, 16S, 12S, 28S, H3 and H4). An ultrametric tree was generated with BEAST and haplotype networks were represented with Popart. Finally, a PCA was built using the most relevant morphological characters. The results show phylogenetic clades of recent divergence and inconsistent with morphological data for the *A. molleri-moebii* species complex, recovering together individuals of *A. molleri*, *A. moebii*, *A. fernandae* and *A. monchicana* in several clades. The PCA slightly differentiates *A. monchicana*, the only diploid species of the complex, from the three remaining taxa, whose individuals form a “continuum” in terms of morphological characters. These results support the *A. molleri-moebii* species complex as a single species with an unusual phenotypic variability, possibly acquired through genomic duplication and epigenetic mechanisms. This, and the fact that their distribution area is included in a latitudinal gradient along the Iberian Peninsula, would highlight this complex as a great candidate for studying the interaction between genotype, phenotype and environment, and therefore, the adaptation of these animals to Climate Change.

Keywords: *Allolobophora molleri-moebii;* phenotypic variability; molecular phylogeny; phylogeography.

ON THE HAPLOTAXIDAE MICHAELSEN, 1900 (ANNELIDA, CLITELLATA)

Patrick Martin

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Being, most of which, greatly different in size and present in distinct environments, aquatic oligochaetes (“microdriles”, paraphyletic) and terrestrial oligochaetes (“megadriles”, or “Crassiclitellata”, monophyletic) have traditionally been the subject of interest and study by distinct scientific communities. In this context, the Haplotaxidae family is an exception in that it has always been considered by both scientific communities as falling within their field of expertise. In this presentation, we will examine the historical reasons for this situation. The family Haplotaxidae is often regarded as a basal lineage leading to the megadriles, a view probably inherited from Michaelsen's ideas in the last century. As a result, its morphological characteristics and ecological preferences have often been interpreted with this a priori, as "primitive" characteristics from which “true” earthworms would have evolved. However, we now know that the Haplotaxidae are genuine freshwater “microdriles” which, in their strict sense, i.e. restricted to the genus *Haplotaxis* (Hartmann in Oken, 1819), are phylogenetically quite distinct from the Crassiclitellata. We will review some of these characteristics, which have been interpreted as preceding the emergence of earthworms and place them in a context that reflects current knowledge. We will show that the family Haplotaxidae, in the broadest sense, is a polyphyletic family, with some members appearing, however, as the sister group to the Crassiclitellata. As far as the Haplotaxidae sensu stricto are concerned, we will try to understand why they could be considered as a basal lineage of the Crassiclitellata and present recent results attesting to the extraordinary diversity within this rare oligochaete group.

Keywords: Haplotaxidae; phylogeny; systematics; definition.

OVARY ORGANIZATION AND ULTRASTRUCTURE OF LUMBRICIDAE EARTHWORMS

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Earthworms, like other oligochaetes, are hermaphrodites. Each individual has female and male reproductive organs and can reproduce by cross-fertilization. The ovaries are inconspicuous paired organs located in the XIIIth segment, whose task is the production of oocytes. So far, only the location and a very general structure of earthworm ovaries are known, so this organ has not been studied in more detail. Our research aimed to thoroughly understand the morphology, ultrastructure, and functioning of earthworm ovaries from the Lumbricidae family and, as a result, to understand the course of oogenesis in these organisms. Specimens for the study were collected in Poland and Hungary. Species were determined based on morphological features and DNA barcoding. We chose species from *Aporrectodea*, *Dendrobaena*, *Eisenia*, *Lumbricus,* and *Octolasion* genera for our study. The material was examined by light, fluorescence, and transmission electron microscopy. We found that ovaries are mostly cone-like structures (sometimes also square-like) with the broader (proximal) end attached to the septum between XII and XIII segments, whereas the distal end is narrow and extends into the body cavity. The proximal part of the ovary comprises numerous, densely packed cells, whereas the distal end has a form of a cord composed of linearly arranged growing oocytes. Ovaries are built of germline cells associated with somatic cells. We divided the internal ovary organization into three zones based on light and electron microscopy analyses. Zone I contains oogonia and early-meiotic cells united into germline cysts. In zone II, oocytes and nurse cells differentiate within cysts. In zone III, oocytes grow intensively and lose contact with the rest of the cyst. It was found that in zone I, all interconnected cells are in the same cell phase (synchronous development), and this synchrony is lost in zone II. The presence of germline cysts equipped with the central cytoplasmic mass (cytophore) and the diversification of germ cells into growing oocytes and supporting nurse cells are common features of oogenesis in clitellates. However, germline cysts found in lumbricid earthworms have poorly developed cytophore with reticular character. After the conducted analyses, we can conclude that the general pattern of organization of earthworm ovaries between different genera of Lumbricidae is very similar, and the differences occur primarily at the level of their shape. Further analyses will concentrate on the details of cyst organization and functioning, such as the number of interconnected germ cells, and also allow to compare Lumbricidae ovaries with other selected families, i.e., Megascolecidae, Hormogastridae, and Eudrilidae.

Keywords: Lumbricidae; earthworm ovaries; germline cysts.

Financial support: The research was supported by National Science Centre, Poland, contract number 2020/37/B/NZ4/00560.

POPULATION GENETIC STRUCTURE AND DIVERSITY OF METAPHIRE REMANENS (OLIGOCHAETA: MEGASCOLECIDAE) BASED ON MITOCHONDRIAL DNA ANALYSIS

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Metaphire remanens Jin & Jiang, 2022 is widely distributed throughout Henan, Hunan and Hubei provinces of China. We sequenced the mitochondrial DNA to investigate its population genetic structure and genetic diversity, including COI, COII, ND1, 12S and 16S, derived from 208 individuals from three geographic locations. The genetic diversity indices showed that populations of *M*. *remanens* have a strong genetic structure and obvious dispersal histories. *M*. *remanens* did not experience population expansion due to its parthenogenesis evolution history. The divergence time estimate shows that *M*. *remanens* originated at Late Oligocene (~27.4 Ma) and then generated two main lineages at Middle Miocene (~16.0 Ma). A distribution map of the ancestral region shows that *M*. *remanens* originated in the China's Central Plain, then spreads to hilly region in eastern and western of Hubei Province. With the end of the Quaternary glaciation (~ Ma), it then spreads to plain region. The results indicate that glaciation, geographic isolation, and dispersal ability are significant factors that influence the differentiation and dispersal of *M*. *remanens*.

