Earthworms

Types, Roles and Research

Clayton G. Horton
Editor

Insects and Other Terrestrial Arthropods: Biology, Chemistry and Behavior

NOVA

Complimentary Contributor Copy

Complimentary Contributor Copy

INSECTS AND OTHER TERRESTRIAL ARTHROPODS:
BIOLOGY, CHEMISTRY AND BEHAVIOR

EARTHWORMS

TYPES, ROLES AND RESEARCH

No part of this digital document may be reproduced, stored in a retrieval system or transmitted in any form or by any means. The publisher has taken reasonable care in the preparation of this digital document, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained herein. This digital document is sold with the clear understanding that the publisher is not engaged in rendering legal, medical or any other professional services.

Complimentary Contributor Copy

INSECTS AND OTHER TERRESTRIAL ARTHROPODS: BIOLOGY, CHEMISTRY AND BEHAVIOR

Additional books in this series can be found on Nova's website under the Series tab.

Additional e-books in this series can be found on Nova's website under the eBooks tab.

Complimentary Contributor Copy

INSECTS AND OTHER TERRESTRIAL ARTHROPODS:
BIOLOGY, CHEMISTRY AND BEHAVIOR

EARTHWORMS

TYPES, ROLES AND RESEARCH

CLAYTON G. HORTON
EDITOR

nova
science publishers
New York

Complimentary Contributor Copy

Copyright © 2017 by Nova Science Publishers, Inc.

All rights reserved. No part of this book may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic, tape, mechanical photocopying, recording or otherwise without the written permission of the Publisher.

We have partnered with Copyright Clearance Center to make it easy for you to obtain permissions to reuse content from this publication. Simply navigate to this publication's page on Nova's website and locate the "Get Permission" button below the title description. This button is linked directly to the title's permission page on copyright.com. Alternatively, you can visit copyright.com and search by title, ISBN, or ISSN.

For further questions about using the service on copyright.com, please contact:
Copyright Clearance Center
Phone: +1-(978) 750-8400 Fax: +1-(978) 750-4470 E-mail: info@copyright.com.

NOTICE TO THE READER

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

Additional color graphics may be available in the e-book version of this book.

Library of Congress Cataloging-in-Publication Data

ISBN: 978-1-53612-196-4 (eBook)

Published by Nova Science Publishers, Inc. † New York

Complimentary Contributor Copy

CONTENTS

Complimentary Contributor Copy

Complimentary Contributor Copy

PREFACE

Earthworms are often recognized as key organisms in soil ecosystems. In Chapter One, the authors propose endozoochory (seed dispersal through ingestion) as a missing mechanism of Oligochaeta dispersal and put forward the fusion-orthogonalization model for the diversification and speciation of the Oligochaeta populations. Chapter Two discusses the biodiversity of earthworms in Madhya Pradesh, a central part of India. Earthworm diversity in some parts of India is still poorly explored, but findings suggest that the Madhya Pradesh region is rich in biodiversity of earthworms. In Chapter Three, a predation pressure is presented as an important variable which can be viewed as another type of pressure on the earthworm population, such as pollution, environmental stress or land management, causing additional or extrinsic mortality to earthworm population. Chapter Four covers the key role played by earthworms as ecosystem engineers through their bioturbation activities involving soil mixing, their influence on the decomposition and mineralization of litter by breaking down organic matter, and their influence on the gas and water exchange or nutrient transfer in the soil. Chapter Five reviews recent research regarding the assessment of various pollutants on earthworms with emphasis on the possible improvement of the investigation in soil pollution monitoring using these organisms.

Complimentary Contributor Copy

Complimentary Contributor Copy

In: Earthworms
Editor: Clayton G. Horton
ISBN: 978-1-53612-176-6
© 2017 Nova Science Publishers, Inc.

Chapter 1

EVOLUTION AND LIFE OF OLIGOCHAETES

Tomáš Pavlíček[1,*], Oren Pearlson[1,2], Tova Cohen[1], Mete Mısırlıoğlu[3], Shweta Yadav[4], Petr Král[5] and Michèle Glasstetter[6]

[1]Institute of Evolution, University of Haifa, Haifa, Israel
[2]School of Science and Technology, Tel Hai Academic College, Upper Galilee, Israel
[3] Biyoloji Bölümü, Fen-Edebiyat Fakültesi, Eskişehir Osmangazi Üniversitesi, Eskişehir, Turkey
[4]School of Biological Sciences, Dr H S Gour Central University, Sagar, India
[5]Departments of Chemistry and Physics, University of Illinois at Chicago, US
[6]Biogeography, Department of Environmental Sciences, University of Basel, Basel, Switzerland

Symbiotic bacteria and other microorganisms are playing an important role in the adaptation of Oligochaeta to their environment. An evolutionary

* Corresponding Author Email: pavlicek@research.haifa.ac.il or contact@tomas-pavlicek-biologie.net.

Complimentary Contributor Copy

unit operating upon the natural selection might be a network-like extended phenotype encompassing worms and symbiotic microorganisms, and characterized by epigenetic regulation. The extended phenotype plays a role in endozoochory. We propose endozoochory as a missing mechanism of Oligochaeta dispersal and in the put forward fusion-orthogonalization model for the diversification and speciation of the Oligochaeta populations. The endozoochory dispersal hypothesis suggests a passage of Oligochaeta cocoons and eggs through the digestive tract of a vector, for example migrating birds. The experimental validation of the endozoochory dispersal hypothesis might contribute to the dismissal of the plate tectonics hypothesis treating Oligochaeta species as passively 'living fossils' whose evolution history is punctuated by tectonic/geologic events. The fusion-orthogonalization model of speciation considers error-prone macro-evolutionary aneuploidy-polyploidy-like and microevolutionary mutation-recombination-like mechanisms generating clouds of quasispecies and ecological species, respectively. Since natural selection operates at the level of clouds and individuals at the population level, the criteria of the evolutionary success are, in a given environment, cloud stability and relative individual fitness. The loss of the most recent polarity layers during quasispeciation can reset the evolutionary process by re-wiring the exposed earlier polarity layers. Depending on the level of stress, the evolution of quasispecies continues via quasispeciation or via orthogonalization towards the establishment of ecological species. Quasispeciation explains, among others, the appearance of morphologically and genotypically simplified parasitic worms and of lineages colonizing extreme environments, as well as the establishment of cyclicity in evolution.

1. OLIGOCHAETA

Oligochaeta, whose origin might be monophyletic (Struck et al., 2011), – for example, free-living earthworms and ectoparasitic leeches – are directly developing (i.e., the larval stage is absent), fundamentally

Complimentary Contributor Copy

hermaphroditic annelids. They inhabit mainly terrestrial soils and freshwater bodies, but rarely a marine environment (Ruehland et al., 2008). The worm together with endosymbiotic microorganisms establishes an extended phenotype, as defined by Dawkins (Dawkins, 1989). The important role of the extended phenotype concept can be illustrated in the taxonomy of two earthworm species *Eisenia lucens* (Waga, 1857) and *E. spealea* (Rosa, 1901). Based on morphology, these two species are synonyms distributed in one continuous geographic area. However, the presence of bioluminescence bacteria in *E. lucens* and their absence in *E. spelaea* led to a physico-geographic separation of their geographic distributions and to the re-establishment of their species ranking (Csuzdi & Zicsi, 2003).

The interactions between host (e.g., an oligochaete) and symbionts, pathogens and parasite might, among others,

1. cause the death of the host (e.g., killing of lumbricid earthworms by planarians (Murchie & Gordon, 2013)),
2. boost immunity (Jinek et al., 2012), and
3. provide the capacity to acquire an ‘extreme’ ecological niche through facilitating food digestion and regulating metabolic and cellular processes. For example, the chemoautotrophic sulphur-reducing bacteria allows the exploitation of hypoxic sea sediments rich in sulphur by gutless marine oligochaetes belonging to the genera *Inanidrilus* and *Olavius* (Oligochaeta: Naididae) (Ruehland et al., 2008).

Nevertheless, the spectrum of potential interactions is more diverse. Predators and prey might co-occur in the same niche (Ponge, 1999). This is the case for *Dendrodrilus rubidus* living in nests of the voracious ant *Formica rufa* (Hymenoptera, Formicidae) (Laakso & Setälä, 1997). Former parasites become macroscopic or microscopic (intra-cellular) symbionts (e.g., centrosome and telomere present in the majority of Metazoa probably of (retro)viral (Chichinadze et al., 2013), and bacterial (Villasante et al., 2007) origin). Currently, the invading parasitic bacterium

Complimentary Contributor Copy

Wolbachia attempts to control and modify the body polarity systems in invertebrates by remodelling, among others, the male chromatin, which, as a consequence, is leading to the disturbance of the mitotic spindle assembly and chromosome behaviour (Riparbelli et al., 2012).

In the present study, we use the terms Polychaeta and Annelida despite their suggested polyphyletic origin (Almeida et al., 2003 and Struck et al., 2011) because the "worms" phylogeny is in disarray. Also, we consider "phylogenetically uncertain" Aeolosomatidae and other taxa for comparative and explanatory purposes.

2. Broadcast Spawning and Spermcast Mating in Soil-Inhabiting Oligochaeta

Spermatophores occur in different animal groups (Mann, 1984) and their acknowledged primary role is the transfer of spermatozoa for the purpose of egg fertilization. One of the first descriptions of Oligochaeta spermatophores was in the fresh-water genus *Tubifex* (Oligochaeta: Tubificidae) (Lankester, 1871). However, direct spermatophore exchange between the body surfaces of two worm individuals was rejected in lumbricid earthworm *Eisenia fetida* (Monroy et al., 2003). Based on this result, doubts were expressed about the maintaining of the spermatophore primary role in soil-inhabiting earthworms. Rightfully, the observed abandoning of spermatophores in a substrate before cocoon deposition (Monroy et al., 2003) is not consistent with the model of direct spermatophore exchange between two individuals and the storing of spermatophore in the partner's spermatecae.

Nevertheless, the rather large inter- and intra-specific variability in the size and shape of spermatophores in Oligochaeta (Lankester, 1871) (Figure 1) suggests that spermatophores might function in different ways. Spermatophores originate either in the segmented nephrostome of nephridia or metanephridia, as documented in Spionid Polychaeta (Rice, 1980) and *Aeolosoma*, or in the atrium that is an expansion of the male

Complimentary Contributor Copy

sperm ducts, for example in Naididae and Tubificidae (Mann, 1984). In Oligochaeta, spermatophores are ejected to the body surface either through nephrophores or through the male pore (Gorgoń et al., 2015). Spatial separation of the place of spermatophore assembly and spermiogenesis might lead to the production of empty spermatophores if the spermiogenesis (e.g., in 3n *Lumbricillus lineatus* (Oligochaeta: Enchytraeidae) (Christensen, 1960) or the spermatozoa intra-body transfer are disrupted. Spermiogenesis occurs in the coelomic cavity in Aeolosomatidae (Bunke, 1986) or in the testes in other Oligochaeta taxa.

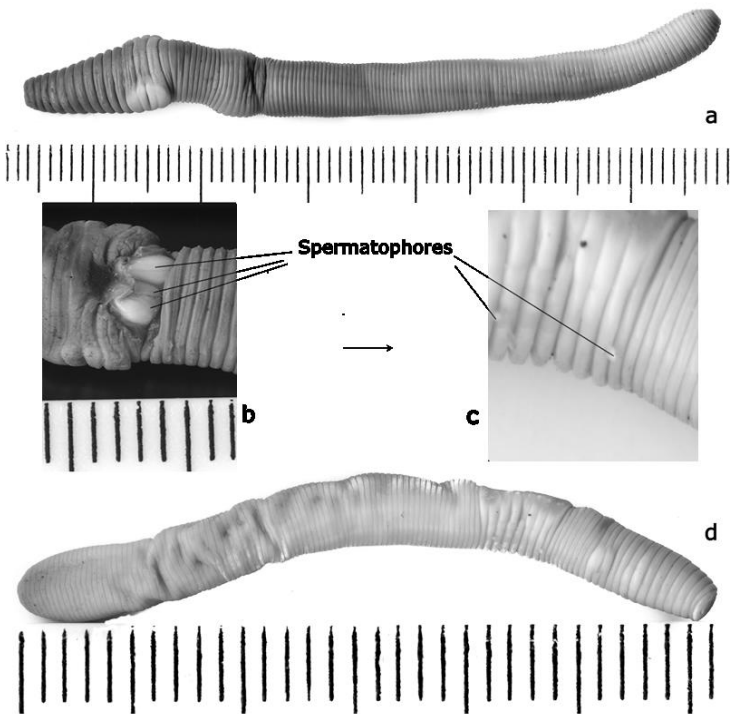

Figure 1. *Dendrobaena samarigera* (a) and *D. veneta* (d) (both Oligochaeta: Lumbricidae) and their spermatophores (b, c). In *D. samarigera* spermatophores are large and usually localized in the furrows of body segments near the male pore. In *D. veneta*, spermatophores are small, abundant and might be present in furrows of different body segments. Presented examples originated from the same locality (Kdoshim, Israel). A millimetre scale is included in the picture.

Complimentary Contributor Copy

It is known that fertilization, assisted by the release of free gametes or spermatophores into the external environment, occurs in aquatic annelids, either by means of broadcast spawning or spermcast mating (Bishop & Pemberton, 2006). In a similar fashion to broadcast spawning, both male and female gametes of soil-inhabiting earthworms could be released from spermatophores into floodwater or to water bodies where an external fertilization takes place. Alternatively to spermcast mating, spermatophores (or spermatozoa) could be released into the floodwater, from where they are gathered and stored in spermatecae. Considering these variants, we might assume that unfertilized cocoons or eggs are released to external environments or that spermatophores are not too large for reuptake to occur (Figure 1). There is no guarantee that released cocoons are fertilized. This is indicated, among others, by the relatively frequent occurrence of parthenogenetic reproduction among Oligochaeta, for example, in Lumbricidae (Jaenike & Selander, 1979), Megascolecidae (Shen et al., 2012), Enchytraeidae (Christensen, 1960) and Tubificinae (Marotta et al., 2014). The release of cocoon-free eggs seems to be rare among Oligochaeta, but it is occurring, for example, among the above-mentioned gutless ones (Giere, 2006).

Clearly, spermatophores of *Dendrobaena samarigera* (Figure 1) cannot be uptaken to spermatecae because of their size, but they still might release sperms participating in fertilization. Regarding the Levantine *D. samarigera*, one could conclude that the large spermatophores and cocoons (Pavlíček and Pearlson, unpublished) are adaptations/exaptations to the droughts they frequently face. According to the endozoochory dispersal hypothesis, described below, egg fertilization could happen in the digestive tract of a host (vector).

3. ENDOZOOCHORY DISPERSAL HYPOTHESIS

One of the challenges facing the first annelid-like inhabitants of the terrestrial soils was the loss of biological dispersal (defined as any movement of organisms with potential consequences for the gene flow

Complimentary Contributor Copy

across a space (Ronce, 2007)). Even today, in the majority of the soil-inhabiting oligochaetes, the rate of the active underground dispersal is low, about 5 - 10 meters per year, as estimated in earthworms *Aporrectodea caliginosa*, *Ap. longa*, *Dendrobaena octaedra*, *Lumbricus terrestris*, and *L. rubellus* (all family Lumbricidae) (Marinissen & Van den Bosch, 1992; Hale et al., 2005 and Emmerling & Strunk, 2012). An active aboveground dispersal of 19 and more meters per night has been observed in some lumbricid species (Blakemore, 1999 and Mather & Christensen, 1988). However, the aboveground dispersal might epitomize an escape behaviour from predators (Darwin, 1881) and other adversities of the environment or a parasite-mediated host suicide. The parasite-mediated suicide might explain the following observation: "*Sick individuals, which are generally affected by the parasitic larvae of a fly… wander about during the day and die on the surface*" (Darwin, 1881). If the relatively distant aboveground dispersal is real, it is hard to imagine how soil-inhabiting oligochaetes could overcome multiple external barriers (e.g., predators, parasites, physiogeographic features like deserts, continental and mountain glaciers, salty lakes and seas, and acidic swamps and so on). Even if stretched over a large number of generations, their journey would cover hundreds and thousands of kilometres above ground.

An attempt to explain the current distribution of Oligochaeta by combination of the continental drift (Wegener, 1912)) and the temporary land bridges hypotheses is known as the tectonic plate hypothesis (= plate tectonics) (Michaelsen, 1922). The continental drift hypothesis alone treats the terrestrial Oligochaeta as sedentary "living fossils". Their evolution history is punctuated by tectonic/geologic/climate events responsible for vicariant patterns of distribution. For example, the age of earthworm genera living on both sides of the Atlantic is inferred, based on the continental drift hypothesis, as equal or superior to the age of the ocean itself (about 180 My or more (Omodeo, 2000)). As such it corresponds to the age of reptiles such as *Plesiosaurus*, *Ichthyosaurus*, and *Tyrannosaurus rex*. Similarly, the world-wide distribution of the current earthworms families is regarded as a mirror of their spread on the most recent super-continent Pangea (Omodeo, 2000). Unfortunately, the continetal drift

Complimentary Contributor Copy

hypothesis cannot explain how the earthworm families achieved to be widely distributed already on Pangea.

In contrast to the continental drift hypothesis, the temporary land bridges hypothesis allows for dispersal (Michaelsen, 1922). The weakness of the temporary land bridges hypothesis is that not all proposed land bridges have been proven to exist and it does not explain how earthworms overcome the dispersal barriers. Some authors did not agree to infer the phylogeny and phylogeography of the soil-inhabiting Oligochaeta from the plate tectonics. They replaced both or one of its mechanisms (the temporary land bridges hypothesis) by passive dispersal such as anemochory (Gislén, 1948), hydrochory (Davies et al., 1982; Subba Rao & Ganapati, 1974; Stephenson, 1923), epizoochory (Davies et al., 1982) and anthropochory in the current inter-glacial. However, the dispersal by all these mechanisms might be coincidental since no particular adaptations to them are known in soil-inhabiting Oligochaeta. Contrastingly, we concluded that the prey/predator "arms-race" could lead to the development of endozoochory. We ascertained adaptations (see below) for the survival passage through the digestive tract of a predator (vector, disperser) in oligochaete cocoons, eggs and possibly in spermatophores.

The dynamic coevolution leading to endozoochory requires the following:

1. Development and maintenance of cocoons, and eggs tolerance to the physico-chemical and biotic properties of the vector digestive tract. The physico-chemical features are mainly fluctuating osmotic pressure, pH and temperature in the digestive tract, as well as the pulverizing action of gizzard. The biotic properties stand for symbiotic microorganisms and the ones absorbed together with food that might influence the digestion process.
2. Establishment of a mechanism allowing eggs fertilization or metasexual reproduction (see below) and activation of embryogenesis.
3. Rewarding of the vector for its predation on cocoons and spermatophores.

Complimentary Contributor Copy

The following evidence supports the endozoochory dispersal hypothesis:

1. *Widespread resistance to high osmotic pressure including cocoons, eggs and, presumably, spermatophores in Oligochaeta*
   The resistance to high osmotic pressure enables oligochaetes, at all or only some stages of their life cycle, to tolerate both fresh and brackish or sea waters (such as the mud and sand loving *Pontodrilus litoralis* ((Oligochaeta, Megascolecidae: (Blakemore, 2007)). It also allows them to live in sea habitats and in the intertidal zone (Brinkhurst, 1973; Timm, 1980 and Erseus, 2005), to resist desiccation or freezing (Holmstrup et al., 2016), and facilitates the passage of cocoons through the digestive track of a host (Davies et al., 1982).
2. *Disruptive innovation in the size of cocoons and eggs*
   The primary function of cocoons is the protection of eggs against harmful effects of the environment, including their passage through the digestive system or part of it. In megadriles (= large-sized Oligochaeta except for leeches, for example, Megascolecidae), a decrease of the egg mass by two or three orders of magnitude has been observed. In the same time, a significant increase has been noted in the cocoon mass compared to what has been observed in microdriles (= small-sized Oligochaeta, for example Enchytraeidae) (Omodeo, 2000). This observation conflicts with the expected allometric relationship between the size of the earthworm body and the size of cocoons, eggs and the developing embryo. Besides, the eggs are spherical and only slightly deformable in the majority of megadriles. On the other hand, in the families Lumbricidae and Megascolecidae, and in some related aquatic taxa, the eggs are highly plastic and irregular (Omodeo, 2000). Our explanation is that, on the one hand, the cocoon is under pressure to grow and provides a larger reward to a potential vector and, on the other hand, the increase in its size builds up its vulnerability to be pulverized in the gizzard of

Complimentary Contributor Copy

a predator. The cocoon destruction releases eggs that are protected from the destruction due to their small size. In the families of phylogenetically most advanced oligochaetes, the eggs might become irregular and more flexible in order to survive the passage through the gizzard. The nutrition reward might consist, apart of albumen, also of bacteria (Davidson & Stahl, 2008) and possibly regulatory substances.

