10th International Oligochaeta Taxonomy Meeting

November 3–7, 2025

Osijek, Croatia

Book of Abstracts

Josip Juraj Strossmayer University of Osijek
DEPARTMENT OF BIOLOGY
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Impressum

Title

Book of Abstracts of the 10th International Oligochaeta Taxonomy Meeting (IOTM 2025)

Editors

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Publisher

SCIOM d.o.o.
Fruškogorska 5
31000 Osijek, Croatia

Place and year of publication: Osijek, 2025

Available online: iotm2025.earthworms.eu

How to cite

[Author(s)] (2025). [Abstract title]. In: Djerdj, T., Hackenberger Kutuzović, D., Hackenberger Kutuzović, B. (Eds.) *Book of Abstracts of the 10th International Oligochaeta Taxonomy Meeting (IOTM 2025)*, November 3–7, 2025, Osijek, Croatia. pp. [page numbers].

ISBN 978-953-46841-0-8

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Acknowledgement

The 10th International Oligochaeta Taxonomy Meeting (IOTM 2025) was supported by the Croatian Science Foundation under the project Functional-ecological characterisation of soils - foundation for ecotoxicological classification (FUNECOSoil, IP-2022-10-3233).

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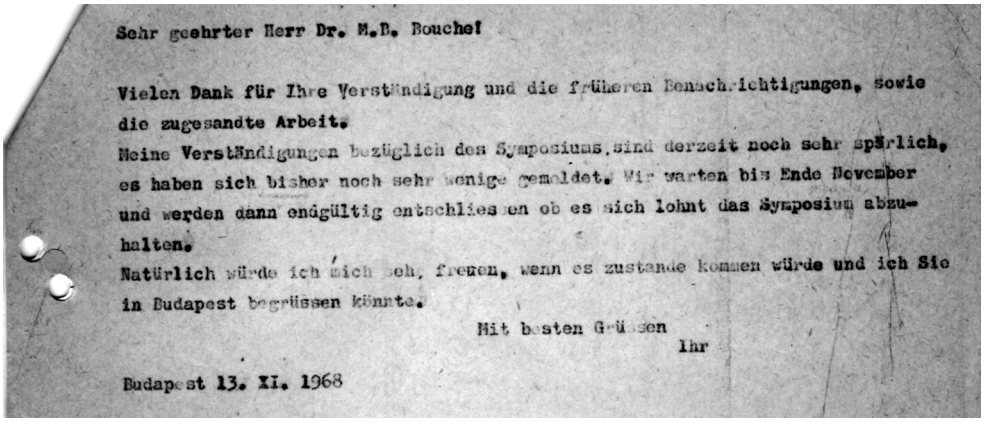
PREFACE

History of the first International Oligochaeta Meetings (IOTMs)

Csaba Csuzdi & Patricia Cardet

The first International Oligochaeta Taxonomy Meeting, organised by Ana Moreno (Complutense University, Madrid) was held in Madrid, Spain, from July 21–25, 2003. However, this conference was not without preceding events. Several conferences were organised from the late 1960s onwards that paved the way for the first IOTM, which later became a regular event and now we are celebrating the 10th conference in Osijek.

The first forerunner of IOTM was the Colloquium held in Nitra (Slovakia) and Aggtelek (Hungary) in 1969, which was organised by Ivo Zajonc and András Zicsi and titled *'Questions of Ecology and Taxonomy of Earthworms'*. The decision to hold this conference in September 1968 was made at the third International Colloquium on Soil Zoology (ICSZ) conference in Braunschweig (Germany) in 1966. However, due to the military intervention in Czechoslovakia on 20–21 August 1968, it was impossible to hold the event on the planned date. The organisers therefore decided to postpone it to 1969. However, due to the unstable circumstances, by the end of 1968, only a few people had registered for the postponed event (Figure 1).



Sehr geehrter Herr Dr. M.D. Bouché!

Vielen Dank für Ihre Verständigung und die früheren Benachrichtigungen, sowie die zugesandte Arbeit.

Meine Verständigungen bezüglich des Symposiums sind derzeit noch sehr spärlich, es haben sich bisher noch sehr wenige gemeldet. Wir warten bis Ende November und werden dann endgültig entschliessen ob es sich lohnt das Symposium abzuhalten.

Natürlich würde ich mich sehr freuen, wenn es zustande kommen würde und ich Sie in Budapest begrüssen könnte.

Mit besten Grüssen

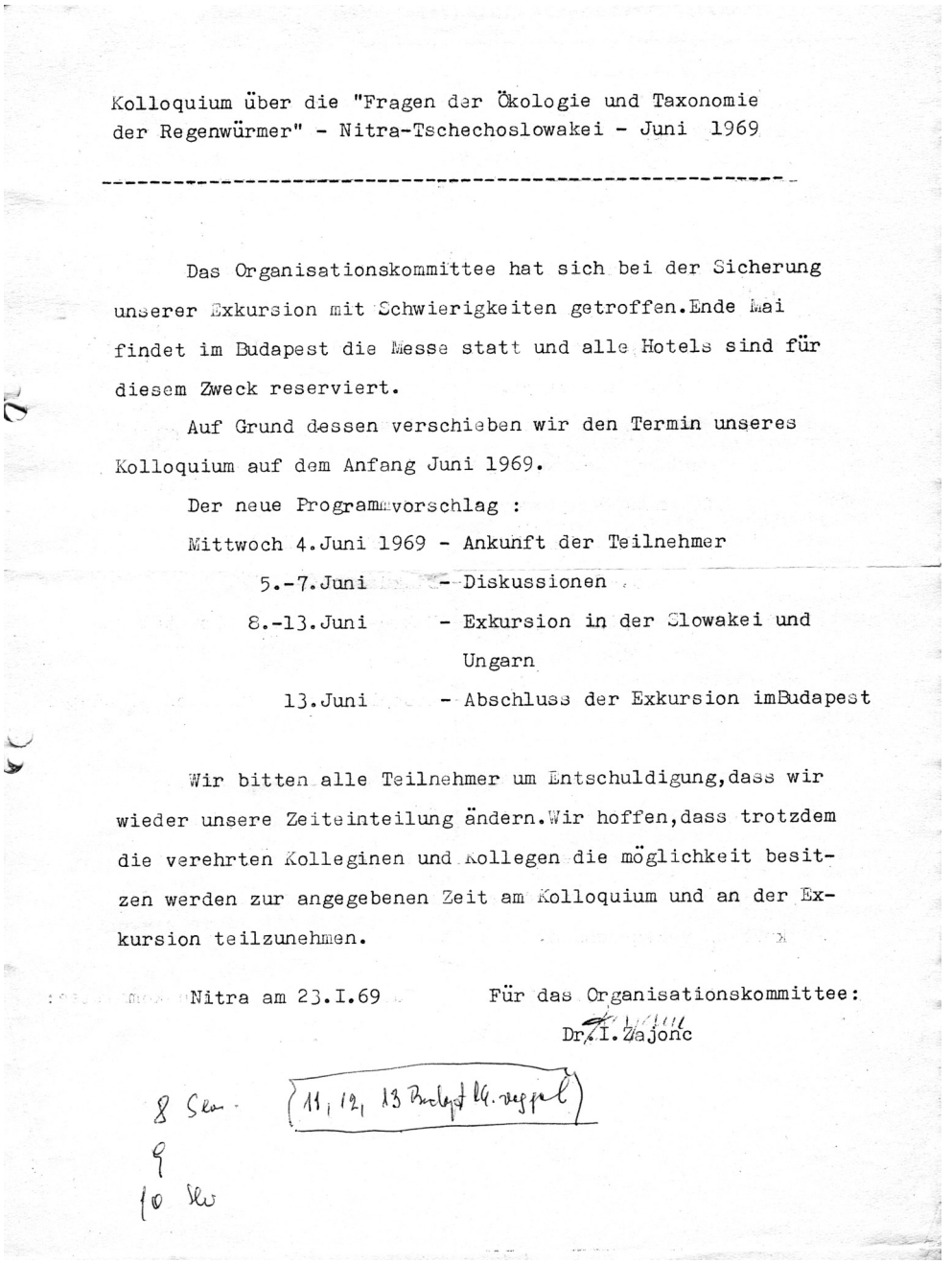
Ihr

Budapest 13. XI. 1968

Figure 1: Letter from Dr. A. Zicsi to M. Bouché regarding the possibility of cancelling the conference due to the low number of registrations.

Finally, about 20 people registered, and a very successful conference was held from 4–13 June 1969 (Figure 2). Among the participants were some of the most prominent earthworm researchers of the time, such as R. W. Sims, P. O. Ljungström, O. Graff, J. D. Plisko, V. V. Pop, J. E. Satchell and M. Bouché.

One of the most important results of this conference was the establishment of the 'Central Collections of Lumbricidae' (Figure 3), which was set up to create permanent and widely accessible depositories of the described earthworm species, serving as a comparative material for further taxonomic and ecological studies. In this way, many specimens, e.g. from Perel and Bouché, found their way into Zicsi's earthworm collection, which is now housed in the Hungarian Natural History Museum.



Kolloquium über die "Fragen der Ökologie und Taxonomie der Regenwürmer" - Nitra-Tschechoslowakei - Juni 1969

Das Organisationskommittee hat sich bei der Sicherung unserer Exkursion mit Schwierigkeiten getroffen. Ende Mai findet im Budapest die Messe statt und alle Hotels sind für diesem Zweck reserviert.

Auf Grund dessen verschieben wir den Termin unseres Kolloquium auf dem Anfang Juni 1969.

Der neue Programmvorschlag :

Mittwoch 4.Juni 1969 - Ankunft der Teilnehmer
5.-7.Juni - Diskussionen
8.-13.Juni - Exkursion in der Slowakei und Ungarn
13.Juni - Abschluss der Exkursion imBudapest

Wir bitten alle Teilnehmer um Entschuldigung, dass wir wieder unsere Zeiteinteilung ändern. Wir hoffen, dass trotzdem die verehrten Kolleginen und Kollegen die möglichkeit besitzen werden zur angegebenen Zeit am Kolloquium und an der Exkursion teilzunehmen.

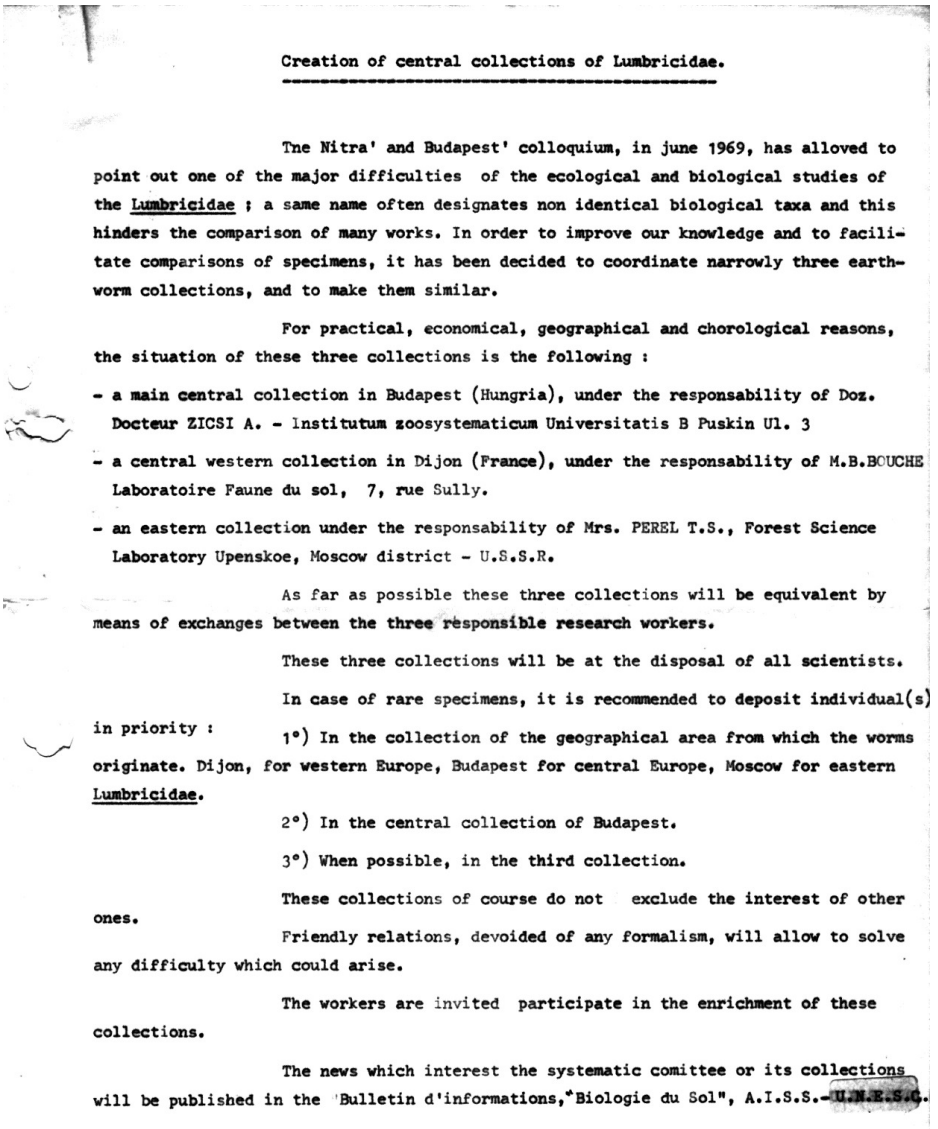
Nitra am 23.I.69

Für das Organisationskommittee:
Dr. I. Zajonc

8 Sa
9
10 So

(11, 12, 13 Budapest Hauptbahnhof)

Figure 2: Letter from Dr. Zajonc to the participants of the new conference schedule (with Dr. Zicsi's hand notes).



Creation of central collections of Lumbricidae.

The Nitra' and Budapest' colloquium, in june 1969, has alloved to point out one of the major difficulties of the ecological and biological studies of the Lumbricidae ; a same name often designates non identical biological taxa and this hinders the comparison of many works. In order to improve our knowledge and to facilitate comparisons of specimens, it has been decided to coordinate narrowly three earthworm collections, and to make them similar.

For practical, economical, geographical and chorological reasons, the situation of these three collections is the following :

- a main central collection in Budapest (Hungria), under the responsability of Doz. Docteur ZICSI A. - Institutum zoosystematicum Universitatis B Puskin Ul. 3
- a central western collection in Dijon (France), under the responsability of M.B.BOUCHE Laboratoire Faune du sol, 7, rue Sully.
- an eastern collection under the responsability of Mrs. PEREL T.S., Forest Science Laboratory Upenskoe, Moscow district - U.S.S.R.

As far as possible these three collections will be equivalent by means of exchanges between the three responsible research workers.

These three collections will be at the disposal of all scientists.

In case of rare specimens, it is recommended to deposit individual(s) in priority :

1°) In the collection of the geographical area from which the worms originate. Dijon, for western Europe, Budapest for central Europe, Moscow for eastern Lumbricidae.

2°) In the central collection of Budapest.

3°) When possible, in the third collection.

These collections of course do not exclude the interest of other ones.

Friendly relations, devoided of any formalism, will allow to solve any difficulty which could arise.