Keywords: earthworm; *Metaphire remanens*; mitochondrial gene; population genetic structure; genetic diversity.

PRESENCE OF SOIL FAUNA IN POST-MINING AND POST-FIRE SITES OVERGROWN WITH VARIOUS TREE SPECIES

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Restoring degraded areas post-human activities such as mining or after catastrophes like fires is crucial. We conducted a thorough exploration of diverse strategies to regenerate and reconstruct forest ecosystems in these settings. Soil organisms play a pivotal role in the reclamation process of newly formed or regenerated soils. Afforestation, facilitating the influx of organic matter, proves instrumental in promoting microorganism development and subsequently facilitating the emergence of soil fauna, fostering the soil formation process. Our primary research focus was to address the question: In post-mining and post-fire soil regeneration, how do soil fauna, earthworms and enchytraeids, contribute to the soil-forming process? We took into account the tree species and the presence of pyrogenic carbon. For our study, we specifically chose sandy soil in sandpit excavations (SM) and post-fire areas (PF). These areas were afforested with a variety of tree species, including Scots pine (Pinus sylvestris L.), European larch (Larix decidua Mill.), Silver birch (Betula pendula Roth), and European oak (Quercus robur). Soil samples were carefully collected for basic soil analysis, encompassing pH, soil organic carbon and nitrogen content, and Cation Exchange Capacity (CEC). Furthermore, we collected earthworms and enchytraeids from all plots to assess the density and species diversity. When comparing soils reclaimed after sand excavation (SM) with those undergoing regeneration after a fire (PF), the latter are characterized by a higher content of carbon, nitrogen, CEC, and soil fauna density. Earthworm density ranged from zero in Larch_SM to 28 ind m^{-2} in Birch_PF. Earthworms belonged species common in Poland such as Dendrobaena octaedra, Bimastos rubidus and Aporrectodea caliginosa. Enchytraeid density ranged from 11890 ind m^{-2} in Pine_SM to 92269 ind m^{-2} in Pine_PF. Enchytraeids belonged to 23 species and included two species of Achaeta to science.

Keywords: enchytraeids, earthworms, tree species, post-fire soil, post-mining soil.

REMARKS ON THE DENDROBAENA ALPINA SPECIES COMPLEX IN THE CARPATHIAN BASIN

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The *Dendrobaena alpina* species group, as originally defined, contained some fifteen nominal species characterized by a clitellar position on segments 26, 27-33, 34, tubercles around 30-32. It was thought that a characteristic synapomorphy of this group was the reduction of the number of hearts with the last pair in segment 10 or 11 and lacking extraoesophageals in 12. These species show a great variability regarding the number of vesicles, the position of the spermathecal pores and the pigmentation. A recent molecular taxonomic study revealed that the original *D. alpina* species group is polyphyletic, because the unpigmented Bulgarian *D. alpina* specimens form a clade together with Anatolian and Levantine species such as *D. orientalis*, *D. pentheri*, *D. orientaloides* and *D. semitica*. Consequently, the unpigmented population from Bulgaria was described as a new species, *Dendrobaena misirlioglui.* Here we reanalysed the available *D. alpina* species group sequences together with the newly collected *D. alpina popi* and *D. alpina armeniaca* and two *D. alpina alpina* specimens from the Retezat Mts, Southern Carpathians, Romania, originally used in the first publication where the species group was defined. Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit from the body wall behind the clitellum. The mitochondrial COI and 16S rRNA genes and the nuclear ITS2 gene were amplified with PCR and sequenced at the Hungarian Natural History Museum. The sequences were aligned with the MAFFT online service. The concatenated sequences of 1656 bp were analysed using Bayesian and ML methods on the CIPRES and IQTree platforms respectively. The Bayesian and ML analyses resulted in almost the same topology and corroborated the previous observations that the unpigmented species *D. misirlioglui* from Bulgaria and *D. alpina popi* from the Northern Balkan and Southern Carpathians do not belong to the heavily pigmented *D. alpina* group clade, but join the Anatolian species (*D. alpina armeniaca*, *D. semitica*, *D. pentheri*, *D. orientalis,* etc.). Interestingly, the Retezat specimens, which are moderately pigmented on the head, are also associated with this Balkan-Anatolian clade and are thus not related to the more northern *D. alpina* proper, and represent a new species. In honour of the work of Victor Pop and Victor V. Pop, this species will be named after them. Moreover, the former subspecies *D. alpina popi and D. alpina armeniaca* are elevated to species rank.

Keywords: *Dendrobaena alpina*, Oligochaeta, molecular taxonomy, new species.

REMARKS ON THE PHYLOGENY OF THE MALAGASY ACANTHODRILID EARTHWORMS (MEGADRILI: ACANTHODRILIDAE)