One can imagine that the pulverizing action on the relatively large spermatophores, if present, could release sperm that might fertilize "free eggs" and initiate embryogenesis. Thus, the breaking down of a cocoon could bring about embryogenesis in a way similar to the induction of germination in plants by the removal of the pericarp or endocarp (Marques & Fischer, 2009). However, the absence of sperm could initiates certain forms of metasexual reproduction (see below).

After passing the glandular stomach and gizzard, the intestine, the caecum and, to a lesser degree, cloaca (anus, hindgut) serve as "fermenters" and "developers" of everything that was digested especially in homeothermic vectors. The underlying assumption that eggs released from the cocoons might develop in the rear part of the digestive tract is plausible. After all, gutless Oligochaeta produce only eggs (without cocoons), in which bacteria are transmitted vertically and horizontally (Giere, 2006).

The reason for the non-detecting of oligochaete eggs/cocoons/ spermatozoa in faeces are: 1. the tiny size of the eggs and spermatozoa, 2. their quick decomposition if they die, 3. the absence of identification keys for their eggs/cocoons, and 4. the seasonality of their appearance in many oligochaetes.

3. *Birds as potential vectors of the widely distributed Oligochaeta*

Among many of the potential predators that could become vectors of Oligochaeta eggs and cocoons, birds were already shown as being the most competent and abundant vectors able to count for short and long distance dispersal (Green & Figuerola, 2005). Every year, huge numbers of birds are migrating between breeding and

Complimentary Contributor Copy

wintering localities. Also, they demonstrate a territorial feeding behavior, sometimes repeatedly visiting the same habitats. Not surprisingly, earthworms have been recorded to be present in bird nesting cavities (Lapied, 2002).

In the field, it might be difficult to differentiate between Oligochaeta dispersed by birds and by other vectors and the ones transmitted anthropochorically. However, the majority of populations established in connection with human activities are expecting to show low genetic and morphological variability due to the founder effect. However, there might be exceptions such as water bodies visited frequently by fisherman throwing out repeatedly different earthworm species used as baits (Tiunov *et al*., 2006), thus maintaining the dynamics of the colonization-extinction-recolonization process. In a contrast to the majority of earthworm populations of anthropogenic origin, the populations established by migrating birds are expecting to show high genetic and clonal variabilities due to a potentially large number of birds involved in the repetitive visits of the same localities and the bringing of different genotypes and lineages.

There are some phenomena in widely distributed earthworms that are easier to explain as the result of a dispersal by birds, but not, or with difficulty, by anthropochory:

1. *Subcosmoplitan and cosmopolitan distribution of earthworms*
   In fact, the distribution of sub-cosmopolitan and cosmopolitan Oligochaeta corresponds to the major bird flyways which are the East Atlantic Flyway, East Asia/Australian Flyway, Central Asian Flyway, Black Sea/Mediterranean Flyway, Atlantic Americas Flyway, Pacific Americas Flyway and Mississippi Americas Flyway (Elphick, 2011).
2. *Colonization and re-colonization of climatically extreme environments by widely distributed earthworms*

Complimentary Contributor Copy

Oligochaeta are present in sub-arctic and arctic islands, some geologically young ones (Terhivuo & Saura, 1997). Many of these islands have never been inhabited by men but are frequented by birds (Prat et al., 2002; Davies et al., 1997 and Terhivuo & Saura, 2006). A high clonal turnover between the years indicating an effective mechanism of dispersal has been recorded (Terhivuo et al., 2002). This shows an intense recolonization process. The noted complete niche overlap between earthworm clones (Terhivuo et al., 2002), haplotypes (James et al., 2010), parthenogenetic and polyploid lineages (Terhivuo & Saura, 2006) is violating the principle of competitive exclusion. This phenomenon might be the consequence of an intense colonization-recolonization process driven by repeated visits, associated with earthworm dispersal, of the same resource-rich localities by birds rather than by humans.

3. *Altitudinal dispersal of oligochaetes*
   Oligochaetes are often present near or on the top of mountains and hills or near of retreating mountain glaciers from where they might be dispersed downstream by running water (Terhivuo et al., 2002). Despite the active vertical (upwards) dispersal on the rock observed in *Eiseniella tetraedra* (Terhivuo et al., 2002), it can hardly explain the colonization of large deep and often isolated mountain valleys and steep slopes.
4. *The patchy distribution of oligochaetes*
   This phenomenon might be easier to explain as a consequence of the feeding or migration behaviour of the potential vector(s) than by the action of men.
5. *The role of the geographic orientation of flyways on earthworm distribution*
   Since the majority of global flyways show north-south orientation, the north-south distributions of peregrine earthworms are more frequent. However, the migration patterns of birds might as well explain the regional phenomena of earthworm distribution. For example, the limited migration between the Atlantic and Pacific coasts of North America (Elphick, 2011) might elucidate the

Complimentary Contributor Copy

absence of earthworms in some regions of North America where earthworms became extinct during the last glaciation period (Tiunov et al., 2006).

4. REPRODUCTION IN OLIGOCHAETA

In Oligochaeta we find gametic reproduction (GR) and agametic cloning (AC). GR involves digametic-amphimictic and/or mono- or digametic-metasexual reproduction (Table 1). AC alone (except embryonic cloning) or AC + GR appear in the lineages with embryonic primordium in which only part of the body segments found in adults is established. That is to say, the segmental growth takes place during the postembryonic period (e.g., Aeolosomatidae, Naididae). In the majority of species exhibiting GR + AC, AC occurs in the earlier developmental stages. During the life cycle, the stages might either not be spatially separated (for instance in directly developing *Marionina weilli* (Lasserre, 2012)) or might be spatially separated (e.g., in some Polychaeta). The intermittent appearance of GR and AC, known in *Cognettia glandulosa* (Enchytraeidae (Christensen, 1959)), is rare.

The lineages reproducing by GR, which is sometimes accompanied by embryonic cloning (Table 1), present all the adult segments in the embryonic primordium (e.g., in "soil earthworms" of the families Lumbricidae, Megascolecidae, Glossoscolecidae).

In the evaluation of reproduction in Oligochaeta and Spiralia, the transmission of the basal body (a form of centrosome) has to be considered. The reason is, among others, that the monastral spindle (e.g., in *Tubifex tubifex* (Shimizu, 1996)) or the diastral spindle that become asymmetric following transient down-regulation of one centriole (e.g., in the leech *Helobdella robusta* (Lyons & Weisblat, 2009)) are essential for the spiral cleavage and development of the Oligochaeta spiral type of embryo. The occurrence of additional mechanisms leading to spiralian embryo indicates the observed division of the primary blastomere into two

Complimentary Contributor Copy

or three secondary blastomeres during embryogenesis in *Ap. caliginosa* (referred as *Lumbricus trapezoides*) (Kleinenberg, 1879).

Table 1. Reproductive modes in Oligochaeta (abbreviations: Ench. = Enchytraeidae, Lumb. = Lumbricidae)

Mode	Example	Mode	Example
Gametic amphimictic reproduction			
Allogamous amphimixis	*Pristina leidyi* (Naididae) (Bely & Wray, 2001) and the large part of oligochaetes	Autogamous amphimixis	*Enchytraeus bucholzi* (Ench.) (Dózsa-Farkas, 1995) *Eisenia foetida* (Lumb.) (André, 1963) *Dendrobaena rubida* f. *subrubicunda* (Lumb.) (André & Davant, 1972) *Tubifex tubifex* (Naididae) (Gavilov, 1935)
Gametic metasexual reproduction			
Sperm independent parthenogenesis	*Octodrilus transpadanus* (Lumb.) (Garbar et al., 2009) *Octolasion cyaneum* (Lumb.) (Lowe & Butt, 2008)	Sperm-dependent parthenogenesis	*Lumbricillus lineatus (Ench.)* (Christensen & O' Conor, 1958), possibly 3n *Fridericia galba* (Christensen et al., 1992)
Sub-amphimictic simultaneous hermaphrodites	*Enchytraeus lacteus* (Ench.) (Christensen & Jensen, 1964), possible in *Aktedrilus* (*Phallodrilus*) *monospermathecus* (Ench.) and *Ilyogenia santixavieri* (Megascolecidae) (Christensen, 1984)		
Agametic cloning			
Architomy (pygidial budding with terminal addition)	*Aeolosoma* (D'Uedekem, 1862) (Aeolosomatidae)	Paratomy	*Pristina leidyi* (Naididae) (Bely & Wray, 2001) (Özpolat & Bely, 2015) *Enchytraeus japonensis* (Ench.) (Yoshida-Noro & Tochinai, 2010)
Embryonic cloning	*Pontoscolex corethrurus* (Glossoscolecidae) (Vijayakumaran et al., 2009), *Octolasion cyaneum* (Lumb.) (Lowe & Butt, 2008), *Aporrectodea caliginosa f. trapezoides* (reported as *Lumbricus trapezoides* (Lumb.) (Kleinenberg, 1879)		

Complimentary Contributor Copy

4.1. Gametic Reproduction in Oligochaeta

4.1.1. Allogamous Amphimixis

Symmetric allogamous amphimixis with present paternal centrosome. This type of GR is characterized by the syngamy of two allogamous (genetically different) gametes. The equal complement of autosomes of two parents (Normark, 2006) recombines during meiosis. A new centrosome is reconstituted in the zygote from the maternal MTOC (microtubule organizing centre – an electron dense material that nucleates most microtubules of the cell) and the paternal centrosome.

Symmetric allogamous amphimixis with present maternal centrosome. This variant probably exists in *Tubifex* in which the centrosome is of maternal origin (Shimizu, 1996), irrespective of reproduction that can be either parthenogenetic or amphimictic (Marotta et al., 2014). In *Tubifex*, the monopolar spindle assembly produces smaller AB and larger CD cells in the first cleavage and macromeres A, B of different sizes and C < D in the second cleavage (Shimizu, 1996). Interestingly, in *Tubifex* the centrosome of maternal origin is behind the spindle assembly splitting C/D macromeres in early embryogenesis and the MTOC not related to centrosome is behind the spindle assembly splitting macromeres A/B (Shimizu, 1996).

Asymmetric allogamous amphimixis. In sub-amphimixis (hybridogenesis), part of the female autosomes (usually of female origin) forms bivalents and the other part (usually of male origin) forms monovalents in meiosis (e.g., in *Enchytraeus lacteus* (Oligochaeta: Enchytraeidae, Table 1), (Christensen & Jensen, 1964). In fact, discarding monovalents and preventing them from entering the germline (Stenberg & Saura, 2013) constitute a violation of the principle of autosomes generation transmission, independently of their origin (Normark, 2006). In the next generation, the missing autosomes and the paternal centrosome are replaced by the paternally transmitted ones.

Autogamous amphimixis (self-fertilization). There is no doubt that simultaneous maturation of male and female gametes might contribute to autogamous amphimixis (Table 1). Its frequency, however, seems to be

Complimentary Contributor Copy

relatively low, except in Enchytraeidae (Dózsa-farkas, 1995). As a matter of fact, Enchytraeidae show a large variability in chromosome numbers, both aneuploid and polyploid (Christensen, 1961), which indicates the occurrence of quasispeciation (see below). Autogamous amphimixis might be quite harmful due to the activation of the Muller's ratchet (Felstenstein, 1974). However, in lineages, in which the embryological development stops in the absence of the paternal centrosome, the reason for autogamous amphimixis might be found in the absence of the allogamous partner.

4.1.2. Metasexual Reproduction

In metasexually reproducing Oligochaeta, the female origin of all autosomes of the progeny constitutes a violation of the principle of the equal complement contribution of autosomes by both parents (Normark, 2006). In sperm-independent parthenogenesis neither genes nor the parental centrosome are transmitted. The degenerated maternal centrosome is replaced by a new one reconstituted *de novo* (Stearns, 2001) from the maternal MTOC in the stage of the one-cell embryo (Delattre & Gönczy, 2004) or, possibly, the maternal centrosome is continuously employed (Shimizu, 1996).

Parthenogenesis might be either permanent or occasional (tychoparthenogenesis).

In gynogenesis (pseudogamy), a kind of sperm-dependent parthenogenesis, the male DNA is absent from the germplasm, but an "unknown" male-by-origin factor (Beukeboom & Vrijenhoek, 1998), identified as a centrosome (basal body) (Neaves & Baumann, 2011) is paternally transmitted. Spermatozoa of the diploid *Lumbricilus linneatus* (Christensen, 1960) are the source of a basal body for the gynogenetic triploid (3n) *L. linneatus* (Oligochaeta: Enchytraeidae, Table 1). The 3n gynogens of *L. linneatus* finalize the embryonic development but are unable to complete the spermiogenesis (Christensen, 1960).

Orthoploid and unorthoploid polyploid lineages possess an even number of chromosome sets, 4n, 6n… (Table 2), alternatively an asymmetric odd number of chromosome sets, 3n, 5n…. Since changes into genomic and morphological structures during quasispeciation are systemic,

Complimentary Contributor Copy

there are, contrary to the generalized expectation (Viktorov, 1997), some orthoploid-polyploid lineages that are metasexual (e.g., Mesenchytraeus *glandulosus*, 2n = 32 in Christensen, 1961 and *Octolasion cyaneum* (Table 1, 2). In fact, associations between parthenogenetic and polyploid lineages (Muldal, 1952) (Omodeo, 1955) indicate that polyploidization is used as a repair mechanism of defects related to segregation of chromatids and chromosomes in mitosis, meiosis, as well as fertility problems caused by parthenogenesis (Seehausen, 2004). However, other problems might be even aggravated by polyploidization, such as the change in the nuclear/cytoplasmatic ratio and the higher risk from the increased accumulation of deleterious mutations (Muller's ratchet), associated with the increase of the genome size.

4.2. Agametic Cloning in Oligochaeta

In Oligochaeta, we identified three kinds of agametic cloning (AC): budding (i.e., paratomy and architomy), body fragmentation and embryonic cloning (Table 1).

4.2.1. Budding (fission)

Reproduction accompanied by the establishment of a budding zone by the new segments is frequent, for example in Aeolosomatidae and Naididae (Lasserre, 2012). The term "architomy" is used to describe the separation of the new individual before the formation of the head on the part that broke away. The term "paratomy" describes the separation of a new individual after the formation of the head (Lasserre, 2012). Body fragmentation accompanied by a regeneration of all segments is rare in Oligochaeta.

The differentiation in the number of body segments between parent and progeny can result from the addition of a new segment to the parent, as in the below mentioned *Amphichaeta*, or by transfer of one parental segment to progeny like in stylarian paratomy (Lasserre, 2012). As a matter of fact, in animals reproducing by pygidial budding with terminal

Complimentary Contributor Copy

addition, the body is prolonged by chains of zooids. For example, in *Nais elinuis*, the separation at the level of the budding (fission) zone takes place when the new zooid has about 15 body segments (Herlant-Meeewis, 1946 in Lasserre, 2012). However, it is not the breaking of the new zooid chains from the parental animal that leads to an increase of the number of body segments in the parent (Özpolat & Bely, 2015).

Table 2. Examples of diploid and polyploid chromosome numbers in Oligochaeta

Species	No. chromosomes (ploidy)	Species	No. chromosomes (ploidy)
Megascolecidae		Lumbricidae	
Diplocardia communis German, 1888	4n = 44 (Murchie, 1966)	*Aporrectodea caliginosa* (Savigny, 1826)	2n = 36, 3n = 54 (Jaenike & Selander, 1979)
Dip. verrucosa Ude, 1895	18n = 198 (Murchie, 1966)	*Ap. rosea* (Savigny, 1826)	2n = 36 (Garbar & Vlasenko, 2007), 3n = 54, 5n = 90, 6n = 108 (Jaenike & Selander, 1979)
Dip. gatesi Murchie, 1965	8n = 88 (Murchie, 1966)	*Dendrobaena octaedra* Savigny, 1826	6n = 108,7n = 124 (Jaenike & Selander, 1979)
Dip. singularis (Ude, 1893)	6n = 66 (Murchie, 1966)	*D. tellermanica* Perel, 1967	4n = 72 (Bakhtadze et al., 2008)
Dip. riparia Smith, 1895	4n = 44 (Murchie, 1966)	*Dendrodrilus rubidus* Savigny, 1826	2n = 34, 4n = 68, 6n = 102 (Jaenike & Selander, 1979)
Dip. ornata Gates, 1943	4n = 44 (Murchie, 1966)	*Dendrodriloides grandis perelaea* Kvavadze, 1973	6n = 108 (Bakhtadze et al., 2008)
Dip. udei Eisen, 1899	4n = 44 (Murchie, 1966)	*Eisenia nordenskioldi* (Eisen, 1879)	2n = 36, 4n = 72, 6n = 96-102,7n = 110-115, 8n = 144, 8n = 142-152 (Viktorov, 1997)
Dip. bivesiculata Murchie, 1961	4n = 44 (Murchie, 1966)	*E. spelaea* Rosa, 1901	4n = 58 (Omodeo, 1984)
Dip. eiseni	4n = 44 (Murchie,	*E. submontana*	6n = 102 (Omodeo, 1984)

Complimentary Contributor Copy

Species	No. chromosomes (ploidy)	Species	No. chromosomes (ploidy)
(Michaelsen), 1894	1966)	(Vejdovský, 1875)	
Dip. alba Gates, 1943	4n = 44 (Murchie, 1966)	*E. fetida* (Savigny, 1826)	2n = 22 (Bakhtadze et al., 2008)
Dip. floridana Smith, 1824	4n = 44 (Murchie, 1966)	*E. iverica* (Kvavadze, 1973)	2n = 36 (Bakhtadze et al., 2008)
Aeolosomatidae		*Lumbricus terrestris* incl. *L. herculeus*	2n = 30-34,36,38 (Walsh, 1954)
Aeolosoma viride Stephenson, 1911	2n = 30 (Marescalchi et al., 2008)	*Octolasion cyaneum* (Savigny, 1826)	6n = 108, 10n = ca. 190 (Muldal, 1952)
Aeol. hemprichi Ehrenberg, 1828	4n = 60 (Marescalchi et al., 2008)	*O. tyrtaeum* (Savigny, 1826)	2n = 38, 3n = 54, 4n = 72 (Jaenike & Selander, 1979)
Enchytraeidae		Tubificidae	
Lumbricillus lineatus	2n = 26, 3n = 39 (Christensen, 1960)	*Tubifex tubifex* (Mueller, 1774)	3n = 75, 4n = 100, 8n = 150 (Marotta et al., 2014)
Fridericia galba	2n = 64, 3n = 96 (Christensen et al., 1992)	*T. blanchardi* Vejdovský, 1891	2n = 50 (Marotta et al., 2014)

An example of architomy is the reproduction by pygidial budding with terminal addition in Amphichaeta:

> "The worm which is not budding consists of seven segments. The first step towards asexual multiplication is the production of an eighth segment, which comes to be separated from the seventh segment by a budding zone. This eighth segment becomes the fourth of the daughter bud, the three anterior being formed from the budding zone and those behind the original segment – (sic) – the eighth of the parent. The two individuals then come apart, leaving a budding zone and eighth segment upon the parent, which gives rise to further division." (Kallstenius quoted and translated in Beddard, 1895)

Complimentary Contributor Copy

4.2.2. Embryonic Cloning

The reported cases of polyembryony or twinning (= occasional polyembryony) in Oligochaeta (Table 1) involve mechanisms associated with agametic cloning such as budding (Weber, 1917). Therefore, polyembryony and twinning in Oligochaeta might be more narrowly identified as embryonic cloning defined as "*the physical splitting of embryonic cells after the first few divisions into separate embryos*" (Craig et al., 1997). The budding involvement, that might lead to the production of large and rudimentary offspring of the same mother, e.g., in *Ap. caliginosa* (Weber, 1917), means that the cloned embryonic cells might be produced in Oligochaeta not only sexually (e.g., *L. terrestris* (Weber, 1917)) but also parthenogenetically (e.g., in *O. cyaneum*, possibly *P. corethrurus*) (Table 1). This detail differentiates the embryonic cloning in Oligochaeta from polyembryony canonically defined as "*splitting one sexually produced embryo into many offspring which are genetically identical one to another but different from their mother*" (Craig et al., 1997). Another interesting observation is that all described cases of polyembryony/twinning are in the lineages reproducing otherwise only by GR.

5. CENTROSOME AND MTOC

The importance of MTOC stems from its ability to nucleate the microtubular cytoskeleton (Azimzadeh & Bornens, 2007). This cytoskeleton is facilitating epigenetic regulation between cell components and receptors derived from microvilli. Establishing localized spatio-temporal motifs of the RNA and other molecules (Regolini, 2014) provides a better spatio-temporal resolution than gradients of diffusible molecules (Pavlíček et al., 2014). The significant role played by the centrosome proceeds from its capacity to control or co-opt older polarity-inducing systems, such as the telomere/centriole, and from its spherical properties. The spherical properties of the centrosome function as the reference system to cell geometry (Regolini, 2014) and are important tools for sensing the

Complimentary Contributor Copy

cell size and shape. Consequently, the centrosome plays an important part in karyokinesis and cytokinesis (Bornens, 2012), as well as in the maintenance of the structural and functional body symmetries (Lyons & Weisblat, 2009) and asymmetries (for example, in frog *Xenopus laevis* the induction of parthenogenesis by the centrosome can be triggered by injection of a heterologous centrosome, isolated from mammalian cells (Bornens, 2012).