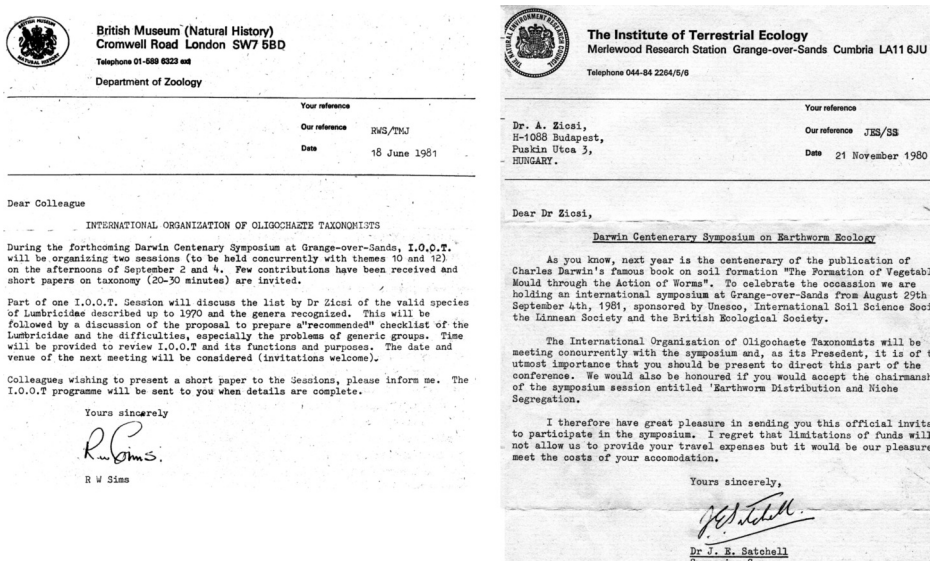
The workers are invited participate in the enrichment of these collections.

The news which interest the systematic comittee or its collections will be published in the 'Bulletin d'informations,"Biologie du Sol", A.I.S.S.-U.N.E.S.C.

Figure 3: Decision on the establishment of the Central Earthworm Collections.

The second conference which could be regarded as forerunner of IOTMs was held from 1–5 September, 1975 in Jaca (Spain). Unfortunately, we did not find much information about this conference, but its most important outcome was the establishment of the International Organization of Oligochaeta Taxonomists (IOOT), chaired by Dr. András Zicsi.

Unfortunately, the organisation's activities were largely formal due to a lack of financial support, and the political divide between the Eastern and Western blocs hindered its effective operation. However, IOOT successfully organised a side-meeting of the Darwin Centenary Symposium held in Grange-over-Sands in 1981. The main topic of the IOOT meeting was the discussion of the valid name of Lumbricidae species described up to 1971 (Figure 4)



British Museum (Natural History)
Cromwell Road London SW7 5BD
Telephone 01-589 6323 ext
Department of Zoology

Your reference
Our reference RWS/TMJ
Date 18 June 1981

Dear Colleague

INTERNATIONAL ORGANIZATION OF OLIGOCHAETE TAXONOMISTS

During the forthcoming Darwin Centenary Symposium at Grange-over-Sands, I.O.O.T. will be organizing two sessions (to be held concurrently with themes 10 and 12) on the afternoons of September 2 and 4. Few contributions have been received and short papers on taxonomy (20-30 minutes) are invited.

Part of one I.O.O.T. Session will discuss the list by Dr Zicsi of the valid species of Lumbricidae described up to 1970 and the genera recognized. This will be followed by a discussion of the proposal to prepare a"recommended" checklist of the Lumbricidae and the difficulties, especially the problems of generic groups. Time will be provided to review I.O.O.T and its functions and purposes. The date and venue of the next meeting will be considered (invitations welcome).

Colleagues wishing to present a short paper to the Sessions, please inform me. The I.O.O.T programme will be sent to you when details are complete.

Yours sincerely

R W Sims

The Institute of Terrestrial Ecology
Merlewood Research Station Grange-over-Sands Cumbria LA11 6JU
Telephone 044-84 2264/5/6

Your reference
Our reference JES/SS
Date 21 November 1980

Dr. A. Zicsi,
H-1088 Budapest,
Puskin Utca 3,
HUNGARY.

Dear Dr Zicsi,

Darwin Centenerary Symposium on Earthworm Ecology

As you know, next year is the centenerary of the publication of Charles Darwin's famous book on soil formation "The Formation of Vegetabl Mould through the Action of Worms". To celebrate the occassion we are holding an international symposium at Grange-over-Sands from August 29th September 4th, 1981, sponsored by Unesco, International Soil Science Soci the Linnean Society and the British Ecological Society.

The International Organization of Oligochaete Taxonomists will be meeting concurrently with the symposium and, as its Presedent, it is of utmost importance that you should be present to direct this part of the conference. We would also be honoured if you would accept the chairmansh of the symposium session entitled 'Earthworm Distribution and Niche Segregation.

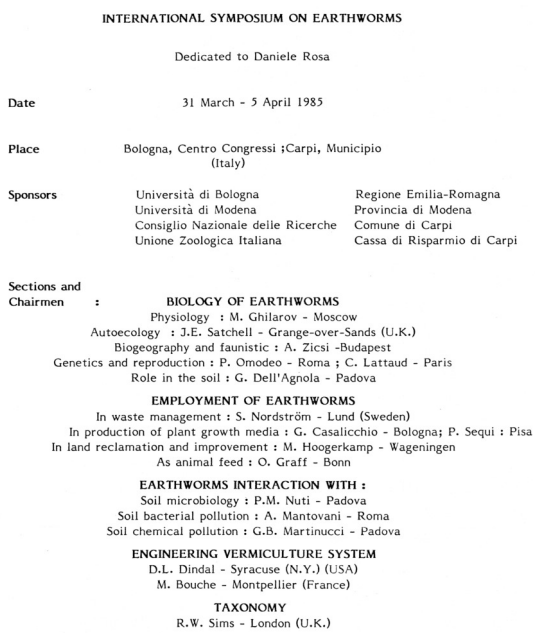
I therefore have great pleasure in sending you this official invit to participate in the symposium. I regret that limitations of funds will not allow us to provide your travel expenses but it would be our pleasur meet the costs of your accomodation.

Yours sincerely,

Dr J. E. Satchell

Figure 4: IOOT circular of the Darwin Symposium and Dr. Satchell's invitation to Dr. A. Zicsi.

Following the Darwin Symposium, another congress was organised: the 'Second International Symposium on Earthworms', dedicated to the work of Daniele Rosa. This took place from 31 March to 5 April 1985 in Bologna. These events were held more or less regularly thereafter and are now known as the International Symposium on Earthworm Ecology (ISEE).



INTERNATIONAL SYMPOSIUM ON EARTHWORMS

Dedicated to Daniele Rosa

Date 31 March - 5 April 1985

Place Bologna, Centro Congressi ;Carpi, Municipio (Italy)

Sponsors
Università di Bologna
Università di Modena
Consiglio Nazionale delle Ricerche
Unione Zoologica Italiana
Regione Emilia-Romagna
Provincia di Modena
Comune di Carpi
Cassa di Risparmio di Carpi

Sections and Chairmen :

BIOLOGY OF EARTHWORMS
Physiology : M. Ghilarov - Moscow
Autoecology : J.E. Satchell - Grange-over-Sands (U.K.)
Biogeography and faunistic : A. Zicsi -Budapest
Genetics and reproduction : P. Omodeo - Roma ; C. Lattaud - Paris
Role in the soil : G. Dell'Agnola - Padova

EMPLOYMENT OF EARTHWORMS
In waste management : S. Nordström - Lund (Sweden)
In production of plant growth media : G. Casalicchio - Bologna; P. Sequi : Pisa
In land reclamation and improvement : M. Hoogerkamp - Wageningen
As animal feed : O. Graff - Bonn

EARTHWORMS INTERACTION WITH :
Soil microbiology : P.M. Nuti - Padova
Soil bacterial pollution : A. Mantovani - Roma
Soil chemical pollution : G.B. Martinucci - Padova

ENGINEERING VERMICULTURE SYSTEM
D.L. Dindal - Syracuse (N.Y.) (USA)
M. Bouche - Montpellier (France)

TAXONOMY
R.W. Sims - London (U.K.)

Figure 5: Circular of the Rosa symposium.

However, conferences dedicated specifically to earthworm taxonomy have ceased to exist until 2003, when the first International Oligochaeta Taxonomy Meeting was organised at the Complutense University in Madrid.

Here, almost twenty years after the Rosa symposium, the taxonomists met again to discuss current issues in earthworm taxonomy and phylogeny, including presentations on the first molecular phylogenetic studies. This conference was very familiar bringing together ten scientists mainly from Europe (Figure 6).

Figure 6: Participants of the 1st IOTM (persons from right to left: Antonia A. Pop, Rob Blakemore, Sonia Borges, Victor V. Pop, Catalina Mischis, Csaba Csuzdi, Ana G. Moreno, Marcel Bouché and Pietro Omodeo as well as three accompanying persons).

The success of the meeting was marked by the publication of the 19 presentations in the first volume of a newly launched series, Advances in Earthworm Taxonomy/Avances en Taxonomía de Lombrices de Tierra, in both English and Spanish. It was also agreed that the next meeting will be held in Cluj, Romania.

The second IOTM was organised by Victor V. Pop and Antonia Pop under the auspices of the ICB (Institutul de Cercetări Biologice) Cluj and took place at the Congress Centre of the Babeș-Bolyai University from 14 to 18 September 2005. This meeting was dedicated to the memory of Victor Pop (1903–1976), the world-renowned earthworm taxonomist.

The filming of the award-winning movie The Worm Hunters, directed by Randall Wood, also began at this meeting.

This second meeting also took place in a very friendly atmosphere, with twelve professional participants in attendance (Figure 7). The conference proceedings were published as 18 articles in the volume entitled 'Advances in Earthworm Taxonomy II'.

Figure 7: Participants of the 2nd IOTM (persons from right to left: Victor V. Pop, Gábor Cech, Antonia Pop, Ana Rożen, Samuel James, Pietro Omodeo, Tomás Pavlíček, Danuta Plisko, Tarmo Timm, Ana G. Moreno, Csaba Csuzdi and Randall Wood).

And then, Dr Tomáš Pavlíček and Patricia Cardet took over. They organized

- The 3rd IOTM in Platres, Cyprus, from April 2 to 6, 2007

- The 4th IOTM in Diyarbakır , Türkiye, from April 20 to 24, 2009

- The 5th IOTM in Beatenberg, Switzerland from April 11 to 15, 2011

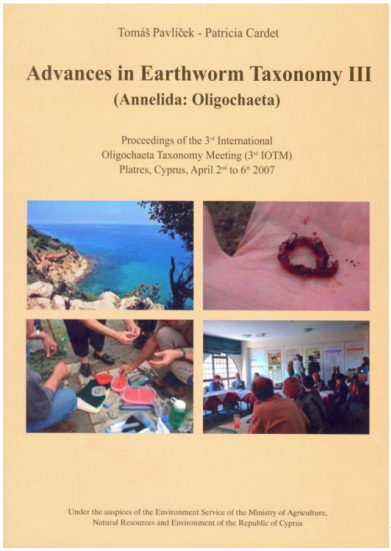
- The 6th IOTM in Palmeira de Faro, Portugal from April 22 to 25, 2013

The idea behind these four conferences was “Experiencing new environments”, i.e. the IOTMs should, of course, help the participants to know more about earthworms, but be also a way to discover a new country, a new culture, foreign scientists, a way to gain knowledge about the local cultural, touristic and gastronomic environment as well as the landscape. A way to meet with local scientists and organizers.

Among the local organizers, let us cite and thank those who spared no efforts to reach these goals:

- Prof. Yüksel Coşkun of Dicle University in Diyarbakır, Türkiye, and his team of colleagues and students. With them, the participants discovered wonderful places in Diyarbakır and other touristic jewels in north-east Türkiye;
- Prof. Renée-Claire Le Bayon, of the Laboratoire Sol & Végétation, Université de Neuchâtel, Switzerland, who not only was the local anchor, but also introduced us to eminent colleagues and Swiss passionate “earthworm hunters”;
- Prof. Maria Teresa Almeida, Prof. Fernanda Cássio and Prof. Cláudia Pascoal, all three from the Universidade do Minho in Braga, who helped with their scientific knowledge, but also imprinted a Portugues touch to the conference.

These four IOTMs produced each a Proceedings Book. A lot of works not only for the authors, for the editors, but the results were fantastic. Have a look at the contributions. All aspects of nowadays earthworm knowledge are represented.



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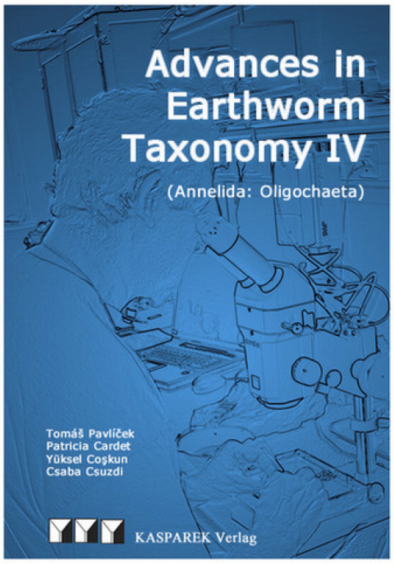

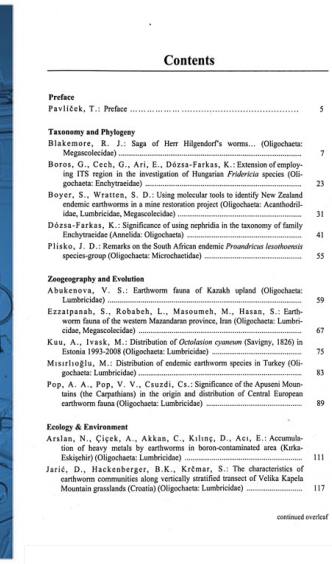

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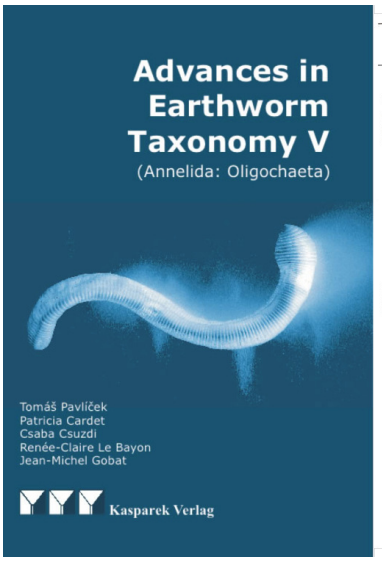

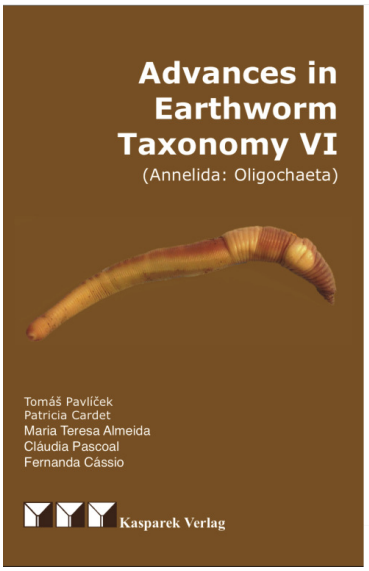

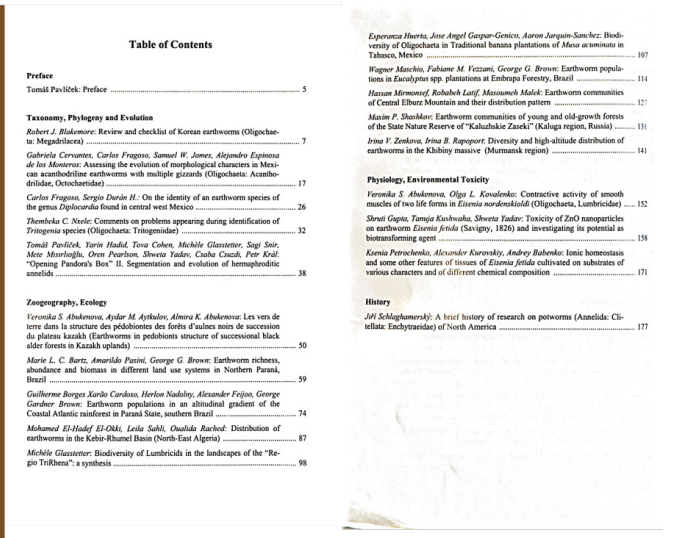

All these IOTM's, the past and the following ones are a mixture of the universality of the desire of knowledge, of the love of nature, of the respect of science and scientists in the field of taxonomy.