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Madagascar, the world's fourth largest island, is one of the 10 biodiversity hotspots originally recognised. Earthworms of this island were only occasionally surveyed between 1887 and 1931. During this period 33 earthworm species were recorded on Madagascar; most of them belong to the native family Kynotidae but one ocnerodrilid and four acanthodrilid species were also reported. In 2008, a new project was launched to explore the earthworm fauna of Madagascar, resulting in the description of 15 new species, six of which belong to the family Acanthodrilidae, bringing the total to 11 species, including the previously described 5 acanthodrilid species. The taxonomy of the Malagasy acanthodrilids is rather complex. Six species comprises the endemic genus *Howascolex* which is characterised by a mixed holoic and meroic nephridial system and well developed calciferous glands in the region of 15–16. Of the other purely holoic species, one belongs to the newly described genus *Vazimbascolex,* which shows a microscolecine reduction of the male apparatus. Of the remaining 4 holoic species, only one is vesiculate with characteristic J-shaped nephridial vesicles therefore this species was provisionally placed in the New Caledonian genus *Acanthodrilus*. The remaining three species are avesiculate and over time, they have been classified into different genera like *Eodrilus*, *Notiodrilus* and later *Diplotrema*. *Diplotrema* is basically an Australian genus therefore for the South African *Eodrilus* like species, a new genus *Eodriloides* was erected on their paired spermathecal diverticula. The Malagasy holoic avesiculate species are similar to those of South Africa in this character; they have therefore recently been placed in *Eodriloides* as well. The proper phylogenetic position of the Malagasy acanthodrilin earthworms can only be determined using molecular methods due to the inadequacy of the morphological characters. We have extracted DNA from four Malagasy acanthodrilid earthworms and two species collected in New Caledonia. Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit from the body wall behind the clitellum. Different regions of the mitochondrial gene 16S rRNA and the nuclear 18S and 28S rRNA genes were amplified using PCR and sequenced by SolGent, South Korea. Sequences were aligned using the MAFFT online service including James & Davidson's Acanthodrilidae sequences downloaded from GenBank. The concatenated sequence of 3083 bp was analysed using Bayesian and ML methods on the CIPRES and IQTree platform respectively. The Bayesian and ML analysis resulted in highly concordant trees. Interestingly, neither the acanthodrilids of Madagascar nor the *Acanthodrilus* species of New

Caledonia formed a monophylum. The New Caledonian *Acanthodrilus ouenghianus* is sister to an Australian *Diplotrema* sp. in a moderately supported clade formed by other acanthodrilid genera like *Microscolex* and *Parachilota*. The Malagasy Howascolex madagascariensis joins to this clade (however, with low support). Another highly supported clade is formed by the remaining Malagasy species (both the meroic *Howascolex* and the holoic *Eodriloides*) and also a New Caledonian species *Acanthodrilus barrieri*. This corroborates the previous findings that meronephry is highly homoplasious as well as the vesiculate-avesiculate condition of the excretory system.

Keywords: Madagascar, Oligochaeta, Crassiclitellata, multilocus phylogeny.

SHOOTING THE PHANTOM: WHAT IS OCTOLASION TYRTAEUM?

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Enterion tyrtaeum was described by Savigny in a group of species now belonging to the genus *Lumbricus*, with characteristics such as closely paired setae, two pairs of spermathecae, three pairs of vesicles, clitellum on segments 30–35. After Dugès, Rosa and Beddard treated *tyrtaeum* as *Lumbricus*, Rosa mentioned it as species inquirenda. Ribaucourt reported a specimen he found in Valais, Switzerland, morphologically identical to *E. tyrtaeum*, under the name *Allolobophora tyrtaea*. He compared this specimen to *A. profuga* (=*O. lacteum*) and emphasized some differences, eg. body size, colourless but has iridescent reflections, closely paired setae. Ribaucourt denoted *A. tyrtaea* as 'nov. species?'. Thus, Michaelsen differentiated two species: *L. tyrtaeus* Savigny, 1826 and *Helodrilus* (*Helodrilus*) *tyrtaeus* (Ribaucourt, 1896). The latter was then regarded as a synonym of *Proctodrilus antipai* (Michaelsen, 1891) by Easton. Zicsi marked both *E. tyrtaeum* and *A. tyrtaea* as species incertae sedis. In the database of Csuzdi the name *tyrtaeum* appears as *Lumbricus* sp. inc. sed. Černosvitov regarded *E. tyrtaeum* identical to *O. lacteum* (Örley, 1881), but mentioning it only in a short footnote. Gates proposed to use the name *tyrtaeum* instead of *lacteum*. Several authors followed Gates however, nobody could examine the type specimen of *E. tyrtaeum* because it disappeared from the collection of Savigny. Easton and Blakemore treated *O. lacteum* and *O. tyrtaeum* separately. To reveal the current status of *tyrtaeum-lacteum* and the relationships within the genus *Octolasion*, we performed a molecular phylogenetic study based on COI sequences. Altogether 122 *Octolasion*, *Octodrilus*, *Lumbricus, Bimastos*/*Dendrodrilus*, and *Aporrectodea* sequences were used. We performed Bayesian inference for the phylogenetic reconstruction, the genetic distances were calculated with the K2P model. According to our results, the morphologically uniform *O. cyaneum* forms a monophyletic group appearing as a descendant group of the *lacteum-tyrtaeum* complex. This latter group appears mixed and is separated into three different lineages (L1, L2, L3). The morphologically quite similar *O. lacteovicinum* joins L3. *O. lacteum* is a parthenogenetic and morphologically diverse species, which manifests in the body size and the size of the male pores. Unfortunately, we do not have data on the morphology of the specimens used for the analysis however, Shekhovtsov et al. proved that on the basis of COI, COII and ITS2 the small and large forms of *O. tyrtaeum* can't be separated well. Parallel to this, our specimens studied can't be separated by their geographic distribution either. The genetic distances within lineages are between 15.8%–18.6%, while the distances between the lineages and *O. cyaneum* are between 10.9%–15.8%. Our study also reveals several misidentifications in the databases, e.g., *B. rubidus* and *Ap. caliginosa* specimens identified as *O. tyrtaeum*, *O. cyaneum* and *Octolasion* sp; and one *O. lacteum* sequence is nested

within the *Lumbricus* species. In summary, *O. cyaneum* is a monophyletic descendant group of *O. lacteum*, without which this latter remains a paraphyletic species. *O. tyrtaeum* and *O. lacteum* can’t be separated genetically moreover, regarding its history, the name *E. tyrtaeum* should be treated as *Lumbricus* species incertae sedis.

Keywords: *tyrtaeum*; *lacteum*; *Octolasion*; Oligochaeta; molecular phylogeny.

