



Station Biologique de Paimpont

France

7-13 November 2016



7th IOTM (International Oligochaete Taxonomy Meeting)

Taxonomy, Phygeny and Ecology of earthworm'communities

Programme of the Scientific Conference

Monday 7 November 2016

9h00-9h15	Thibaud Decaëns & Daniel Cluzeau	General introduction
9h15-10h15	Sam W. James	Diversity and classification of earthworms
10h15-10h45	Jiang Jibao	A comprehensive phylogeny resolves the evolutionary timing and pattern of Megascolecidae earthworms from China
	Coffee break	
11h15-11h45	Daniel Fernández Marchán	Integrative systematic revision of the family Hormogastridae (Annelida, Oligochaeta)
11h45-12h00	Daniel Fernández Marchán	Eiseniella tetraedra Project
	Lunch	
14h00-14h30	Gabriela Cervantes	Preliminar Molecular Phylogeny of Acanthodrilid Earthworms from Mexico (Crassiclitellata: Acanthodrilidae)
14h30-15h00	Yan Dong	Phylogeography of Three Common Species in Pheretimid Earthworms (Oligochaeta: Megascolecidae) in China
15h00-15h30	Thembeke Nxele	The first proandric species of Tritogenia from Botswana; Tritogenia talana sp.n. (Oligochaeta, Tritogeniidae)
	Coffee break	
16h00-17h30	Working group	<i>Towards a global earthworm diversity observatory</i>
17h30-18h30	First Synthesis	

Thursday 8 November 2016

9h00-9h45	George Brown	Earthworm taxonomy, biology and ecology. An overview of recent advances (2013-2016) in Brazil
9h45-10h15	Qi Zhao	How earthworms disperse between Hainan Island and China mainland? ----take two Amynthes species as an example
	Coffee break	
10h45-11h15	Liangliang Zhang	Fifteen new earthworm mitogenomes shed new light on phylogeny within the Pheretima complex
11h15-11h45	Sam W. James	<i>Pontoscolex</i> phylogeny
11h45-12h15	Muriel Guernion	French collaborative science network on earthworm communities
	Visit the pet monkey	
	Lunch (13h)	
14h00-14h30	Shweta Yadav	Exploring earthworms of Central India (Sagar, MP) using DNA barcodes
14h30-15h00	George Brown	Use of DNA barcoding for Brazilian earthworm biodiversity assessment: untangling taxonomy for conservation
15h00-15h30	Thibaud Decaëns	Combining training programs and DNA barcoding to explore the diversity of tropical earthworm communities - feedbacks from the ECOTROP field school
	Coffee break	
16h00-17h30	Working group	<i>Towards a global earthworm diversity observatory</i>
17h30-18h30	Second Synthesis	

Wednesday 9 November 2016

9h00-9h30	Malalatiانا Razafindrakoto	State of Knowledge of the Malagasy endemic Family, <i>Kynotidae</i> (Oligochaeta) from Madagascar
9h30-10h00	Oren Pearson	Earthworm Populations in Mature Pinus halepensis Forest Subjected to different Forestial Thinning Treatments
10h00-10h30	Daniel Cluzeau	Earthworms distribution in agricultural and urban french soils
	Coffee break	
11h00-11h30	Marcel Koken	Bioluminescence in earthworms: a short review
11h30-12h00	Satyendra M. Singh	Phorate toxicity may reduce species diversity of earthworms
	Lunch	
14h00-14h30	Thibaud Decaëns	Earthworm diversity in Amazonian ecosystems of French Guiana
15h00-15h30	George Brown	Earthworms and Amazonian Dark Earths: improving understanding of the relationships between soil management, biodiversity and function*
15h30-16h00	Rüdiger Schmelz	The Edaphobase Nationwide Field Monitoring ? A survey of soil oligochaete (Lumbricidae, Enchytraeidae) assemblages in different habitat types in Germany
16h00-16h30	Open discussion	(included other examples of DataBase : DriloBASE, EcoBioSOIL, ...)
	Coffee break	
17h00-18h30	Working group Final Synthesis	<i>Final proposals and time organization</i>

Additional communications (Posters)

Annegret Nicolai	Molecular phylogeny of the six most common European Lumbricus species
Bazri Kamel-Eddine	The earthworm biodiversity in eastern Algeria
Veronika Abukenova	Some aspects of earthworm fauna in Kazakhstan (Oligochaeta: Lumbricidae)
Robabeh Latif	Comparison of life cycle and reproductive traits of eight populations of Eisenia fetida (Savigny, 1826) from Zagros Mountain

7th IOTM (*International Oligochaete Taxonomy Meeting*)

Post-conference workshop - Earthworm taxonomy course (Sam JAMES)

Friday 11 November 2016

9h00-12h00		Exploration of external anatomy
	Lunch	
14h00-17h30		Describing external anatomy and using it in identification <i>Discussion of the main traits of external anatomy in the main earthworm families</i>

Saturday 12 November 2016

9h00-12h00		Beginning dissection and exploration of internal anatomy
	Lunch	
14h00-17h30		Exploration of internal anatomy and describing internal anatomy <i>Discussion of the main traits of internal anatomy in the main earthworm families</i>

Sunday 13 November 2016

9h00-12h00		Continue with internal anatomy; identification and description of earthworms brought by participants.
	Lunch	
14h00-17h30		Sampling methods in the field, or continued identification. <i>Synthesis on the morphological and anatomical traits in the main earthworm families</i>

A comprehensive phylogeny resolves the evolutionary timing and pattern of Megascolecidae earthworms from China

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Abstract

Earthworms belonging to the family Megascolecidae are the most important and widely distributed in China. However, the evolutionary history of Megascolecidae is still unresolved. A robust taxonomy and molecular phylogenetic analysis are of significant value to understand their evolution.