Apropos: We would like to remind you of the fascinating documentary by Randall Wood: **"The Worm Hunters"** (https://vimeo.com/134693448).

Oral Presentations

Earthworms in urban and peri-urban sites in Brazil: How these locations contribute to the maintenance and diversity of species

George G. Brown [a], Rafaela T. Dudas [a], Jerome Mathieu [b], Marie L.C. Bartz [c], Thibaud Decaëns [d]

[a]Brazilian Agricultural Research Corporation EMBRAPA Florestas, Brazil; [b]Université Sorbonne, France; [c]Universidade Federal de Santa Catarina, Brazil; [d]Université Montpellier (CEFE), France

Urbanization is reshaping soils across Brazil–a continental country (8.5 million km^2) spanning multiple biomes–yet the built-up urban footprint remains relatively small (37.000 km^2 in 2022). Although earthworms are among the most familiar soil organisms to society, the evidence on earthworm diversity in cities and at their edges is still scattered across case studies. Here we compile data on earthworms diversity recorded in urban and peri-urban sites across Brazil between 1970 and 2025, with the aim of analyzing how anthropogenic activity influences this component of earthworm diversity. For the purposes of this study, we define urban sites as locations within city limits, including gardens, lawns, institutional green spaces (e.g., corporate or university campuses), experimental institutional sites, and public parks. And peri-urban areas are considered transitional zones between urban and rural settings, where natural landscapes and human settlements coexist and interact. For this dataset, we operationally consider sites located 5-10 km from the nearest city boundary. Overall, there are 182 sites in urban perimeter and 45 in peri-urban, sites distributed across major Brazilian biomes and states–e.g., Acre and Pará (Amazon), Distrito Federal (Cerrado), Rio de Janeiro, São Paulo, Paraná, Santa Catarina (Atlantic Forest) and Rio Grande do Sul (Pampa), with land-use systems including intensive pasture, fragments of native vegetation, annual and perennial crops, and lawns/grasslands. As for earthworm diversity, urban areas presented overall 80 species and peri-urban areas 28 species, and only 14 species are shared between both contexts, indicating substantial turnover along the urban–peri-urban gradient. Species include widespread exotics–e.g., *Pontoscolex corethrurus*, *Amynthas* spp., *Dichogaster* spp., Ocnerodrilidae spp.–alongside native Glossoscolecidae (e.g., *Glossoscolex*, *Fimoscolex*) and Rhinodrilidae (*Andiorrhinus*, *Urobenus*), suggesting that a mixture of introduction history, habitat filtering, and legacy land use shapes community composition. More broadly, our results suggest that urban green spaces can harbor a substantial share of recorded earthworm diversity while exhibiting high turnover relative to nearby peri-urban areas-an insight with implications for monitoring design, biodiversity valuation in city planning, and the management of green infrastructures.

Keywords: urbanization, soil diversity, peri-urban ecology, earthworm assemblages, Oligochaeta

25 years of the *Metaphire formosae* species group research: where it started and is taking us

Chih-Han Chang, Po-Wei Yu

Department of Life Science and Institute of Ecology and Evolutionary Biology, Taipei, Taiwan

The *Metaphire formosae* species group is a clade of giant earthworms endemic to Taiwan and the Southern Ryukyus of Japan. This clade is currently composed of 13 species, including 11 species in Taiwan. Recorded at 66 cm long and 1.7 cm wide, *Metaphire taiwanensis* is the largest earthworm in Taiwan as well as in the species group. Species in this group generally have an allopatric distribution. However, sympatry is not uncommon. Before 2000, only three species were described, two from Taiwan and one from the Southern Ryukyus. Subsequently, ten more species new to science were added into the species group during 2000-2025, the last one in the Yonaguni-jima of the Southern Ryukyus in 2022. This study documents the research history of the *M. formosae* species group, reports recent advances, and highlights ongoing and future research directions.

Keywords: *Metaphire formosae* species group, phylogenomics, speciation

Linking Soil Structure to Earthworm Activity Using 3D X-ray CT and Information Theory

Enrico Chiesa [a], *Filipa Reis* [b], *Jose Paulo Sousa* [b], *Riccardo Rigon* [a], *Luís Cunha* [b]

[a]Department of Cellular, Computational and Integrated Biology (CIBIO), Trento University (Italy); [b]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra (Portugal)

Over the last three decades, 3D X-ray Computed Tomography (CT) has emerged as a powerful tool in soil sciences, allowing researchers to investigate the complex soil structure without compromising the samples. It opened new possibilities for modelling soil structure and dynamics, water behavior in the 3D porous matrix of soil, and even soil fauna activity, enabling researchers to study soil in an unprecedented way. This approach provides a three-dimensional voxel grid where intensity values reflect local density, offering potential to distinguish between soil particles, pore spaces, and biological features.

Here we propose an innovative method to analyse 3D X-ray CT images of soil using concepts from Information Theory. Specifically, we apply thethe Fisher-Shannon method to jointly capture global and local properties of the voxel intensity distributions without the need for binarization. By working directly with greyscale images, we avoid the information loss and arbitrariness associated with thresholding, while still recovering key structural insights.

Our results show that our method is able to consistently detect depth-related alterations in the soil structure, yielding results in accord with our understanding of soil physical properties, ensuring that our method is robust and well- adjusted to describe soil systems. We found that two indicators, Shannon Entropy Power (SEP) and Fisher Information Measure (FIM). SEP distinguished soils under wet conditions (55% of Maximum Water-holding Capacity (MWHC), versus dry conditions (45% of MWHC), while FIM was able to discriminate efficiently between soils with and without different earthworm species. Remarkably, FIM values correlated strongly (r^2 = 0.9) with the final earthworm biomass of the earthworms *Aporrectodea rosea* and *A. trapezoides*.

This study demonstrates the potential of coupling 3D X-ray CT with Information Theory to non-invasively detect physical and earthworm-driven alterations in soil structure. Our findings highlight a promising pathway for monitoring earthworm populations and activity, with broad applications for soil ecology and management. While further validation across soil types is required, this work provides a strong foundation for the use of Information Theory in soil biophysics and soil fauna research.

Keywords: 3D X-ray CT, soil structure, Information Theory, earthworms, soil biophysics

Are earthworms a useful tool to prioritize areas for conservation?

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In recent years, the impact of human-induced environmental degradation and global climate change, along with their consequences, has become a central focus of scientific research and conservation efforts. As a result, preserving existing biodiversity and establishing a solid scientific foundation for these efforts have become more crucial than ever. Two complementary approaches have been developed to address the problem of biodiversity loss: taxon-based and area-based conservation. The first method is mainly effective for the protection of vertebrate species, while the second approach provides a more comprehensive solution by protecting entire communities. For the soil fauna, the area-based method appears to be a more suitable approach. A pivotal question: by what criteria are protected areas selected? Conventional practice has historically focused on the protection of habitats of selected species, with a particular emphasis on vertebrates. However, there is often a scarcity of information available regarding the invertebrate fauna of these areas, particularly the soil fauna. Various area-selection algorithms have been developed. One method classifies the species in the given area into discretely scored groups based on their conservation biology traits, frequency, and distribution, forming an index whose species-specific totals reflect the conservation priority of the area. The other includes indices based on continuous scales. In this project, we aimed to identify conservation biology hotspots in Hungary based on the earthworm fauna. We then compared these hotspots with the existing network of protected areas to answer two main questions: How suitable is earthworm fauna for predicting the conservation value of a given area? To what extent do our current protected areas also serve to protect soil fauna, including earthworms? We analyzed data from approximately 7,000 records which span nearly 50 years and cover almost the entire country. We determined the distribution of all earthworm species found in Hungary and created UTM-based distribution maps. In evaluating the earthworm fauna we used a composite index that combines global endemism and local rarity, which are then summed to produce a conservation value index (CV) characterizing each specie. Then the individual CV values were summed up in each UTM grid to produce a cumulative CV index (CCV) representing the conservation value of the given UTM cell. The top 5% (13 UTM squares) had CCV values above 13.5. These areas can be considered earthworm “hotspots”, but the top 25% (65 UTM squares) also deserve attention. It is clearly visible that with only one exception areas in the upper percentiles already benefit from some form of protection. Similarly, many UTM squares with slightly lower CCV values also correlate with currently protected areas. This suggests that earthworm fauna is a suitable predictor for estimating the conservation value of different regions.

Keywords: earthworms, conservation, area selection, rarity, endemism

The first attempt to place the Indian acanthodrilids in a phylogenetic context

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The systematics of the family Acanthodrilidae has long been debated. In his monograph on Oligochaeta, Michaelsen treated it as a subfamily of Megascolecidae and a sister taxon of Octochaetinae. The main difference between the two subfamilies was the structure of the excretory system, being holoic in Acanthodrilinae and meroic in Octochaetinae. The ranks of the subfamilies within Megascolecidae have shifted over time, from tribes to full family status. Despite these changes, the principal diagnostic character–the structure of the excretory system— has remained consistent. The first major taxonomic change was reinstating another subfamily, Benhamiinae, which was later elevated to family rank. Since the 2000s, molecular phylogenetic studies have consistently refuted the separation of Acanthodrilidae/-nae and Octochaetidae/-nae based on excretory systems. These analyses support Lee's view that meroic nephridia evolved independently several times. Consequently, holoic–meroic taxon pairs occur repeatedly, and the separation of Octochaetidae and Acanthodrilidae is purely artificial. Therefore, the meroic taxa were merged into Acanthodrilidae/-nae. However, these molecular phylogenetic studies have focused mainly on material from New Zealand, Australia, and the Americas, while the rich "octochaetid" fauna of the Indian subcontinent remained unstudied. From a taxonomic perspective, these Indian "octochaetid" taxa cannot be assigned to Octochaetidae, as the type species of the family is described from New Zealand, and the molecular phylogenetic analyses published to date clearly place the New Zealand Octochaetus species within Acanthodrilidae, rendering Octochaetidae/-nae a synonym of Acanthodrilidae/-nae. If in future research, the Indian 'octochaetids' are found to be of monophyletic origin, they could be grouped together in the family Eutyphoeidae. This family was initially proposed by Benham as Typoeidae. Here we present the first molecular phylogenetic study of megascolecoid earthworms, including some Indian "octochaetids" such as *Barogaster prashadi*, *Octochaetona beatrix*, *Octochaetona paliensis*, and a *Ramiella* sp. supplemented with sequences from the previous works. Unfortunately, our dataset (28S, 18S, 16S, 12S) and that of the other studies overlapped only partially, with several markers missing. As a result, the analyses remain preliminary, and several nodes show low support, although some cautious conclusions can still be drawn. First, the Indian samples formed a monophyletic clade only in the Bayesian tree with relatively low support. Surprisingly, all analyses placed them within the clade comprising megascolecids and diplocardiids, rather than near the meroic or holoic Australasian acanthodrilids. On the ML tree, the Indian samples analysed formed two distinct clades: the two *Octochaetona* and *Basrogaster+Ramiella*. A broader sampling of taxa and markers will be required to resolve the exact phylogeny of the Indian "octochaetid" taxa.

Keywords: Octochaetidae, Acanthodrilidae, phylogeny, polyphyletic, India

Microannelid assemblages at the eastern edge of the Podyjí National Park (Czechia): effect of coppice restoration and specific habitats

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In 2021-2023 we studied microannelids in acidophilous, thermophilous oak-dominated forests of the Podyjí National Park in south-eastern Czechia. These forests had been traditionally coppiced, but had turned into neglected coppices since the end of WWII. In general, coppicing has been promoted as a way to preserve forest biodiversity, in particular of flora and fauna adapted to open woodlands. So far, the effect of coppicing on soil fauna has been poorly investigated and no study has looked at assemblages of small soil-dwelling annelids in this context. In 2016-2021, the administration of the Podyjí National Park (Czechia) had restored coppicing management by cutting a total of ten forest stands in two areas, near the villages of Hnanice and Popice, respectively (five each). At each site, three quadrats were delimited within the restored coppice stand and three quadrats within the surrounding neglected coppice, primarily to study vegetation development. We collected one soil core from each quadrat in spring and again in autumn 2021. Additional soil samples were taken for soil texture measurement, data on soil chemistry, moisture and vegetation were available from other research teams. In total, we found 23 species of small soil-dwelling annelids, the sites at Hnanice being more species-rich. The dominant species were *Fridericia brunensis*, *Cognettia chlorophila*, *Buchholzia appendiculata*, and *Achaeta affinis* at both sites, and *Oconnorella cambrensis* at the Hnanice sites (all Enchytraeidae). *Hrabeiella periglandulata* ("Polychaeta": Hrabeillidae) was one of the dominant species at Popice. The mean total density of small soil annelids at the sites in both areas was 30 718 $\pm$ 5 692 ind·m^{-2}, the mean total dry biomass 3.35 $\pm$ 0.8 g·m^{-2}. The difference in mean densities between treatments was significant. There was a significant effect of coppicing on the assemblages overall (db-RDA), but especially at the Popice site, where also species richness was higher. Abiotic factors with significant effects were soil pH, moisture and organic carbon content, marginally also soil texture, which correlated positively with microannelid abundance (Pearson). In 2023, several additional (micro)habitats were sampled to complement information on the area's species diversity: Havraníky heathland, floodplain and slope of the Daníž brook valley, shallow soil on granite boulders, and rotten wood from the Daníž valley. Six soil cores (or wood samples) were taken per (micro)habitat in spring and then again in autumn 2023. In the heathland and shallow soil on boulders *F. brunensis* was dominant, whereas *A. affinis* was dominant in the alluvial soil along the Daníž brook and on the slope of its valley. In the dead wood *Marionina clavata*, *Mesenchytraeus pelicensis*, *C. chlorophila*, and *A. affinis* were most abundant. We also took an additional sample from the bottom of the Daníž brook, where we found *Fridericia perrieri* and *Marionina argentea* s.l.