TAXONOMY, MORPHOLOGICAL VARIATIONS, AND PHENOLOGY OF A GROUP OF LARGE-BODIED EPI-ENDOGEIC EARTHWORMS ENDEMIC TO TAIWAN

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The *Amynthas sexpectatus* species complex is a group of large-bodied pheretimoid earthworms endemic to Taiwan. With a mean body length of 20 cm (95% confidence interval = 12–28 cm) and brownish or greenish yellow coloration, these organisms have a unique appearance among epi-endogeic species. Our field surveys suggest that these organisms have a continuous, north-south distribution throughout the hill regions in western Taiwan. So far, five species in this species complex have been described, but they cannot be reliably identified using diagnostic characters documented in the literature. In this study, we aim to revisit the taxonomy of this species complex and investigate its phenology. Molecular phylogeny based on mitochondrial genes suggests seven lineages with an allopatric distribution, with five of which corresponding to five nominal species. However, nuclear gene haplotype networks show a pattern indicating incomplete lineage sorting, providing little support for the seven-species hypothesis. Three molecular species delimitation methods (Assemble Species by Automatic Partitioning, Bayesian implementation of the Poisson tree processes, and Bayesian Phylogenetics and Phylogeography) result in inconsistent outcomes. Factor analysis of mixed data (FAMD) based on external and internal morphological characters failed to separate the seven mitochondrial lineages, indicating that these lineages are morphologically indistinguishable. We conclude that there is only one species in the *A. sexpectatus* species complex, and *A. binoculatus*, *A. fusing*, *A. lioujia*, and *A. majia* are all junior synonyms of *A. sexpectatus*. Monthly field sampling during 2021–2022 indicates that this giant earthworm is an annual species. Individuals can be found for 6–7 months throughout spring, summer, and fall, with the first mature individual appearing in as early as June in northern populations. Presumably, the species survives through winter-early spring (from December to March/April/May) as cocoons. Populations in the south have a “delayed” phenology compared to those in the north, likely due to different temporal patterns in precipitation.

Keywords: *Amynthas sexpectatus* species complex; annual species; phylogeny; molecular species delimitation; factor analysis of mixed data.

WHEN, WHY AND HOW TO BECOME A GIANT? EVOLUTIONARY DRIVERS, MOLECULAR MECHANISMS AND ECOLOGICAL IMPACT OF LARGE SIZED SCHEROTHECA, OCTODRILUS AND HORMOGASTRIDAE

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An evolutionary trend towards gigantism developed independently in at least three unrelated European earthworm groups (*Scherotheca*, *Octodrilus* and several Hormogastridae) whose ranges partially overlap in the Western Mediterranean. They are an ideal opportunity to understand the evolutionary mechanisms behind the origin of large body size. This complex trait could have evolved convergently as a response to similar or different environmental pressures, and at different moments of geological history or synchronically. The impact of these animals on soil ecosystem functioning is expected to be even larger than that of other earthworms, but is still poorly understood. To answer these questions, a multidisciplinary approach joining taxonomy, molecular biology and community ecology will be followed. The first objective is to establish a robust phylogenetic background based on Anchored Hybrid Enrichment (a targeted Next-Generation sequencing method) as a base for an explicit spatio-temporal frame. The second objective is to characterize the genes involved in the evolution of giant body size by identifying selection signatures and differences in gene expression (using transcriptomes). The third objective is to study the effect on giant earthworms on the diversity and composition of their communities, and the effect of their activity on their habitat (including soil physical characteristics, hydrological properties and organic matter availability) using X-ray tomography. The combination of targeted phylogenomics, transcriptomics, community ecology and burrow X-ray imaging represents an original and innovative approach within the field of soil invertebrate fauna. Results will allow the incorporation of large earthworms in soil management, direct soil diversity conservation efforts and open potential lines of research in applied molecular biology. Additionally, this approach could be extended to other earthworm clades with similar evolutionary trends to obtain more general and robust conclusions.

Keywords: molecular phylogenetics, molecular evolution, body size, Lumbricidae, Hormogastridae
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POSTER PRESENTATIONS

ADDITION OF THREE NEW RECORDS FOR MARINE OLIGOCHAETA (ANNELIDA: TUBIFICIDAE) FROM THE COASTAL FAUNA IN KOREA

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A survey was conducted to confirm the diversity of marine life residing at a depth of 20-50 meters around Ulleungdo and Dokdo in the East Sea and Jeju Island in the South Sea of Korea. During the survey, both fine and coarse sand samples were collected from various areas. As a result of the sample collection, three species were identified as new marine Oligochaeta records for the Korean fauna. The morphological features of these species were meticulously examined under a light microscope, and detailed photographs were taken to provide an accurate description of their characteristics.

Keywords: marine Oligochaeta; Limnodriloidinae; Rhyacodrilinae¿ South Korea.

COMBINED ANALYSIS OF GUADELOUPE AND MARTINIQUE EARTHWORMS INDICATES COMPLEX COLONIZATION HISTORY

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The presence of diverse endemic earthworms on several of the Lesser Antilles islands raises the question of how earthworms were able to colonize small islands presently surrounded by salt water. Here we sampled earthworms from Guadeloupe and Martinique, obtained the CO1 DNA barcode sequences from them and used molecular species delimitation tools and maximum likelihood phylogenetic analysis to ask if the colonization of each island could be explained by a single colonization event per island. *Dichogaster* s.l. species were the primary focus, because the are abundant and diverse on both islands. Maximum Likelihood *CO1* tree topology indicates that the two faunas are not based on a single or simple colonization event(s) per island. Instead, either 1) that some lineages diverged prior to arrival on the islands, resulting in members of these more deeply divergent lineages being present in both locations, or 2) that some inter-island exchange has taken place. A mainly soil-dwelling clade on Guadeloupe is derived within the main clade of Martinique species, and an arboreal Guadeloupe clade is sister to the widespread, abundant MART-sp. 6, which may be derived from Guadeloupe ancestors. The basal-most 2 branches are all Guadeloupean species, of epigeic and/or arboreal habitats. While the oldest divergences are within Guadeloupe lineages, there are more recent divergences between Martinican ancestors and Guadeloupe offshoots. It is possible that a third land area was involved, on which some diversification took place, and this area was the source for earthworm colonization of the central Lesser Antilles. Dominica, which lies between Guadeloupe and Martinique, has *Dichogaster* species morphologically similar to the main group of Guadeloupe arboreal species. While we cannot say why the soil-dwellers of Martinique and Guadeloupe have no known relatives on Dominica, it will be interesting to obtain genetic data from Dominican *Dichogaster* species. We predict they will show affinities to the main group of arboreal Guadeloupe species.