In 2010-2014, 321 species were collected from twenty more nature reserves of China. We reconstructed phylogenetic trees with an unprecedented scale of data: 5 mitochondrial genes (COI, COII, ND1, 12S & 16S) from 584 specimens of Megascolecidae earthworms. Bayesian and maximum likelihood analysis yielded highly supported and nearly identical phylogenetic trees for the Pheretimid lineages. Fourteen major clades form successive sister groups. The phylogenetic trees congruenced with taxonomy research indicated that neither of the two main genera (*Amyntas* and *Metaphire*) were supported as monophyletic.

The result of the divergence time analyses estimated by Bayesian method suggesting a speciation radiation of Megascolecidae earthworms after the Cretaceous–Palaeogene mass extinction. Species richness increased sustainedly during Cenozoic period. Our result conjecture the copulatory pouch of *Metaphire* species is polyphyletic and originated no later than Early Miocene, while many parthenogenetic species are traceable from Middle or Late Miocene.

Ancestors area reconstructed analysis shows that, the ancestors of Megascolecidae in China might come from Indo-China Peninsula, then spread through China in the lengthy Geologic Time, resulting in radiations into vast numbers of species and evolving different morphological character types.

*Speaker

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Integrative systematic revision of the family Hormogastridae (Annelida, Oligochaeta)

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Abstract

For decades, scientific endeavors aiming to resolve the phylogenetic relationships of earthworms have resulted in discrepant hypotheses, with different authors proposing discordant topologies based both on molecules and on morphology. Even though works as James & Davidson (2012) or Domínguez et al. (2015) have outlined the potential of molecular phylogenies as the backbone of a reviewed earthworm taxonomy, it was not without their own pitfalls. The usual molecular markers (i.e., COI/COII, 16S, 18S, 28S, H3, H4, ITS) were insufficient to fully resolve above-species level relationships, and still left the difficult task of assigning morphological or ecological diagnoses to the different taxonomic entities. Integrative systematics combine all available sources of evolutionary information in an attempt to reflect phylogenetic relationships more accurately. This methodology has been embraced by many researchers showing great success, but has been rarely applied to oligochaetes at family level.

The family Hormogastridae Michaelsen, 1900 appears to be the ideal subject for an integrative systematic revision built upon the promising advances of molecular phylogenetics. The relatively low number of species-level taxa (32 including subspecies), restricted distribution area (western Mediterranean basin) and low nomenclatorial conflict constitute an advantage over other earthworm families as Lumbricidae. On the other hand, their deep-burrowing lifestyle and relatively dry habitats explain the slow advance on their research. Novo et al. (2011) revealed the paraphyly of the catch-all genus *Hormogaster* (containing at least four deeply divergent clades), the polyphyly of the species *Hormogaster pretiosa* and the widespread presence of cryptic diversity. After said cornerstone work, significant advances have been achieved: the discovery of new species of phylogenetic relevance, the application of phylogenomic reconstruction to infer deep phylogenetic relationships, the ecological characterization of different clades through Ecological Niche Modelling, and the reassessment of the phylogenetic usefulness of several morphological characters.

In this contribution, we integrate these different sources of information to generate a revised taxonomy that better reflects the biological and evolutionary diversity of the family.

^{*}Speaker

It includes the proposal of four new genera, with the establishment of morpho-ecological diagnoses for all of them.

Eiseniella tetraedra Project

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Abstract

Eiseniella tetraedra is a parthenogenetic, ripicolous and cosmopolitan earthworm. Due to this last characteristic we started the "Eiseniella tetraedra Project". Phylogeographic studies on different cosmopolitan earthworm species have emerged in the last few years. However, they were focused on some specific areas of the world, never including a whole continent. That is our challenge, to reconstruct the biogeographic history of *E. tetraedra* and study its genetic and morphological variability across Europe. At the moment we have fully sampled the Iberian Peninsula, and with the collaboration of some colleagues, Sweden, Norway and France are also well represented. Our initial phylogeographic data indicate that *E. tetraedra* presents a great clonal and morphological variability in Spain and point to the possibility of a postglacial recolonization from Iberian Peninsula to Northern Europe. However, recolonization from other shelters cannot be discarded and more data would be necessary to confirm our hypothesis. In order to fill the geographic gaps, we would like to appeal for help to all oligoquetologists. We would really appreciate *Eiseniella tetraedra* specimens from anywhere in Europe. Only with your help, we would be able to complete the phylogeographic story of this fascinating earthworm. Thank you!

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†Speaker

Preliminar Molecular Phylogeny of Acanthodrilid Earthworms from Mexico (Crassiclitellata: Acanthodrilidae)

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Abstract

We conducted a Bayesian analysis made from sequences of 16S rDNA gene to clarify the phylogenetic relationships of the Mexican acanthodrilid earthworms (subfamilies Acanthodrilinae, Benhamiinae and Octochaetinae). The result of this phylogenetic analysis indicates that earthworms of the family Acanthodrilidae are placed in three subfamilies: 1) Acanthodrilinae, earthworms with acanthodrilid genital arrangement, single oesophageal gizzard, calciferous glands present or absent, and holonephridia (genera *Kaxdrilus*, *Lavellodrilus*) or meronephridia (*Ramiellona* and a new genus related to *Ramiellona*); 2) Benhamiinae, with acanthodrilid genital arrangement, two oesophageal gizzards, calciferous glands and meronephridia (genera *Dichogaster* and *Eutrigaster*); and 3) Diplocardiinae, with acanthodrilid genital arrangement, multiple oesophageal gizzards, without calciferous glands, and with holonephridia (*Diplocardia*, *Prozotapotecia*, *Zapotecia*) or meronephridia (*Zapatadrilus*). Our results indicate that the family Diplocardiinae, as suggested by James and Davidson (2012), should be restored and that the highly discussed subfamily Octochaetinae (represented by the genera *Neotrigaster*, *Zapatadrilus*, *Ramiellona*, and a new genus related to *Ramiellona*), by its non-monophyletic nature should be rejected. Further analyses using more genes will be required to confirm the topology of the obtained phylogenetic tree.