Each centrosome is composed of mother and daughter centrioles of different age, embedded in the hub of an electron-dense MTOC that nucleates most microtubules of the cell (Delattre & Gönczy, 2004). The epigenetic capabilities are provided by the centrosome microtubular connections to microvilli, produced by the mother centrosome (Bornens, 2012). In Annelida, microvilli are establishing the respiration, absorption and secretion epithelia. They play a fundamental role in the geometric organization of cilia and chaetae (Tilic et al., 2015), and constitute major components of baro-, photo- and thermo-receptors. For example, in *Lumbricus terrestris*, photoreceptors at the base of the epidermis are composed of a large number of microvilli and of several cilia with a microtubular pattern of 9 x 2 + 0 (i.e., it is composed of nine peripheral doublet microtubules and no central single tubule) (Myhrberg, 1979). This pattern is similar to the microtubular patterns of the basal body/centriole.

The modification of the embryonic development by changing the position of the spindle apparatus and by a time- and place-dependent positioning of substances within the embryonic cells, as described in roundworm Ascaris (Boveri & Stevens, 1904) constitutes the epigenetic regulation of development (Satzinger, 2008).

An example of the positioning and interconnections between cell structures might provide a spermatozoon in *A. singular*e. In spermatozoa of this species, a dense material containing tube-like structures spirally enwraps the acrosome (a cap-like structure in which the Golgi apparatus is involved in its development, and which is positioned anteriorly on the head of the spermatozoa) and probably spirally coil-like structures around the nucleus (Marotta et al., 2003). The shuttle-like proximal centriole is connecting the nucleus with the flagellum. The basal body, aligned with

Complimentary Contributor Copy

the proximal centriole, is anchored to the plasma membrane. The tail is formed by an axoneme with a 9 x 2 + 2 arrangement (i.e., nine peripheral doublet microtubules and two central single tubules). The central doublet of axonemal microtubules runs inside the basal body (Marotta et al., 2003).

The (retro)viral origin of the centrosome, deduced from the presence of the viral reverse transcriptase and viral RNA (Chichinadze et al., 2013), allows a bypassing of the central dogma of molecular biology by the retrotransposition of RNA into genomic DNA. In addition, the basal body supporting the flagellum provides a "propelling engine" for flagellated spermatozoa in Metazoa. The importance of the paternal centrosome transmission might result from the ability of self-assembly, given by the fact that the centrosome (and basal body) might be established *de novo* from MTOC (Stearns, 2001) or from the directive role of pre-existing structures and organization known in *Paramecium* and other ciliates (Beisson, 2008).

6. Evolution and Speciation in Oligochaeta

The evolutionary stage before the appearance of the centrosome coincides with the emergence of telomere and centromere. The telomeric sequence (TTAGGG)n has been identified in *E. fetida* and *Octodrilus complanatus* (Vitturi et al., 2000). The telomere is associated with the linearization of chromosomes, the protection of chromosome ends against damages, the marking of chromosome polarities (= establishing an order in which chromosomes are expressed during development) and, the molecular clock (Gomes et al., 2010). The centromere, probably derived from the telomere (Villasante et al., 2007), is associated with the structure of the mitotic apparatus and the kinetochore responsible for the separation of chromosomes. However, the close relationship of the centrosome (centriole/basal body) duplication with the mitotic cycle (e.g., in *T. tubifex* (Shimizu, 1996)) and the importance of the trans-nuclear contact indicate a co-option between these two systems, resulting in hierarchically structured polarity layers. The trans-nuclear contact between centromere/telomere and

Complimentary Contributor Copy

centrosome in the spindle formation is facilitated by the cytoplasmatic microtubules (Fennell et al., 2015).

6.1. Evidence for Quasispeciation in Oligochaeta

6.1.1. The Character of Morphological Variability

It is clear that morphological differences in Oligochaeta show the influence of quasispeciation, i.e., of the macroevolutionary process. Let us take as an example the origin of the spiral embryo by the employment of the monastral spindle (e.g., in *Tubifex tubifex* (Shimizu, 1996)) and the diastral spindle that become asymmetric following transient down-regulation of one centriole (e.g., in the leech *Helobdella robusta* (Lyons & Weisblat, 2009)). We have either two independent origins, and then Oligochaeta and Hirudinea represent two independent clades or their body and processes were reorganized, as we suggest, and then leeches could be regarded as a branch of Oligochaeta as indicated by molecular methods of deep phylogeny (Struck et al., 2011). Another example is the loss of centrosome in planarians. The centrosome is rarely lost in Metazoa, but this can happen in planarians ((Azimzadeh et al., 2012). Due to the loss of the centrosome (Azimzadeh et al., 2012) (but not of the centrioles), of some polarity layers, centrosome-associated genes (Egger et al., 2015) and septa, planarians presumably adopted a simpler life form than their unknown predecessor. Today, planarians occupy marine, fresh-water and humid terrestrial habitats similar to those of annelids on which some planarians voraciously prey (Blakemore, 1987). Among others, the removal of the centrosome-associated polarities exposed the earlier ones. The rewiring of the latter led to almost endless regeneration abilities and immortality associated with the infinite somatic telomerase activity in proliferating stem cells during regeneration or fission (Tan et al., 2012). This example shows that planarians went through quasispeciation associated with a removal of the most recent polarity layers and a reorganization of body structures and functions. It might be therefore

Complimentary Contributor Copy

worth to look into the more complex spiralian taxa for the predecessors of planarians.

Another example of the same trend is the appearance of a high number of the relatively morphologically and genetically simplified parasitic lineages among segmented invertebrates and lineages colonizing extreme environments such as hydrocarbon sites. Morphological simplification is known for example in endoparasites, such as Cestoda (tapeworms), Trematoda (flukes), Monogenea, and Nematoda (roundworms). They do not show clear phylogenetic affinities. Given the fact that quasispeciation seems to be quite frequent among Annelida, the estimated age of 170-250 years in the amphimictic, sedentary, non-clonal, and large (ca. 2 m) tubeworm *Lamellibrachia luymesi* (Polychaeta: Siboglinidae) inhabiting deep marine hydrocarbon sites (Bergquist et al., 2000) is quite an evolutionary achievement. Contrastingly, the age of the oldest specimen of *Eisenia Andrei* (Oligochaeta: Lumbricidae) exceeds barely 8 years in laboratory experiments (Mulder et al., 2007).

6.1.2. Clouds of Aneuploid Quasispecies

The understanding of the role played by the inter- and intra-specific aneuploid variability has been hampered by low sample sizes (often one specimen per species or per population) and by the view that the aneuploidy observed on microscope slides is a technical error (Pavlíček et al., 2016). Therefore, only invariable values per species or the "corrected" averages are usually published. Clouds of quasispecies with different aneuploidy numbers were nevertheless published, for example, in *E. nordenskioldi*, *L. terrestris/L. herculeus* (Pavlíček et al., 2016) (Table 2). However, this conclusion also implicates that the current effort to claim species level differences between *L. terrestris* and *L. herculeus*, based on mtDNA gene variability (James et al., 2010), might be premature since "large" genetic distances do not guarantee reproductive isolation in quasispecies. This might be the case in *L. terrestris*/*L. herculeus* that seem to be part of the same cloud of quasispecies as indicated by an aneuploidy cloud in *L. terrestris/L. herculeus* (Table 2), overlapping geographical distribution and identical habitat requirements.

Complimentary Contributor Copy

6.1.3. High Rate of Monstrosities

A high rate of monstrosities is expected in species that have undergone recently quasispeciation. This might happen during the crossing of the valley of instability between two stability peaks in which symbionts and parasites are involved. In fact, a lot of asymmetric "monsters" (incl. biclitellate homeosis) were reported in peregrine and widely spread species such as *Ap. caliginosa*, *E. fetida*, *L. terrestris*, *L. rubellus* (Foxon, 1933; Gates, 1958a; Gates, 1958b; Gates, 1956 & Weber, 1917). The appearance of biclitellate homeosis, counting for part of the asymmetries, was explained by irregularities in the crossing-over following polyploidization induced by a hybridization event, and the "monsters" were regarded as part of a hybrid swarm (Pavlíček et al., 2012). The concepts of hybridization (Figure 2) and hybrid swarm are similar to the concepts of quasispeciation and clouds of quasispecies, but they disregard the details, as shown above.

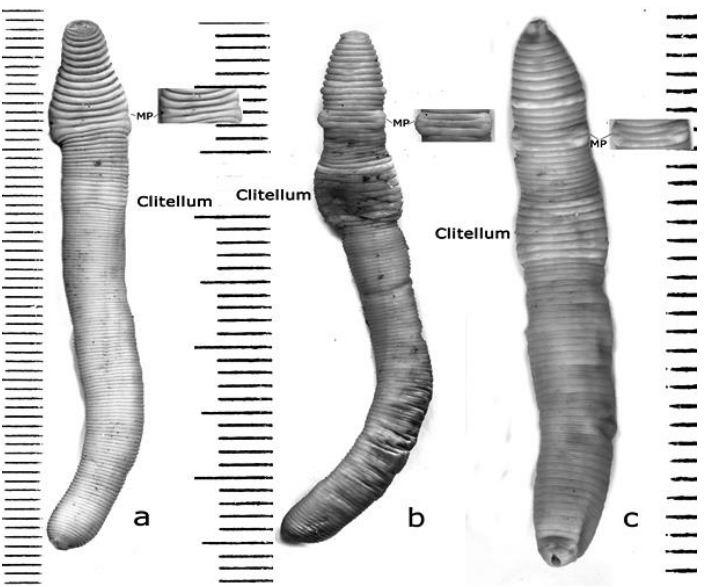

Figure 2. Comparison of typical *D. samarigera* (left), and *D. veneta* (right) with the presumed "hybrid" *D. samarigera* x *D. veneta* (middle) at the same locality (Kdoshim, Israel). A millimetre scale has been included in the picture.

Complimentary Contributor Copy

The proposed fusion-orthogonalization model might be useful to explain 1) the concept of punctuated equilibria in evolution which proposes that once a species appears its evolution slows down considerably (stasis), measured in the geological time of the fossil record (Gould & Eldredge, 1977), 2) the phylogenetic pattern as discussed by Pavlíček et al. (2012, 2014), and 3) last but not least the appearance of immortality in evolution.

7. Fusion-Orthogonalization Model of Evolution

The co-option between the centrosome and the telomere/centriole system, combined with epigenetic control, established error-prone aneuploidisation-polyploidisation mechanisms. The aneuploidy-like mechanisms, frequently leading to a diminution of the number of chromosomes (e.g., by Robertsonian translocations), are associated with the control of the mitotic spindle by kinetochore (Pavelka et al., 2010) and by centrosome. Such a mechanism could be documented in oocytes in the triploid *L. lineatus* (3n=39) (Christensen, 1960). During the final maturation divisions, in 3n *L. lineatus*, oocytes freely float in the coelomic cavity of segments Nos. 13 and 14. After spindle formation, the polar movement before the first anaphase establishes two groups of undivided univalents differing in the number of chromosomes. The observed double-groups were: 19-20 or 18-21 or **17-22** or **16-23** or **15-24** or 14-25 or 13-26 (the most frequent double-groups were the ones marked in bold) (Christensen, 1960). The sum of chromosomes in each double-group is 39 and corresponds to 3n = 39. Probably, one group of chromosomes from each double group develops and the other one degenerates. The elimination of super-numeral blastocells has been described (Shankland, 1984).

Another solution to triploidy might be polyploidization of each chromosome group. This, produces chromosome groups ranging from 26 to 52 chromosomes, and interestingly enough, closely overlapping with the ploidy in the majority of the cytogenetically studied lineages of Enchytraeidae and other oligochaetes, with the exception of polyploids of higher order (Christensen, 1961). However, polyploidization cannot run

Complimentary Contributor Copy

upward endlessly because the danger of Muller's ratchet increases with the active genome size. In Oligochaeta, the risk of Muller's ratchet is partially counter-balanced by the maintenance of relatively small chromosomes. Currently, the highest known number of chromosomes in Oligochaeta is in octodecaploid *Diplocardia verrucosa*, 18n = 198 (Table 2).

The occurrence of error-prone mechanisms, as mentioned above, implies that the microevolutionary changes will appear as coincidental from the position of an internal observer. However, this is not true from the perspective of an external observer. For example, speciation in mole rats might be triggered by desertification of the steppe during inter-pluvial periods in the Middle East or by the replacing of steppe by forest during pluvial periods in Pannonia (Hadid et al., 2012). For the external observer, there will be a causality link between the observed speciation and astronomical forcing (Muller, 1997) that is behind the pluvial/interpluvial cycles in the Middle East. The background rates of the error-prone aneuploidy-polyploidy and the mutations-recombinations can be modulated. Importantly, the competitive interactions with other members of the viral cloud of quasispecies might result in the preference of a genotype with lower replication rate than the one with a higher replication rate (Wilke et al., 2001). Similarly, the outcome of co-option might favour Oligochaeta quasispecies located on the fitness landscape at the highly connected (flat) lower fitness peak rather than a quasispecies located at a higher but narrower individual fitness peak. In Oligochaeta, "highly connected" refers to the extent of the interaction network of the cellular symbionts (organelles, MTOC) and parasites establishing layers of polarity. In such a case, the more suitable comparative parameter is stability.

As a matter of fact, even a "monster", subjectively defined as such by a human eye, can colonize a new life zone if the interaction network it is connected to compensates for the fitness loss. For example, quasispeciation explains the origin of the ectoparasitic leeches (Hirudinea), derived from Oligochaeta as indicated by molecular data (Struck et al., 2011). Leeches, in comparison to Oligochaeta, are characterized by the loss of symmetry between external segmentation (invariable number of 32 body segments

Complimentary Contributor Copy

(Shankland, 1984)) and internal intestine segmentation, by the loss of bristles, by the presence of anterior and posterior sucking organs, by the loss of regeneration ability and by the compacting of the coelom cavity by connective tissues. It could be impossible for the non-connected predecessor to cross the fitness valley between two peaks in the individual fitness landscape without the "help" of symbionts that re-wire the polarity.

After quasispeciation, clouds of 1st order composed of similar quasispecies are established. The reproductively more distant lineages compose another cloud separated by ecological affinities. For example, among *Eisenia*/*Eiseniella* lineages, one can identify a cloud of quasispecies with limnological affinities (Table 3), a cloud of species with affinities to inhabit rich organic substrates (e.g., *E. fetida*, *E. lucens*, possibly *E. andrei*) and a cloud of species inhabitings the soil (e.g., *E. malevici*, *E. muganiensis*, *E. patriciae* and *E. transcaucasica* (Szederjesi et al., 2014)). Occasionally, the quasispecies of different clouds can react one with another and establish new clouds of quasispecies. Possibly, *E. spelaea* that inhabits submerged litter in streams represents such a case (Csuzdi & Zicsi, 2003). A decrease in the chromosome number in comparison with the expetected ancestral ploidy, the presence of autogamous amphimixis (Table 1), tachyparthenogenesis and peregrine behaviour (Csuzdi & Zicsi, 2003) indicate the quasispecies character of the *E. fetida* cloud.

The observed hybrids between *Amynthas* sp. (Oligochaeta: Megascolecidae) and the unrelated *Pontoscolex corethrurus* (Oligochaeta; Glossoscolecidae) near Paracou in French Guiana (Pavlíček et al., 2014) constitute another example. The cloud of *Amynthas* sp. is composed of the of the tropical and subtropical moisture-loving epigeic quasispecies introduced probably by man to French Guiana from native South-East Asia. The cloud of *P. corethrurus* is composed of tropical and subtropical soil inhabiting lineages. The quasispeciation between these two quasispecies is facilitated by the fact that they occupy the same habitats (the flat grass lawns of the rest areas laid out near roads) but a slightly different niche (*P. corethrurus* was encountered in the lawn soil whereas *Amynthas* was found in the leaf and organic material sediments in shallow ditches built to remove excess rainwater). Both, *Amynthas sp*. and *P.*

Complimentary Contributor Copy

corethrurus are widely distributed quasispecies composed of polyploid and parthenogenetic lineages and are highly variable as regards AFLP markers (Dupont et al., 2012) with changes in polarity layers. If judged according to plate tectonics, these two species would have been isolated for 180 my by the fragmentation of Pangea.

Table 3. The "phylogeny" of the genus *Eiseniella* (Oligochaeta: Lumbricidae). Abbreviations: ante-MP = number of segments before male pore, b = beginning, Cl = clitellum, e = end, MP = male pore, Cl-Mp = number of segments between MP and Cl, Tp = tubercula pubertatis

G	ante Mp	Mp	Cl – Mp	Cl-b	Cl-e	Tp-b	Tp-e	Taxon
A	12	13	1	15	22	20	21	*Eiseniella t. macrura* (Friend, 1892)
B	11	12	5	18	22	19	21	*Tetragonurus pupa* Eisen, 1874
	12=(11+1)	13	4	18	22	19	21	*E. t. tetragonura* (Friend, 1892)
	14=(12+2)	15	5=(4+1)	21	25	22	24	*E. neapolitana ninnii* (Rosa, 1886)
	14	15	6=(5+1)	22	26	23	25	*E. t. hercynia* (Michaelsen, 1900) (a)
	13=(12+1)	14	8=(6+2)	23	27	24	26	*E. t. intermedia* (Cernosvitov, 1934)
C	12	13	6	20	24	21	24	*E. neapolitana* (Örley, 1885) (a)
	12	13	8=(6+2)	22	26	23	26	*E. t. tetraedra* (Savigny, 1826) (a)
	14=(12+2)	15	6	22	26	23	26	*E. t. hercynia* (b)
	12	13	10=(8+2)	24	28	25	28	*E. t. tetraedra* (d)
D	12	13	6	20	25	21	24	*E. neapolitana* (b)
	12	13	7=(6+1)	21	26	22	25	*E. t. sewelli* Stephenson, 1824
	12	13	8=(7+1)	22	27	23	26	*E. t. tetraedra* (b)
	14=(12+2)	15	9=(8+1)	25	30	26	29	*Eisenia balatonica* (Pop, 1943)(a)
E	12	13	7	21	24	21	24	*E. neapolitana* (c)
	12	13	9=(7+2)	23	26	23	26	*E. t. tetraedra* (c)
F	14	13	7	21	25	21	24	*E. neapolitana* (d)

Quasispecies in the same cloud are genetically and morphologically nearer one to another, they interact one with another and speciate thanks to a micro-evolutionary orthogonalization mechanism (Král, 2001) facilitated by mutations and recombinations. Since structural and functional changes are relatively small and not connected with the removal of polarities layers, but rather with their modifications, fitness, as the comparative criterion of the evolutionary success, might be useful but stability is more precise.

Complimentary Contributor Copy

Speciation by orthogonalization results in a niche/ecological zone partitioning.

The name of the fusion-quasispeciation model is derived from the fact that the quasispeciation resembles nuclear fusion and radioactive decay. The quasispeciation rate is decreasing over time. The main difference with other models of evolution and speciation is that the fusion-quasispeciation model separates between macro- and micro-evolutionary processes. It is not possible to predict evolutionary trajectories from the characteristic of quasispecies or regular species at the highest relative fitness peak.

ACKNOWLEDGEMENTS

This study was made possible by the special assistance of the JNF (research grant 10-08-022-14). We thank Patricia Cardet (Haifa, Israel) and Csaba Csuzdi (Budapest, Hungary) for comments on the manuscript and assistance for and during the expedition to French Guiana. In French Guiana, we would like to thank Philippe Gaucher (CNRS), Bruno Hérault (CIRAD) and Stéphane Traissac (EcoFoG) for their support of our collections in French Guiana.

REFERENCES

Almeida, W. D. O., Christoffersen, M. L., Amorim, D. D. S., Garraffoni, A. R. S. & Silva, G. S. (2003). Polychaeta, Annelida, and Articulata are not monophyletic: articulating the Metameria (Metazoa, Coelomata). *Rev. Bras. Zool.* 20: 23–57.

André, F. (1963). Contribution à l'étude expérimentale de la reproduction des Lombriciens. *Bull. Biol. Fr. Belg.* 97: 4–101.

André, F. & Davant, N. (1972). L'autofécondation chez les Lombriciens. Observation d'un cas d'autoinsémination chez *Dendrobaena rubida* f. *subrubicunda* Eisen. *Bull. Soc. Zool. Fr.* 97: 725–728.

Complimentary Contributor Copy

Azimzadeh, J. & Bornens, M. (2007). Structure and duplication of the centrosome. *J. Cell Sci.* 120: 2139–2142.

Azimzadeh, J., Wong, M. L., Downhour, D. M., Alvarado, A. S. & Marshal, W. S. (2012). Centrosome loss and evolution of planarians. *Science* 335: 461–463.