Keywords: microannelids, Enchytraeidae, *Hrabeiella periglandulata*, oak forest, coppicing

Morphological and ecological radiation of crown Lumbricidae

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In the last 10 years, our knowledge about the phylogenetic relationships within Lumbricidae has increased significantly thanks to increased taxon sampling and refinement of analyses. Within the family, the tribe Prosellodrilini mainly comprises morpho-ecologically similar species (endogeic) relegated to the Iberian Peninsula, southern France and Sardinia. Meanwhile, the tribe Lumbricini has expanded its range across the whole Palearctic, and underwent a remarkable radiation on their functional traits with the emergence of the epigeic, epianecic and anecic ecological categories. This was specially true for *Octodrilus*, *Dendrobaena*, *Eisenia*, *Aporrectodea*, *Lumbricus* and *Scherotheca*.

In this presentation, I will focus on these diverse, highly successful genera and employ different approaches such as time-calibrated trees and ancestral state reconstruction to test hypotheses about their evolution. Did they evolve in isolation both in time and space, or did their ancestral ranges and age of origin overlap? Did epigeic, epianecic and anecic trait syndromes evolve convergently several times or where they already present in their common ancestors? Was the emergence of those novel ecological categories a key factor in the strong diversification and success of these genera?

In spite of the forthcoming conclusions, we are far from fully understanding these macroevolutionary questions: a more robust phylogenetic reconstruction of deep nodes as well as replicating these analyses with functional groups will provide even more answers in the future.

Keywords: macroevolution, earthworms, molecular phylogenetics, morphology, ecology

Functional-ecological characterization of soils: the role of earthworms in assessing biotic potential and soil health

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Soil is a complex ecosystem whose functionality depends on the diversity and activity of soil organisms. Among these, earthworms (Annelida: Crassiclitellata) are crucial bioindicators and ecosystem engineers essential for soil health. In line with growing legislative demand for biological indicators in soil monitoring, this presentation addresses the challenge of quantifying Biotic Potential (BP) and functional biodiversity using earthworms as model organisms. BP is defined as the maximum sustainable living biomass and ecological function a soil can support, integrating biological structure and resilience. However, the conventional classification of earthworms (epigeic, endogeic, anecic) is insufficient for precise functional assessment due to high species-level variability in burrowing architecture, hydraulic conductivity, and stress tolerance. For example, two endogeic species may differ substantially in their contribution to soil porosity or organic matter turnover. Therefore, taxonomic precision is a prerequisite for developing robust functional indicators. Our ongoing project establishes a measurable link between xenobiotic pollution, earthworm community responses, and soil health metrics. The approach involves creating an integrated functional catalog linking species identity with autecological profiles (tolerance ranges, soil-mixing intensity, sensitivity thresholds). We are developing an Extended Biotic Potential Index (BPIext), a quantitative tool combining density, biomass, functional diversity, infiltration capacity, and community chemical sensitivity. The experimental design combines field sampling, controlled microcosm experiments, and computational modeling to test how BP and earthworm tolerance to model pollutants (e.g., arsenic, cadmium, imidacloprid) vary across soil types and abiotic conditions. Microcosms allow standardized exposure assessments, while field data provide ecological validation under realistic conditions. By integrating faunistic, functional, and ecotoxicological data, we aim to establish standardized indicators of soil biotic potential applicable across different soil types. These indicators will complement classical physicochemical analyses and provide a biologically meaningful basis for soil classification, ecological risk assessment, and soil-health monitoring. This presentation will discuss the theoretical framework, methodological challenges, and expected contributions of this functional-ecological approach to sustainable soil management.

Keywords: earthworms, soil health, biotic potential, functional diversity, taxonomy, ecotoxicology, soil monitoring

An overview of the earthworm fauna of Madagascar

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Madagascar, with a territory of about 600,000 km^2, is the fourth-largest island in the world. After its separation from Africa around 160 million years ago and from India about 88 million years ago, its position some 400 km off the East African coast has remained more or less stable. Due to its long isolation, varied topography and diverse climate, Madagascar is home to a remarkable biodiversity, with an endemism rate of around 90%, making it one of the world's most important biodiversity hotspots.

Despite this, the Malagasy earthworm fauna remains highly underexplored. Until the 2000s, only a couple of publications had dealt with it, most dating back to the late 1800s and early 1900s. By that time, a total of 31 earthworm species had been recorded from the island, 18 of which were endemic. These included the family Kynotidae with 13 valid species, and the acanthodrilid genus *Howascolex* represented by a single species.

A new phase of research on Madagascan earthworms began in the early 2000s with the launch of the Faune-M project (IRD, France) in 2008, later joined by colleagues from Jeonbuk National University, South Korea. Over the past 18 years, we have collected earthworms from about 50 locations across the island. As a result, the number of known Malagasy earthworm species was increased from 31 to 54. In addition to the numerous species previously reported, we have collected and described 14 species new to science, including a new genus, *Vazimbascolex*, and nine species new to the Malagasy earthworm fauna.

The Malagasy earthworm fauna is dominated by the endemic Kynotidae family, which comprises 22 species, nine of which have newly been described. Another significant group is the Madagascan acanthodrilids, with 14 species, nine of which are endemic to the island. These are distributed across six genera, including the recently described *Vazimbascolex*, as well as the problematic 'Eodriloides' species, which exhibits clear South African affinities. The third most speciose group is the Megascolecidae family, which includes several well-known tropical peregrine species, as well as *Polypheretima pentacystis*. This species was first described from Mahé Island in the Seychelles, and was later reported from Nosy Bé Island in Madagascar by Michaelsen, and has not been reported since 1899.

Despite recent advances, the Malagasy earthworm fauna remains poorly known. This was underscored during the 2025 expedition, when we re-collected the type species of the genus *Kynotus* (*Kynotus darwini*) and *Polypheretima pentacystis* for the first time in our surveys, along with an undescribed *Kynotus* species.

Keywords: Madagascar, check-list, Oligochaeta, fauna

Taxonomic history of the "Glossoscolecidae" family and its paradigms

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This study addresses the taxonomic history of earthworms, focusing specifically on the Glossoscolecidae, a group that was considered the main family of Crassiclitellata in the Neotropical region. It presents the main figures who contributed to the taxonomy of Glossoscolecidae and aims to fill an important gap in the knowledge of oligochaete taxonomy. To analyze the history of earthworm taxonomy, this work applies Thomas Kuhn's theory of scientific revolutions. Based on Kuhn's concepts, the history of Glossoscolecidae is divided into five paradigmatic periods, which may also reflect the general history of earthworm taxonomy.

The first period is the "Pre-paradigmatic Period," beginning with the description of *Glossoscolex giganteus* Leuckart, 1836, the first Neotropical earthworm known to science. Other early descriptions followed, such as *Pontoscolex corethrurus* F. Müller, 1857, from south of Brazil. These early works were characterized by descriptions focused on external morphology. The first paradigm was established by the French Edmond Perrier (1844-1921), who classified global earthworm diversity based on the position of the male pores in relation to the clitellum. This stage is referred to as "Perrier's Period." Perrier described three neotropical genera: *Anteus* Perrier, 1872, *Titanus* Perrier, 1872, *Rhinodrilus* Perrier, 1872. Other naturalists from the late 19th century, including Frank Evers Beddard (1858-1925), Daniele Rosa (1857-1944), and William Benham (1860-1950), followed or modified Perrier's system.

The third period is the "Michaelsen Period." German naturalist Wilhelm Michaelsen (1860-1937) redefined oligochaete classification, focusing on the variation in reproductive structures. For the "Glossoscolecidae" Michaelsen described new taxas, created new diagnoses for the genera using as reference the reproductive system (holandric and meroandric condition) and the structure of calciferous glands. His system became a reference for later taxonomists, such as Gilberto Righi (1937-1999). Righi, who dedicated his entire career to studying Latin American earthworms, followed Michaelsen's method, classifying species based on the number of testes and the structure of the calciferous glands.

The fourth and fifth periods are, respectively, the "Cladistic Period" and the "Molecular Period." Cladistics introduced a broader methodological framework, but in earthworm taxonomy, it did not completely replace earlier paradigms, as it still relied heavily on morphological traits, particularly the reproductive system. The molecular paradigm, however, brought a more profound shift, revealing that some traditional groupings were polyphyletic. As a result, the Glossoscolecidae was split by James and Davidson (2012) into Rhinodrilidae and Glossoscolecidae.

Today, the challenge in earthworm taxonomy is to integrate molecular data with morphological approaches, ensuring a more accurate classification system.

Keywords: history of biology, Michaelsen, Glossoscolecidae

From resistance to resilience: Tackling earthworm stress limits under exposure to tank mixtures

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The use of pesticide tank mixtures (TMs) has become routine in modern agriculture, yet their impact on soil organisms remains poorly understood. Earthworms, essential engineers of soil ecosystems, play a vital role in maintaining healthy soils and are often exposed to these chemical combinations during their life cycle. Standard ecotoxicological tests usually assess survival and reproduction, but these measures can overlook subtle, long-lasting effects. Here, we investigate the responses of *Eisenia andrei* under semi-natural mesocosm conditions, simulating four successive TM applications, commonly used in soybean cultivation, followed by a recovery period. Alongside classical endpoints such as survival and reproduction, transcriptomics (RNA-Seq) were applied to capture molecular-level changes. Results showed that while adult survival was largely unaffected, reproduction dropped sharply after repeated applications and did not recover after two months without applications (so-called resilience period). The earthworms also lost weight over time, showing signs of sustained physiological stress, which traditional ecotoxicological assessments might easily underestimate. Transcriptomic analysis revealed that essential cellular functions, including DNA repair and protein production, were suppressed from the first application onward, with cumulative disruption across treatments. Although some genes were altered to compensate, particularly those linked to rebuilding tissue and maintaining neuromuscular function, these adjustments failed to restore normal function by the end of the recovery period. In other words, our results demonstrate that survival alone gave a misleading impression of earthworm resilience. These results highlight two important points. First, repeated exposure to mixtures can have long-lasting, unnoticeable effects on soil organisms, even when survival and reproduction seem unaffected, threatening earthworm populations and, ultimately, the soil functions they sustain. Second, if we rely only on standard ecotoxicological endpoints, we risk underestimating these impacts. By integrating traditional ecotoxicological measures with molecular tools, we can better understand how intensive agricultural practices compromise the ecological role of earthworms and move forward to more realistic soil health risk assessments. This is exactly the kind of progress needed to protect soil biodiversity and the essential ecological processes it underpins, especially in a world that continues to rely heavily on pesticides.

Keywords: *Eisenia andrei*, pesticide mixtures, soil ecotoxicology, transcriptomics, RNA-seq

Using integrative taxonomy to assess the *Allolobophora chlorotica* species complex in Southwestern Europe

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Allolobophora chlorotica (Savigny, 1826) (Lumbricidae) is an endogeic earthworm widespread in Europe, with numerous introductions reported outside its native range. This species exhibits color dimorphism, with green and pink morphs normally associated with different ecological preferences: the green morph is more common in moist soils, while the pink is often found in drier environments. Previous studies have shown that these morphs are linked to distinct mitochondrial lineages, namely L1-L5, possibly reflecting the existence of different cryptic species. Some subspecies have also been described based on morphological differences (e.g., *A. chlorotica postepheba*, *A. chlorotica waldensis*...). Moreover, mitonuclear discordance and reproductive isolation have been detected between some of the lineages, pointing to historical hybridization or incomplete reproductive isolation within the complex.

In this work, we barcoded ~250 individuals of this species complex across the western Mediterranean, complemented with additional sequences retrieved from public databases. Multilocus data (COI, 12S, 16S, ND1 and 28S) were also generated for representatives of each lineage to refine their phylogenetic relationships and assess their taxonomic status. Some morphological traits were assessed to test for congruence with the molecular results (difference in clitellum position, length, weight, internal characters...). Together, these analyses support a clear separation between lineage L5 and the rest of the lineages (L1-L4), as well as for *A. c. waldensis*, and *A. c. postepheba* (the latter appears as a sister taxon to an outgroup species, *A. burgondiae*).

To explore ecological preferences, we carried out Species Distribution Models under both present and future climate scenarios. Models were constructed both separately for each lineage, giving special attention to those present in the Iberian Peninsula (L2 and L5) and jointly for the entire complex, using different modelling methods. Results highlight distinct ecological niches and potential shifts under climate change for the different lineages, indicating that cryptic taxa might be differentially vulnerable.

Our results comprise an integrative taxonomic revision of the *A. chlorotica* species complex, combining genetic, ecological, and morphological data. To understand the evolutionary history of the complex and develop adequate conservation strategies in a rapidly changing environment, it is essential to understand its hidden diversity.

Keywords: *Allolobophora chlorotica*, integrative taxonomy, phylogeography, SDMs

Unusual (diffused) oogenesis in the ovo-spermathecal apparatus of *Eudrilus eugeniae*

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The family Eudrilidae is remarkable among earthworms for the occurrence of internal fertilization. In eudrilid earthworms, an extensive reorganization of the structure of the genital organs in some species of the ovo-spermathecal system occurs. In *Eudrilus eugeniae*, the best-known representative of the family, the ovo-spermathecal system consists of three main elements: 1) spermatheca with two subunits (diverticulum and ampulle), 2) ovary with ovo-spermathecal duct, and 3) ovisac with ovisac duct. We used light, transmission, and scanning electron microscopy to describe the organization and micromorphology of the ovo-spermathecal apparatus.

The ovo-spermathecal apparatus is paired and connected via the ovaries to the septum of the XII/XIII segments. A single ovary is a tiny, pear-shaped structure that appears as a vesicle surrounded by an envelope containing oogonia and germ cells at the onset of meiosis. We discovered that the cysts of germline cells detach from the ovary and move via the ovo-spermathecal duct toward the spermatheca, where they continue oogenesis to some extent (early vitellogenic oocytes were observed here) within small outgrowths of the spermathecal wall termed bulges. The full oogenesis occurs in ovisacs, which are massive and cauliflower-like structures. Here, all generations of female germ cells were observed, from oogonia to late vitellogenic oocytes.

Through ultrastructural analyses, we found that the germline cells within the ovaries and ovisacs are organized similarly to those found in other earthworms and other clitellates, i.e., they form syncytial cysts, where each cell is connected to the central cytoplasm (cytophore) via one intercellular bridge. The cyst architecture is similar to other earthworms – the cytophore is poorly developed and has a reticular character. Surprisingly, the ultrastructure of late vitellogenic oocytes differs significantly from other earthworm species because of the very well-developed vitelline envelope.

Oogenesis in *E. eugeniae* is diffuse and takes place partly in the ovary and spermathecal bulges. In contrast, full oogenesis occurs only in the ovisacs, which can be considered a functional ovary.

This work was supported by the National Science Centre, Poland: Contract grant number 2020/37/B/NZ4/00560.