*Speaker

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Phylogeography of Three Common Species in Pheretimoid Earthworms (Oligochaeta: Megascolecidae) in China

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Abstract

Amyntas morrisoni (Beddard, 1892), *Amyntas gracilis* (Kinberg, 1867) and *Metaphire californica* (Kinberg, 1867) are three common pheretimoid species in China. Previous studies showed that all of them originated from South China. However, their dispersal are still unknown at present.

In order to understand their evolutionary history, we collected earthworms systematically in South China during 2006-2015. 19 populations of *A. morrisoni*, 17 of *A. gracilis*, 30 of *M. californica* were found. In this study, the phylogeny of these three populations were reconstructed separately by five mitochondrial genes (COI, COII, ND1, 16S and 12S) using Maximum likelihood (ML) and Bayesian (BI) method. The divergent time of different populations were estimated by Beast.

Our results showed that *A. morrisoni* and *A. gracilis* dispersed in different directions in 6.46-9.78 Ma (Pleistocene). *A. morrisoni* dispersed in three directions. One expanded east to Guangdong province along Nanling mountainous and Wuyishan Mountain. The second dispersing direction is directly to Yunnan-Guizhou Plateau, and then arrived at Yunnan province. The third way is to the north along the east of Xuefeng mountain, Wushan and Qinling mountainous, such as Hunan province, Guizhou province, Anhui province. *A. gracilis* dispersed in four directions. One expanded to Hainan province. The second and third route expanded to Yunnan province, Guangdong province, Fujian province and so on, similar as *A. morrisoni*. The last one directly dispersed to Zhejiang province. Unfortunately, the dispersal of *M. californica* was still nebulous in present study.

*Speaker

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The first proandric species of *Tritogenia* from Botswana; *Tritogenia talana* sp.n. (Oligochaeta, Tritogeniidae)

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Abstract

The earthworm fauna of Botswana is unknown. A collecting trip to never investigated regions of Botswana (Chobe and Tuli Block) resulted in the discovery of a new species, *Tritogenia talana* sp.n. Its reproductive organs showed some level of degeneration when compared to other *Tritogenia*. The spermathecae were not observed, testes and sperm funnels are in proandric condition and lack of the seminal vesicles noted. This is the first record of earthworm species from Botswana.

*Speaker

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Earthworm taxonomy, biology and ecology. An overview of recent advances (2013-2016) in Brazil*

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¹⁹Universidade Estadual do Maranhao – Brazil

²⁰MPEG – Brazil

Abstract

*Speaker

As done in the past two IOTM's, the present talk synthesizes recent (2013-2016) advances in earthworm taxonomy, biology and ecology in Brazil. The work presented includes mostly unpublished results of research performed over the last 3 years by graduate and undergraduate students and researchers belonging to various collaborating institutions, both in Brazil and abroad. Earthworm populations were quantitatively evaluated by handsorting soil monoliths (generally using the TSBF-method) in 11 locations (total 98 sites) including agricultural, pastoral, agroforestry, regenerating and old forest systems. Furthermore, at these and at > 10 other locations (140 sites total) scattered throughout Brazil, earthworms were collected using qualitative methods, by searching in various niches (soil, litter, under rocks, next to streams, swamplands, rotten logs, bromeliads). Many of these worms were identified to species level, but much material must still be processed. Over 1000 individuals were barcoded, of which ~50% were *Pontoscolex corethrurus*, for which neotypes were collected at the type locality (Blumenau). The genetic variation and DNA size of this species was found to be more variable than previously assumed. The Fritz Müller Oligochaete Collection at Embrapa Forestry now contains over 1000 glass vials with earthworms and over 1500 tubes with tissue samples or whole worms, representing well over 200 species, all preserved in alcohol 80%. Over 60 new species were found, many in the *Glossoscolex* and *Fimoscolex* genera, but also in several Ocnodrilidae genera. Overall 100 new CO1 barcode MOTUs were generated for BOLD. Five taxonomy courses were given, training over 60 persons in earthworm identification. Additionally, a molecular phylogeny course trained 10 students in these techniques. For the first time on record, sperm was found in the nephridia of *Glossoscolex*, shedding some light on how this genus lacking spermathecae may actually reproduce. The biology of some *Glossoscolex*, *Rhinodrilus* and *Andiorrhinus* species were studied, revealing differences in growth, casting rates and cast properties. The gut and cast bacteria of *Perionyx excavatus* and *Dichogaster annae* were studied, and the impacts of *P. corethrurus* on sugarcane rhizosphere microbial diversity were evaluated. The use of mustard, onion and formalin were compared, revealing formalin as more efficient for population and biodiversity assessment. Earthworms were found to be important components of Amazonian Dark Earths, although their populations were greatly affected by land use system in these soils. The role of earthworms as environmental and soil quality bioindicators was further elucidated, indicating the need for methodological and theoretical adaptations in order for optimal adoption of this practice. *Funded by CNPq, Newton-CONFAP, NERC, EU, Fapesp, Fapema, Fapemig, Fapeam, Fundação Araucária, Embrapa and CAPES

How earthworms disperse between Hainan Island and China mainland? —take two *Amyntas* species as an example

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²School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai – China

Abstract

Amyntas species distributes mainly in the southern part in China. After the investigation of earthworms in South China from 2009-2016, *Amyntas instabilis* Qiu & Jiang, 2014 was found only in Hainan Island, Guangdong and Guangxi province. Previous study had already presented that *A. instabilis* originated from Hainan Island. However, how it disperses between Hainan Island and China mainland is still a mystery.