Bakhtadze, N. G., Bakhtadze, G. I. & Kvavadze, E. (2008). The chromosome numbers of Georgian earthworms (Oligochaeta: Lumbricidae). *Comp. Cytogenet.* 2: 79–83.

Beddard, F. E. 1895. *A Monograph of the Order of Oligochaeta*. The Clarendon Press, Oxford.

Beisson, J. (2008). Preformed cell structure and cell heredity. *Prion* 2: 1–8.

Bely, A. E. & Wray, G. A. (2001). Evolution of regeneration and fission in annelids: insights from engrailed and orthodenticle class gene expression. *Development* 128: 2781–2791.

Bergquist, D. C., Williams, F. M. & Fisher, C. R. (2000). Longevity record for deep-sea invertebrate. *Nature* 403: 499–500.

Beukeboom, L. W. & Vrijenhoek, R. C. (1998). Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *J. Evol. Biol.* 11: 755–782.

Bishop, J. D. D. & Pemberton, A. J. (2006). The third way: Spermcast mating in sessile marine invertebrates. *Integr. Comp. Biol.* 46: 398–406.

Blakemore, R. J. (1999). Diversity of exotic earthworms in Australia - a status report. In: *The Other 99% - The Conservation and Biodiversity in Invertebrates.* (W. Ponder & D. Lunney, eds), pp. 182–187. Transactions of the Royal Society New South Wallles; Mosman, Australia.

Blakemore, R. J. (2007). Origin and means of dispersal of cosmopolitan *Pontodrilus litoralis* (Oligochaeta: Megascolecidae). *Eur. J. Soil Biol.* 43: 3–8.

Blakemore, R. J. 1987. Vermicology I. Ecological considerations of the earthworms used in vermiculture - a review of the species. *http://www.annelida.net/earthworm/Vermillennium%202000/Vermicology%20I.pdf* 1–25.

Complimentary Contributor Copy

Bornens, M. (2012). The centrosome in cells and organisms. *Science* 335: 422–426.

Boveri, T. & Stevens, N. M. (1904). Über die Entwicklung dispermer Ascariseier. *Zool. Anzeiger* 26: 406–417.

Brinkhurst, R. O. (1973). Marine and brackish water Oligochaeta. *Tech. Rep.* 420: 1–9.

Bunke, D. (1986). Ultrastructural investigation of the spermatozoon and its genesis in *Aelosoma litorale* with consideration on the phylogenetical implication for the Aeolosomatidae (Annelida). *J. Ultrastruct. Mol. Struct. Res.* 95: 113–130.

Chichinadze, K., Lazarashvili, A. & Tkemaladze, J. (2013). RNA in centrosomes: structure and possible functions. *Protoplasma* 250: 397–405.

Christensen, B. (1960). A comparative cytological investigation of the reproductive cycle of an amphimictic diploid and a parthenogenetic triploid form of *Lumbricillus lineatus* (O.F.M.) (Oligochaeta, Enchytraeidae). *Chromosoma* 11: 365–379.

Christensen, B. (1984). Asexual propagation and reproductive strategies in aquatic Oligochaeta. *Hydrobiologia* 115: 91–95.

Christensen, B. (1959). Asexual reproduction in the Enchytraeidae (Olig.). *Nature* 184: 1159–1160.

Christensen, B. (1961). Studies on the cyto-taxonomy and reproduction in the Enchytraeidae. *Hereditas* 47: 387–450.

Christensen, B., Huilsom, M. & Pedersen, B. V. (1992). Genetic variation in coexisting sexual diploid + parthenogenetic triploid forms of *Fridericia galba* (E,O) in a heterogenous environment. *Hereditas* 117: 153–162.

Christensen, B. & Jensen, J. (1964). Sub-amphimictic reproduction in a polyploid cytotype of *Enchytraeus lacteus* Nielsen and Christensen (Oligochaeta, Enchytraeidae). *Hereditas* 52: 106–118.

Christensen, B. & O' Conor, F. B. (1958). Pseudofertilization in the genus *Lumbricillus* (Enchytreidae). *Nature* 181: 1085–1086.

Complimentary Contributor Copy

Craig, S. F., Slobodkin, L. B., Wray, G. A. & Biermann, C. H. (1997). The "paradox" of polyembryony : A review of the cases and a hypothesis for its evolution. *Evol. Ecol.* 11: 127–143.

Csuzdi, Cs. & Zicsi, A. (2003). *Earthworms of Hungary (Annelida: Oligochaeta, Lumbricidae)* (Cs. Csuzdi & S. Mahunka, eds). Natural History Museum, Budapest.

D'Uedekem, J. (1862). Notice sur les organes génitaux de *Aeolosoma*. *Bull. l'Académie R. Belg.* 22: 533.

Darwin, C. (1881). *The Formation of Vegetable Mould through the Action of Worms with Observations of their Habits*. Kessinger Publisher (reprint).

Davidson, S. K. & Stahl, D. A. (2008). Selective recruitment of bacteria during embryogenesis of an earthworm. *ISME J.* 2: 510–518.

Davies, K. F., Greenslade, P. & Melbourne, B. A. (1997). The invertebrates of sub-Antarctic Bishop Island. *Polar Biol.* 17: 455–458.

Davies, R. W., Linton, L. R. & Wrona, F. J. (1982). Passive dispersal of four species of freshwater leeches (Hirudinoidea) by ducks. *Freshw. Invertebr. Biol.* 1: 40–44.

Dawkins, R. (1989). *The Extended Phenotype*. Oxford University Press, Oxford.

Delattre, M. & Gönczy, P. (2004). The arithmetic of centrosome biogenesis. *J. Cell Sci.* 117: 1619–1630.

Dózsa-Farkas, K. (1995). Self-fertilization: An adaptive strategy in widespread enchytraeids. *Eur. J. Soil Biol.* 31: 207–215.

Dupont, L., Decaëns, T., Lapied, E., Chassany, V., Marichal, R., Dubs, F., et al. (2012). Genetic signature of accidental transfer of the peregrine earthworm *Pontoscolex corethrurus* (Clitellata, Glossoscolecidae) in French Guiana. *Eur. J. Soil Biol.* 53: 70–75.

Egger, B., Lapraz, F., Tomiczek, B., Müller, S., Dessimoz, C., Girstmair, J., et al. (2015). A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms. *Curr. Biol.* 25: 1347–1353.

Elphick, J. (2011). *Atlas of Bird Migration*. Firefly Books, Ltd., Buffalo.

Complimentary Contributor Copy

Emmerling, C. & Strunk, H. (2012). Active dispersal of the endo-anecic earthworm *Aporrectodea longa* (Ude) in an experimental box. *Soil Org*. 84: 491–498.

Erseus, C. (2005). Phylogeny of oligochaetous Clitellata. *Hydrobiologia* 535/536: 357–372.

Felstenstein, J. (1974). The evolutionary advantage of recombination. *Genetics* 78: 737–756.

Fennell, A., Fernandez-Alvares, A., Tomita, K. & Promisel Cooper, J. (2015). Telomeres and centromeres have interchangeable roles in promoting meiotic spindle formation. *J. Cell Biol*. 208: 415–428.

Foxon, G. H. E. (1933). XXX - *Lumbricus terrestris* Linn., a specimen with asymmetrical genital organs. *J. Nat. Hist.* 12: 284–287.

Garbar, A. V., Onyschuk, I. P. & Mezhzherin, S. V. (2009). Polyploid races, genetic structure and morphological features of the earthworm *Octodrilus transpadanus* (Rosa, 1884) (Oligochaeta: Lumbricidae) in the Ukraine. *Comp. Cytogenet.* 3: 131–141.

Garbar, A. V. & Vlasenko, R. P. (2007). Karyotypes of three species of the genus *Aporrectodea* Örley (Oligochaeta: Lumbricidae) from the Ukraine. *Comp. Cytogenet.* 1: 59–62.

Gates, G. E. (1958a). On another biclitellate earthworm. *Am. Midl. Nat.* 63: 418–423.

Gates, G. E. (1958b). On homoeosis, as well as other aberrations, and their origin in an earthworms species, *Eisenia foetida* (Savigny, 1826), along with some deductions as to morphogenesis in the Lumbricidae. *Am. Midl. Nat.* 59: 452–464.

Gates, G. E. (1956). On the origin of the biclitellate condition in lumbricid earthworms. *Ann. Mag. Nat. Hist.* 9: 577–581.

Gavilov, K. (1935). Contribution à l'étude de l'autofécondation chez les Oligochaetes. *Acta Zool.* 16: 21–64.

Giere, O. (2006). Ecology and biology of marine Oligochaeta - An inventory rather than another review. *Hydrobiologia* 564: 103–116.

Gislén, T. (1948). Aerial plankton and its conditions of life. *Biol. Rev. Camb. Philos. Soc*. 23: 109–126.

Complimentary Contributor Copy

Gomes, N. M. V, Shay, J. W. & Wright, W. E. (2010). Telomere biology in Metazoa. *FEBS Lett.* 584: 3741–3751.

Gorgoń, S., Krodkiewska, M. & Świątek, P. (2015). Ovary ultrastructure and oogenesis in *Propappus volki* Michaelsen, 1916 (Annelida: Clitellata). *Zool. Anzeiger - A J. Comp. Zool.* 257: 110–118.

Gould, S. J. & Eldredge, N. (1977). Punctuated equilibria: the tempo and mode of evolution reconsidered. *Paleobiology* 3: 115–151.

Green, A.J. & Figuerola, J. (2005). Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. *Divers. Distrib.* 11: 149–156.

Hadid, Y., Németh, A., Snir, S., Pavlíček, T., Csorba, G., Kázmér, M., et al. (2012). Is evolution of blind mole rats determined by climate oscillations? *PlosOne* 7: e30043.

Hale, C. M., Frelich, L. E. & Reich, P. B. (2005). Exotic european earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. *Ecol. Appl.* 15: 848–860.

Holmstrup, M., Slotsbo, S., Henriksen, P. G. & Bayley, M. (2016). Earthworms accumulate alanine in response to drought. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 199: 8–13.

Jaenike, J. & Selander, R. K. (1979). Evolution and ecology of parthenogenesis in earthworm. *Am. Zool.* 19: 729–737.

James, S. W., Porco, D., Decaëns, T., Richard, B., Rougerie, R. & Erséus, C. (2010). DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): resurrection of *L. herculeus* (Savigny, 1826). *PLoS One* 5: e15629.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A. & Charpentier, E. (2012). A programmable dual-RNA – guided DNA endonuclease in adaptive bacterial immunity. *Science* 337: 816–821.

Kleinenberg, N. (1879). The development of the earth-worm *Lumbricus trapezoides* Dugés. *Q. J. Microsc. Sci.* XIX: 206–244.

Král, P. (2001). Species orthogonalization. *J. Theor. Biol.* 212: 355–366.

Laakso, J. & Setälä, H. (1997). Nest mounds of red wood ants (*Formica aquilonia*): hot spots for litter-dwelling earthworms. *Oecologia* 111: 565–569.

Complimentary Contributor Copy

Lankester, E. R. (1871). On the origin and structure of the spermatophors, or sperm ropes, of two species of *Tubifex*. *Q. J. Microsc. Sci.* 10: 180–187.

Lapied, E. (2002). Earthworms in Black Woodpacker (*Dryocopus martius*) tree cavities. In: *Book of Abstracts: The 7th International Symposium on Earthworm Ecology*, p. 368. Cardiff University, Cardiff, Walles, UK.

Lasserre, P. (2012). Clitellata. In: *Reproduction of Marine Invertebrates. V3 Annelids and Echiurans* (A. Giese, ed), p. 358. Elsevier.

Lowe, C. N. & Butt, K. R. (2008). Life cycle traits of the parthenogenetic earthworm *Octolasion cyaneun* (Savigny, 1826). *Eur. J. Soil Biol.* 44: 541–544.

Lyons, D. C. & Weisblat, D. A. (2009). D quadrant specification in the leech *Helobdella*: actomyosin contractility controls the unequal cleavage of the CD blastomere. *Dev. Biol.* 334: 46–58.

Mann, T. (1984). *Spermatophores. Development, Structure, Biochemical Attributes and Role in the Tranfer of Spermatozoa*. Springer-Verlag.

Marescalchi, O., Gugnali, A. & Falconi, R. (2008). First report on the chromosomes of *Aeolosoma viride* and *Aeolosoma hemprichi* (Aeolosomatidae; Annelida). *Zoolog. Sci.* 25: 904–906. Zoological Society of Japan.

Marinissen, J. & Van den Bosch, F. (1992). Colonization of new habitats by earthworms. *Oecologia* 91: 371–376.

Marotta, R., Crottini, A., Raimondi, E., Fondello, C. & Ferraguti, M. (2014). Alike but different: the evolution of the *Tubifex tubifex* species complex (Annelida, Clitellata) through polyploidization. *BMC Evol. Biol*. 2014: 14–73.

Marotta, R., Ferraguti, M. & Martin, P. (2003). Spermiogenesis and seminal receptacles in *Aeolosoma singulare* (Annelida, Polychaeta, Aeolosomatidae). *Ital. J. Zool.* 70: 123–132.

Marques, M. C. M. & Fischer, E. (2009). Effect of bats on seed distribution and germination of *Calophyllum brasiliense* (Clusiaceae). *Ecotropica* 15: 1–6.

Complimentary Contributor Copy

Mather, J. D. & Christensen, O. (1988). Surface movement of earthworms in agricultural land. *Pedobiologia (Jena).* 32: 399–405.

Michaelsen, W. (1922). Die Verbreitung der Oligochäten im Lichte der Wegenerischen Theorie der Kontinentenverschiebung und andere Fragen zur Stammgeschichte und Verbreitungen dieser Tiergruppen. *Verhandlungen des Naturwissentschaft-lichen Vereins Hambg.* 29: 45–79.

Monroy, F., Aira, M., Velando, A. & Domínguez, J. (2003). Have spermatophores in *Eisenia fetida* (Oligochaeta, Lumbricidae) any reproductive role? *Pedobiologia (Jena).* 47: 526–529.

Muldal, S. (1952). The chromosomes of the earthworms. I. The evolution of polyploidy. *Heredity (Edinb.).* 6: 55–76.

Mulder, C., Hendriks, J., Baerselman, R. & Posthuma, L. (2007). Age structure and senescence in long-term cohorts of *Eisenia andrei* (Oligochaeta: Lumbricidae). *Journals Gerontol.* **62**: 1361–1363.

Muller, R. A. (1997). Glacial cycles and astronomical forcing. *Science* 277: 215–218.

Murchie, A. K. & Gordon, A. W. (2013). The impact of the "New Zealand flatworm", *Arthurdendyus triangulatus*, on earthworm populations in the field. *Biol. Invasions* 15: 569–586.

Murchie, W. R. (1966). Chromosome numbers of some diplocardian earthworms (Megascolecidae-Oligochaeta). *Am. Midl. Nat.* 78: 534–537.

Myhrberg, H. E. (1979). Cell and tissue fine structural analysis of the basal epidermal receptor cells in the earthworm (*Lumbricus terrestris* L.). *Cell Tissue Res.* 203: 257–266.

Neaves, W. B. & Baumann, P. (2011). Unisexual reproduction among vertebrates. *Trends Genet.* 27: 81–88.

Normark, B. B. (2006). Perspective: Maternal kin groups and the origins of asymmetric genetic systems-genomic imprinting, haplodiploidy, and parthenogenesis. *Evolution (N. Y).* 60: 631–642.

Omodeo, P. (1955). Cariologia dei Lumbricidae II Contributo. *Caryologia* 8: 135–178.

Complimentary Contributor Copy

Omodeo, P. (2000). Evolution and biogeography of megadriles (Annelida, Clitellata). *Ital. J. Zool.* 67: 179–201.

Omodeo, P. (1984). On aquatic Oligochaeta Lumbricomorpha in Europe. *Hydrobiologia* 115: 187–190.

Özpolat, B. D. & Bely, A. E. (2015). Gonad establishment during asexual reproduction in the annelid *Pristina leidyi*. *Dev. Biol.* 405: 123–136.

Pavelka, N., Rancati, G. & Li, R. (2010). Dr Jekyll and Mr Hyde: role of aneuploidy in cellular adaptation and cancer. *Curr. Opin. Cell Biol.* 22: 809–815.

Pavlíček, T., Cohen, T., Yadav, S., Glasstetter, M., Král, P. & Pearlson, O. (2016). Aneuploidy occurrence in Oligochaeta. *Ecol. Evol. Biol.* 1: 57–63.

Pavlíček, T., Hadid, Y., Cohen, T., Glasstetter, M., Snir, S., Mısırlıoğlu, M., et al., (2014). "Opening Pandora's Box": II. Segmentation and evolution of hermaphroditic annelids. In: *Advances in Earthworm Taxonomy VI. (Annelida: Oligochaeta). Proceedings of the 6th International Oligochaeta Taxonomy Meeting (6th IOTM), Palmeira de Faro, Portugal, 22-25 April, 2013* (T. Pavlíček et al., eds), pp. 38–49. Kasparek Verlag, Heidelberg.

Pavlíček, T., Hadid, Y. & Csuzdi, Cs. (2012). Opening Pandora's box : Clitellum in phylogeny and taxonomy of earthworms. *Zool. Middle East.* Supplement: 31–46.

Ponge, J.-F. (1999). Interaction between soil fauna and their environment. In: *Going underground. Ecological studies in forest soils.*, pp. 45–76.

Prat, P., Charrier, M., Deleporte, S. & Frenot, Y. (2002). Digestive carbohydrases in two epigeic earthworm species of the Kerguelen Islands (Subantarctic). *Pedobiologia (Jena).* 46: 417–427.

Regolini, M. (2014). Centrosome : The cell spherical reference system organizer. *Indian J. Res.* 3: 80–83.

Rice, S. A. (1980). Ultrastructure of the male nephridium and its role in spermatophore formation in Spionid Polychaetes (Annelida). *Zoomorphologie* 95: 181–194.

Complimentary Contributor Copy

Riparbelli, M. G., Giordano, R., Ueyama, M. & Callaini, G. (2012). *Wolbachia*-mediated male killing is associated with defective chromatin remodeling. *PLoS One* 7: e30045.

Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu. Rev. Ecol. Evol. Syst.* 38: 231–253.

Ruehland, C., Blazejak, A., Lott, C., Loy, A., Erséus, C. & Dubilier, N. (2008). Multiple bacterial symbionts in two species of co-occurring gutless oligochaete worms from Mediterranean sea grass sediments. *Environ. Microbiol.* 10: 3404–3416.

Satzinger, H. (2008). Theodor and Marcella Boveri: chromosomes and cytoplasm in heredity and development. *Nat. Rev. Genet.* 9: 231–238.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends Ecol. Evol.* 19: 198–207.

Shankland, M. (1984). Positional determination of supernumerary blast cell death in the leech embryo. *Nature* 307: 541–543.

Shen, H.-P., Yu, H.-T. & Chen, J.-H. (2012). Parthenogenesis in two Taiwanese mountain earthworms *Amynthas catenus* Tsai et al., 2001 and *Amynthas hohuanmontis* Tsai et al., 2002 (Oligochaeta, Megascolecidae) revealed by AFLP. *Eur. J. Soil Biol.* 51: 30–36.

Shimizu, T. (1996). Behaviour of centrosomes in early *Tubifex* embryos: asymmetric segregation and mitotic cycle-dependent duplication. *Roux's Arch. Dev. Biol.* 205: 290–299.

Stearns, T. (2001). Centrosome duplication. a centriolar pas de deux. *Cell* 105: 417–420.

Stenberg, P. & Saura, A. (2013). Meiosis and its deviations in polyploid animals. *Cytogenet. Genome Res.* 140: 185–203.

Stephenson, J. (1923). *The Fauna of British India including Ceylon and Burma. Oligochaeta*. Taylor and Francis, London.

Struck, T. H., Paul, C., Hill, N., Hartmann, S., Hösel, C., Kube, M., et al., (2011). Phylogenomic analyses unravel annelid evolution. *Nature* 471: 95–98.

Complimentary Contributor Copy

Subba Rao, B. V. S. S. R. & Ganapati, P. N. (1974). On the breeding and cocoons of a littoral oligochaete *Pontodrilus bermudensis* Beddard. *Proc. Ind. Ac. Sci.* 1: 18.

Szederjesi, T., Pavlíček, T., Robabeh, L. & Csuzdi, Cs. (2014). Review of the *Eisenia muganiensis* (Michaelsen, 1910) species group with description of two new species (Oligochaeta: Lumbricidae). *Zootaxa* 3884: 282–288.

Tan, T. C. J., Rahman, R., Jaber-Hijazi, F., Felix, D. A., Chen, C. & Louis, E. J. 2012. Telomere maintenance and telomerase activity are differentially regulated in asexual and sexual worms. *Proc. Natl. Acad. Sci.* 109: 4209–4214.