Keywords: phylogeography; *Dichogaster*; DNA barcode; species delimitation; Caribbean islands.

EARTHWORMS OF KAMENJAK PENINSULA (CROATIA)

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Mršić (1991) previously designated Donji Kamenjak as the l*ocus typicus* for the species *Octodrilus istrianus* (Mršić, 1988). However, the area remained largely unexplored since that time. Given that *O. istrianus* is an endemic species to the Istrian region, this fact inspired our research in this unique location.Field samplings were conducted over a two-year period (2020-2021), with biannual collections in both spring and autumn. In total, we identified 11 earthworm species, including ten from the Lumbricidae family and one from the Acanthodrilidae family. We confirmed the presence of *O. istrianus* (Mršić, 1988) and, for the first time in Croatia, documented the presence of *Microscolex dubius* (Dugès, 1837) and *Helodrilus ospensis* (Mršić, 1990).The endemic species *O. istrianus* in this surveyed area faces threats from mechanical land cultivation practices and the impact of intensive tourism during the summer months.

Keywords: *Octodrilus istrianus*; protected area; Istria, endemic species.

ERIK2 SERBIA - MACHINE LEARNING-BASED EARTHWORM IDENTIFICATION KEY AND GIS PLATFORM (WEB AND MOBILE APPLICATION)

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Present research represents a seamless evolution of a software-based earthworm identification system previously developed for Croatian species. Taxonomic species identification plays a pivotal role in biodiversity research. Despite the continuous advancements in computer technology, there remains a notable absence of user-friendly software tools for earthworm species identification, alongside the concurrent storage of location data. Our research is dedicated to addressing this gap by introducing a machine learning-based earthworm identification key and a Geographic Information System (GIS) platform, referred to as ErIK2. The ErIK2 platform is a comprehensive solution, combining an earthworm species identification key with a sophisticated GIS component. The machine learning-powered tabular key enables precise species identification through an analysis of morphological and anatomical characteristics of individual earthworms. The GIS component facilitates the input and visualization of data stored in a dedicated geospatial database. Users can conveniently record the time and location of various earthworm species, either during the identification process within the ErIK2 platform or independently.ErIK2 Serbia, a specialized edition of the platform, currently supports the identification of 78 earthworm species found within Serbian territory. To ensure widespread accessibility, the platform has been rigorously tested and proven to be compatible with various operating systems, web browsers, and smartphone devices. You can access the ErIK2 Serbia web application at http://earthworms.eu/erik2Serbia, or download the ErIK2 Serbia Application from Google Play. ErIK2 is designed with ease of use in mind, poised to emerge as a pivotal tool for mapping the distribution of earthworm fauna across Europe. It holds immense potential for both scientific research and citizen science projects. Our future endeavors include integrating additional keys and expanding our database with species and findings from various countries through collaborative efforts.

Keywords: taxonomic identification; machine learning; geospatial database; Balkan.

F24/AWE - THE FOOD PALATABILITY INDEX OF EARTHWORMS.
THE CASE STUDY OF L. TERRESTRIS L. IN RELATION TO FOUR PLANT SPECIES (X TRITICOSECALE, SINAPIS ALBA L., FAGOPYRUM ESCULENTUM MOENCH, PHACELIA TANACETIFOLIA BENTH.)

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Lumbricus terrestris L. is an anecic earthworm species ubiquitously inhabiting an unploughed soil across temperate climate. The type of mulch (i.e. plant species) left on the surface may or may not be an attracting factor for this species. In consequence, the preferred plant litter may lead to an increase in the population size of this species in the agricultural field. In the assessment of food palatability and dynamic of its intake can be measured using a simple F24/AWE index. It means the averaged fresh mass of food consumed by the earthworms within 24 hours (F24) compared to the average fresh weight of the individual (AWE). The higher the value of the indicator, the greater the palatability of the food eaten by the earthworms and the greater dynamics of the intake and processing of litter into coprolites. In broader ecosystem-based context this index informs about the efficiency of substrate decomposition by individual actors of the ecosystem, i.e. indicates the effectiveness of saprophagous macrofauna in processing organic matter. In the laboratory experiment the F24/AWE index for four plant species (spring triticale ×*Triticosecale* Wittm. ex A. Camus, white mustard *Sinapis alba* L., buckwheat *Fagopyrum esculentum* Moench and lacy phacelia *Phacelia tanacetifolia* Benth.) was assessed using *L. terrestris* L. specimens. The ratio of food intake per day to the average body weight of one earthworm exceeded the unity threshold (1.41) only in the case of the soil object. In other cases (non-cereal plants) it oscillated around 0.22 and in triticale reached three times lower value (0.07). The most preferred stubble crop species were white mustard (0.27) and buckwheat (0.24).

Keywords: earthworm; evolution; phylogeny; speciation; dispersal.

GEOGRAPHICAL PARTHENOGENESIS OF THE SPECIES DENDROBAENA ILLYRICA (COGNETTI DI MARTIIS, 1906) AND PROPOSAL FOR SYNONYMIZING OF SOME BALKAN SPECIES