Keywords: oogenesis, germline cysts, reproductive system, histology, ultrastructure

Responses of two earthworm species to soil moisture variation: implications for soil properties and bacterial communities

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Earthworms are key soil ecosystem engineers, enhancing soil structure, fertility, and providing habitat for other soil organisms. Yet, earthworms are highly sensitive to fluctuations in soil conditions, such as temperature and moisture, especially under climatic extremes. In Mediterranean agroforestry systems, prone to long summer droughts, little is known about species-specific responses to variable moisture conditions and the ensuing effects they may have on soil properties and processes. Here, we tested the effects of *Aporrectodea trapezoides* and *Aporrectodea rosea*, alone and in combination, on litter decomposition, soil aggregate stability, soil chemistry, and bacterial communities under drought and moist treatments in a controlled microcosm experiment. After two months, earthworm activity declined and survival was lower under drought conditions, particularly for *A. rosea*. Under moist conditions, earthworm presence consistently enhanced litter mass loss and aggregate stability. When both species were present, soil organic carbon, nitrogen, organic matter, and pH decreased, particularly under drought. Bacterial diversity also declined under drought, except in the presence of both species. These results indicate that the effects of earthworms on soil are strongly shaped by soil moisture, species identity and species interactions. In drought-prone Mediterranean agroforestry systems conserving earthworm diversity is essential for sustaining soil functioning and resilience, but also to safeguarding nutrient cycling, maintaining microbial diversity, and supporting the long-term productivity and stability of agroecosystems under climate change.

Keywords: soil moisture, earthworm ecology, bacterial communities, soil properties, climate change

Earthworm Genomics at the Crossroads: From Scarce Resources to Mechanistic Insights into Adaptation

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Earthworms are regarded as keystone soil invertebrates and have long been employed as model organisms in ecotoxicology and soil ecology, yet progress in their genomics has lagged far behind that achieved for other invertebrate systems. Despite the ecological and economic importance of earthworm species in driving nutrient cycling, carbon storage, and soil structure, genomic resources have remained scarce. Only a limited number of draft genomes have been produced, often with variable completeness and annotation quality. Therefore, the molecular base of earthworm adaptation, evolution, and ecosystem function have remained insufficiently understood. Recent advances in sequencing and bioinformatics are transforming earthworm genomics, enabling robust assemblies, comparative analyses, and integrated multi-omics. These tools now allow fundamental questions of plasticity, local adaptation, and resilience under environmental change to be addressed, with emphasis placed on linking genomic, transcriptomic, and epigenomic responses to ecological performance under both natural and anthropogenic stressors.

Here an overview of the current state of earthworm genomics will be provided, with attention directed to progress in genome sequencing, epigenetic profiling, and functional annotation. As an illustrative case study, recent work on the pantropical species *Amynthas gracilis* will be presented. This earthworm has been observed to thrive in volcanic soils of the Azores that are characterised by simultaneous exposure to extreme temperature, hypoxia, and elevated CO_2 and metal levels. In a reciprocal transplant experiment, epidermal morphology was shown to rapidly converge on local optima. Through the application of RNA-seq, DNA methylation profiling, and miRNA sequencing, coordinated regulatory networks underpinning this plastic response were revealed, thereby providing one of the first mechanistic frameworks for earthworm acclimation to multiple abiotic extremes. This case study is used to demonstrate how earthworm genomics can be leveraged to uncover molecular pathways of resilience. By extending such approaches across diverse species and ecological contexts, earthworms can be firmly established as model systems for understanding how genomes interact with environments to shape ecosystem processes. The integration of genomic data with soil ecology promises not only to deepen biological understanding but also to provide predictive frameworks for assessing ecosystem responses to global change.

Keywords: genomics, phenotypic plasticity, epigenetic regulation, adaptation, extreme environment

Distribution of *Euenchytraeus* spp. (Annelida: Clitellata: Enchytraeidae) in Europe: How many species are there and are they glacial relicts?

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In 1906 Bretscher described *Euenchytraeus bisetosus* from the Bernina Pass in southeastern Switzerland. Though he had only juvenile specimens at his disposal, the species seemed unique by nephridia starting already in segments II/III, and this first pair ("head nephridia") bearing peculiar characters. It took a long time before this species was reported again. Based on the description, Cernosvitov (1937) synonymized *Euenchytraeus* with *Marionina* but also considered *E. bisetosus* a species *dubia* because of the improbable position of nephridia in the head part. Later, Nurminen (1977) described *Mesenchytraeus franzi* from the Grossglockner Massiv in Austria and Bauer (1977) *Cognettia clarae* from a site in Styria, eastern Austria. Schmelz and Collado (2010) suggested that all three above-mentioned species might be identical (there was no mention of head nephridia in *M. franzi*, but some other characters were strikingly alike) and also suggested a wider distribution and mentioned morphologically somewhat differing specimens from Galicia, Spain. Martinsson (2017) obtained material of *Euenchytraeus* (originally identified as *Cognettia clarae*) from the eastern edge of the Alps in western-most Hungary, established phylogenetic relations between *Euenchytraeus* and *Cognettia* (= *Chamaedrilus*), proposed to keep *Euenchytraeus* in a separate genus, but hesitated to synonymize *E. clarae* and *E. bisetosus*, due to the lack of *E. bisetosus* types or specimens from its type locality. Triggered by own finds of *Euenchytraeus* in the Czech Giant Mountains (Krkonoše) in 2020, a mountain range known for its glacial relicts and well separated by distance and lowlands from the Alps, we obtained and processed soil samples from several sites within and north of the Alps (including type localities of *E. bisetosus*, *C. clarae* and *M. franzi*) and northwestern Spain. We also collected further data from literature and collections. For sites from which we obtained live specimens, we established phylogenetic relationships based on a selection of six molecular markers (COI, 16S, 28S, H3, ITSA, ITSB). Based on this analysis and morphological examination, we established the existence of three *Euenchytraeus* species in Europe: two in Galicia, Spain (to be described) and one distributed over a considerable area in the Alps (from western Switzerland to western-most Hungary) but also north of the Alps in Bavaria (Germany), Bohemia and Moravia (Czechia). Altogether we have knowledge of ca 25 localities in total, many hitherto not published. Though we did not succeed in obtaining specimens from the type locality of *E. bisetosus*, it is highly probable that both *C. clarae* and *M. franzi* are synonyms of *E. bisetosus* (we found *Euenchytraeus* at several sites in the Grossglockner Massiv sampled by Nurminen, whereas no *Mesenchytraeus* specimens matching the description of *M. franzi* were found in any of our samples). Though the Central European *Euenchytraeus* species has a distribution exceeding the Alps, also its other localities confirm that it is probably a glacial relict. The more so, as another apparently closely related species with head nephridia, *Cognettia piperi*, was described from northeastern Siberia (Christensen & Dózsa-Farkas, 1999). Work on the description of the new species is underway.

Keywords: Enchytraeidae, *Euenchytraeus*, taxonomy, ecology, European range

Is the setal distance ratio a useful morphological character for distinguishing cryptic earthworm species? A case study of the *Aporrectodea caliginosa* species group

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The *Aporrectodea caliginosa* species group has been a taxonomic riddle for a number of years and still remains unresolved on a morphological basis. Members of this species group represent one of the most common earthworms in Europe and yet the various forms within it can be very diverse. One form in particular, *Ap. nocturna*, even belongs to an entirely different ecological group, namely anecic. Whilst numerous molecular studies have successfully separated *Ap. longa*, *Ap. terrestris*, *Ap. nocturna*, *Ap. borellii*, *Ap. trapezoides* and *Ap. caliginosa* from each other, no definitive morphological character has been found to date to really make this a feasible split in the field.

Although classical morphological characters such as pigmentation, clitellum position, and the position and shape of the tubercula pubertatis are useful for initial morphological groupings, these characters are not always definitive due to the presence of several transitional forms. Since molecular techniques are not always readily available and financial constraints also exist, reliable morphological characters would support the work of non-taxonomist colleagues.

One potentially useful character could be the setal distance ratio. Setal distances have been widely used in earthworm taxonomy since Sr. Victor Pop separated lumbricid genera using a combination of setal arrangements, pigmentation, and prostomium form however, it was rarely used in distinguishing closely related earthworm species. One of the first attempts was by Omodeo and Rota, who used setal distances to distinguish between *Healyella kosswigi* and *He. syriaca*. However, they only compared average setal distances without using any statistical tools.

The first study to compare setal distances between populations of two closely related species was conducted by Paoletti et al., who used NMDS multivariate analysis of setal distances in *Eophila tellinii* and *E. crodabepis*, and found significant differences, particularly in the setal distances dd.

Here we applied the same methods to most taxa of the *Aporrectodea caliginosa* species group (except *Ap. terrestris* and *Ap. borellii*). According to the NMDS analysis, *Ap. trapezoides* and *Ap. caliginosa* samples overlap partially, while *Ap. longa* specimens are nested within the *Ap. caliginosa* cluster. In contrast, *Ap. nocturna* is completely separated mainly due to its much larger dd distances. This character, when combined with classical morphological features, proved to be a useful tool for distinguishing species within the *Ap. caliginosa* species group.

Keywords: *Aporrectodea caliginosa* specie group, morphology, setal distances

Mission possible: an attempt toward a phylogenetic understanding of the genus *Dendrobaena* (Crassiclitellata, Lumbricidae)

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The earthworm genus *Dendrobaena* is the most species-rich genus within the family Lumbricidae and is probably also the most heterogeneous, both in terms of morphological characteristics and geographic distribution. Among its species, notable differences exist in the structure of the longitudinal musculature and calciferous glands, the position of the last pair of hearts, pigmentation, and setal arrangement. This high degree of heterogeneity should have been the subject of a thorough taxonomic review long ago. In his morphology-based revision, Csuzdi distinguished six main species groups within the genus: the *Dendrobaena octaedra*, *D. veneta*, *D. byblica*, *D. schmidti*, and the *D. mammalis* groups, along with a group of uncertain species. *Dendrobaena veneta* and its close relatives are characterized by fasciculated musculature and poorly developed calciferous glands without diverticula. Some species also exhibit a closer setal arrangement. Based on these features, Csuzdi suggested that the veneta group may represent a transitional form toward the genus *Eisenia* and may be related to *E. kattoulasi*. He also emphasized that *Eisenia grandis* should be included in the *D. veneta* group and that this group may merit recognition as a separate genus. Kvavadze established the genus *Dendrodriloides* for the *Eisenia grandis* group distributed in the Caucasus. These species are remarkable for their tetrahedral genital setae, spermathecal openings, and closely paired setae. Kvavadze further proposed that other Balkan *Eisenia* species (e.g., *E. ebneri*) and the Anatolian *D. montana* should also be assigned to this genus. Later, Szederjesi & Csuzdi demonstrated that these Balkan *Eisenia* species, along with *E. oreophila*, indeed possess tetrahedral genital setae and, based on COI sequences, form a well-separated clade within *Eisenia*. In a more recent large-scale study, Shekhovtsov et al. using mitochondrial genomes found that *E. grandis* is nested within *Dendrobaena* and is most closely related to the *D. veneta–D. hortensis* clade. In the present study, we sequenced a broader range of *Dendrobaena* species for multiple markers (COI, 16S, ITS2). Our results indicate that both *Eisenia* and *Dendrobaena* are polyphyletic. The southern Balkan *Eisenia ebneri* group falls outside *Eisenia sensu stricto* and instead forms a clade with the Caucasian *Dendrodriloides* species and *D. hrabei*. Their sister clade comprises *D. veneta*, *D. succinta*, *D. hortensis*, *D. pavliceki*, and *D. karacadagi*. Within *Dendrobaena proper*, six clades are recovered. The type species, *D. octaedra*, clusters basally with *D. attemsi*. The Alpine–Carpathian–Balkanic and Balkanic–Anatolian–Levantine groups previously identified by Szederjesi et al. are clearly supported here as well. The peculiar *D. pantaleonis* and *D. skipetarica*, the former *Fitzingeria* with some similar species, as well as the *D. byblica* group together with the recently described *Philomontanus baloutchi* fall outside the "classical" *Dendrobaena* clade.

Keywords: earthworms, *Dendrobaena*, molecular phylogenetics, Balkan, Caucasus

Genomic Polymorphism and Characterization of earthworms with an Emphasis on Native and Exotic Assemblage in Reserve Forests of India

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This study presents an integrative assessment of earthworm biodiversity, genomic variation, and assemblage dynamics across two contrasting ecosystems of India: Nauradehi Wildlife Sanctuary (Madhya Pradesh) with minimal anthropogenic pressure, and the Andaman & Nicobar Islands, characterized by high disturbance. Combining morpho-anatomical taxonomy with COI-based DNA barcoding, the work pursued four objectives: establishing a taxonomic database, mapping native-exotic distribution, compiling authoritative checklists, and evaluating genetic diversity, population structure, and cryptic taxa.

Field surveys and classical identification were complemented by molecular sequencing, Maximum Likelihood phylogenies, and species delimitation algorithms (ASAP, ABGD). A total of 23 species were documented from the Andamans, three new national records, and several distributional extensions. Regional checklists serve as the first authoritative baselines, resolving nomenclatural ambiguities and clarifying ecological status.

Results revealed sharp biogeographic contrasts: Nauradehi was dominated by natives (146 native vs. 27 exotic individuals), while the Andamans showed severe exotic invasion (160 exotic vs. 40 native), with complete native displacement at Diglipur. Molecular analyses confirmed evolutionary relationships, revealed deep intraspecific divergence, and uncovered cryptic lineages in *Drawida*, *Barogaster*, and *Metaphire*. The generated COI barcode library establishes a genomic reference for Indian earthworms. The study highlights three key implications: integrative taxonomy is vital to uncover India's hidden earthworm diversity; exotic invasions drive faunal homogenization and threaten soil ecosystem functions; and conservation requires protecting mainland endemics while enforcing urgent biosecurity on islands. Together, these findings provide a genomic-ecological framework for biodiversity monitoring and conservation policy.

Keywords: native, exotic assemblage, reserve forest, genetic polymorphism, invasion

Comparative analysis of ovarian organization in *Drawida* and *Delaya*, close relatives of Crassiclitellata

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Drawida, belonging to the family Moniligastridae, represents terrestrial oligochaetes considered the sister group to Crassiclitellata (Moniligastridae + Crassiclitellata = Metagynophora). Our analysis reveals that the *Drawida* species possess paired, feathery ovaries located in the 11th segment, each giving rise to long ovisacs that extend into subsequent segments. The ovaries are composed of several lobes, in which germ cells are located at successive stages of oogenesis (oogonia, early meiotic cells, and growing oocytes). Early germ cells are united into syncytial germline cysts, where each germ cell is connected to a common cytoplasmic mass (cytophore) via intercellular bridges. The cytophore in *Drawida* is underdeveloped, forming only thin, branched projections, similar to crassiclitellates. Oogenesis progresses from oogonia to yolky oocytes, which are deposited into the ovisacs for further maturation. Notably, nurse cells have not been confirmed, as all developing germ cells (oocytes) are morphologically similar.

According to recent molecular analyses, *Delaya*, a cave-dwelling member of the Pelodrilidae family, is closely allied to Metagynophora. In *Delaya*, two pairs of ovaries are situated in consecutive body segments (XII and XIII), each subdivided into 3-5 functional subunits. These subunits display spatial polarization: apical regions contain oogonia and early meiotic cells, while distal regions contain growing oocytes and nurse cells. Initially, germline cysts develop synchronously; later, oocytes detach and mature individually. Vitellogenic oocytes, rich in yolk and nutrient reserves, are transferred to ovisacs, where maturation continues. Notably, comparison of two *Delaya* species (from Greece and France) revealed both conserved clitellate features and evolutionary novelties, such as the multiplication of functional ovarian subunits and species-specific cytophore structure.