Terhivuo, J., Lundqvist, E. & Saura, A. 2002. Clone diversity of *Eiseniella tetraedra* (Oligochaeta : Lumbricidae) along regulated and free-flowing boreal rivers. *Ecography (Cop.).* 25: 714–720.

Terhivuo, J. & Saura, A. (2006). Dispersal and clonal diversity of north-european parthenogenetic earthworms. *Biol. Invasions* 8: 5–18.

Terhivuo, J. & Saura, A. (1997). Island biogeography of North European parthenogenetic Lumbricidae. 1. Clone pool affinities and morphometric differentiation of Aland populations. *Ecography (Cop.).* 20: 185–196.

Tilic, E., von Döhren, J., Quast, B., Beckers, P. & Bartolomaeus, T. (2015). Phylogenetic significance of chaetal arrangement and chaetogenesis in Maldanidae (Annelida). *Zoomorphology* 134: 383–401.

Timm, T. (1980). Distribution of Aquatic Oligochaetes. In: *Aquatic Oligochaete Biology*, pp. 55–77. Springer US, Boston, MA.

Tiunov, A. V., Hale, C. M., Holdsworth, A. R. & Vsevolodova-Perel, T. S. 2006. Invasion patterns of Lumbricidae into the previously earthworm-free areas of Northeastern Europe and the Western Great Lakes Region of North America. *Biol. Invasions* 8: 1223–1234.

Vijayakumaran, N. K., Manazhy, J., Manazhy, A. & Reynolds, J. W. (2009). Biology of cocoons of five species of earthworms (Annelida: Oligochaeta) from Kerala, India. *Megadriologica* 13: 1–8.

Complimentary Contributor Copy

Viktorov, A. G. (1997). Diversity of polyploid races in the family Lumbricidae. *Soil Biol. Biochem.* 29: 217–221.

Villasante, A., Abad, J. P. & Méndez-Lago, M. (2007). Centromeres were derived from telomeres during the evolution of the eukaryotic chromosome. *Proc. Natl. Acad. Sci. U. S. A.* 104: 10542–10547.

Vitturi, R., Colomba, M. S., Pirrone, A. & Libertini, A. (2000). Physical mapping of rDNA genes, (TTAGGG)n telomeric sequence and other karyological features in two earthworms of the family Lumbricidae (Annelida : Oligochaeta). *Heredity (Edinb).* 85: 203–207.

Walsh, M. P. (1954). A chromosome study of *Lumbricus terrestris* (L.). *Trans. Am. Microsc. Soc.* 73: 164–167.

Weber, R. A. (1917). Observations on the structure of double monsters in the earthworms. *Biol. Bull.* 33: 339-348-354.

Wegener, A. (1912). Die Herausbildung der Grossformen der Erdrinde (Kontinente und Ozeane), auf geophysikalischer Grundlage. *Petermanns Geogr. Mitt.* 63: 185–195, 253–256, 305–309.

Wilke, C. O., Wang, J. L., Ofria, C., Lenski, R. E. & Adami, C. (2001). Evolution of digital organisms at high mutation rates leads to survival of the flattest. *Nature* 412: 331–333.

Yoshida-Noro, C. & Tochinai, S. (2010). Stem cell system in asexual and sexual reproduction of *Enchytraeus japonensis* (Oligochaeta, Annelida). *Dev. Growth Differ.* 52: 43–55.

Complimentary Contributor Copy

Complimentary Contributor Copy

In: Earthworms
Editor: Clayton G. Horton
ISBN: 978-1-53612-176-6
© 2017 Nova Science Publishers, Inc.

Chapter 2

A CONTRIBUTION TO EARTHWORM DIVERSITY OF CENTRAL INDIA (MADHYA PRADESH)

Ashutosh Vishwakarma and Shweta Yadav*
Department of Zoology, School of Biological Sciences,
Dr. Harisingh Gour Vishwavidyalaya (A Central University),
Sagar, MP, India

ABSTRACT

The earthworm fauna of India is well reported as compared to other Asian Countries (excluding Myanmar). Presently, 505 valid species/ subspecies of earthworms under 69 genera are known from the Indian territory, including the islands of Andaman, Nicobar and Lakshadweep. Endemism, both at genera and species level, is very high; about 71% of genera and 89% of species are endemic. Some exotic peregrine species of earthworms are also found and these are now widespread in disturbed habitats following deforestation and intensive cultivation practices. The earthworm biodiversity in India predominantly represented by native

* Corresponding author email: kmshweta3@yahoo.com.

Complimentary Contributor Copy

species (357species), which constitute 88.8% of total earthworm diversity of India. However, earthworm diversity in some parts of India including central part of country (Madhya Pradesh) is still poorly explored. Madhya Pradesh lies between latitude 21°17'-26°52' N and longitude 74°08'-82°49' E, has 51 district (10 divisions) covering an area of 3, 08, 245 km^2 constituting 9.38% of geographic area of the country. The current study deals with biodiversity of earthworms in Madhya Pradesh, a central part of India. Finding suggested that Madhya Pradesh region is rich in biodiversity of earthworms. Collections were made during rainy and humid season according to Indian seasonal calendar. Thirty one species were identified belonging to genera 15 and 4 families. Collected specimens were identified with morpho-taxonomy and molecular biomarker cytochrome oxidase (*coi-1*) gene. *Lampito mauritii* and *Eudichogaster prashadi* were predominantly found in different parts of Madhya Pradesh. Study is an attempt to enrich the existing information on earthworm biodiversity with use of molecular markers.

Keywords: biodiversity, COI gene, DNA barcode, molecular taxonomy

1. INTRODUCTION

Earthworms are ancient and they have been on our planet for 600 million years. They have survived through five mass extinctions, and helped life to sustain on the earth and human civilization by ploughing and fertilizing the soil. As far back as 1881, Charles Darwin, the great biologist, observed '*It may be doubted whether there are many other animals which have played so important a part in the history of the world as have these lowly organised creatures and every inch of top soil on the entire planet had surely passed through the belly of earthworms several times.*'

For the first time, Darwin's experiments on earthworms brought to light the importance of earthworms in burial of surface lying organic matter and opened a new line of research in Soil Biology. Although before Darwin (1881), White (1789), Hansen (1877) and Muller (1878) had recognized the role of earthworms in the formation of soil and humus. Serious interest in earthworm biology, however, emerged only in the second half of the last century with the realization of the role of

Complimentary Contributor Copy

earthworms in soil mineralization, soil fertility, plant productivity and vermicomposting (Kuhnelt, 1950; Satchell, 1967, 1983; Edwards and Lofty, 1977; Hartenstein et al., 1979; Lee, 1985; Lavelle, 1988).

Earthworms constitute a major component of the invertebrate biomass (>80%) in most terrestrial ecosystems of the world contributing considerably to soil physico-chemical properties and processes (Senapati and Dash, 1982). Thus, earthworms play an important role in the soil ecosystem spending their life eating through any organic matter which is passed through their intestines and deposited back in the form of nutrient rich humus. Although living below ground, they are vital in running all the above ground ecosystems and even the world, and are at the heart of a healthy environment. Earthworms are known as natural bio-reactor which converts the organic waste in to organic manure and enhances soil fertility, porosity, health and productivity. There are some ecological types of earthworm have been recognized *viz*. epigeic, endogeic, anecic. In agro-ecosystem earthworms play an important role in cultivation. Their habitat of burrows and swallows helps to increase the fertility of agriculture field in many ways. Their burrow permits the aeration and moistures in the porous soil and improves the water holding capacity. The earthworms are continuously ploughing the soil and eat dead leaves. They are digested and mixed with the castings. They provide optimum conditions for plant growth and microbial productivities by reducing both alkalinity and acidity of soil. Plant growth stimulants such as auxin hormones are produced in the earthworm castings.

The earthworm belongs to the phylum Annelida and class Oligochaeta. The current classification of the class Oligochaeta recognizes three constituent orders; Lumbriculida, Moniligastrida (Soil dwelling, earthworm group of India, Southeast Asia, southern and central China), Haplotaxida (all other oligochaeta). Earthworms of India belong to two orders (Moniligastrida and Haplotaxida) and nine families (Moniligastridae, Almidae, Rhinodrilidae (earlier known as Glossoscolecidae), Lumbricidae, Ocnerodrilidae, Acanthodrilidae

Complimentary Contributor Copy

Octochaetidae, Megascolecidae, Eudrilidae). Approximately 3700 species of earthworms are known in the world (Reynolds, 1994) but the number of estimated species may be as high as 8000 (Fragoso et al., 1997). In India, 505 species and subspecies belonging to 69 genera have been described so far (Blakemore, 2006). This number is expected to rise to about 800 with extensive surveys of large unexplored areas of 'biodiversity hotspots' of the Western Ghats and Eastern Himalaya. High earthworm diversity in our country is primarily due to its geographical location with a wide latitudinal range (located 8.4°-37.6°N latitude with covered area 3,287,797 km), complex topography, varied climate (ranging from temperate to arctic in the Himalaya to tropical in the peninsular India) and past geological history that is linked to ancient super continent of Gondwanaland from which it separated in the late Jurassic and drifted to collide with the Asian mainland in the Eocene. India has been divided into six well-defined physiographic regions (depending upon topography, climate and vegetation) *viz.* the Northern Mountains, the Peninsular pateau, Indo-Gangetic plain, Thar Desert, the coastal plains, the Islands. The scientific exploration of earthworm's diversity in India dates back to the nineteenth century. The credit for naming the first earthworm species in the Indian subcontinent goes to Templeton (1844) when he discovered *Megascolex caeruleus* from Sri Lanka. However, Perrier (1872) was the first to describe earthworm species from the Indian mainland.

Endemism, both at the genus and species level, is very high; about 71 per cent of genera and 89 per cent of species are endemic. Some exotic peregrine species of earthworms are also found, and these are now widespread in disturbed habitats following deforestation and intensive cultivation. It is estimated that as many as 45 exotic species have been brought to India, mostly unintentionally, from other zoogeographical realms. They were possibly introduced to the area due to human activity such as accidental transportation in soil around roots of plants or in soil stuck on feet of animals and birds.

Complimentary Contributor Copy

The most diverse families of earthworms in India are the Megascolecidae with 136 species in 13 genera, Octochaetidae with 134 species in 30 genera and Moniligastridae with 79 species in 3 genera. At generic level, the moniligastrid *Drawida* with 68 species is the most diverse, followed by the megascolecids *Perionyx* (46 species) and *Megascolex* (33 species), and the octochaetids *Eutyphoeus* (22 species) and *Hoplochaetella* (19 species). The Western Ghats and West Coast agro-climatic region is the richest with 219 species comprising of 52.4 per cent of Indian earthworm diversity, followed by Eastern Himalaya and Northeast Hills region with 110 species. The present study is based on earthworms collected from Madhya Pradesh (a central part of India).The earthworm study of Madhya Pradesh is known from the works of Stephenson (1920), Gates (1939,1949,1956,1960), Julka (1988) and Paliwal (2008). Stephenson (1920) studied the collections from Central India and 4 species of earthworms were reported from Jabalpur of Madhya Pradesh.Gates (1945,1949) explored the area and discovered 3 new species from the surroundings of Jabalpur.Subsequent contribution by Gates (1956) revealed the occurrence of 25 species from the area.Julka (1988) revised the taxonomic status of oligochaetes belonging to family Octochaetidae from Madhya Pradesh,So far, 26 species belonging to 18 genera spread over five families have been recognized from Jabalpur district of Madhya Pradesh by Paliwal (2008).Present study enriches the existing information on earthworm biodiversity in the study area with use of modern trend of molecular taxonomy.

2. MATERIAL AND METHODS

Earthworms for the present taxonomic study were collected by digging and hand sorting method during July 2015 to March 2016. The total earthworm collections were carried out in nine divisions of 35 districts Madhya Pradesh (Table 1, Figure 1).

Complimentary Contributor Copy

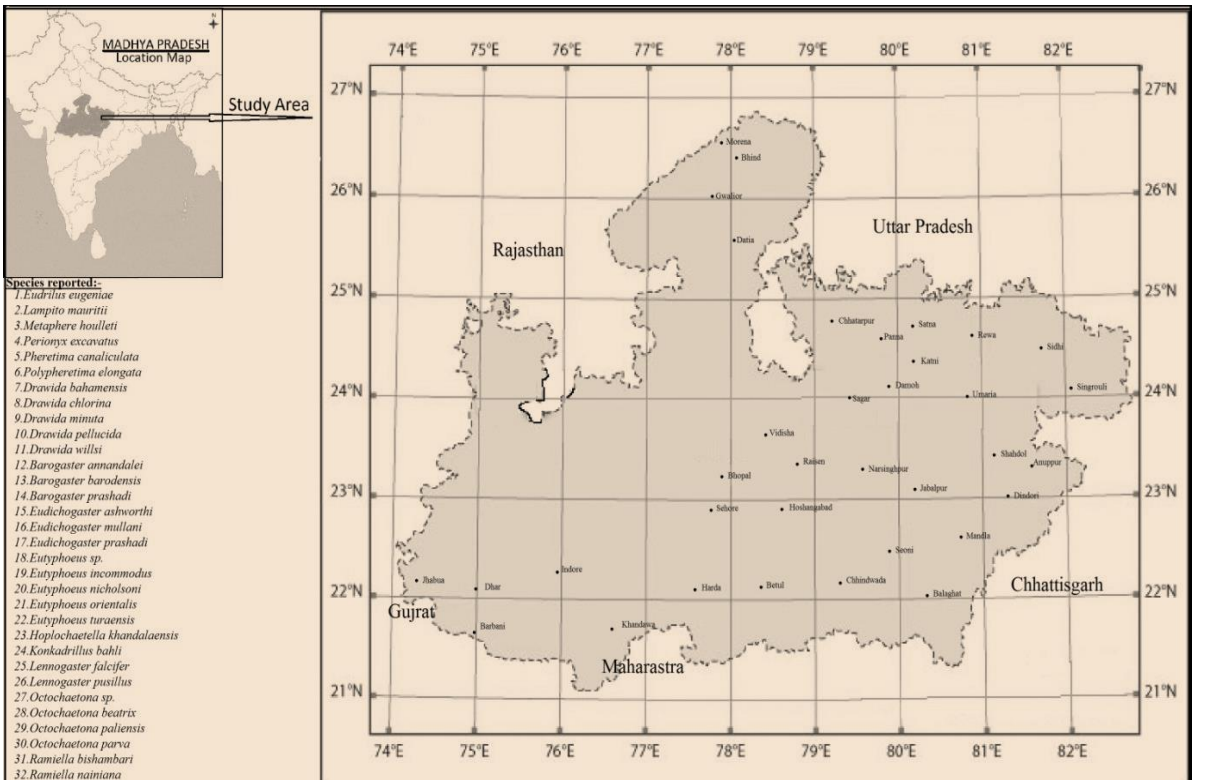

Figure 1. Study area to record earthworm diversity in the Madhya Pradesh region of India.

Complimentary Contributor Copy

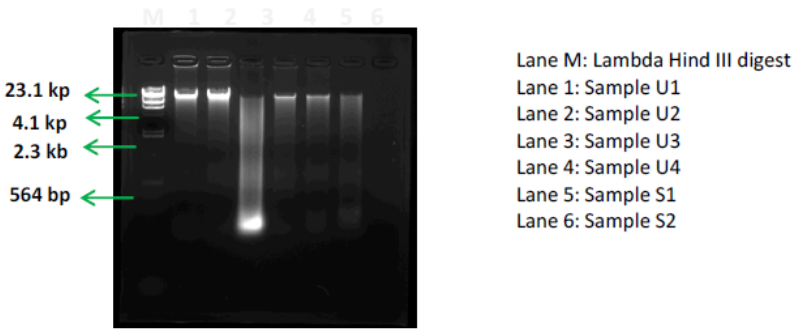

Figure 3. High molecular weight total DNA extraction from earthworm tissue.

Table 1. Sampling site of study area in Madhya Pradesh, India

No	Divisions	Districts
1.	Bhopal division	Bhopal, Raisen, Sehore, Vidisha
2.	Chambal division	Morena, Bhind
3.	Gwalior division	Gwalior, Datia
4.	Indore division	Barwani, Dhar, Indore, Jhabua, Khandwa
5.	Jabalpur division	Balaghat, Chhindwara, Jabalpur, Katni, Mandla, Dindori, Narsinghpur, Seoni
6.	Narmadapuram division	Betul, Harda, Hoshangabad
7.	Rewa division	Rewa, Satna, Sidhi, Singrauli
8.	Sagar division	Chhatarpur, Damoh, Panna, Sagar
9.	Shahdol division	Anuppur, Shahdol, Umaria

Earthworms were preserved following Julka (1988) and Kushwaha et al. (2015) for morpho-taxonomy and molecular studies. All specimens were deposited in Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar (Madhya Pradesh) India. Earthworms were identified under a stereoscopic zoom microscope (Leica Model No. M60 with available literature (Gates 1972, Stephenson 1923, Julka 1988). At present, species identification of adult earthworms is only possible by dissection of the genitalia (male gonopore mp, female gonopore fp); however, this method is labor intensive, time consuming and very difficult for non-specialists, particularly when dealing with field collections consisting of several different earthworm species. Furthermore, identification is limited to adult worms, as most life stages were

Complimentary Contributor Copy

unidentifiable. Many morphological and anatomical characteristics of earthworms were variable, and the degree of variability was differed and features overlapped between taxa, therefore molecular identification techniques were used following amplification of *coi*-gene.LCO1490 FP and LCO1490 FP primers used for the amplification of gene (Table 2). The high molecular weight PCR grade total DNA from earthworm tissue was extracted using CTAB and ran on 0.7% agarose electrophoresis gel containing ethidium bromide, DNA staining dye and the photograph was taken under tranilluminator emitting UV light (Figure 3). The partial sequences of mitochondrial cytochrome c oxidase I (COI) gene containing 710 bp has been amplified using LCO1490 FP and HCO2198 gene specific primers at an annealing temperature of 45°C. The PCR amplified products were ran on 1% agarose gel electrophoresis containing ethidium bromide, DNA staining dye and the photograph was taken under transilluminator emitting UV light. Obtained sequences (an example of chromatogram of *Eutyphoeus* sp is shown in Figure 4) of amplified were aligned, edited and analysed in MEGS v5.

3. RESULTS AND DISCUSSION

Earthworm survey conducted in 35 different districts of Madhya Pradesh revealed occurrence of 15 genera and 30 species belonging to family Eudrilidae, Megascolecidae, Moniligastridae and Octochaetidae collected from different habitats (Table 3). Earthworm species *Eudrilus eugeniae, Lampito mauritii, Metaphere houlleti, Perionyx excavatus, P. sansibaricus Pheretima canaliculata, Polypheretima elongata, Drawida bahamensis, D. chlorina, D. minuta, D. pellucida, D. willsi, Barogaster annandalei, B. barodensis, B. prashadi, Eudichogaster ashworthi, E. mullani, E. prashadi, Eutyphoeus incommodus, E. nicholsoni, E.orientalis, Hoplochaetella khandalaensis, Konkadrilus bahli, Lennogaster falcifer, L. pusillus, Octochaetona sp., O. Beatrix, O. paliensis, O. parva, Ramiella bishambari, R. nainiana* were collected from the study area. *Lampito mauritii* and *Eudichogaster prashadi* were abundantly found in the study area.

Complimentary Contributor Copy

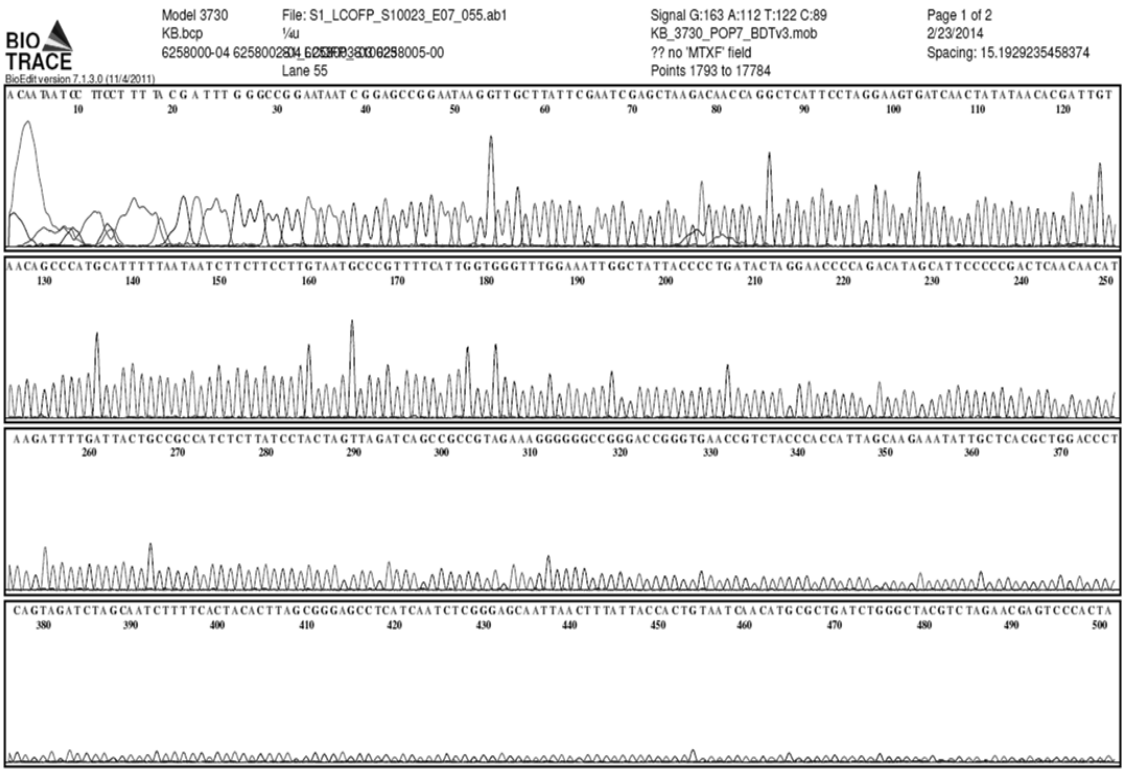

Figure 4. Sequencing chromatogram of sample *Eutyphoeus* sp. using LCO1490 foreword primer showing clear peaks without mixed/uninterrupted reads.