In order to solve the puzzle, we studied the phylogenetical relationship of five *A. instabilis minusculus* populations and seven *A. instabilis instabilis* populations. Meanwhile, *Metaphire californica* was considered as an outgroup. The phylogeny tree was reconstructed by five mitochondrial genes (COI, COII, ND1, 16S and 12S) using Maximum likelihood (ML) and Bayesian (BI) method. Beast was used to calculate the divergent time of earthworm populations.

Our results showed that *A. instabilis minusculus* and *A. instabilis instabilis* was separated clearly into two clades. *A. instabilis minusculus* showed the dispersing route from Hainan Island to Guangdong and Guangxi province. This may be due to the land bridge between the island and the mainland. *A. instabilis instabilis* showed an opposite direction from Guangdong and Guangxi province to Hainan Island. It may be explained by the several connections and separations between Hainan Island and the mainland during the Quaternary.

*Speaker

†Corresponding author:

Fifteen new earthworm mitogenomes shed new light on phylogeny within the *Pheretima* complex

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Abstract

The *Pheretima* complex within the Megascolecidae family is a major earthworm group. Recently, the systematic status of the *Pheretima* complex based on morphology was challenged by molecular studies. In this study, we carry out the first comparative mitogenomic study in oligochaetes. The mitogenomes of 15 earthworm species were sequenced and compared with other 9 available earthworm mitogenomes, with the main aim to explore their phylogenetic relationships and test different analytical approaches on phylogeny reconstruction. The general earthworm mitogenomic features revealed to be conservative: all genes encoded on the same strand, all the protein coding loci shared the same initiation codon (ATG), and tRNA genes showed conserved structures. The *Drawida japonica* mitogenome displayed the highest A + T content, reversed AT/GC-skews and the highest genetic diversity. Genetic distances among protein coding genes displayed their maximum and minimum interspecific values in the *ATP8* and *CO1* genes, respectively. The 22 tRNAs showed variable substitution patterns between the considered earthworm mitogenomes. The inclusion of rRNAs positively increased phylogenetic support. Furthermore, we tested different trimming tools for alignment improvement. Our analyses rejected reciprocal monophyly among *Amyntas* and *Metaphire* and indicated that the two genera should be systematically classified into one.

*Speaker

† Corresponding author:

When citizens and scientists work together : a French collaborative science network on earthworms communities (the OPVT)

Muriel Guernion^{*1}, Kevin Hoeffner¹, Sarah Guillocheau¹, Hoël Hotte¹, Daniel Cylly¹, Yoann Vourc'h¹, Denis Piron¹, Jennifer Scimia¹, Aurélien Briard¹, Guenola Pérès², and Daniel Cluzeau¹

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Abstract

Scientists have become more and more interested in earthworms because of their impact on soil functioning and their importance in provision of many ecosystem services. In order to improve the knowledge on soil biodiversity and integrate earthworms in soil quality diagnostics, it appeared necessary to gain a large amount of data on their distribution. However, despite the huge number of research programs to collect these data, efforts are still needed. In order to compensate for these missed data and accelerate their collect, the University of Rennes 1 developed since 2011 a collaborative science project called Observatoire Participatif des Vers de Terre (participative earthworm observatory). It has several purposes : i) to offer, through earthworm assessment, a simple tool for soil biodiversity evaluation in natural and anthropic soils, ii) to offer trainings to farmers, territory managers, gardeners, pupils..., iii) to build a databank of reference values on earthworms iv) to propose a website (https://ecobiosoil.univ-rennes1.fr/OPVT_accueil.php) providing for example general scientific background (earthworm ecology and impacts of soil management), sampling protocols and online visualisation of results (data processing and earthworms mapping).

This project, initially available to anyone on a voluntary basis, is also used by the French ministry of agriculture to carry out a scientific watch throughout the French territory. Up to now, more than 4500 plots have been prospected since the opening of the project in 2011. Details of this collaborative project will be presented, meaning adaptation of the sampling protocols, tools for training, results and returns from end-users.

^{*}Speaker

Exploring earthworms of Central India (Sagar, MP) using DNA barcodes

Shweta Yadav*¹

¹Department of Zoology Dr H S Gour Vishwavidyalaya (A Central University) Sagar MP, India –
Department of Zoology Dr H S Gour Vishwavidyalaya (A Central University) Sagar MP, India, India

Abstract

India is located 8.40 – 37.60N latitude with covered area 3,287,797 km² and is rich country as far as its biodiversity is concerned. Considering the past geological history of the Indian subcontinent excluding (Burma, Nepal and Pakistan) has been divided into six well-defined physiographic regions depending upon topography, climate and vegetation viz. Western Himalayas, Eastern Himalayas, and Northeast ranges, Indo-Gangatic Plains, Central high lands, Peninsular plateaus and Western Ghats including Sahyadri and Nilgiri hills. The most diverse families of earthworm species in India Megascolecidae comprise 136 species (119 native) of 13 genera; Octochaetidae with 134 species (129 native) of 30 genera and Moniligastridae with 79 species (all native) of 3 genera. Especially diverse earthworm genera of India belongs to Moniligastridae *Drawida* (68 species); the Megascolecidae *Perionyx* (46 species) and *Megascolex* (33 species); the Acanthodrilidae *Agrilopilus* (22 species) the Octochaetidae *Eutyphoeus* (22 species), *Haplochaetella* (19 species) and *Octochaetona* (15 species). The diversity of Oligochaete is poorly known from central part of India. Earthworm fauna of Sagar district of Madhya Pradesh is known from the works of Stephenson and Gates. Stephenson (1923) studied the earthworms of Central India and explored four species from surroundings of Sagar (formerly called Saugor). Subsequent contribution by Gates (1945) revealed the occurrence of 25 species from Madhya Pradesh. Julka (1988) revealed the taxonomic status of oligochaetes belonging to the family Octochaetidae from Madhya Pradesh. So far, 2 species belonging to one genera (*Lennogaster*) of Octochaetidae have been recognized from the Saugor district. Present study revealed the description of 17 species belonging to 12 genera spread over 4 families (description of *Drawida* sp. not included due to pending results of molecular analysis) using integrated approach of taxonomy *i.e.* morpho-anatomy, ecological and molecular. The study enriches the existing information on earthworm biodiversity in the study area with use of modern trend of molecular taxonomy.