In conclusion, our comparative analysis reveals that oogenesis in *Drawida* and Delaya shares fundamental clitellate characters such as syncytial germline cysts equipped with the cytophore. However, they differ notably in ovary structure, with *Delaya* exhibiting more complex ovarian subunits and species-specific cytophore variations, while *Drawida* displays a simpler ovarian organization without nurse cells. The yolky-rich eggs are produced and stored in prominent ovisacs in both groups. These similarities and differences provide essential insights into the evolutionary diversification of reproductive systems among crassiclitellate earthworms and their relatives.

This research was supported by the National Science Centre, Poland, contract number 2020/37/B/NZ4/00560.

Keywords: oogenesis, earthworms, germline cyst, oocyte

Earthworm-Mediated investigation of Polyethylene Microplastics with Metabolomic Insights into Antioxidant, and Neurotoxic biomarker

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Microplastic particles (< 5 mm) are now ubiquitous across terrestrial ecosystems, with polyethylene (PE) a widely used polymer particularly prevalent in agricultural soils. Soil-dwelling invertebrates, especially earthworms, thus encounter PE microplastics frequently. Earthworms of the species *Eudrilus eugeniae* were exposed to adjacent environmentally realistic concentrations of polyethylene microplastics (0.05-1.0% w/w) over a 56-day period to investigate biochemical and metabolomic effects. While survival, and growth, remained largely normal than and reproduction, significant molecular disruptions emerged on exceeding concentration tested from 0.5% w/w. Key antioxidant enzymes including, superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST) were significantly changed, accompanied by markedly increased lipid peroxidation (LPO), signalling pronounced oxidative stress. Neurotoxic effects were also observed, as evidenced by altered acetylcholinesterase (AChE) activity. Untargeted GC-MS metabolomic profiling revealed widespread perturbations across multiple metabolic pathways: energy metabolism was disrupted, with altered glycolytic intermediates and tricarboxylic acid (TCA) cycle metabolites such as decreased succinic acid and malic acid, indicating impaired ATP production; amino acid metabolism was affected, with dysregulation of glycine, valine, phenylalanine, leucine, aspartic acid and glutamic acid and also other amino acids alongside purine metabolic changes; lipid homeostasis was disturbed, with notable fluctuations in membrane-associated lipids linoleic acid, valeric acid, Palmitic acid, Stearic acid, Myristic acid, Oleic acid, and Myoinositol as well as previously reported shifts in Arachidonic acid, methyl malonic acid, pointing to compromised detoxification mechanisms were weakened by diminished antioxidant capacity and altered lipid- and energy-related biochemical pathways. Together, these findings demonstrate that even sub-lethal PE microplastic exposure initiates a cascade of biomolecular dysfunctions oxidative damage, neurotoxicity, energy deficit, destabilized lipid membranes, and disrupted detoxification. By integrating traditional biochemical assays with high-resolution metabolomics, this study identifies early biomarkers of soil microplastic toxicity, elucidates mechanistic pathways in earthworm stress responses, and supports the refinement of ecological risk assessments as well as the design of targeted soil remediation and pollution mitigation strategies.

Keywords: antioxidant enzyme microplastic, earthworm, GC-MS, metabolomics, polyethylene

Poster Presentations

What Came First: Copulatory Bulbs or Their Absence? Insights from an Integrative Approach

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The internal structures known as copulatory bulbs (CB) – also called chambers or pouches - occur across multiple earthworm lineages, including Megascolecidae (*Metaphire*, *Pheretima*), Eudrilidae (*Eudrilus*), and Glossoscolecidae (*Glossoscolex*). In the Asian species of Megascolecidae the CB often everts during mating, and facilitates sperm transfer to the spermathecae as they may have genital markings or pads inside the CB. In *Glossoscolex*, the CB is more like an ejaculatory muscle. *Eudrilus* shows a similar pattern, shooting into the female opening and directing sperm toward the spermathecae and a fertilization chamber attached to the oviduct. Their role is understood to be assisting in the ejaculation of sperm produced by the seminal vesicles, acting as a fundamental structure during reproduction. In the family Glossoscolecidae, widely distributed in South America and one of the best-known earthworm families in Brazil, the presence and number of copulatory bulbs are key morphological characters used to distinguish genera and species. These bulbs are associated with the external male pores. Traditionally, the two principal genera in Brazil, *Glossoscolex* and *Fimoscolex*, are separated by the arrangement of male pores and copulatory bulbs: one male pore with one bulb corresponds to *Fimoscolex*, whereas two (or one) male pores with two bulbs correspond to *Glossoscolex*. However, what happens when new species of this family present male pores but no copulatory bulbs? We report one species of *Glossoscolex* collected in southeastern Brazil (Minas Gerais), a relatively large worm (~190 mm length), with two distinct male pores in segment XVII but lacking any copulatory bulb. Similarly, a *Fimoscolex* species from southern Brazil (Rio Grande do Sul) was found with a single large male pore occupying most of segment XVII, but no copulatory bulb. To investigate evolutionary relationships among these patterns, we analyzed 40 COI sequences representing different arrangements of male pores and copulatory bulbs. Our dataset included *Glossoscolex* with one male pore/two bulbs, two pores/two bulbs, and two pores/no bulbs, and *Fimoscolex* with one male pore plus either one or no copulatory bulb. Preliminary analyses show that the separation between *Glossoscolex* and *Fimoscolex* is blurred in the phylogenetic tree, and morphological variation complicates the distinction even further. Our results blur the traditional boundaries between *Glossoscolex* and *Fimoscolex* and raise new questions about the evolutionary role of copulatory bulbs. These remain open issues that we intend to explore further through an integrative approach combining molecular, morphological, and ecological evidence.

Keywords: Glossoscolecidae, phylogenetics, evolutionary relationship

Functional groups keep growing: burrowing, casting and feeding behaviour of large bodied Hormogastridae, *Aporrectodea*, *Octodrilus* and *Scherotheca*

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Since Marcel Bouché's seminal work in 1972 large, strongly pigmented earthworms have usually been assigned to the anecic ecological category. Species belonging to this group are thought to create deep, vertical burrows and to consume abundant leaf litter in the soil surface. Thus, it is expected that their activity has a strong impact in soils and the provision of ecosystemic services. Capowiez et al. (2024), instead of relying on morphological traits to infer earthworm behaviour in soils, analysed burrowing, casting and feeding behaviour itself (through soil cores CT scanning). This new approach allowed to create a new functional group classification which mostly overlapped with ecological categories. However, it was made clear that morphology alone cannot predict earthworm behaviour accurately. Within the genera *Scherotheca*, *Octodrilus*, *Aporrectodea* (Lumbricidae), *Norana* and *Boucheona* (Hormogastridae) several species reach large or giant body size. The lack of knowledge of their burrowing, casting and feeding behaviour (and thus of their ecological group assignment) precludes the understanding of their effect in their habitats. In this work, representatives of those large bodied species together with smaller relatives were included in the methodological framework of Capowiez et al. (2024). Soil cores were scanned, and all associated parameters were quantified. After analysing the different functions associated with their behaviour, it was found that size and pigmentation were two of the key factors explaining their assignment to functional groups. The increased knowledge of the role of these large earthworms in the soil habitat, in the context of their smaller congenerics, highlights the importance of their conservation to preserve their valuable ecosystem service provision.

Keywords: bioturbation, ecology, lumbricidae, micro CT scanning

Phylogenomic tree of Lumbricoidea keeps growing: Anchored Hybrid Enrichment phylogeny with a focus on the large-bodied *Scherotheca*, *Octodrilus* and Hormogastridae

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Molecular phylogenetics based on legacy (Sanger sequenced) markers have allowed significant advances in the systematics of Lumbricidae and Hormogastridae, but some relationships between genera have remained unresolved. The use of Anchored Hybrid Enrichment (AHE), a phylogenomic approach that provides a good balance of % of taxa and genome representation already showed promise by improving the resolution of the backbone of Lumbricidae. Here, 75 new representatives have been added to the previous 43, including 26 species of *Scherotheca*, 23 species of *Octodrilus*-Octodriloides, 12 species of 7 genera of Hormogastridae and several unrepresented genera and rogue taxa (such as *Panoniona*, *Murchieona*, *Flabellodrilus*, *Coventina* or *Imetescolex*). In order to root the phylogeny and to help obtain divergence time estimates, an Errantia annelid, a hirudinean and a microdrile oligochaete, as well as representatives from Megascolecidae, Acanthodrilidae and Rhinodrilidae were also included.
The resulting trees reinforce our knowledge on the most conflictive relationships between these taxa and constitute the base for future studies on the selective pressures behind the evolution of large body size within *Scherotheca*, Lumbricidae and Hormogastridae.

Keywords: phylogenomics, Lumbricidae, Hormogastridae, systematics, macroevolution

Transcriptomic data as a source of mitochondrial sequences for taxonomy and phylogenetics of Oligochaeta

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Mitochondrial DNA sequences, including fragments of genes such as cytochrome c oxidase I (COI), 16S rRNA, and 12S rRNA, are widely used for species identification and phylogenetic studies of Oligochaeta. However, obtaining these markers can be challenging for certain taxa using classical PCR, since so-called "universal" primers are often not truly universal. Transcriptomic data, primarily generated for gene expression studies, represent an underutilized resource for recovering mitochondrial sequences. We found that the coding sequence of the key gene for DNA barcoding, COI, is readily available from assembled transcriptomes. Moreover, with sufficiently deep sequencing and additional bioinformatic steps, it is possible to reconstruct large portions of the mitochondrial genome. Here, we report the successful recovery of more than 10,000 bp of the mitogenome of the enchytraeid worm *Enchytraeus albidus* based on RNA-seq reads generated in our previous studies. Such reuse of transcriptomic data not only provides access to valuable mitochondrial markers but also enables the retrospective identification of individuals used in transcriptome sequencing projects.

Keywords: RNA-Seq, mitogenome, genetic markers, DNA barcoding, pipeline

Tracking anecic earthworms by gene amplification from soil DNA

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Soil DNA represents an essential component of environmental DNA (eDNA) research, encompassing genetic material from microorganisms, plants, and soil fauna. This study aimed to define and validate species-specific primers for anecics, key earthworm species, to assess their importance in soil structure and organic matter decomposition, using a quantitative PCR (qPCR) to detect their DNA traces. Primer pairs targeting the mitochondrial Cytochrome Oxidase I (COI) gene were designed for the most representative anecic species in Italian agricultural soils, *Aporrectodea nocturna*, *Octodrilus complanatus*, and *Lumbricus terrestris*. Field and controlled soil mesocosms with the three anecic species were established to test the selected primers for direct detection of the target species in soil and cast samples. The primers' performance was evaluated by running qPCR on DNA from earthworm tissues, as a positive control, and soil using the QIAGEN DNeasy PowerSoil kit. In cross-reactivity assays against non-target earthworm species (heterologous tissue) and against soil from mesocosms that had hosted non-target species (heterologous soil). Optimizing thermal conditions for the qPCR by raising the annealing temperature to 64℃ and limiting amplification to 35 cycles enhanced reaction specificity and reduced nonspecific amplification. Each primer pair demonstrated the highest specificity with its target species (homologous DNA), while tests with non-target species (heterologous DNA) showed either no amplification or a delay of more than 10 threshold cycles. The cycle thresholds for the positive controls on DNA from pure tissue, for *Oc. complanatus* and *Ap. nocturna* showed similar responses (Ct:12-16), while the signal for *L. terrestris* was slightly weaker, having higher Ct (Ct:20-22). Analysis of DNA from field and microcosm soil confirmed that the primer pairs retained sufficient sensitivity and specificity. Detection in environmental soil substrates that had hosted those earthworms required 4 to 18 more PCR cycles compared to the pure tissue DNA controls. Lower Ct values were detected in casts compared to soil where the target species were present, except for *Ap. nocturna*, which appeared to be more spatially confined. In casts of *L. terrestris* collected from natural soil, DNA was detected for both *L. terrestris* and *Ap. nocturna*. The Ct values for the target species (*L. terrestris*) were lower (25-30 cycles), indicating a stronger signal. The unexpected detection of *Ap. nocturna* in *L. terrestris* casts could be due to residual environmental DNA, cohabitation, or potential nonspecific binding, warranting further investigation. Despite some limitations, these results provide, as a preliminary proof of principle, initial support for the potential applicability of the primers in indirect species detection through environmental DNA analysis, showing soil DNA-based qPCR is a sensitive and reliable tool for tracking soil fauna biodiversity and guiding sustainable agriculture.

Keywords: soil DNA, qPCR, anecics, primer design, sustainable agriculture

First insights into the earthworm diversity (Annelida: Crassiclitellata) of the Kalnik Mountain (Croatia)

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Although the earthworm fauna of Croatia has been relatively well studied, the Kalnik Mountain area had not been previously investigated. This thesis presents the first faunistic analysis of earthworms (Annelida: Crassiclitellata) from the Kalnik Mountain region, providing new data on species diversity, ecological groups, and zoogeographical patterns. Field sampling was conducted at 32 sites across different habitat types during spring 2025 using the excavation and hand-sorting method. In total, 18 species belonging to 11 genera were identified, with *Aporrectodea rosea* (Savigny, 1826) and *Octodriloides karawankensis* (Zicsi, 1969) as the most abundant. Species were classified into ecological categories following Bouché (1977): endogeic, epigeic, and anecic. Endogeic species dominated most sites, particularly in meadow and shrub habitats, while epigeic species were characteristic of Illyrian oak–hornbeam forests. Anecic species were rare and localized. Zoogeographical analysis revealed six distribution types: peregrine, South Alpine, Central European, Trans-Aegean, Illyrian, and Holo-Mediterranean. The greatest species richness and diversity (Shannon index = 1.10; Simpson = 0.67) were recorded in Central European mesophilic beech forests on neutral to slightly acidic soils. In contrast, acidophilic oak and birch forests supported the lowest diversity. Species composition clearly differentiated between open habitats, dominated by cosmopolitan peregrine taxa, and forest habitats, which hosted regionally restricted South Alpine and Illyrian elements. Soil physicochemical parameters–pH, conductivity, and organic matter content–were measured to explore their relationship with earthworm abundance and biomass. Although no statistically significant correlations were detected, trends indicated higher biomass in soils with moderate pH (6.0-7.0) and higher organic matter. These results suggest that soil conditions influence local abundance but that broader habitat characteristics and vegetation type are stronger determinants of community composition. Altogether, 424 individuals were collected (268 juveniles and 156 adults), corresponding to an average density of 147 ind m^{-2} and mean biomass of 80.5 g m^{-2}. The faunal composition of Kalnik Mountain is characterized by a mixture of widespread European species and several regionally significant taxa, reflecting the area's transitional biogeographical position between the Pannonian Plain and the Dinaric Alps. The presence of both forest-specialist and anthropotolerant species indicates a mosaic of relatively well-preserved forest habitats interspersed with human-modified landscapes.The results highlight Kalnik Mountain as an important hotspot of earthworm biodiversity in continental Croatia, contributing to national faunistic databases and forming a basis for future ecological and conservation studies. The study emphasizes the need for continued monitoring using standardized sampling and functional-trait approaches to better understand how soil parameters, land use, and climatic gradients shape earthworm assemblages in Central European ecosystems.