Complimentary Contributor Copy

Table 2. Primers used for the amplification of mitochondrial *cytochrome c oxidase I (COI)* gene

S. No	Primer Name	Sequence (mer)	Tm/Ta	GC %	Reference
1	LCO1490 FP	GGTCAACAAATCATAAAGATATTGG (25 mer)	51	32	Folmer et al. (1994)
2	HCO2198 RP	TAAACTTCAGGGTGACCAAAAAATCA (26 mer)	55	34	Folmer et al. (1994)
3	COI-E RP	TATACTTCTGGGTGTCCGAAGAATCA (26 mer)	55	42	Bely and Wray (2004)

Table 3. Earthworm diversity recorded in study area

Eudrilidae	Megascolecidae	Moniligastridae	Octochaetidae
1.*Eudrilus eugeniae*	2.*Lampito mauritii* 3.*Metaphere houlleti* 4.*Perionyx excavatus* *5. P. sansibaricus* 5.*Pheretima canaliculata* 6.*Polypheretima elongata*	7. *Drawida bahamensis* 8. *D. chlorina* 9. *D.minuta* 10. *D.pellucida* 11. *D.willsi*	12.*Barogaster annandalei* 13.*B. barodensis* 14.*B. prashadi* 15.*Eudichogaster ashworthi* 16.*E. mullani* 17.*Eudichogaster prashadi* 18. *Eutyphoeus incommodus* 19. *E. nicholsoni* 20. *E. orientalis* 21. *Hoplochaetella khandalaensis* 22. *Konkadrilus bahli* 23. *Lennogaster falcifer* 24. *L. pusillus* 25. *Octochaetona sp.*[*] 26.*Octochaetona beatrix* 27. *O. paliensis* 28.*O.parva* 29. *Ramiella bishambari* 30.*R. nainiana*

[*] Represent new species.

Complimentary Contributor Copy

4. SYSTEMATIC ENUMERATION

The earthworm species collected and identified from the study area are arranged family-wise in alphabetical order. Each entry gives the information in sequence: collected and identified from the study area are arranged family-wise in alphabetical order, Earthworms' scientific name, voucher specimen number, date of collection and general habitat. A brief introductory note on each family also precedes the text.

4.1. Family: Eudrilidae

Originated in tropical West Africa and has been extended throughout the tropics, presumably as a result of transportation of one species by man since 1500A.D.

1 Eudrilus eugeniae Kinberg

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal, EWS280F1 (21.03.2016); Indore, EWS277F1 (20.03.2016), EWS177F2 (20.03.2016).
3. Diagnosis: Length 90-180 mm, diameter 4-5 mm, segments 200-220. Clitellum, inter-segmental furrows faintly indicated and setae retained, xiii, xiv-xviii. Setae closely paired. Nephridiopores lateral to *c*. Prostomium epilobic, tongue open, color reddish-blue. Septa all present from 4/5, 6/7 and several subsequent septa slightly strengthened. Gizzard in v; intestine origin close to 14/15. Typhlosole and caeca absent. Supra-intestinal glands small, paired, postseptal, in 8-42 consecutive segments of lxii-cxxxii. Testis sacs unpaired, ventral. Copulatory chamber, large, containing penis and Y-gland porophore, a groove from Y-gland pore passing up the porophore and then down nearly to the tip of the penis (Figure 5).

Complimentary Contributor Copy

4. General habitat: Mostly found in debris place, vermicompost bin, leaf litter, etc.

4.2. Family: Megascolecidae

The Megasclecidae, considerably the largest family of the oligochaeta, can be trucked back to their evolutionary staring point, which is represented by worms of the genus *Notiodrilus* as defined by Michaelsen. Its distributional range extends between warm-temperate Asia and Australia. Megascolecid distributed widely including India. Ceylon, Korea, Japan, Malaysia, New Zealand, Australia, Burma, Malay Peninsula.

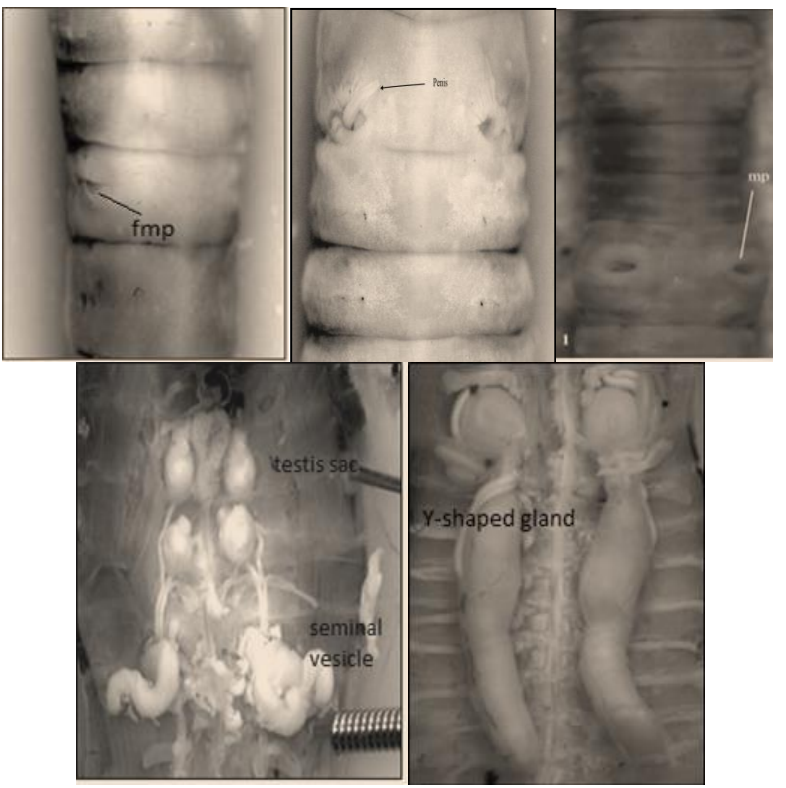

Figure 5. Diagnostic features of *Eudrilus eugeniae*.

Complimentary Contributor Copy

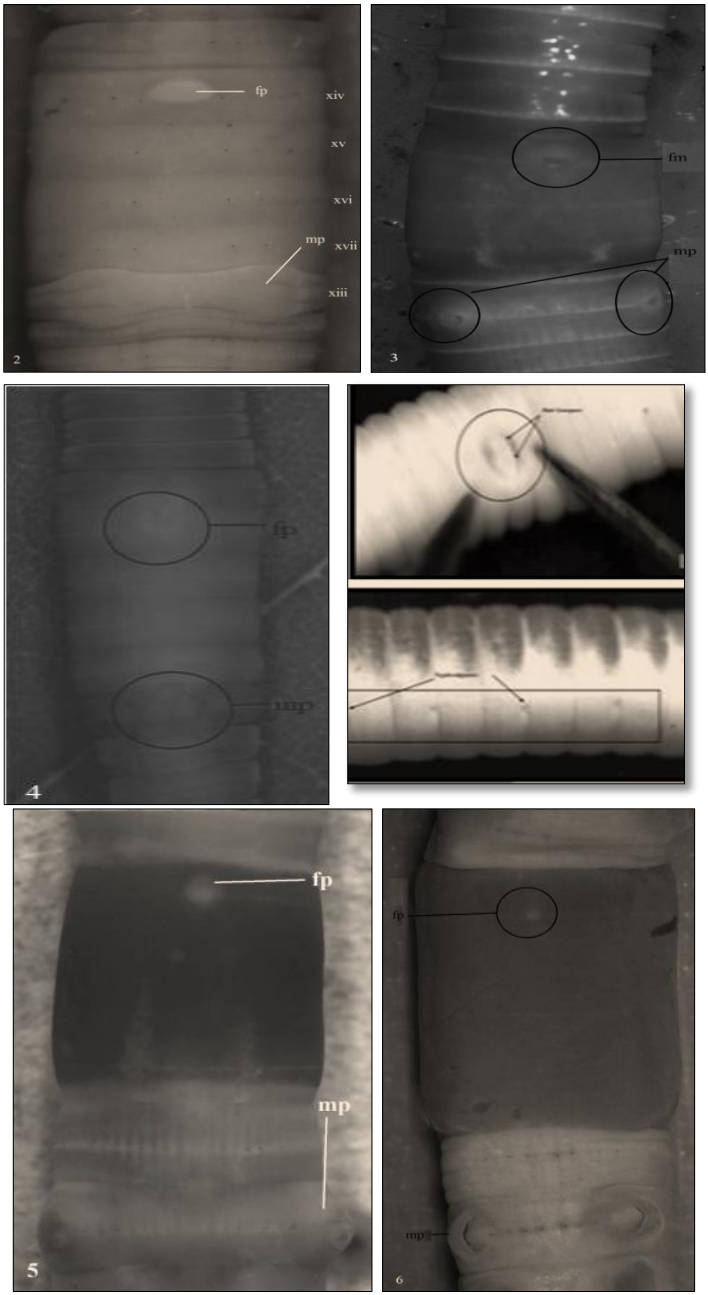

Figure 6. Genital region of *Lampito mauritii*, *Metaphere houlleti*, *Perionyx excavatus*, *P.sansibaricus*, *Pheretima canaliculata*, *Polypheretima elongate*.

Complimentary Contributor Copy

Figure 6 depicts diagnostic feature of Megascolecid of the study area.

1 *Lampito mauritii* Kinberg

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Raisen: EWS128F (12.08.2015), Vidisha: EWS129F (12.08.2015); Chambal division; Morena: EWS175F1 (29.09.2015); Gwalior division; Datia: EWS178F1 (01.10.2015); Indore division; Dhar: EWS136F2 (19.08.2015), Jhabua: EWS138F4 (20.08.2015); Jabalpur division; Katni: EWS193F1 (19.09.2015), EWS193F2 (19.09.2015); Narsinghpur: EWS139F1 (15.08.2015), EWS141F1 (15.08.2015); Narmadapuram division; Betul: EWS132F3 (15.08.2015); Rewa division; Satna: EWS191F2 (18.09.2015), Singrouli: EWS188F1 (17.09.2015); Sagar division; Chhatarpur: EWS179F1 (14.09.2015), EWS181F1 (14.09.2015), EWS181F2 (14.09.2015), EWS182F1 (14.09.2015), EWS182F2 (14.09.2015); Damoh: EWS117F2 (25.07.2015), EWS118F3 (25.07.2015), EWS120F2 (25.07.2015), EWS121F2 (25.07.2015), EWS122F1 (25.07.2015); Panna: EWS183F1 (15.09.2015), EWS184F1 (15.09.2015), EWS184F2 (15.09.2015), EWS185F3 (15.09.2015); Sagar: EWS110F2 (22.07.2015), EWS111F1 (22.07.2015), EWS112F2 (22.07.2015), EWS115F2 (25.07.2015), EWS123F3 (25.07.2015), EWS125F1 (27.07.2015), EWS126F2 (27.07.2015), EWS126F3 (12.08.2015), EWS205F3 (19.08.2015), EWS206F2 (19.08.2015), EWS207F4 (19.08.2015), EWS208F2 (21.08.2015), EWS208F3 (21.08.2015), EWS209F2 (21.08.2015), EWS218F1 (27.08.2015), EWS219F1 (28.08.2015), EWS226F2 (01.09.2015), EWS227F1 (01.09.2015), EWS230F3 (02.09.2015), EWS233F1 (04.09.2015), EWS240F (14.09.2015), EWS253F (28.09.2015), EWS254F (28.09.2015).

Complimentary Contributor Copy

3. Diagnosis: Colour greyish brown. Length 95-155 mm, diameter 3-6mm, 157-195 segments. Prostomium prolobic, tongue closed. First dorsal pore 11/12. Clitellum annular xiv-xvii. Setae perichaetine. Male pores in xviii on slightly raised porophores, at or lateral to *b*. Female pores in xiv presetal within *aa*. Spermathecal pores paired 6/7/8/9 (3 pairs). Genital markings absent. Septa present from 4/5, 7/8-12/13 muscular. Last pair of hearts in xiii. Spermathecae bidiverticulate paired in vii-ix, each with a median and a lateral digitiform diverticula. Holandric; seminal vesicles in ix and xii. Pineal setae ornamented with closely crowded circles of triangular teeth, tip horseshoe shaped, 1.32-2mm long, 24-31µ diameter. Ovaries, fan shaped, with several eggs- strings. Digestive system, with a single gizzard in v segment, longitudinal calciferous lamellae with free median margins in x-xii, intestinal origin in region of xv-xvi, with a typhlosole but without caeca and supra-intestinal glands.
4. General habitat: Usually reported in soil with high organic matter.

2 *Metaphere houlleti* Michaelsen

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Jabalpur division; Balaghat: EWS156F1 (18.08.2015), EWS157F1 (18.08.2015); Mandla: EWS203F2 (23.11.2015); Seoni: EWS202F2 (04.11.2015); Sagar division; Panna: EWS199F1 (25.10.2015), EWS200F1 (26.10.2015), EWS201F2 (27.10.2015); Sagar: EWS208F4 (21.08.2015), EWS213F3 (25.08.2015), EWS246F1 (21.09.2015), EWS261F1 (30.09.2015); Shahdol division; Umaria: EWS196F2 (21.10.2015), EWS198F1 (22.10.2015).
3. Diagnosis: Length 70-200 mm, diameter 4-6mm, segments 100-110. Colour purplish brown on dorsum, with still darker median strip, pale on ventral surface, clitellum pale, prostomium epilobous. First dorsal pore from 12/13. Clitellum

Complimentary Contributor Copy

xiv-xvi (=3). Male pores on papillae, about one-third of circumference apart, in line with *b*. Spermathecal pores three pairs in 6/7/8/9. No genital papillae. Septa 5/6-7/8 thickened, 8/9 and 9/10 wanting, 10/11-13/14 thickened. Caeca originating in xxvii with constriction. Testis sacs in x and xi, those in xi united ventrally, those in x apparently separate. Seminal vesicle in xi and xii of a considerable size much cut up into lobes. Spermathecal ampulla irregularly shaped, duct straight, as long as ampulla, thick, narrowing a little towards ectal end, diverticulum arising from near ectal end of duct, long, tubular, its ental portion much convoluted.

4. General habitat: Abundantly found in *Dalbergia sisoo* leaf litter rich soil.

3 *Perionyx excavatus* Perrier

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Jabalpur division; Jabalpur: EWS144F2 (16.08.2015); Seoni: EWS151F1 (17.08.2015); Sagar division; Sagar: EWS115F3 (25.07.2015), EWS211F2 (24.08.2015), EWS211F2 (24.08.2015), EWS252F1 (28.09.2015).
3. Diagnosis: Length 40-170 mm, diameter 3-7 mm, 123-180 segments. Prostomium epilobic, tongue open. First dorsal pore in 4/5. Setal arrangement perichaetine. Quadrithecal, pores near *mV*, at 7/8-8/9. Clitellum annular xiii-xvii. Male pores on small papillae in a single male field. Spermathecal pores paired near mid-ventral line in 7/8/9. Genital marking absent. Septa all present from 4/5. Gizzard muscular on v segment. Intestinal origin in xv. Last heart in xii. Nephridia avesiculate, Holandric. Seminal vesicles in xi and xii, last pair often continued in pockets of 12/13 back to level of 14/15. Prostates in xviii, ducts short and straight. Spermathecae large, duct short and stout.
4. General habitat: Abundantly found in organic matter rich soil.

4 *Perionyx sansibaricus* Michaelsen

Complimentary Contributor Copy

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s) Sagar division; Sagar: Patheria village EW-48 (17.08.2015); Dr H.S. Gour University campus EW-61 (24.08.2015), EW-64 (28.09.2015); Naura Dehi Reserve Forest EW-63 (24.08.2015); Semra village, Rehli EW-90(28.09.2015); Juna village, Rehli EW-91 (24.08.2015).
3. Diagnosis: Figure 2. Length 60-90 mm; body segments 110-165. Colour purple dorsally, pale ventrally. Prostomium epilobic. First dorsal pore in any furrows 3/4/5/6. Setae many per segments. Clitellum annular from segment xiii-xvii. Male pore on segment xviii, close together in a median depressed area. Female pore single median, presetal on segment xiv. Spermathecal pores 3 pairs on 6/7/8/9 close to mid ventral line. Nephridiopores at two levels regularly alternating between dorsolateral and ventrolateral positions on each side. Holonephric, Nephridia stomate with preseptal funnel. Penial setae absent.

5 *Pheretima canaliculata* Gates

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Bhopal: EWS279F1 (21.03.2016).
3. Diagnosis: Length 140-150 mm, diameter 4-5 mm, segments 110-140. Color in dorsum, slate anterior to clitellum, posteriorly reddish. Prostomium, epilobic, tongue open. Male pore in xviii, minute, superficial. Female pore median. Genital markings one pair, between equators of xvii and xviii, slightly median to male pore levels. First dorsal pore 7/8. Intestinal origin in xv. Holandric, testis sacs, unpaired, ventral. Seminal vesicles, large in xi,xii. Prostates, in xvii-xx, ducts short, stout, L-shaped.
4. General habitat: Abundantly found in organic matter rich soil.

Complimentary Contributor Copy

6 *Polypheretima elongata* Perrier

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Jabalpur division; Balaghat: EWS154F2 (18.08.2015); Mandla: EWS158F1 (19.08.2015), EWS159F1 (19.08.2015); Seoni: EWS151F3 (17.08.2015), EWS152F1 (17.08.2015), EWS202F1 (04.11.2015); Narmadapuram division; Hoshangabad: EWS202F (04.11.2015); Sagar division; Damoh: EWS194F3 (20.09.2015), EWS195F (20.09.2015); Panna: EWS200F2 (26.10.2015); Sagar: EWS244F2 (17.09.2015), EWS245F1 (18.09.2015); Shahdol division; Anuppur: EWS164F2 (20.08.2015); Umaria: EWS170F2 (21.08.2015), EWS171F1 (21.08.2015).
3. Diagnosis: Length 100-235 mm, diameter 4-5 mm, body segments 221. Colour greyish yellow. Prostomium rudimentary, first dorsal pore in 11/12. Setal rings closed dorsally, ventral setae enlarged in anterior part of the body but diminishing regularly from the middle line. Clitellum usually without setae annular xiv-xvi. Male pores superficial on xviii segment. Female pores on xiv. Spermathecal pore two pairs in 5/6-6/7. Septa 5/6 and 6/7 much and 7/8 very much thickened. No intestinal caeca, last heart in xii. Seminal vesicles in xi, xii and xiii. Prostates fairly large glandular on xvi xxi.
4. General habitat: Rotting leaves, soil in wet ravines.

4.3. Family: Moniligastridae

They are distributed from Southeast Asia and eastern Asia form South India to Manchuria, Korea also Japan, the Philippines, Borneo, Sumatra.

Complimentary Contributor Copy

Gates (1972) stated this range by autochthonous forms has been extended as a result of transportation of species of *Drawida*.The genital region of recorded *Drawida* species of study area shown in Figure 7.