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Dendrobaena Eisen, 1873 has always been a problematic group for earthworm taxonomists. The history of this genus is long and complicated by the fact that includes a large number of taxonomically challenging species pending revision (especially in the Balkan Peninsula) possibly due to the considerable number of species and their great morphological variation. Balkanic earthworms are taxonomically similar species that are difficult to distinguish; some have been described as new species, which raises doubts about their identity. *Dendrobaena illyrica* (Cognetti de Martiis, 1906) is a species group with high taxonomic variability in terms of pigmentation, position of the first dorsal pore, position of clitellum and *tubercula pubertatis*, position of calciferous glands, and position of spermathecae. Its varieties have been described several times under different specific names, and erroneous identifications are still occurring. *Dendrobaena illyrica* has been considered a parthenogenetic species in the pertinent literature, and the present contribution is a confirmation of the geographical parthenogenesis in this species (e.g. *Dendrobaena serbica* Karaman, 1973). The taxonomic status of Balkanic endemic species *Dendrobaena sasensis* Šapkarev, 1983 and *D. serbica* was clarified by comparing specimens from our collections and literature data, to determine the intraspecific variations of taxonomic characters. The individuals of *D. serbica* that we found appear externally imperfect given the narrower glandular atrium of their male aperture, lack of *tubercula pubertatis*, and in some cases a shortened clitellum, corresponding to the parthenogenetic form of the species. Given the high polymorphism of taxonomic characters at the population level, we support geographical parthenogenesis for *D. illyrica*, mainly because their study in several distant populations revealed individuals in the border area lacking *tubercula pubertatis* . On the other hand, an interesting species is *D. sasensis* described by Šapkarev (1983to North Macedonia only, and considered by Mršić (1991) as *nomen nudum*. Based on the literature data, we noticed that *D. sasensis* differ from *D. illyrica* by shorter *tubercula pubertatis* (1-2 segments) and body size (65-76 vs. 76-112). Considering that these characters could vary to some extent during maturation and that these differences are within the usual intraspecific variation in species of this group, they are taxonomically relevant. Also, the area occupied by the aforementioned species is found in the territory of Western Balkan. Therefore, the taxonomic and biogeographic similarity of the mentioned species suggests that *D. sasensis* and *D. serbica* are junior synonyms of the species *D. illyrica*. The species of genus *Dendrobaena* in Balkan still need to be studied in a molecular phylogenetic context and molecular markers need to be standardized to combine the fragmentary data. As little is known about the origin of parthenogenetic earthworms, integrative approaches that incorporate molecular, geographical and morphological information will b neccessary to solve the problem of the phylogenetic position of disputed species.

Keywords: Balkan; *Dendrobaena illyrica*; geographical parthenogenesis; junior synonyms.

INSIGHT INTO THE REPRODUCTION OF OLIGOCHAETES: EVOLUTIONARY MECHANISMS RELATED TO PARTHENOGENESIS

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Parthenogenesis is a very common phenomenon in the animal kingdom and in most animal groups there are parthenogenetic forms. Although studies of isolated cases of parthenogenesis in groups of normally sexual animals can contribute to our understanding of the evolution of reproductive systems, work with taxonomic groups in which parthenogenesis has occurred independently in a large number of species may provide a better approach. This is the case with earthworms, which are considered hermaphrodites with cross-fertilisation, as this is the most common mode of reproduction. However, hermaphroditism is not the only reproductive mechanism in oligochaetes. Nowadays, numerous parthenogenetic species are discovered, most of which are polyploid. There are even species that maintain both modes of reproduction. This makes them excellent candidates for studying the molecular and evolutionary mechanisms associated with this type of reproduction. Because parthenogenetic lineages can arise from species with biparental reproduction in a variety of ways, including bacterial infection, spontaneous loss of sex due to mutations in genes related to mating or in genes involved in sexual forms, contagion, or hybridization between individuals of the same or closely related species, the possible evolutionary mechanisms have been studied in three different ways. The first approach is to examine the transcriptional changes that occur in individuals of two earthworm species (one sexual and one parthenogenetic) at the time of reproduction to understand which molecular mechanisms are the same and which are different for carrying out these two reproductive processes. The second goal is to establish and study the general cell atlas of individuals of the two species. Thus, the expression of the different cell types of both species will be studied in order to determine differences and similarities between the two types of reproduction. Since the gut microbiome has been extensively studied in earthworms, the third objective involves its study and the possible relationship between the differences observed in the two species and the two modes of reproduction. We studied three populations with sexual and parthenogenetic specimens: Argelia, Plasencia and Mallorca. We found 5297 ZOTUs grouped by populations. The phyla most present were Actinobacteriota, Firmicutes and Proteobacteria. Algerian earthworms presented more than 500 ZOTUs with differential abundance (314 sexual and 440 parthenogenetic). Thus, some earthworm's microbiome seems to be related with different reproduction modes.

Keywords: earthworm; RNA; single cell; microbiome.

INVASION PATTERNS OF EXOTIC EARTHWORMS IN GALAPAGOS: RELATIONSHIPS WITH ENVIRONMENTAL FACTORS, LAND USE AND TORTOISE CORRIDORS

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Invasive species are a global threat to native fauna and flora, and their effects are exacerbated in islands' ecosystems. The Galapagos archipelago has been the focus of evolutionary biology since Darwin's observations on finches, seminal for developing his theory of natural selection. Galapagos' unique biodiversity is threatened by species introduced for agricultural, livestock and ornamental purposes. On the Island of Santa Cruz, the forests of *Scalesia pedunculata*, a species of endemic tree, are threatened by another introduced plant species, *Rubus niveus*, which invades from the nearby agricultural area. Exotic earthworms are known to favor the establishment of these invasive plants, which in turn may displace endemic vegetation. This study aimed at understanding the earthworm colonization routes and patterns in Galapagos and the role of environmental factors, considering human disturbance but also with a focus on the migratory corridors of endemic giant tortoises (complex *Chelonoidis nigra*). They could be facilitating the dispersal of earthworm cocoons during their migratory movements within the islands and the open corridors created could facilitate earthworms' natural ability to disperse.We collected earthworms by hand-sorting small (25 × 25 cm) soil monoliths, excavated to a depth of 30 cm in Santa Cruz Island. Sampling quadrats were distributed in areas showing different anthropogenic disturbance degrees from protected forests within the Galapagos National Park, dominated by the endemic tree *S. pedunculata* to highly managed agricultural or urban areas, passing through buffer areas. Moreover, samples were collected along the migration corridors of giant tortoises to clarify whether they could be acting as dispersal vectors of these invasive earthworms between protected and not protected areas. Environmental information (plan species, land use, conservation degree, altitude) was recorded and soil samples were analyzed (pH, organic matter, nitrogen, texture). DNA was extracted from the collected specimens and the mitochondrial marker cytochrome oxidase I (COI) was sequenced. The results gathered will help identify habitat preferences of exotic earthworms, their relationship with other species and potential for invasion meltdown. Then, it will be possible to propose measures for invasion management as well as strategies to prevent further spread. This information will be relevant for Galapagos and island ecosystems elsewhere. We have uncovered new earthworm records for Galapagos and possibly new species for science.