Keywords: Kalnik Mountain, earthworms, faunistic analysis, biodiversity, zoogeography, soil fauna

Variation in morphological characters in northern populations of *Eisenia nordenskioldi* (Lumbricidae, Annelida)

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Earthworm taxonomy relies on morphological traits to distinguish species. However, recent molecular studies have revealed that many earthworm taxa, particularly those with wide geographic ranges, harbor cryptic genetic diversity that often escapes detection through traditional morphological methods. This study investigates the morphological variation within lineage 9 of the *Eisenia nordenskioldi* complex, distributed across Siberian tundra and taiga zones. Rather than treating morphological traits as fixed diagnostic markers, we adopt a population-level approach to document the extent, nature, and ecological correlates of phenotypic variation within this lineage, and to assess whether morphological differences can reliably distinguish it from a co-occurring lineage (lineage 1). We analyzed 132 individuals from 17 northern populations, combining detailed morphological examination with DNA barcoding (cox1). Our results reveal that morphological variation within lineage 9 frequently exceeds the boundaries of the species' formal diagnosis. For example, while the standard description places the first dorsal pore in the 4/5 intersegmental groove, nearly half of specimens from certain locations exhibited pores shifted to the 3/4, 5/6, or even 6/7 grooves. Similarly, the clitellum, typically described as spanning segments 27–33, varied from 26–34, with 33% of individuals showing deviations of half a segment or more. The tuberculae pubertatis, another key diagnostic feature, began as early as segment 28 and extended to segment 32 in some cases, contradicting the canonical range of 29–31. Prostomium morphology was equally variable: although traditionally classified as epilobous, we observed both "open" and "closed" variants, with depth ranging from 25% to 75% of the peristomium, and in one case, a fully tanylobous form.Internal anatomy, by contrast, was remarkably stable: nephridial sac shape, testis number, and calciferous gland position showed no variation, except for rare developmental anomalies such as duplicated sperm receptacles. When comparing lineage 9 with lineage 1 in sympatric populations, lineage 1 consistently exhibited a closed prostomium, whereas lineage 9 displayed both open and closed forms equally. Tuberculae pubertatis in lineage 1 were more frequently irregularly shaped and initiated earlier (segment 28), while lineage 9 predominantly had triangular tuberculae starting at segment 29. Lineage 1 individuals were also slightly larger and more pigmented. The results of this study underscore the importance of characterizing morphological variation in the study of earthworm diversity, ideally coupled with genetic verification. Our findings indicate that misidentifications of earthworms may be more common than previously thought. Additionally, they emphasize the necessity of morphologically characterizing earthworm genetic lineages, which may not be as cryptic as presumed.

Keywords: earthworms, genetic lineages, morphologic diversity

Facing the challenges in re-assessing our knowledge on Austrian earthworm diversity

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The first documented collections of earthworms in Austria were made around the turn of the 20th century. Along with other written records, this formed the basis for the first identification key in 1965. The most recent countrywide classification dates from 1999 and comprises 62 species. Apart from occasional surveys limited to specific areas, no systematic large-scale survey of earthworms for Austria has been carried out until now. Our aim was to fill this knowledge gap and initiate a country-wide monitoring, to compile an up-to-date checklist of earthworms, and to draw up a provisional Red List for Austria based on both historical and current observations. Firstly, all known historical earthworm findings in Austria were summarized as part of an extensive literature search. A major challenge was to manage the numerous synonyms of species names mentioned in the literature and to adjust them according to the current nomenclature. For this, three different databases (DriloBASE, GBIF, Earthworm species - A searchable database) were used. Secondly, to determine the current state of earthworm diversity, we conducted a country-wide survey in 2024 in open agricultural land, which was supplemented in 2025 by sampling riparian forest sites and other niche habitats. All collected earthworms were identified in the laboratory whenever possible. By combining historical and recently collected data, we were able to compile an updated checklist of earthworms in Austria, which now includes 65 species. In addition, we mapped the distribution of all earthworm species within the country. As a next step, the data will be used to create a preliminary Red List of earthworms. Finally, the recent country-wide field study is intended to serve as a motivation to establish a long-term monitoring of earthworms in Austria and to ensure their protection, as they are crucial for a healthy soil ecosystem.

Keywords: Lumbricidae, earthworms, checklist, Austria

Morphological variation in the parthenogenetic earthworm *Eiseniella tetraedra* across different habitat

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The stenotopic semiaquatic species *Eiseniella tetraedra* typically inhabits water-rich environments, including the bottoms and banks of rivers and lakes, as well as brackish waters. It is generally characterized by low mobility but can swim through undulating body movements when disturbed. Populations of the parthenogenetic and polyploid earthworm were collected from habitats differing in moisture levels to investigate habitat-related variation. Samples were obtained from three distinct habitat types: fully submerged river sediments, waterside habitats with relatively high humidity, and humid vegetated sites located farther from the water's edge. Morphological traits showing the greatest variability – including clitellum and tubercle shape, body size in the post-clitellar region, setae length in the post-clitellar region, and coloration of preserved specimens – were examined to compare populations across habitats. No significant differences were detected between specimens from submerged and waterside habitats; however, populations from vegetated terrestrial habitats exhibited distinct morphological divergence. Whether this divergence arises from ecological factors or reflects underlying genetic differentiation remains to be determined.

Keywords: parthenogenesis, poliploidy, ecotype, morphological diversity

Calciferous Glands in Rhinodrilidae and Glossoscolecidae

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Calciferous glands are esophageal pouches capable of secreting calcium carbonate into the gut of earthworms. These structures are important in earthworms taxonomy and have been studied since the 19th century. More recently, Briones et al. (2008) suggested that their main function is to fix CO_2. In Neotropical worms–(Rhinodrilidae and Glossoscolecidae)–calciferous glands have been studied primarily for their systematic value. Michaelsen proposed a classification system that defined at least seven morphological categories of calciferous glands for glossoscolecid genera. Examples include Kompositenschlauchtasche (tubular compound) and Rispenschlauchtasche (tubular branched). Righi later expanded this classification to ten categories. The aim of this study is to reanalyze the calciferous gland structures in major Neotropical genera such as *Andiorrhinus* Cognetti, 1908 Chibui Righi & Guerra, 1985, *Fimoscolex* Michaelsen, 1900, *Glossoscolex* Leuckart, 1836 *Pontoscolex* Scharmda, 1861 and *Rhinodrilus* Perrier, 1872 with a critical examination of the categories Kompositenschlauchtasche, Rispenschlauchtasche, and Lamellentasche (lamellar). The material analyzed comes from the MZUSP collection, particularly the Gilberto Righi collection—one of the most important earthworm collections in Latin America. This study provides new images using photomontage techniques. Errors in the interpretation of calcium glands have resulted in taxonomic mistakes. The "tubular compound" structure is described as tubes attached to one another. This type reportedly occurs in several genera, such as *Glossoscolex*, *Fimoscolex*, and *Rhinodrilus*. However, detailed analysis reveals important differences between genera, raising concerns about grouping them into a single category. For instance, species in the *Glossoscolex giganteus* group have glands with a hollow, wide central cavity, while *Fimoscolex* and *Glossoscolex truncatus* group species have smaller cavities with internal vascular tissue. In *Rhinodrilus*, what is described as "tubular compound" is a structurally distinct type, featuring radial tubes connected to a central cavity, which varies in size among species. The "tubular branched" structure is primarily found in species of *Rhinodrilus* and related genera from northern South America. These glands are characterized by large tubes in the ectal region and smaller tubes in the ental region. One example is *Rhinodrilus paradoxus* Perrier, 1872 – the type species of the genus. "Lamellar" glands show significant variation. Some species, such *Andiorrhinus kuika* Righi, 1993 exhibit regular lamellae with straight walls. Others, like *Andiorrhinus duseni* (Michaelsen, 1918) display lamellae with tortuous walls–representing a transitional form between lamellar and tubular structures, and suggesting morphological overlap between the two genera. The study of the main types of calciferous glands shows that the current categories are insufficient.

Keywords: Glossoscolecidae, calciferous glands, *Glossoscolex*

Genomic signatures of environmental adaptation and phenotypic variation in the polyploid *Allolobophora molleri* species complex

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Earthworms play a fundamental role in terrestrial ecosystems. Adaptation to the soil environment has resulted in a simplified body plan across all earthworm species, leading to the emergence of numerous cryptic species and species complexes. The *Allolobophora molleri* species complex comprises five green-pigmented species of mixed ploidy, whose differences are primarily based on a single morphological trait: the position of the clitellum. Previous phylogenetic studies have supported the synonymy of this complex into a single species, presenting an unusual case of high phenotypic plasticity in a key morphological trait. This study aims to investigate the interplay between genotype, phenotype, and environment within this species complex. To this end, 192 individuals from 43 populations collected across the Iberian Peninsula and northern Morocco were genotyped using double-digest RAD sequencing. Genomic diversity analyses were performed both through de novo assembly and with the reference genome of a closely related species (*Allolobophora icterica*), applying software designed to account for polyploidy. The analyses revealed geographically cohesive genetic clusters at both large (Morocco vs. Iberian Peninsula) and regional scales. At the regional level, the genetic clusters followed a latitudinal gradient across the Iberian Peninsula. These patterns contradict the currently described morphospecies. Furthermore, genomic associations with climatic and phenotypic variation in terms of the position of the clitellum were explored using Gradient Forest (GF), Redundancy Analysis (RDA), and Genome-Wide Association Studies (GWAS). Based on a set of non-collinear climatic variables, GF analysis predicted genomic composition across the species' distribution range, revealing three main geographic areas shaped by precipitation and temperature. RDA indicated that population structure, geography, and climate significantly contributed to genomic variance, and identified SNPs associated with climatic variation. These findings provide insights into earthworm adaptation to climate and resilience under changing conditions. GWAS analyses identified a subset of SNPs with additive effects on clitellum position under a Mixed Linear Model. The discovery of a molecular basis for clitellum variation has important implications for molecular systematics.

Keywords: earthworms, species complex, genomic diversity, phenotypic variation, adaptation to climate

Diversity of earthworms in urban and peri-urban areas of southern Brazil as indicators of disturbance

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Earthworms (Oligochaeta) contribute to nutrient cycling and soil structure improvement, being widely recognized as indicators of soil health. Urban parks, serve as important biodiversity refuges, but there is still a lack of information linking soil fauna to levels of conservation and anthropogenic disturbance in urban areas. Therefore, this study assessed earthworm species composition in urban and peri-urban areas of the metropolitan region of Curitiba, relating them to levels of disturbance and insertion in the urban matrix. Sampling was conducted in April and May 2025 in two sites with native forest (Capão da Imbuia Woods and Embrapa Forestry experimental station) and four sites with both forest and grass vegetation (Passaúna Park, Botanical Garden, Barigui Park, and Tingui Park). Earthworms were sampled using the standard TSBF method (five monoliths 25 x 25 cm to 20 cm deep), qualitative hand-sorting, and formalin extraction. A total of 15 species of six families were found, including five native Glossoscolecidae species, all of which may be new to science (*Glossoscolex* sp.1 and sp.2, *Fimoscolex* sp.1, sp.2, and sp.3), as well as the cosmopolitan species *Pontoscolex corethrurus* (Rhinodrilidae), *Amynthas gracilis*, *Amynthas corticis*, *Amynthas morrisi*, and *Metaphire californica* (Megascolecidae), *Bimastos parvus*, *Aporrectodea rosea*, *Octalasion tyrtaeum* (Lumbricidae), and juveniles of an Ocnerodrilidae species and of a Benhamiidae species (probabaly a *Dichogaster* sp.). The number of species found per method at each site was as follows (the number after each site represents the three methods, i.e., TSBF, qualitative and formalin extraction, followed by the total species richness): Passaúna 4, 1, 2, and 5 spp.; Botanic Gardens 6, 4, 4, and 9 spp.; Barigui 6, 4, 0, and 6 spp.; Tingui 8, 4, 1, and 8 spp.; Capão da Imbuia 1, 1, 1, and 1 spp.; and Embrapa 3, 1, 2, and 4 spp. Species richness in the forests and grass lawns (with native/exotic species in parentheses each richness) were, respectively: Passaúna 4 (1/3) and 2 (0/2) spp.; Botanic Gardens 5 (1/4) and 6 (0/6), spp.; Barigui 2 (1/1) and 5 (0/5) spp.; Tingui 3 (1/2) and 7 (1/6) spp. Hence, the grass lawns tended to have higher species richness than the forests, and exotic species dominated in all sites. The TSBF method was more efficient than the qualitative method and formalin. The parks with higher ecological connectivity (Tingui and Barigui) presented the greatest species richness (6, 8 spp.), while the more peripheral site (Passauna) had lower (5 spp.), although the most disturbed site (Botanical Garden) had the highest richness (9 spp). These results highlight the important role of urban parks in maintaining earthworm diversity, but also the invasion of many exotic species. Further research is needed to better understand the reason for the origin and maintenance of native species in urban areas in order to foster biodiversity conservation and reduce exotic species invasion risks.

Keywords: forests, lawns, parks, soil

How can we explain the current distribution of earthworms in Central Pacific?

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The New Caledonian biodiversity endemism in earthworms has been observed at the species and genera phylogenetic levels. We organized three one-month expeditions (in 2014, 2023 and 2024) in New Caledonia for earthworm sampling. Traditionally, speciation on Grande Terre (the main island of New Caledonia) was considered the result of long-term cladogenesis and tectonically largest volcanic events in the Earth history, including the break-up of Gondwana. However, endemic earthworms in the tropical New Caledonia do not show the expected pattern of the trans-Pacific-(sub)tropical groups distributed between Australia, New Caledonia and the Americas. An example of the trans-Pacific (sub)tropical group is the land crocodile Mekosuchus inexpectatus that went extinct about 3000 years ago.

Five earthworm species – *Acanthodrilus kermadecensis*, *A. mereensis*, *Pithemera sedgwicki*, *Metapheretima speiseri* and *Polypheretima fida* – were described as endemic species of smaller islands (parts of five archipelagos) in the central Pacific. However, the fact that different islands belong to one of these archipelagos does not mean that they are of the same origin, the same age and that they have the same taxonomic composition. For instance, the northern Lau islands of the Fiji Archipelago are of volcanic origin, whereas the southern ones of the same archipelago are made of sedimentary carbonate or of uplifted coral reefs.

We concluded suggest that the trans-tropical Pacific distributions in animals might be related to the central Pacific tectonics which is responsible for the formation of large igneous plateaus and not to the Gondwana break up. The observed earthworm distributions might result from the emplacement of large igneous plateaus in the central Pacific during the Cretaceous, one of the largest volcanic events in the history of Earth. The mostly subaerial eruptions in the Ontong Java plateau and the Manihiki and Hikuragi plateaus indicate that distributions could be explained on an ad hoc basis by the extinction in other areas and not necessarily as the result of dispersal.

However, more work will be needed to explain the observed pattern in New Caledonian earthworms.