1 *Drawida bahamensis* Beddard
   1. Origin: Exotic
   2. Locality and voucher specimens with collections no (s) and date (s): Rewa division; Satna: EWS190F2 (18.09.2015); Sagar division; Sagar: EWS232F2 (04.09.2015).
   3. Diagnosis: Length 30 mm, diameter 2 mm, segments 150. Dorsal pore absent. Clitellum from 10^{th}-13^{th} Segment (=4). Male pores and spermathecal pore between the setal lines *b* and *c*; segment 5/6-8/9 thickened. 3 gizzards in the 13^{th}-15^{th} segment. Ovaries in segment xi. Spermatheca with pear-shaped ampullae, long, tortuous duct and easier fairly large atrium-like enlargement at the distal end.
   4. General habitat: Loam soil rich in moisture and organic matter.
2 *Drawida chlorina* Bourne
   1. Origin: Exotic
   2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Panna: EWS183F2 (15.09.2015).
   3. Diagnosis: Length 120 mm, diameter 3.5 mm, 140 segments. Body colour greenish. Nephridiopores in *cd*. Dorsal pore absent. Male pores between *b* and *c* or nearer to c. Female pores in *ab*. Spermathecal pores in *cd*. Septa 5/6-8/9 thickened. Four gizzards, in xiv-xvii. Testis sacs ovoid, with rather pointed ends. Prostates hemispherical, of glandular appearance. Ovaries free in xi segment. Spermathecae with pear shaped or ovoid ampulla and small atrial dilatation at ectal end.
   4. General habitat: Mostly found sandy soil.
3 *Drawida minuta* Bourne
   1. Origin: Exotic

Complimentary Contributor Copy

2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS262F2 (01.10.2015).
3. Diagnosis: Length 40 mm, diameter 1.5 mm, 140segments. Strongly pigmented. Nephridippores in *cd*. Dorsal pore missing. Male pores, female pores and spermathecal pores in *ab*. Septa 5/6-8/9 very slightly thickened. 2 or 3 gizzards, in xii to xiii or xiv. Testis sacs ovoid. Prostates hemispherical. Ovaries freely segment xi, without ovarian chamber; ovisacs extend back at least to xv. Spermathecal ampulla ovoid; atrium a bifid widening of the duct at its ectal end.
4. General habitat: Generally found in sandy soil.

4 *Drawida pellucida* Bourne

1. Origin: Exotic
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS123F1 (25.07.2015), EWS123F2 (25.07.2015).
3. Diagnosis: Length 65-180 mm, diameter 3-4 mm, segments 130-160. Without pigment; body-wall very transparent. Male pores between b and c; in segment x. Female pores inconspicuous. Spermathecal pores in between b and c but nearer to a, on segment 8/9. Septa 5, 6-8, 9 thickened. Nephridiopores in cd. Gizzards 4-5. Testis sacs spherical or ovoid, mainly in x. Prostates as flattened hemispheres. Ovaries free in segment xi, without ovarian chamber; ovisacs present.
4. General habitat: Mostly found in sandy moisture soil.

5 *Drawida willsi* Michaelsen

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS124F3 (27.07.2015), EWS233F2 (04.09.2015), EWS230F1 (02.09.2015), EWS262F3 (01.10.2015).
3. Diagnosis: Length 50-65 mm, maximum diameter 2.5 mm, segments 155-180. Colour bluish grey. Prostomium prolobous. Nephridiopores in *cd*. Clitellum ring-shaped, x-xiii (=4). Male

Complimentary Contributor Copy

pores in segment 10/11 on setae ab. Genital marking paired on 09/10 setae *ab*. Female pores and spermathecal pores inconspicuous. Septa 6/7-8/9 thickened. Gizzards in 2-4 on segment xii-xvi.

4. General habitat: Mostly found in sandy moist soil.

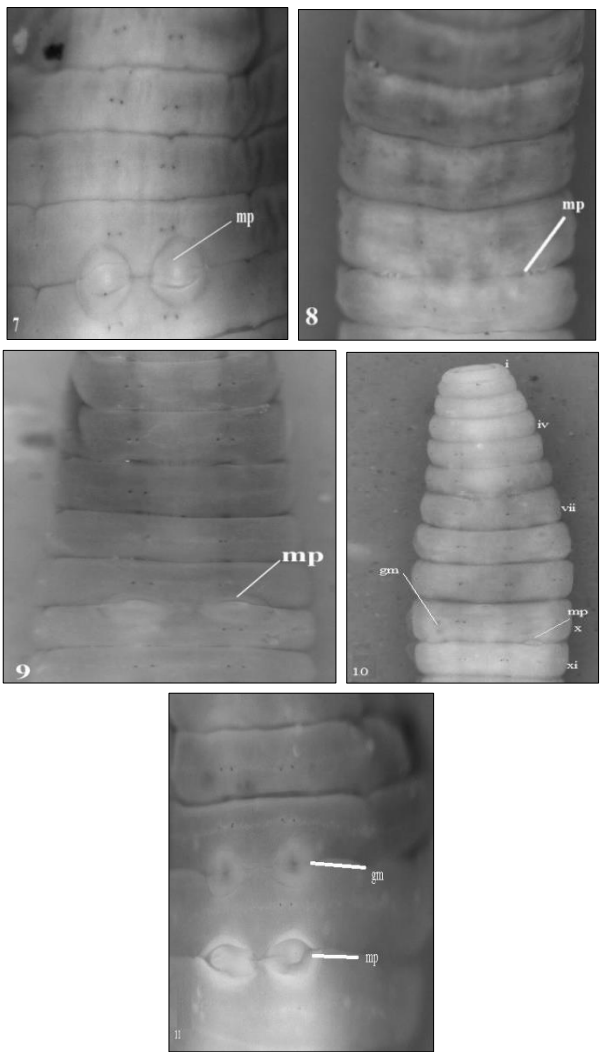

Figure 7. Genital region of *Drawida bahamensis, D. chlorina, D. minuta,D. pellucida, D. willsi*.

Complimentary Contributor Copy

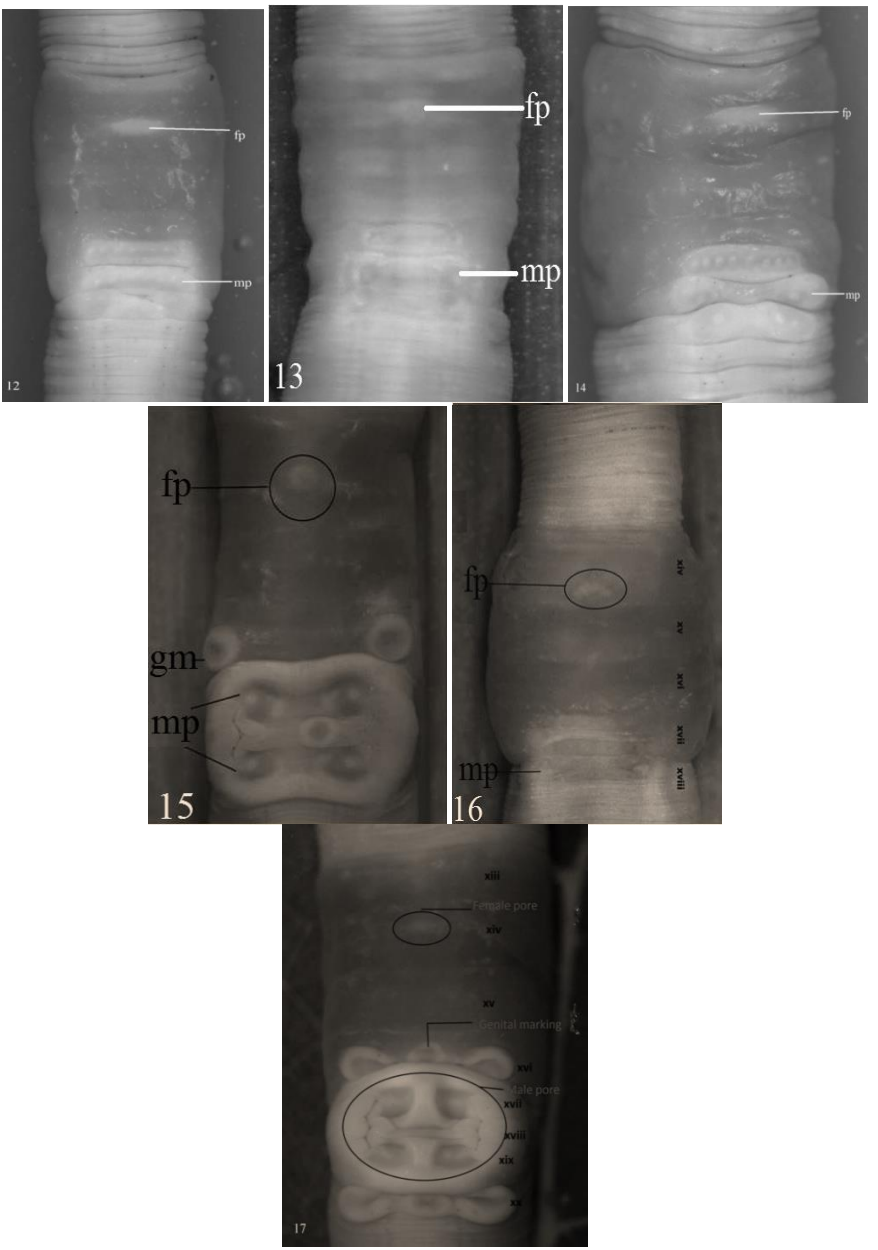

Figure 8. Genital region of *Barogaster annandalei*, *B. barodensis*, *B. prashadi*, *Eudichogaster ashworthi*, *E. mullani*, *E. prashadi*.

Complimentary Contributor Copy

4.4. Family: Octochaetidae

The Indian Octochaetidae comprises 26 genera, of which 25 are restricted in their distribution to the Indian subcontinent (excluding peregrine species) and one genus *Dichogaster*, with endemicity in Africa and South America is represented by widely-distributed peregrine species. The diagnostic features (genital margin) of Octochaetid of the study area shown in Figure 8-13.

1 *Barogaster annandalei* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Damoh: EWS118F1 (25.07.2015); Sagar: EWS113F1 (25.07.2015), EWS114F (25.07.2015), EWS115F1 (25.07.2015).
3. Diagnosis: Length 95-115 mm, diameter 4-5 mm, 130-150 segments. Prostomium prolobic retracted, first dorsal pore 12/13. Clitellum xiii-xvii. Setal arrangement lumbricine, Setae aa = 2.4-3.6 ab = 1.2-2.1 bc = 2.2-3.6 cd = 0.18 dd on xii, aa = 3.6-4.7 ab = 1.5-1.6 bc = 2.5-3.5 cd = 0.2 dd on xxiv. Male genital field transversely depressed on xviii. Combined male and prostatic pores minute, discharging on oval porophores at b. Female pores slightly anterior to the setal arc. Spermathecal pores minute, on anterior margin of viii, slightly posterior to 7/8, at or just lateral to *b*. Genital markings small oval, 3-6 in transverse rows, at *bb*, 2 rows, pre and postsetal on xviii, sometimes additional single rows, presetal on vii, post setal on viii and xvii. Oesophagus with two gizzards. Supra-intestinal ‘grid like’ thickening extends through 6(+) or 10 segments, in lxi or lxxxvi to lxvi (+) or xcv, typhlosole in xxvii-xxix to the last ‘grid’ segment. Testes and male funnels free in x and xi, seminal vesicles in ix and xii. Penial setae unornamented 0.58-0.66 mm long, 16-22µ diameter. Each spermatheca with a

Complimentary Contributor Copy

median flattened ental diverticulum adherent to the duct, which is shorter than ampulla. Genital marking glands absent.

4. General habitat: Found in moist soil.

2 *Barogaster barodensis* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS205F2 (19.08.2015), EWS207F3 (19.08.2015), EWS218F2 (27.08.2015), EWS221F2 (29.08.2015), EWS225F2 (31.08.2015), EWS226F4 (01.09.2015), EWS229F2 (02.09.2015), EWS231F2 (02.09.2015).
3. Diagnosis: Length 60-105 mm, diameter 3-4 mm, 138-170 segments. Prostomium prolobic. First dorsal pore 12/13. Clitellum xiii-xvii, it covers a little portion of xii and xviii segment. Setal arrangement lumbricine, Setae aa=4.1 ab=1.4 bc=2.4 cd=0.24 dd on xii, aa=5.3 ab=1.4 bc=2.7 cd-=0.24 dd on xxiv. Combined male and prostatic pores minute, discharging on oval porophores at b. Female pores presetal. Spermathecal pores minute, on oval tumescence, in 7/8, at or just lateral to b. Genital markings small oval in shape in single transverse rows. Oesophagus with two gizzards. Supra-intestinal 'grid like' thickening extends through 8-12 segments, in 1xviii-1xxix to 1xxviii-1xxxix; typhlosole in xxiv-xxvi to the last segment. Testes and male funnels free, in x and xi; seminal vesicles in ix, x and xii. Each spermatheca with a cauliflower-shaped sessile ental diverticulum, duct shorter than ampulla. Genital marking gland absent.
4. General habitat: Found in soil around compost pits and rotten wood moist soil, thatched roof of a house.

3 *Barogaster prashadi* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Bhopal: EWS130F2 (13.08.2015), EWS130F3 (13.08.2015), EWS130F4 (13.08.2015),

Complimentary Contributor Copy

EWS130F5 (13.08.2015); Sehor: EWS131F2 (14.08.2015), EWS131F3 (14.08.2015), EWS131F4 (14.08.2015); Indore division; Barbani: EWS137F2 (19.08.2015), EWS137F3 (19.08.2015); Khandwa: EWS135F1 (18.08.2015), EWS135F2 (18.08.2015); Jabalpur division; Chhindwara: EWS147F2 (17.08.2015), EWS149F2 (17.08.2015), EWS150F (17.08.2015); Narsinghpur: EWS142F1 (15.08.2015); Narmadapuram division; Betul: EWS132F5 (15.08.2015); Sagar division; Damoh: EWS121F1 (25.07.2015); Panna: EWS201F1 (27.10.2015); Sagar: EWS110F1 (22.07.2015), EWS111F2 (22.07.2015), EWS112F1 (22.07.2015), EWS124F1 (27.07.2015), EWS124F5 (27.07.2015), EWS127F (27.07.2015), EWS219F2 (28.08.2015), EWS220F (28.08.2015), EWS221F1 (29.08.2015), EWS226F3 (01.09.2015), EWS227F2 (01.09.2015), EWS228F1 (01.09.2015); Shahdol division; Umaria: EWS197F (21.10.2015).

3. Diagnosis: Length 40-43 mm, diameter 4-5 mm, 140 segments. Prostomium prolobic, first dorsal pores 12/13, clitellum xiii-xvii. Setal arrangement lumbricine, setae aa =3.6 ab=bc=3 cd=0.23 dd on xii, aa=3.8 ab=bc=2.5 cd=0.21 dd on xxiv. Combined male and prostatic pores minute, on oval slightly raised porophores at b. Female pore on xiv presetal. Spermathecal pores tiny, transverse slits in 7/8, a little lateral to b. Genital marking small 4-7 in single transverse rows, at about bb, post setal on viii, xix and xx, on setal annulus on xvii. Testes and male funnels free in x and xi, seminal vesicles in ix, x and xii. Penial setae absent. Genital marking glands absent.
4. General habitat: Found in moist soil.

4 *Eudichogaster ashworthi* Michaelsen

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Gwalior division; Gwalior: EWS177F1 (30.09.2015);

Complimentary Contributor Copy

Jabalpur division; Chhindwara: EWS148F1 (17.08.2015), EWS149F1 (17.08.2015); Dindori: EWS162F1 (19.08.2015); Seoni: EWS151F2 (17.08.2015), EWS152F3 (17.08.2015), EWS153F1 (17.08.2015); Sagar division; Damoh: EWS194F2 (20.09.2015); Sagar: EWS206F1 (19.08.2015), EWS215F1 (25.08.2015), EWS216F2 (25.08.2015), EWS217F1 (27.08.2015), EWS217F2 (27.08.2015), EWS244F1 (17.09.2015), EWS256F (29.09.2015); Shahdol division; Anuppur: EWS166F2 (20.08.2015).

3. Diagnosis: Length 90-120mm, diameter 5-7mm, 163-198 segments. Unpigmented, yellowish grey in appearance. Prostomium prolobic. First dorsal pore 11/12 or 12/13. Clitellum ring shaped, but ventrally less developed than dorsally; ½ xiii-xvi. The rectangular male field comprises xvii-xix, extends outwards beyond the line of b, and is somewhat raised. Prostatic pore on xvii and xix, on small papillae in b; the pores connected by E-shaped seminal grooves, with a double convexity outwards. Spermathecal pores minute at ab, those of viii on setal annulus (equatorial), those of ix on presetal annulus, slightly nearer to 8/9 than to the setal arc and within genital markings. Area of female pore fairly large, median, transversely oval, situated anteriorly on xiv. Spermathecal pore two pairs on papillae in ab on the anterior annulus of viii and ix. Septa 5/6-7/8 very strong, 8/9-10/11 successively less strong. Two almost spherical gizzards in v and vi. Two pairs of retort- shaped calciferous glands. In xi and xii. Intestine begins in xiv. Last heart in xii. A pair of large nephridia in addition to the micronephridia, near the nerve cord in each segment. Two pairs of funnels, the anterior rather smaller in x and xi. Supra-intestinal glands 8 pairs, in lxxxviii-1xxxvii to lxxxv-xciv. Testes and male funnels in x and xi, male funnels all of same size; seminal vesicles in ix, x and xii. Each spermatheca with a flat, disc-like, vertically placed ental diverticulum. Enlarged setae a, b on viii-xxxiv ornamented

Complimentary Contributor Copy

with short transversely placed ridges or rows of very fine thorn-like teeth, 0.20-0.47mm long. Genital marking glands absent.

4. General habitat: Found in soil around compost pits; rotten woods and in domestic garbage.

5 *Eudichogaster mullani* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS205F1 (19.08.2015), EWS207F1 (19.08.2015).
3. Diagnosis: Length 95-130, diameter 6 mm, segments 187-200, Grey in Colour. Prostomium prolobic. First dorsal pore 11/12 or 12/13. Clitellum xiii-xvii. Segments xvii-xix depressed mid-ventrally. Prostatic pores apparently on four small papillae at the angles of the depression in line with b, slightly in front of the setal zone of xvii and behind that of xix. Spermathecal pores minute in front of the setal zones of viii and ix slightly lateral to a. Genital markings circular, unpaired and median. Male genital field indistinct. Male pores minute, in seminal grooves on xviii at b. Septa 5/6-10/11 moderately strong, 8/9 and 9/10 the thickest, 11/12 also somewhat thickened. Gizzard in v and vi, the posterior of the two rather smaller. Calciferous glands in xi and xii, attached by one edge to the esophagus. Last heart in xii. Micronephridia behind genital region in a transverse row in each segment, about nine on each side. Seminal vesicles in ix, x and xii.Prostates small in xvii and xix, the glandular part in a few loose loops: duct thin, shinning, of same diameter as glandular part. Supra-intestinal glands 10 pairs, in xc-xcix. Holandric, testes and male funnels free in x and xi. Each spermathecae with a small wart-like ental diverticulum, duct much shorter than ampulla. Genital marking gland absent.

Complimentary Contributor Copy

4. General habitat: Found in sandy loam and clay loam soils in grasslands, upland crop fields and around roots of potted plants.

6 *Eudichogaster prashadi* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Chambal division; Morena: EWS176F1 (29.09.2015); Gwalior division; Datia: EWS178F2 (01.10.2015); Gwalior: EWS177F2 (30.09.2015); Indore division; Jhabua: EWS138F1 (20.08.2015), EWS138F2 (20.08.2015), EWS138F3 (20.08.2015); Khandwa: EWS134F1 (17.08.2015), EWS134F2 (17.08.2015); Jabalpur division; Chhindawara: EWS147F1 (17.08.2015); Mandla: EWS158F2 (19.08.2015), EWS160F1 (19.08.2015), EWS158F2 (19.08.2015), EWS203F1 (23.11.2015); Dindori: EWS161F1 (19.08.2015), EWS163F1 (19.08.2015); Narsinghpur: EWS139F2 (15.08.2015), EWS140F2 (15.08.2015), EWS141F2 (15.08.2015); Seoni: EWS151F4 (17.08.2015), EWS202F3 (04.11.2015); Narmadapuram division; Harda: EWS133F1 (16.08.2015), EWS133F2 (16.08.2015); Sagar division; Sagar: EWS212F2 (24.08.2015), EWS212F3 (24.08.2015), EWS212F4 (24.08.2015), EWS213F1 (25.08.2015), EWS213F2 (25.08.2015), EWS215F1 (25.08.2015), EWS216F2 (25.08.2015), EWS218F3 (27.08.2015), EWS218F5 (27.08.2015), EWS222F1 (31.08.2015), EWS223F1 (31.08.2015), EWS224F1 (31.08.2015), EWS224F4 (31.08.2015), EWS226F1 (01.09.2015), EWS244F3 (17.09.2015), EWS247F2 (21.09.2015), EWS250F2 (24.09.2015), EWS251F (24.09.2015), EWS255F (28.09.2015), EWS257F (29.09.2015), EWS258F (30.09.2015), EWS259F (30.09.2015), EWS260F (30.09.2015); Shahdol division; Anuppur: EWS164F1 (20.08.2015), EWS165F1 (20.08.2015), EWS166F1 (20.08.2015); Shahdol: EWS167F1 (21.08.2015), EWS168F1

Complimentary Contributor Copy

(21.08.2015), EWS169F1 (21.08.2015); Umaria: EWS170F1 (21.08.2015), EWS170F2 (21.08.2015), EWS196F1 (21.10.2015), EWS198F2 (22.10.2015).