Keywords: invasive species; barcoding; anthropogenic disturbance.

IS APORRECTODEA CALIGINOSA SLOWING DISAPPEARING AND COULD IT BE REPLACED BY MICROSCOLEX DUBIUS IN PROVENCE UNDER THE INFLUENCE OF CLIMATE CHANGE?

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Climate change will have drastic effects in the mediterranean zone with more severe and frequent droughts. However, Provence in SE of France shows a great variability regarding climate and soil types. For example, earthworm communities are very different between Avignon and Aix-en-Provence with a lot of paleartic earthworm species found in Avignon but very rarely in Aix but both zones sharing a common pool of mediterranean earthworm species. We assume that (i) the main driver of the species distribution is soil humidity that defined the duration of the activity window for earthworm species and that (ii) with ongoing climate change, soil conditions will change and may exclude most sensitive species characterized by their soil water content requirements. We select three current earthworm species with different ecological needs: *Aporrectodea caliginosa*, *Allolobophora chlorotica* (pink morph) and *Microscolex dubius*. The first is generally found in wetter environment, the second one has a wide distribution in Provence and the last one had a vey limited distribution 50 years ago (with two occurrences in the most southern parts of Provence according to Bouché 1972). We thus first compared the 3 species distribution 50 years ago (data from Bouché 1972) to the nowadays distribution (data from the #Vers2022 operation). Then, we collected 200 additional sampling points obtained after 2000 in the region (mostly agricultural plots). Using distribution maps and some landscape descriptors, we observed that (i) *A. caliginosa* abundance decreases when the sampling point distance to the nearest river increases, (ii) the distribution of *M. dubius* greatly increased and this species is observed more than 100 km northward between 1972 and 2022. These observations will be completed by laboratory experiments to characterize the sensitivity of the three species, assessed by phenological and ecophysiological parameters, to soil water content variability.

Keywords: climate change; biogeography; behaviour; endogeic.

NEW RECORDS OF EARTHWORMS SPECIES IN THE SOUTHERN REGION OF BRAZIL

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Latin America has estimated around 1000 species of earthworms, of which approximately 340 spp. are found in Brazil, but many more still need to be described and discovered. In the current study we evaluated the earthworm's population in three different Land Use Systems (LUS): Native Vegetation (NV), annual crops under No-Tillage System (NTS) and agricultural site under bad soil management practices (BM) in seven municipalities (Mangueirinha in Paraná State, Faxinal dos Guedes in Santa Catarina State and Panambi, Ajuricaba, Cruz Alta, Muitos Capões e Bom Jesus in Rio Grande do Sul State), totalizing 21 sites. Earthworms were sampled in August of 2023, using the quantitative method Tropical Soil Biology Fertility (TSBF) and qualitative method. Overall, 689 individuals were found, 390 on TSBF and 299 on qualitative. Regarding the LUS, NTS presented the higher total number of individuals (362). BM and NV had similar number of individuals (167 and 160, respectively). For the species identificationwork is still in progress. Preliminary results show 21 spp., of which eight are exotic spp. and belong to Acanthodrilidae (1), Megascolecidae (2 spp.), Lumbricidae (3 spp.) and Ocnerodrilidae (2) families. The extant 13 native spp. belong to Rhinodrilidae (2), Glossoscolecidae (10) and Ocnerodrilidae (1) families. In regard to the LUS, NV had 7 spp., BM 9 spp. and NTS 15 spp. The exotic species were found only the agricultural sites (BM and NTS), although the NTS had higher proportion of native species. There are still samples to be identified, but from these preliminary results it is possible to anticipate a high diversity in the sampled sites and high potential for the development of native species in NTS sites.

Keywords: taxonomy; ecology; No-Tillage; native species.

PHYLOGENETIC POSITION OF THE FRENCH-IBERIAN ENDEMISMS PROSELLODRILUS AND CATALADRILUS (LUMBRICIDAE) AND ANCESTRAL RANGE RECONSTRUCTION OF THE GENUS PROSELLODRILUS

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The Iberian Peninsula and the South of France are a center of diversification of the Lumbricidae family, which has a large number of endemisms and basal genera in the phylogeny of lumbricids in that area. Two basal and endemic genera of this area are *Cataladrilus* and *Prosellodrilus*. These genera are very poorly studied and there are very few phylogenetic studies in which representatives of these genera have been included. In the case of *Prosellodrilus,* the type species has never been included in phylogenetic analysis. Therefore, in this study, after capturing specimens of different species of *Cataladrilus* and *Prosellodrilus* in their type localities (including the type species of both genera), a phylogenetic analysis was carried out using the molecular markers COI, 16S (mitochondrial) and 28S (nuclear) of several species of both genera and sequences from other genera obtained in previous studies, with the purpose of demonstrating or rejecting the fact that *Prosellodrilus* and *Cataladrilus* were two monophyletic genera and, observing the phylogenetic relationships of these genera with the rest of the basal genera in the phylogeny. As a result, it was possible to demonstrate that both genera are monophyletic and, therefore, valid genera. Both genera are evolutionarily very close and are found very close to other basal genera within Lumbricidae such as *Castellodrilus* and *Zophoscolex*. Furthermore, by calculating the ancestral area of *Prosellodrilus* it was possible to observe that the origin of the genus is located in an area between Sardinia and eastern France, and that the genus predates the separation of Sardinia from France and the formation of the Pyrenees, which could explain the presence of *P. festae* in Sardinia and the presence of this genus on both sides of the Pyrenees. Furthermore, if the geological age of the Pyrenees and its formation process are studied in detail, it can be observed how the different stages of formation of this mountain range largely coincide with the distribution of the different species of *Prosellodrilus.*

Keywords: Molecular phylogenetics, systematics, historical biogeography, evolution.