*Speaker

Use of DNA barcoding for Brazilian earthworm biodiversity assessment: untangling taxonomy for conservation*

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Abstract

Worldwide, soils and their biodiversity are threatened by land use changes and management practices that can have profound effects on soil organisms that perform important ecosystem services. However, our ability to adequately assess biodiversity in soils is greatly hindered by taxonomic impediment. The present research work addresses these issues/limitations, by focusing on DNA barcoding of Brazilian earthworms, a major soil ecosystem engineer.

Around 850 specimens from 175 sites (mainly in S Brazil) were DNA barcoded, to delimit molecular operational taxonomic units (MOTUs), and MOTU number and composition were used to estimate species richness. In total, barcodes have been generated for approx. 232 MOTUs with > 10-14% genetic distances, most of them representing species new to science. In S and SE Brazil, approximately 75 species-level lineages of *Glossoscolex* and 29 of *Fimoscolex* were found, most of which belonged to undescribed species. They were found mostly in forested sites and some different species were also found in tree plantations (*Araucaria* and *Eucaliptus*) and pastures. While the barcode sequence itself is not sufficient for robust phylogenetic tree generation, it allowed the detection of cryptic species as the native species *Urobenus brasiliensis* with > 12% of genetic distance. Furthermore, *DNA barcoding* has helped to separate individuals with more complicated taxonomy such as for the Ocnero-drilidae family recently sampled in Southern Bahia.

The *DNA barcoding* has been shown to be very effective for Brazilian earthworms, allowing

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species-level identification of adults, juveniles and cocoons. It is a valuable tool for preliminary species delineation (MOTUs) and therefore the estimation of earthworm biodiversity. While there are still restrictions to the extensive use of barcodes for identifying species, we still expect that a comprehensive database can be a powerful taxonomic tool that merits further development and will help identify species, their distribution ranges and intraspecific diversity, which ultimately can be used to assess biodiversity in areas under pressure for development or conservation in Brazil.

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Combining training programs and DNA barcoding to explore the diversity of tropical earthworm communities - feedbacks from the ECOTROP field school

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Abstract

In Gabon, natural ecosystems are renowned for their charismatic species of vertebrates and plants. By contrast, invertebrates are poorly known and generally absent from conservation plans, despite being several orders of magnitude more diverse and dominant in biomass. This shortfall is critical and largely caused by our inability to sort and identify these organisms.

DNA barcodes offer a way of documenting basic descriptive metrics of invertebrate diversity, even in the absence of a taxonomic framework. By integrating their use into the research and educational program ECOTROP, scientific objectives are paired with the training of students whose participation and increased awareness is expected to promote the consideration and description of these organisms.

To illustrate the potential of this approach, we present the results of a sampling campaign focused on earthworms from three regions of Gabon: in Libreville and the National Parks of La Lopé and Ivindo. So far, we generated DNA barcodes for almost 1400 specimens representing 117 Molecular Operational Taxonomic Units. Using these as surrogates for species diversity and assemblage composition, we describe earthworm diversity at local and regional scales, with special attention to impacts of land use and forest dynamics, and to the spread of invasive species.

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State of Knowledge of the Malagasy endemic Family, Kynotidae (Oligochaeta)

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Abstract

Madagascar has been poorly considered regarding earthworm (Oligochaeta) taxonomy up to recently. The first species were recorded between 1887 and 1931 from casual explorations. The first kynotid species was reported by the German zoologist Prof. Konrad Keller under the name *Geophagus darwini* in 1887. As the genus name was already given to the cichlid fish *Geophagus Heckel*, Michaelsen proposed the replacement name *Kynotus* to accommodate Keller’s *darwini* and several other, newly described species. The genus was placed in Glossoscolecidae by Michaelsen until Jamieson created the subfamily Kynotinae for it, because of the presence of a pair of eversible copulatory organs with attached prostate-like glands. Later, Jamieson elevated the subfamily to family rank in 1980. Morphologically, *Kynotus* species show the presence of biannulate on 3-10, Male pore on 16, ranks of spermathecae, presence of genital setal glands and prostatelike gland. Taxonomic tools used to distinguish kynotid species are the size, seta lacking, number of segments occupied by the clitellum, Spermathecal rank number, the number of ampulla in each row, genital seatal shape. However, taxonomical methods based on morphological criteria are limited if two species are very close morphologically, In this case, DNA barcoding is a useful method for discriminating *Kynotus* species.