Keywords: earthworms, Central Pacific, speciation, Gondwana, New Caledonia

Hunting the ghost: evidence for a new basal endemic earthworm genus from the Iberian Peninsula

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The genus *Dendrobaena* has traditionally served as a wastebasket taxon, as several earthworm species have been assigned to it based on putatively homoplastic morphological characters (such as red pigmentation and separate chaetae). In the Iberian Peninsula, some endemic species have been described in this genus, such as *D. osellai* Zicsi, 1970, *D. ruffoi* Zicsi, 1970 or *D. pseudorrosea* Moreno et al., 1982. These taxa have been repeatedly assigned to other genera on the basis of different morphological interpretations, and some authors have included them in *Kritodrilus* or *Iberoscolex*. Recent phylogenetic studies suggest that some of these species (*D. osellai* and *D. pseudorrosea*) do not belong to their current genera and constitute an independent, early divergent lineage within the Lumbricidae, possibly deserving recognition as a new genus endemic to the Iberian Peninsula. In this work, we revised and compiled all available morphological data from the literature and complemented it with new observations from freshly collected populations across some Iberian sites. External and internal characters (e.g., position of the clitellum and tubercula pubertatis, number and position of spermathecae and seminal vesicles, etc.) were examined and compared with type descriptions. Our results confirm the previously reported high variability between and within putative species, which could have led to this taxonomic instability. For a more integrative study, we conducted multilocus phylogenetic analyses (based on 5 molecular markers: mitochondrial COI, 12S, 16S, ND1 and the nuclear 28S), including ~20 newly sequenced individuals from 6 sites in Spain and morphologically assigned to different putative species of the complex. Preliminary results confirm that *Dendrobaena s.l. osellai* and *D. s.l. pseudorrosea* consistently cluster together in a well-supported, early-branching clade of Lumbricidae that is distant from the type species of *Dendrobaena*, *Kritodrilus* and the former *Iberoscolex* (*D. octaedra*, *K. calarensis* and *I. microepigeus*, respectively). The phylogenetic trees and genetic distances between lineages suggest the presence of several distinct species. Pending additional data (e.g. the molecular addition of some other putative species, such as *D. ruffoi* or other possibly related taxa that have been treated differently, such as *Cataladrilus microendogeus*), we propose to treat these species provisionally as *Dendrobaena sensu lato*, but highlight their potential recognition as a new Iberian endemic genus. This adds more information to the evidence that the Iberian Peninsula represents a major centre of diversification for basal Lumbricidae.

Keywords: Iberian Peninsula, endemism, taxonomy, phylogeny

Ovary organization and ultrastructure in earthworms belonging to the family Acanthodrilidae

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Earthworms (megadriles) are hermaphrodites with both female and male gonads. So far, little attention has been paid to ovarian organization and oogenesis. Therefore, this research is part of a larger project that studies the microorganization and functioning of ovaries in many earthworm families. The representatives of Hormogastridae, Megascolecidae, Lumbricidae, and Eudrilidae have already been analyzed. Another taxon chosen for the study is the species-rich and widely distributed family Acanthodrilidae. We used light and transmission electron microscopy to describe in detail the histological and ultrastructural organization of acanthodrilid ovaries from the genera *Barogaster*, *Diplocardia*, *Lennogaster*, *Octochaetona*, and *Ramiella*.

Studies at the level of gross morphology indicate that ovaries are located in the XIII segment, are fan to rosette-shaped, and consist of numerous rows of germ cells (egg strings) at different stages of oogenesis and enveloped by thin somatic cells. According to the histological results, ovaries can be divided into two zones. Zone I contains oogonia and early meiotic cells; in zone II, growing oocytes accompanied by nurse cells and vitellogenic oocytes. This ovarian organization seems similar to ovaries observed in the Megascolecidae family. Still, acanthodrilid ovaries are composed only of egg strings uniting cells at different stages of meiosis, whereas megascolecid ovaries have a common part (ovarian center) with numerous radiating egg strings.

Ultrastructural analysis revealed the occurrence of germline cysts that are also present in the ovaries of other studied earthworm families. Within the cyst, each clustering cell is connected by a stable intercellular bridge to the central cytoplasmic mass – cytophore, which seems to be poorly developed (the reticular cytophore). As oocytes grow, they detach from the cysts, but the rest of the cells are still interconnected via cytophore and do not continue oogenesis. These cells are nurse cells, so that the acanthodrilid ovaries can be classified as meroistic. The occurrence of germline cysts equipped with the reticular cytophore seems to be a conservative feature of earthworm oogenesis.

This work was supported by the National Science Centre, Poland. Grant 2020/37/B/ NZ4/00560. We are grateful to Shweta Yadaw (Department of Zoology, Dr. Harisingh Gour University, Sagar, India) and Samuel James (Regenerative Organic Agriculture Department, Maharishi International University, Fairfield, USA) for providing earthworms.

Keywords: oogenesis, female gonads, germline cysts, histology

Updated checklist of earthworms (Annelidae: Lumbricidae) of Serbia

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In Serbia, earthworms from the Lumbricidae family began to be studied in the first half of the XX century. The first complete summary of the earthworms of Serbia was published by Šapkarev (1980), who registered a total of 36 taxa. During the 1990s, until today, research on this fauna has been intensified. Taking into account new data and the revised status of individual taxa, the number of taxa has grown, 71, 74, 77, respectively (Stojanović et al., 2008; Stojanović et al., 2018; Stojanović-Petrović et al., 2020). In this paper, updated checklist of earthworms (Annelidae: Lumbricidae) of Serbia is presented. By checking the reliability of taxonomic characters using molecular methods, by establishing phylogenetic relationships, a list was reached containing of 84 species, belonging to 15 genera. The difference in the number of species is a result of several reasons. Either the species were synonyms or they were misidentified. And the most important reason is the field research in which new species were found. Of particular importance are the new six endemic taxa which were recorded for the first time on the territory of Serbia. Almost half of the found species are endemic. The impressive diversity of lumbricids shows that Serbia is a territory of considerable earthworm richness, which is both unique and complex. Also, these data point to the space for further faunal research in these areas.

Keywords: earthworm, biodiversity, distribution, Serbia

Invasions of the european earthworm species in the island forests of the Karkaraly mountains, Central Kazakhstan

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The intercontinental invasion of European earthworms has been studied extensively for decades. Invasions are also ongoing within the Eurasian continent itself, which receives much less attention. The territory of Central Kazakhstan is of particular interest, where suitable habitats for earthworms are settlements and sparse forest patches enclosed by vast areas of dry grasslands, which are unsuitable for them. Larger forest areas are associated with local mountains. The Karkaraly Mountains has become a territory for researching the process of earthworm invasions in Central Kazakhstan. It was assumed that the leading factor responsible for the composition of the earthworm populations in the region is the pattern of land use and its history. The settlers from the European part of the Russian Empire began to arrive in the regions of traditional nomadic cattle herding in Central Asia in the mid-XIXth century. European species of earthworms could have been brought here along with introduced planting stock. One of the migration centres was the town of Karkaraly, in the vicinity of which and on the adjusted territory of the Karkaraly National Park (49.42745°N, 75.35269°E), the research was conducted. A total of 22 forest sites were sampled for earthworms, 8 of them in the Kendara Valley within the mountain range and 14 outside of the mountains. Earthworms were collected by hand-sorting soil samples in 2023-25. Eight species were recorded, of which two were found in both territories of the mountain valley and near settlements: *Dendrobaena octaedra* (Savigny, 1826) and *Lumbricus rubellus* Hoffmeister, 1843. *Aporrectodea caliginosa* (Savigny, 1826) and *Eisenia pallida* (Malevic, 1956) were recorded only outside of mountains; *Aporrectodea rosea* (Savigny, 1826), *Bimastos rubidus* (Savigny, 1826), *Eisenia nordenskioldi* (Eisen, 1878), and *Octolasion lacteum* (Örley, 1881) only within the mountainous area. The earthworm density in the Kendara Valley ranged mostly from 20 to 52 ind./m^2, with a maximum biomass of 25 g/m^2, while outside of the valley the abundance was several times higher: > 100 ind./m^2 for most areas and up to 261 g/m^2 in biomass. This difference is mainly due to *Aporrectodea caliginosa*, which accounts for about 90% of the total density and even more regarding biomass. It can be concluded that there is a widespread invasion of the earthworm *Aporrectodea caliginosa* in the surveyed territory near the residential area, and it has not yet invaded the biotopes within the mountain range. Perhaps this species has colonised this territory over the past 30-40 years, as, according to literature data (1989), it was found sporadically near Karkaraly. Two more species were not recorded here before and are considered invasive as well. The species *Octolasion lacteum* is notably found in an abandoned vegetable garden near the national park forest cabin in the centre of the mountain range. One *Lumbricus rubellus* specimen was found almost in the same location, and dozens were found in five forest sites near settlements outside the mountain range.

This study was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan (project #AP26199585)

Keywords: forest ecology, land use history, soil samples, hand sorting, Lumbricidae

New Records of Two Species of Metaphire Sims and Easton, 1972, from Nepal Using Integrative Taxonomic Approaches

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Earthworms of the genus *Metaphire* (Clitellata: Megascolecidae) are distributed throughout Southeast and South Asia. They are among the least studied organisms in Nepal, with known records of only two species in the country to date. Taxonomic explorations of Nepalese earthworms using integrative approaches that combine morphological, anatomical, and molecular data are currently in their early stages. This study was conducted in central Nepal to explore the taxonomic status of *Metaphire* earthworms using an integrative approach that includes field sampling, morpho-anatomical characterization, and molecular phylogenetic analysis based on mitochondrial NADH dehydrogenase subunit 1 (ND1) gene sequences. The two *Metaphire* species, *Metaphire posthuma* (Vaillant, 1868) and *M. birmanica* (Rosa, 1888), were recorded for the first time in Nepal from the Chitwan and Nuwakot districts in the natural habitats of the Trishuli River Basin. These species have previously been reported from China, India, Myanmar, Pakistan, Laos, and Vietnam. The morpho-anatomical resemblance of specimens from Nepal to the holotypic description of *M. birmanica* from Bhamo, Myanmar, was observed with the following diagnostic characters: three pairs of ventrolateral intersegmental spermathecal pores at V/VI-VII/VIII; female pore midventral at xiv segment; male pore in copulatory pouch bounded by tumescent lip at XVIII segment; genital marking absent; testes sac paired in X and XI segments; seminal vesicle paired in XI and XII; racemose prostate extend from XVII-XX, manicate intestinal caeca originated from XXVII extending up to XXIV segment. Similarly, other specimens from Nepal had morpho-anatomical characters matching with the holotype description of *M. posthuma* from Java, Indonesia with following diagnostic characters: four pairs spermathecal pores at V-VI/VI-VII/VII-VIII/VIII-IX; female pore at mid ventral of XIV; male pore minute at XVII having small disc; two pairs genital marking present at XVII & XIX segment; testes sac paired in X and XI segment; seminal vesical paired in XI and XII; racemose prostate XVII-XX; simple intestinal caeca originated from XXVII extending up to the XXV segment. The BLAST searches of the ND1 gene sequences (943 bp) of specimens from Nepal in NCBI GenBank confirmed the specimens as *M. posthuma* and *M. birmanica*, with sequence similarity of 97.34% (with the *M. posthuma* sequence of GenBank accession number MW222472.1) and 99.56% (with the *M. birmanica* sequence of accession number MK098808.1), respectively. The Maximum Likelihood (ML) phylogenetic analyses using the ND1 gene sequences of *M. posthuma* and *M. birmanica* specimens from central Nepal produced monophyletic clades with the conspecific sequences retrieved from GenBank with strong bootstrap values and confirmed the identity of *M. posthuma* and *M. birmanica* from central Nepal. This new record of *Metaphire* species from Nepal necessitates detailed exploration to document earthworm species.

Keywords: biodiversity inventory, integrative taxonomy, *Metaphire birmanica*, *M. posthuma*, Himalaya biodiversity hotspot

Intraspecific morphological and morphometric variation in earthworm *Dendrobaena veneta* (Rosa, 1886)

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This study provides a detailed morphological and morphometric analysis of the epigeic earthworm *Dendrobaena veneta* (Rosa, 1886), a widespread peregrine species introduced across Europe. Although traditionally considered a single species, *D. veneta* displays marked morpho-anatomical variability, which has led to the historical description of multiple subspecies and varieties. The main aim of this study was to quantify phenotypic variability within a single population by evaluating a set of quantitative traits, to assess the extent of intraspecific differentiation. A total of 330 specimens (300 adults and 30 subadults) were analyzed. Measured traits included body length and width, clitellum and post clitellum length, number of segments, prostomium, dorsal pore, glandular glands, clitellum, tubercula pubertatis, sexual aperature (left and right), seminal vesicles and spermathecae, setae arrangement on the preclitelum and postclitelum part (aa; ab; bc; cd; dd). Spearman's and Pearson's correlation analyses were used to assess trait relationships and group differentiation. Substantial variation was observed in body length (45-102 mm), segment number (95-155), clitellum position (segments 26-33), and setae distance. Adults and subadults showed similar segment counts, suggesting that growth occurs primarily via segment elongation rather than addition. The prostomium was epilobic in 58.9% of adults and tanylobic in 41.1%. No evidence of parthenogenesis was found, as male pores were present on segment 15 in both age groups. Spermathecae were consistently located in segments 9 and 10, and seminal vesicles in segments 9-12. A strong correlation was found between body length and both postclitellum length ($r = 0.962$) and segment number ($r = 0.530$), suggesting size-related developmental changes. In contrast, clitellum width and length were negatively correlated ($r = -0.147$), indicating opposing growth trends. Setae arrangement remained symmetrical across age categories, confirming its potential taxonomic value. Three statistically distinct morphometric groups were identified within the population, possibly reflecting cryptic lineages or high phenotypic plasticity. Overall, *Dendrobaena veneta* exhibits significant morphological variability within a localized population. These findings highlight the importance of integrating morphometric data into future taxonomic and phylogeographic research.

Keywords: *Dendrobaena veneta*, morphometrics, earthworm, variability, phenotypic plasticity

The ovary morphology and internal organization in representatives of Brazilian Ocnerodrilidae

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Using light and electron microscopy techniques, we analyzed the ovary micromorphology in four representatives of the family Ocnerodrilidae. The analyzed earthworms were *Nematogenia panamaensis* and three undetermined species from the genus *Kerriona*. In all species studied, we have found one pair of ovaries attached to the septum between the XIIth and XIIIth segments. The ovary morphology differs between the studied species. In *N. panamaensis* the ovaries are flattened and triangular-like, with the narrow end connected to the septum, whereas growing oocytes form one egg string on the opposite ovary end. In all studied *Kerriona* species, the ovaries are flattened and fan-shaped. The narrow part is connected to the septum and, contrary to *Nematogenia*, oocytes form numerous egg strings at the opposite ovarian end. Despite different morphologies, ovaries in all species studied have the same pattern of internal organization. Their narrow parts, close to the septum, contain oogonia and early germ cells, whereas growing oogonia occupy the broader ovary end. Oogonia and early meiotic cells (till diplotene) are interconnected in all species studied and form germline cysts. The cyst organization is the same as in other crassiclitellate earthworms, i.e., each cell has one intercellular bridge connecting it to the reticular cytophore. At the onset of yolk absorption (vitellogenesis), oocytes detach from cysts. The amount of nutrients in oocytes is moderate; they accumulate mainly glycogen and lipids. The rest of the interconnected cells (i.e., nurse cells) do not accumulate nutrients and do not grow. They are associated with growing oocytes and probably die in the distal part of the ovary.

Two main conclusions can be drawn: 1) ovary localization and internal organization are similar to those of the other Crassiclitellata; 2) surprisingly, we found two different ovary morphologies: an ovary with one egg string in *N. panamaensis* and fan-shaped ovaries with numerous strings in *Kerriona*.

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

Keywords: histology, ultrastructure, oogenesis

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