SYSTEMATICS, BIOGEOGRAPHY, AND EVOLUTION OF THE ENDEMIC METAPHIRE FORMOSAE SPECIES GROUP (CRASSICLITELLATA: MEGASCOLECIDAE) IN TAIWAN AND THE SOUTHERN RYUKYUS

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The *Metaphire formosae* species group is a clade of giant earthworms with 17 described species or subspecies endemic to Taiwan and the Southern Ryukyus. Previous studies have suggested that the endemism and diversity of these earthworms resulted from a series of speciation events taking place during the formation of the Taiwan Island, presumably several million years ago. However, their phylogeny is still poorly resolved, hindering divergence time estimation and our understanding on the mechanisms leading to this diversity. In addition, although some potential hybrid individuals were previously collected, whether there is inter-specific gene flow or not remains unknown. In this study, we applied double-digest restriction-site associated DNA sequencing (ddRAD-seq) techniques to unearth the phylogeny, the biogeography, and the evolutionary history of the *Metaphire formosae* species group. We discovered two potential new species among immature specimens using DNA barcoding, and obtained a well-supported phylogenetic tree using 804,601 SNPs from ddRAD-seq data. Furthermore, population structure inferred using fineRADstructure revealed that there was higher coancestry between *M. paiwanna liliumfordi* and *M. glareosa*, as well as between *M. feijani* and *M. tengjhihensis*, suggesting the occurrence of potential incomplete lineage sorting (ILS) and/or ongoing gene flow among these closely relative species. In addition, Procrustes analysis indicated that geographical barriers (Central Mountain Range) and altitudes-related adaption might be the main factors that shaped the geographical structure of this species group. Future analyses will focus on divergence time estimation, ecological niche modeling, and evaluating mechanisms leading to speciation events. We anticipate that future research endeavors will further enhance our understanding on the historical biogeography of these earthworms.

Keywords: *Metaphire formosae* species group; taxonomy; phylogeography; reticulate evolution; double-digest restriction-site associated DNA sequencing.

THE FIRST RECORD OF THE VARDAR ENDEMIC SPECIES ALLOLOBOPHORA (S.L.) JONCESAPKAREVI (BLAKEMORE, 2004) (CLITELLATA: LUMBRICIDAE) IN SERBIA

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The Vardar endemic species *Allolobophora* (*s.l.*) *joncesapkarevi* (Blakemore, 2004) is poorly known and still insufficiently studied in taxonomic, faunistic and ecological aspects. The individuals found in Lukovska Banja (Kopaonik Mountain) are the first documented record of the species in Serbia. Thus, *All*. (*s.l.*) *joncesapkarevi* represents a new species for the Serbian earthworm fauna. The present record is the northernmost part of the geographical range of this species. Currently, this species has an uncertain status within the genus *Allolobophora* (*s.l.*) Eisen, 1873. Future molecular phylogenetic research will try to solve the taxonomic status of this species by applying integrative systematics. Our studied specimens were identical to the previous description in the position of the clitellum and *tubercula pubertatis*, the number and position of seminal vesicles and spermathecae, and other characters. Its distinction from the taxonomic similar species *Cernosvitovia dofleini* (Ude, 1922), *Cernosvitovia strumicae* (Šapkarev, 1973), *Cernosvitovia gestroides* (Zicsi, 1970) and *Cernosvitovia zicsica* (Szederjesi, Pop & Csuzdi, 2016) is discussed. *Allolobophora* (s.*l.*) joncesapkarevi *is* close to *C. dofleini*/*C. strumicae* but differs in the number of seminal vesicles (11, 12 vs. 9-12), and a smaller number of segments (80-148 vs. 120-250/151-218), justify its specific status. Also, it differs from the species *C. gestroides* and *C. zicsica* by the number of seminal vesicles (11, 12 vs. 9-12), the shape of the nephridial bladders (U-shaped vs. J-shaped) and distribution (Vardar endemic vs. Dacian endemic). Regarding ecological categories, it belongs to the deep-burrowing-endogeic species, which has a remarkable adaptation to life in the deep soil and strong development of the capacities of displacement in the soil. This species has been found in hill meadows and pastures at altitudes of 600 to 1000 m a.s.l. The results of our study provide new faunistic data to expand the knowledge about the distribution of *All*. (*s.l.*) *joncesapkarevi* on the Balkan Peninsula. The finding of endemic species in Serbia confirms the rich and remarkable and biodiversity in this country as well as the importance of defining mitigation measures for minimizing the negative anthropogenic impacts on the habitats of these species. On the other hand, as many Balkan earthworms are stenoendemics found in fairly fragile hill-mountain habitats, there is a great risk of their extinction. Therefore, every new record of these earthworms is important from taxonomical, ecological and faunistic perspectives.

Keywords: *Allolobophora* (*s.l.*) *joncesapkarevi*; Balkan; new record; Serbia; Vardar endemic.

TWO NEW SPECIES OF *ACHAETA* (ENCHYTRAEIDAE, OLIGOCHAETA) FROM POST-MINING AND POST-FIRE SITES IN POLAN

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Achaeta is a cosmopolitan enchytraeid genus of mostly soil-dwelling annelids with currently 48 known and accepted species. In Europe, 28 species are known, seven of them in Poland. Here we describe two further species from Poland and new to science. They were discovered in samples from a post-mining and a post-fire area in southern Poland, in the framework of a project that investigates the role of soil biota in the soil-forming processes with the presence of additional carbon, i.e. wildfire charcoal and lignite remains (see abstract of Józefowska et al.). Specimens were investigated alive and as stained and unstained whole mounts, and COI barcode gene fragments were generated from some of the types, the holotype of both species included. In Species A shape and distribution pattern of epidermal and clitellar gland cells is unique in the genus. Species B has no peculiar characters but a combination of characters which differs from the rest of species in the genus. Genetic distance (uncorrected p-distance based on COI) between the two new species is 19,9%, distances of the new species from other *Achaeta* species with published COI barcodes range between 15,7% and 26,1%. Identity of sequences between species A and a that of a specimen labelled as "A. cf. brevivasa" allowed us to rectify the identification of the latter. Species A was very abundant at acid sites, and thousands of specimens were identified. Both species are probably common in Europe, a detailed comparison of specimens from further unpublished records is under way.

Keywords: Clitellata; taxonomy; soil biodiversity; new species.

Financial support: The research was funded by The National Science Centre, Poland, grant No. 2021/42/E/ST10/00248.

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