From 2008 the Fauna-M Project (Global change and soil macrofauna diversity in Madagascar) paid a close attention to these species and made surveys at different locations, crossing soil types and climates as criteria for choosing places of research. Between 2008 and 2016, 35 species belonging to different families: Acanthodrilidae, Megascolecidae, Glossoscolecidae, Eudrilidae, Ocnodrilidae, Octochaetidae, Moniligastridae and Kynotidae. Regarding the endemic family Kynotidae, 7 new species (*K. giganteus*, *K. proboscideus*, *K. parvus*, *K. sihanakus*, *K. minimus*, *K. sakafotsy*, *H. farafangana*), were characterized using the classical method, based on external and internal morphology, since we do not yet have genetic data banks for them. An identification key to the *Kynotus* species has been provided in 2016. By the recent use of barcoding 3 new species have been discovered and described: *K. vohimanus*, *K. ankisiranus* and *K. blancharti*. During this project, we covered a very small surface of the Island, We can estimate that a large number of species remain to be discovered and described from Madagascar. Unfortunately, every day, Malagasy soil fauna is threatened by Ecosystem degradation, deforestation, Fire, agriculture and land use change, soil erosion,

Keywords : Madagascar, Endemism, Kynotidae, Kynotus, Barcoding

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Earthworm Populations in Mature *Pinus halepensis* Forest Subjected to different Forestial Thinning Treatments

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Abstract

Changes in the density of a pine forest cover might have an effect on the composition of the soil fauna, including the community of earthworms. We evaluated the density and diversity of earthworms in a Long Term Ecology Research (LTER) in the Kedoshim Forest located on the low western slopes of the Jerusalem Mountains (35°02'56E, 31°79'25N) of Israel, where, according to the Israel Meteorological Service (IMS) data, the average annual rainfall amount is 550 mm, concentrated mainly between December and March. The dry and rainless season is long, typically about 6-8 months.

23 plots (70x70 m) were subjected to different thinning treatments that were applied in six or five replicates, using a complete randomized design: 1) intense thinning (100 trees/ha); 2) moderate thinning (300 trees/ha); 3) clear-cut; 4) control (no thinning, ~560 trees/ha). The impact of the thinning treatments on earthworm community structure was investigated over two winter (rainy) seasons, by taking samples with quadrats (25x25x25 cm). Earthworms were hand-sorted in the forest, counted, preserved in ethanol and taken to the laboratory for further analysis.

Only three species were found in the different sampling plots, *Dendrobaena samarigera*, *Dendrobaena veneta* and *Aporrectodea rosea* (some damaged or juvenile specimens could not be identified). Both species *D. samarigera* and *D. veneta* are common in Israel forests, *D. samarigera* in olive orchards and *D. veneta* in open oak-park forests.

On average, the highest abundance was found in the intense thinning plots and lowest in the clear-cut plots (44 indiv./m², SE±17.1; 17 indiv./m², SE±6.5; respectively), ranging from 18-144 indiv./m²; 0-48 indiv./m² (respectively). In the moderate thinning and control plots, the average abundance was 33 indiv./m², SE±10.5; 37 indiv./m², SE±8.4 (respectively),

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ranging from 0-64 indiv./m² in both treatments. In some cases, specimens were found in local clusters (i.e. patchy distribution indicating metapopulation structure). However, in some places that looked ideal (soil profile and humidity) for their occurrence, we did not find any earthworms.

All three species were found only in one plot (treatment 4). In two plots, we found only *D. veneta* (treatment 3 and 4) and in two others only *D. samarigera* (treatments 2 and 4). We collected *D. veneta* and *D. samarigera* together in five plots (treatments 1,2,3 and 4), *D. samarigera* and *A. rosea* together in two plots (treatment 2) and *D. veneta* and *A. rosea* together in one plot (treatment 1).

From our preliminary results, it seems that clear-cut treatment is the least favorable for earthworms. As we observed aestivating earthworms, in the different treatments, not only in the dry season, but also in the dry periods of the rainy season, we presume that in the clear-cut plots, where there are no trees and shade, the semi-arid conditions in the area do not allow the level of moisture to reach high enough for the earthworms to survive.

French earthworms distribution in agricultural and urban soils

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Abstract

Following the work associated with our national network of earthworm observation (OPVT, see Guernion & al communication), we have 1,600 new observations of French earthworm communities in different habitats grouped into 6 main categories: Garden (231) - Crop (788) - Vineyard (252) - Grassland (218) - Agroforestry (92) - Natural area (51). We analyzed the distribution of 66 earthworm taxa identified in terms of community structure, species relationship using network graphs and diversity index vs richness. Citizen science approaches have allowed us to increase the number of observed taxa extant in some habitats. Community composition varied between 16 and 30 very common, common, rare or very rare taxa in different habitats, possibly associated with specific environmental conditions.

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Bioluminescence in earthworms: a short review

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Abstract

Amongst the about 7000 known species of earthworms, thus far only 37 or 38 are known to be able to produce light. A short review will be given reporting what is known about these worms and the way that some of them produce their "living" light. Also some hypotheses concerning the function of producing underground light will be discussed and illustrated with some preliminary data obtained on a recently re-discovered worm in France.

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Phorate toxicity may reduce species diversity of earthworms

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Abstract

PHORATE TOXICITY MAY REDUCE SPECIES DIVERSITY OF EARTHWORMS **S.M.SINGH**

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ABSTRACT

Regular usage of agro-chemicals on or in the crops has created adverse effects on the soil biota. The level of mortality of soil animals varied as per the level of toxicity of the chemical used. Phorate – an organo - phosphate which is widely applied on the pests of standing crops has created alarming situation for the Indian earthworms. Extensive artificial soil tests were conducted for three species of earthworms, viz. *Metaphire posthuma*, *Eutyphoeus waltoni* and *Lampito mauritii* as per OECD (1984) guidelines. The results showed the LC50 for these three experimental worms were 20.5, 24.7 and 26.0 mg/kg, respectively. The findings indicate that even the minimum dose of Phorate in the crops lowers the population of different species of earthworms.

Keywords – Phorate, toxicity, earthworms, mortality

*Speaker

Earthworm diversity in Amazonian ecosystems of French Guiana

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Abstract

Despite being recognized as important actors of soil functioning, earthworms have been poorly considered from a taxonomic perspective. As a consequence, the nearly 6000 species currently recognized worldwide probably represent at best half the actual biodiversity of the group. This taxonomic deficit is particularly critical in the tropics, resulting in difficult species identifications and a lack of ecological studies on earthworm communities.

Earthworm communities were sampled in eight study sites of French Guiana (grants from CNRS-Nouagues and Labex CEBA). In each site, a rapid screening of communities was achieved in a selection of habitats using a standardized protocol based on the systematic harvesting of specimens in all types of microhabitats available in a 1 ha area. DNA barcodes (COI gene) obtained for a selection of specimens were used to delimit molecular operational taxonomic units (MOTUs), the number and composition of which was further used to describe community diversity and structure at different spatial scales.

DNA barcodes produced for 2826 specimens clustered into 166 MOTUs, resulting in a great improvement of our knowledge of regional diversity, as compared to the 22 species that were reported for French Guiana in a recent checklist. Beta-diversity among sites was high, with up to 70% of the MOTUs only found in a single study site. As a consequence, the number of species accumulates steadily with the number of study sites sampled, and a rough estimates suggests that at least 400 species could be found in French Guiana. This region of Amazonian forests could therefore represent one of the richest hotspots for earthworm diversity, and

*Speaker

additional research is critically needed to progress toward documenting the actual number of species in this region.

At a local scale, assemblages seem to be dominated by specialist species, with only a small fraction of generalists able to colonize a broad range of habitats or microhabitats. The number of species co-existing in a given habitat never exceeded 15 MOTUs, suggesting that interspecific competition may drive niche saturation during the process of community assembly. The ongoing development of a functional trait database will allow combining functional, phylogenetic and taxonomic diversity approaches in order to disentangle the relative contribution of habitat filters, biotic interactions and neutral processes in the structuring of earthworm communities in the rainforest of French Guiana.

Earthworms and Amazonian Dark Earths: improving understanding of the relationships between soil management, biodiversity and function*

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Abstract

Amazonian Dark Earths (ADEs) are highly fertile soils created by human activities over millennia and found throughout Amazonia. The distinguishing dark color and high nutrient content (particularly for available Ca and P) make these soils prime areas for agricultural activities. In fact, many ADEs have been used for this purpose to varying degrees for decades, but little is known about the effects of ADE formation and management on soil biodiversity. Therefore, 9 sites with ADE and adjacent soils (reference soil) were sampled for soil fauna, including the assessment of earthworm populations (species composition and abundance) using the ISO standard method (n= five 25x25 cm x 30 cm deep soil monoliths per site). Additionally, qualitative sampling using 50x50 cm formalin extraction and hand-sorting from various niches such as within and under litter & decaying wood and under stones was also used. Soil chemical parameters and micromorphology assessment was performed on samples from the 0-30 cm layer, in order to evaluate the relationship of animals to bioturbation and soil fertility. Study sites included three old-growth forests, three young (secondary & regenerating) forests, and three agricultural systems (pasture, maize & soybean) in the regions of Belterra (PA), Manaus (AM) and Porto Velho (RO). A total of 37 species were

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found, many of them species that are new to science. The worms belonged to five families (Acanthodrilidae, Octochaetidae, Ocnerodrilidae, Rhinodrilidae and Glossoscolecidae), and included the genera *Pontoscolex* (at least 2 spp.), *Diachaeta* (at least 3 spp.), *Atatina* (at least 5 spp.), as well as *Rhinodrilus*, *Urobentus*, *Righiodrilus* and *Glossodrilus*. Formalin extraction was efficient for obtaining surface-dwelling epigeic and epi-endogeic species but generally resulted in lower abundance values when compared to hand-sorting. Abundance ranged from a mean of 86 indiv. m⁻² in adjacent soils up to 216 indiv. m⁻² in ADEs. Species richness ranged from a minimum of 1 to a maximum of 7 species at each site, and young and old forests had higher richness (4-4.5 spp.) than agricultural systems (2.2 spp.). ADEs had slightly higher overall species richness (24 spp.) than adjacent soils (22 spp.), and species composition was conspicuously different, with 15 unique ADE species and 13 unique adjacent soil species. Earthworm biomass ranged from 10.4 g m⁻² in adjacent soils to 17.4 g m⁻² in ADEs. The higher earthworm abundance associated with ADEs was tightly related to the presence of biogenic aggregates as well pottery shards. Furthermore, the sampled areas represent only a fraction of the ADEs present in Amazonia, however, the results suggest that earthworms are an important component of ADEs, representing 28% of the total macrofauna abundance and 58% of the overall biomass. Therefore, we expect that they may contribute significantly to the functioning and engineering of these human-modified ecosystem. *Funded by CNPq, Newton-CONFAP, NERC, EU and CAPES

The Edaphobase Nationwide Field Monitoring – A survey of soil oligochaete (Lumbricidae, Enchytraeidae) assemblages in different habitat types in Germany

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Abstract

EDAPHOBASE is a database-project in Germany where ecological and taxonomic data on soil fauna are collected and made available: <http://portal.edaphobase.org>. As part of this project, the oligochaete (Lumbricidae, Enchytraeidae) assemblages in soils of different habitat types in four regions of Germany were systematically surveyed using standard (ISO) methods. Sampled habitat types were forest, grassland and farmed land, on both acidic and calcareous soils. Three sites per each habitat type were sampled with 5 (Lumbricidae) or 10 (Enchytraeidae) replicate samples, each with sampling in spring and autumn of 2014 and 2015. The overall aims are to evaluate the usability of the database regarding issues of nature protection and soil monitoring in general, to further develop the database and to generate data for habitat types that were underrepresented in the database so far.

Thirteen earthworm species (incl. the first record of *Bimastos parvus* for Germany) and 75 enchytraeid species (plus c. 20 new to science) were found. Earthworm number of species and abundance and enchytraeid diversity are highest in grassland and lowest at farmed and coniferous forest sites due to tillage and low soil pH, respectively. Detrended correspondence analysis of earthworm communities reveals close similarity of sites with the same habitat type in 9 out of 12 cases. The main drivers of habitat type-specific differences appear to be soil pH and C/N ratio. Enchytraeidae species also reflect soil pH, sometimes even within a land use type. Most diverse (35 species in 13 genera) are enchytraeid assemblages at plant species-rich, moderately acidic mesophilic montane grassland sites.

Evaluation of the whole project data is still ongoing; especially community evaluations including all sampled organism groups is pending.

*Speaker

Molecular phylogeny of the six most common European *Lumbricus* species

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Abstract

The *Lumbricus* genus is well-documented as it is used as a functional model in ecological studies. Some *Lumbricus* species have a wide range and are very common in Europe. Recent molecular phylogenies have often revealed taxonomic incongruence in some Lumbricidae genera, even the *Lumbricus* genus showed several lineages within species or cryptic diversity. The taxonomic status of each species needs to be confirmed, as well as their phylogenetic relationships. For this purpose, we sequenced the COI (568 bp), COII (559 bp), 16S (432 bp), ND1 (731 bp), and 28S (422 bp) gene regions for 85 French earthworms from 10 different localities belonging to the six most common *Lumbricus* species and ten outgroup taxa. DNA sequences were analyzed using maximum likelihood and Bayesian inference. The multiloci phylogeny showed that *Lumbricus* genus formed a well-supported monophyletic group. Our molecular analyses showed the first divergence of epigeic species *Lumbricus castaneus* and *L. rubellus*, followed by the divergence of the four anecic species: *L. centralis* close to *L. friendi*, then *L. festivus* and *L. terrestris*. We thus confirm their species status, and the absence of intra-species lineages suggesting the existence of cryptic diversity within these taxa.

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The earthworm biodiversity in eastern Algeria

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Abstract

The objective of our work is to describe the biodiversity of earthworm fauna in Eastern Algeria, along a gradient from the coast to the desert and their relations with soil (pH, electrical conductivity, organic matter and soil texture) and climatic factors. Eighteen species are identified in this study: *Aporrectodea trapezoides*, *Aporrectodea rosea*, *Allolobophora molleri*, *Aporrectodea monticola*, *Octodrilus complanatus*, *Aporrectodea carochensis*, *Octodrilus maghrebini*, *Eisenia fetida*, *Dendrobaena byblica*, *Aporrectodea tetramammalis*, *Eiseniella tetraedra*, *Proctodrilus antipae*, *Octolasion lacteum*, *Aporrectodea caliginosa*, *Allolobophora chlorotica*, *Microscolex dubius*, *Microscolex phosphoreus* and *Hormogaster redii*.

Regarding the demoeology, the average values of density and earthworm biomass, reduce from the north ($40,50 \pm 11,86$ individuals / m² and $6,92 \pm 4,88$ g / m²) to the south ($3,89 \pm 2,80$ individuals / m² and $1,07 \pm 0,83$ g / m²) of the study transect.

The species *Ap. trapezoids* dominates throughout the field of study (PDC = 50,37%).

It seems that both species *Aporrectodea rosea* and *Aporrectodea trapezoides* can withstand hot, dry climate like that of Algeria. They are found on the entire field of study. However, other taxa are occasional; they are linked to the northern part wettest and most humid.

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Some aspects of earthworm fauna in Kazakhstan (Oligochaeta: Lumbricidae)

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Abstract

Faunistical recording during the 60 years shows that Kazakhstan species composition of earthworms comprises twenty four species: *Allolobophora sokolovi*, *A. microtheca*, *A. chloracephala*, *A. bouchei*, *A. albicauda*, *A. umbrophila*, *A. stenosoma*, *A. longoclitellata*, *A. parva*, *Perelia ophiomorpha*, *Dendrodrilus rubidus*, *Octolasion cyaneum*, *Aporrectodea caliginosa*, *A. longa*, *A. rosea*, *Lumbricus rubellus*, *L. terrestris*, *Eisenia magnifica*, *E. foetida*, *E. nordenskioldi*, *E. jungarica*, *E. kusenkoi*, *Eiseniella tetraedra*, *Dendrobaena octaedra*. Earthworms belong to eight genera. The most widely distributed species are the eurytopic *A. caliginosa* and *D. rubidus* which have the antropochoric dispersion along the river system. Lumbricids mountains populations in the Eastern and the Southern Kazakhstan are the most diverse. The majority of local species belong to ancient genus *Allolobophora*, which formed in the Mesozoic era. The boreal species play a key role in the structure and function of the earthworm assemblages in the in forest biotopes of the northern parts of Kazakhstan and in the intrazonal alder forest communities of the Central Kazakhstan. Key words: Kazakhstan region, earthworms, local species, boreal species, *Allolobophora* genus

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Comparison of life cycle and reproductive traits of eight populations of *Eisenia fetida* (Savigny, 1826) from Zagros Mountain

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Abstract

Abstract

Eisenia fetida originated somewhere around the Caucasus region and distributed in Central Asia including the forest steppe zone of Russia and the Caucasus region. These sibling species commonly used in standardized acute and reproduction toxicity tests. *E. fetida* and *E. andrei* are not only closely related but are very similar ecotypes (epigeic) which fill almost the same ecological niche. They have also been used as a model species pair to test evolutionary and ecological hypotheses at a molecular level. In this investigation, The growth and reproduction of *Eisenia fetida* from different parts of western Iran along the Zagros Mountains as a native range of this species were measured in order to uptake intraspecific variations by studying parameters, such as: gain in body weight, number of cocoon production, number of hatchlings and hatchling success. The results showed significant differences between means of viable cocoon, incubation time, reproductive rate, growth rate and final weight between different populations. Understanding of its optimal environmental necessities is required in order to optimize variations of reproduction and growth among different populations.

Key words: Zagros; Population; *Eisenia fetida/andrei*; life cycle

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