3. Diagnosis: Length 75-105 mm, diameter 5-9mm, 140-152 segments. Colour yellowish brown, with only a slight difference between dorsal and ventral surfaces, prostomium prolobic. Dorsal pores from 11/12. In general setae aa =3.2-3.6, ab=1.3-1.6, bc= 2.5-2.6, cd=0.27-0.28 dd on xii, aa=4.5-4.6, ab=1.3-1.6, bc= 2.3-2.7, cd=0.22 dd on xxiv. Male genital field tumescent without special demarcation, on xvii-xix, with or without a deep slit-like depression along xiii, and with a deep longitudinal depression on xvii and xix. Male pores minutes in seminal grooves on the setal arc of xviii at *ab*; prostatic pores minute, at the ends of seminal grooves, on xvii and xix, at ab, seminal groove biconcave. Spermathecal pores minute, on the setal arcs of viii and ix, at ab. Genital markings circular to oval. Septum 4/5 thin, 5/6-9/10 moderately strengthened, 10/11 slightly so, 11/12 still less so. Gizzards in v and vi, large, rounded and firm. Calciferous glands shortly stalked in xi and xii. Intestine begins in xv. Last heart in xii. Nephridia in five longitudinal rows on each side of the body. Supra-intestinal glands 7-9 pairs, in 1xxvi-1xxxix. Holandric, testes and male funnels free in x and xi; seminal vesicle in ix and xii, Each spermatheca with a flat, disc-like, multiloculate ental diverticulum, duct about as long as or slightly shorter than ampulla.
4. General habitat: Found in moist and organic matter rich soil.

7 *Eutyphoeus incommodus* Beddard

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Jabalpur division; Katni: EWS192F1 (19.09.2015), EWS193F3 (19.09.2015); Rewa division; Satna: EWS191F1 (18.09.2015); Sidhi: EWS187F1 (17.09.2015), EWS187F2

Complimentary Contributor Copy

(17.09.2015); Singrauli: EWS188F2 (17.09.2015), EWS189F1 (17.09.2015).

3. Diagnosis: Length 95-120 mm, diameter 4 mm. Segments 120-16. Colour brownish olive. Dorsal pores from 11/12 or 12/13. Prostomium combined pro- and epilobous. Setae all ventral; in middle of the body ab=1/3 or 2/3aa=4/7bc=3/4cd; in front of genital region ab=1/2aa=1/2-4/7bc=2/3cd or more. Clitellum xiii-xvii or ½ xvii. Female pores in front of setae a. Spermathecal pores slit-like in 7/8, between b and c. Genital papillae four pairs, close to the posterior border of their respective segments, on xiii-xvi (almost on grooves 13/14, 16/17), almost circular with a rim of white surrounding a darker central area in ab, their diameter equals to ab. Septa 4/5, 5/6, 8/9-10/11 strengthened, 6/7 and 7/8 absent: 11/12 present. Gizzard large. Calciferous glands in xii and extending in xi also. Intestinal caeca in middle of body. Last heart in xiii; dorsal vessel continued forewords on to pharynx. Testes and funnels free in x and xi, those in x usually smaller than those in xi.Seminal vesicles in ix and xii. Prostatic duct much bent once or twice. Spermathecal ampulla large, globular, diverticula forming a complete frill of seminal chambers round duct. Penial setae 1mm long and almost straight.
4. General habitat: Recorded in agricultural fields, plant nurseries, gardens, grasslands, understones on the banks of ponds.

8 *Eutyphoeus nicholsoni* Beddard

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Chambal division; Bhind: EWS173F1 (28.09.2015), EWS174F2 (28.09.2015); Rewa division; Rewa: EWS186F1 (16.09.2015), EWS186F3 (16.09.2015), EWS186F4 (16.09.2015).
3. Diagnosis: Length up to 185 mm; diameter up to 5mm. Segments 190-225; secondary annulations behind iii; in some

Complimentary Contributor Copy

pre-clitellar segments as many as four secondary annuli, behind clitellum three. Colour dorsally brownish to violet-grey, ventrally yellowish grey. Prostomium combined pro and tanylobous. Dorsal pores apparently begins in front of clitellum. Clitellum 1/3 xiii or all xiii to xvii (=4 1/3 to 5). Male pores near together, surrounded by a common ridge. Female pore single on left side in front of seta *a* of xi. Spermathecal pores in *a*. Genital papillae circular or slightly oval in 15/16, close together, surrounded by a common wall or groove, an separated from each other in the middle line by a groove; occupying most of the spaces between setal zones of xv and xvi, and laterally extending beyond the line of b. Septa 4/5 and 8/9-10/11 very strong; 5/6-7/8 absent. Intestine begins in xv; intestinal pouches five pairs, beginning about lxxxiv. Seminal vesicles long, extending back to xiv, flattened, the margins somewhat lobulated. Prostates tightly coiled; duct muscular, in an S-like curve, of fair length, much thinner than the glandular part. Spermathecal ampulla broad and short, somewhat lobed, the lobes showing a number of small lobular protuberances; duct long half as thick as ampulla, diverticulum fan shaped. Penial setae about 4 mm long.

4. General habitat: Recorded in paddy fields.

10. Eutyphoeus orientalis Beddard

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Chambal division; Bhind: EWS173F2 (28.09.2015), EWS174F1 (28.09.2015); Rewa division; Rewa: EWS186F5 (16.09.2015).
3. Diagnosis: Length 160-250 mm; diameter 5-8 mm. Segments 190. Dorsal pores present behind clitellum. Clitellum includes xiv and a small part of xiii to xvii (=more than 4). The male area on xvii, presents a pair of bracket shaped grooves, each overhung on its outer side by a thickened ridge; male pores in the posterior corner of each bracket, a little outside *b*.

Complimentary Contributor Copy

Spermathecal pores slit-like, between b and c, but nearer c. Three pairs of genital papillae, inter-segmental, in front of the male pores, transversely oval, depressed in the centre; another pair in 18/19, sometimes papillae in 19/20 and 13/14; papillae in the line with ab. Seminal vesicles extend back to xv. Prostates as large coiled tubes; ducts thinner. Spermathecal ampulla an ovoid sac, with crenate margins; duct from under surface of ampulla, short, stout, muscular; two diverticula, one on each side, each with one, two or three seminal chambers. Penial setae 2-5 mm long, 26 µ thick in middle, shaft almost straight; curved, bluntly pointed and flattened tip.

4. General habitat: Recorded in soil around compost pits.

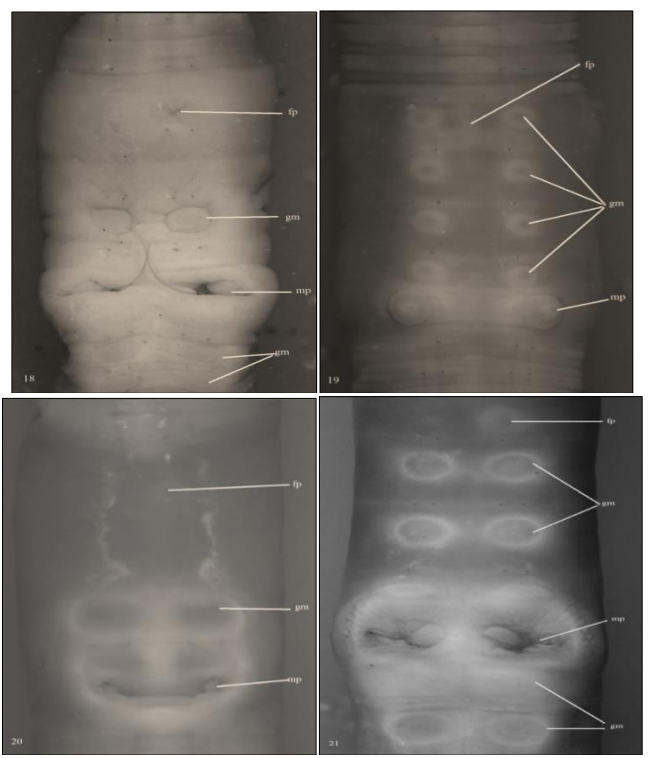

Figure 9. Genital region of *Eutyphoeus prashadi*, *E. incommodus*, *E.nicholsoni*, *E.orientalis.*

Complimentary Contributor Copy

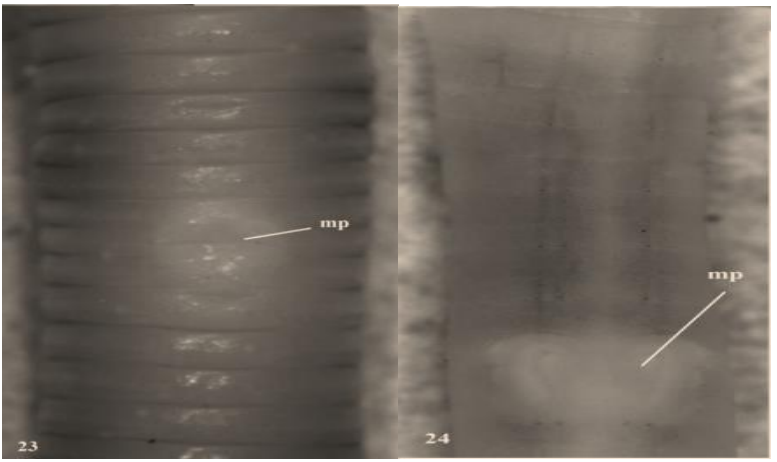

Figure 10. Genital region of *Hoplochaetella khandalaensis* and *Konkadrilus bahli*.

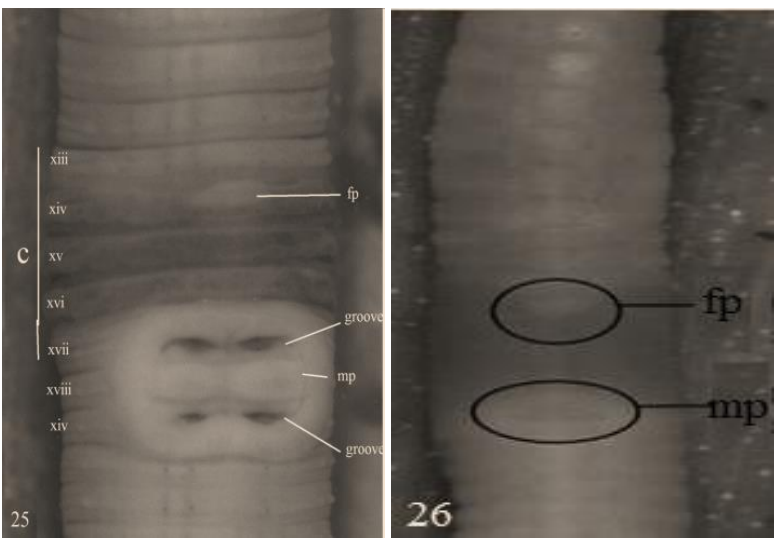

Figure 11. Genital region of *Lennogaster falcifer* and *L. pusillus*.

11 *Hoplochaetella khandalaensis* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS212F1 (24.08.2015), EWS230F2 (02.09.2015), EWS237F (08.09.2015), EWS238F (08.09.2015), EWS239F (10.09.2015).

Complimentary Contributor Copy

3. Diagnosis: Length 80-210 mm, 4-7 mm, 90-140 segments. Prostomium epilobic, tongue open. First dorsal pore 4/5 or 5/6. Clitellum ½ xiii-xvi. Setal arrangement lumbricine, setae aa=2-2.1 ab=2.1-2.3 bc= 1.2-1.5 yz=0.3-0.4 zz on xxiv; 28-41 on ii, 44-60 on vii, 48-65 on xii, 45-70 on xx; setae a-c on viii copulatory, usually shifted around anterior pair of spermathecal pores. Combined male and prostatic pores minute, at about centres of oval porophores, on or close to sites of 17/18 and 18/19 at bc. Spermathecal pores minute, 2 pairs, on viii at ac. Genital markings oval tiny slits-like apertures, unpaired and median. Septa 4/5 slightly muscular, 5/6/7/8 delicate, 8/9-12/13 muscular. Gizzard single in vi. Intestinal caeca absent; typhlosole xxiv-xxv to cviii. Spermathecae 2 pairs in viii, each with a circle of 8-17 ventrally directed digitiform ental diverticula. Testes and male funnels in x and xi, enclosed in unpaired sacs, formed by the peripheral union of septa 9/10/11/12; seminal vesicles in ix, x and xii.
4. General habitat: Found in moist soil.

12 *Konkadrilus bahli* Soota and Julka

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Bhopal: EWS278F (20.03.2016).
3. Diagnosis: Length 40 mm, diameter 1-1.5 mm, 100 segments, prostomium epilobic and tongue closed. First dorsal pore 10/11.Spermathecal pores inter-segmental in 7/8/9, female pores paired. Calciferous glands are not discrete. Oesophagus with calciferous lamellae in xvi, slightly extending to adjacent segments. Penial setae ornamented with a few transverse rows of fine spines or teeth, tip pointed. Each spermatheca with a shortly stalked, spheroidal, ental diverticulum, duct longer than ampulla. Genital marking glands absent.
4. General habitat:Recorded around compost pits and agriculture land.

13 *Lennogaster falcifer* Stephenson

Complimentary Contributor Copy

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Bhopal: EWS130F1 (13.08.2015); Jabalpur division; Jabalpur: EWS143F1 (15.08.2015), EWS145F2 (16.08.2015), EWS146F2 (16.08.2015); Sagar division; Sagar: EWS225F1 (31.08.2015), EWS230F2 (02.09.2015), EWS233F3 (04.09.2015), EWS238F (08.09.2015).
3. Diagnosis: Length 45-60mm, Diameter 2-3mm, 135-160 segment. Prostomium epilobic. Setal arrangement lumbricine, setae aa=1.9 ab=1.2 bc=1.3-1.4 cd=0.14 dd on xii, aa=2.3-2.4 ab=1-1.1 bc= 1.4-1.7 cd=0.14 dd on xxiv, a on vii copulatory. Male genital field shortly oval between 16/17 and 19/20. Male pores minute, in seminal grooves on the setal arc of xviii, prostatic pores minute, at the ends of seminal grooves on the setal arc of xvii and xix, at a; seminal grooves concave. Spermathecal pores minute, on circular tumescent areas, presetal on viii and ix, at or slightly lateral to. Genital markings oval at aa, usually paired on xviii, sometimes unpaired and median areas of tumescence on 20/21. Septa 4/5/6/7 delicate, 7/8 slightly muscular, 8/9-12/13 muscular. Oesophagus with two gizzards on v-vi segment. Calciferous gland anterior to xiv in x-xii segment three in pairs. Spermathecae 2 pairs, in viii-ix, each with a digitiform, ental diverticula, shorter than ampulla. Typhlosole in xix-xx to 1x-1xxxiii. Holandric, testes and male funnel free in x and xi; seminal vesicle in ix and xii. Prostate two pair in xvii and xix. Penial setae ornamented with marginal triangular spines, tip chisel shaped, pointed with a short hair or spines.
4. General habitat: Recorded in loamy and clay loam soils.

14 *Lennogaster pusillus* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Indore division; Khandwa: EWS134F3 (17.08.2015);

Complimentary Contributor Copy

Jabalpur division; Katni: EWS192F3 (19.09.2015); Narsinghpur: EWS140F1 (15.07.2015); Narmadapuram division; Betul: EWS132F7 (15.08.2015); Rewa division; Satna: EWS190F3 (18.09.2015); Sagar division; Chhatarpur: EWS180F2 (14.09.2015); Sagar: EWS124F4 (27.07.2015), EWS125F2 (27.07.2015), EWS126F1 (27.07.2015), EWS208F3 (21.08.2015), EWS211F3 (24.08.2015).

3. Diagnosis: Length 18-53 mm, diameter 1-2.2 mm, 103-128 segments. Prostomium pro-epilobic. First dorsal pore 12/13. Clitellum xiii-xvii. Setal arrangement lumbricine, Setae aa=1.5-1.7 ab=0.8 bc=1-1.1 cd=0.11-0.13 dd on xii, aa=2.3-2.5 ab= 1.3 bc=1.5-1.8 cd= 0.14-0.16 dd on xxiv, no setae copulatory. Male genital field transversely thickened, on xvii; male pores paired, minute, in or near 17/18 at posterior ends of seminal grooves at b. Female pore on xiv, presetal, median. Prostatic pores paired, minute, on the setal arc of xvii at anterior ends of seminal grooves at a. Seminal grooves crescentic. Spermathecal pores paired minute on viii at a. Genital marking absent. Septa 4/5-7/8 delicate, 8/9-12/13 slightly muscular. Oesophagus with two gizzards. Calciferous gland anterior to xiv more than two pair. Typhlosole in xvii-xviii to 1xx-1xxvi. Seminal vesicles absent. Prostate one pair in xvii. Penial setae ornamented with scattered small triangular teeth, tip almost membranous, Spermathecae one pair in viii, each with a sessile, spheroidal ental diverticulum, ampulla longer than the duct.
4. General habitat: Recorded in soils rich in organic matter.

15 *Octochaetona sp*.

1. Origin: Exotic
2. Locality and voucher specimens with collections no (s) and date (s): Jabalpur division; Balaghat: EWS155F2 (18.08.2015); Jabalpur: EWS144F1 (16.08.2015), EWS145F1 (16.08.2015), EWS146F1 (16.08.2015); Narsinghpur: EWS142F2 (15.08.2015); Sagar division; Damoh: EWS117F1

Complimentary Contributor Copy

(25.07.2015), EWS118F2 (25.07.2015), EWS119F (25.07.2015), EWS120F1 (25.07.2015), EWS122F2 (25.07.2015); Sagar: EWS109F (22.07.2015), EWS113F2 (25.07.2015), EWS116F1 (25.07.2015), EWS116F2 (25.07.2015), EWS124F2 (27.07.2015).

3. Diagnosis: Length 80-155mm, diameter 3-6mm, 130-200 segments. Prostomium epilobic. First dorsal pore 12/13. Male genital field slightly depressed. Male pores minute, at or just median to a; prostatic pores minute, median to a; seminal grooves concave between setal arcs of xvii and xix. Female pores paired on xiv presetal. Spermathecal pores minute on or slightly anterior to the setal arcs of viii and ix, median to a. Discrete genital markings present, but paired circular thickened area present lateral to seminal grooves on xviii and xix. Septa 4/5, 8/9-10/11 muscular. Gizzard single between seta 4/5 and 7/8. Intestine begins in xvii, typhlosole in xxii-xxiii to lxxxix-xcviii. Last pair of hearts in xiii. Holandric, testes and male funnels free in x and xi; seminal vesicles small, in ix and xii. Penial setae ornamented with triangular teeth, tip pointed. Spermathecae paired in viii and ix, each with a spheroidal, shortly stalked ental diverticulum.
4. General habitat: Recorded in in soil with high organic matter.

16 *Octochaetona beatrix* Beddard

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Narmadapuram division; Betul: EWS132F4 (15.08.2015), EWS132F6 (15.08.2015); Sagar division; Sagar: EWS214F2 (25.08.2015), EWS222F2 (31.08.2015), EWS224F2 (31.08.2015), EWS231F (02.09.2015), EWS249F (23.09.2015).
3. Diagnosis: Length 50-80mm, diameter 3-4.5mm, 130-200 segments. Prostomium epilobic. First dorsal pore 11/12 or 12/13. Setal arrangement lumbricine, setae aa=2-2.4 ab=0.5-0.7 bc=1.1-1.4 cd= 0.09-0.1 dd on xii, aa =2.4-2.7 ab=0.8-0.9

Complimentary Contributor Copy

bc=1.3-1.5 cd=0.11 dd on xxiv, a, a on viii and ix slightly sigmoid and enlarged. Male genital field slightly depressed. Male pores minute, at or just median to a; prostatic pores minute, median to a; seminal grooves concave between setal arcs of xvii and xix. Female pores paired, on xiv presetal. Spermathecal pores minute, on or slightly anterior to the setal arcs of viii and ix, median to a. Discrete genital markings absent, but paired circular thickened area present lateral to seminal grooves, on xviii and xix. Septa 4/5, 8/9-10/11 muscular. Gizzard single between seta 4/5. Intestine begins in xvii, typhlosole in xxv to civ-cxii. Last pair of hearts in xiii. Metandric. Testes and male funnels enclosed in a sub-oesophageal, u-shaped sac, in xi, male funnels present in x; seminal vesicles small, in xii. Penial setae ornamented with triangular teeth, tip pointed. Spermathecae paired, in viii and ix, each with a spheroidal, shortly stalked ental diverticulum.

4. General habitat: Sandy loam and clay loam soils in lawns, boreal forests and grasslands.

17 *Octochaetona paliensis* Stephenson

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Indore division; Barbani: EWS137F1 (19.08.2015).
3. Diagnosis: Length 35 - 92 mm, diameter 2 - 3.5mm, 119-182 segments. Prostomium epilobic, tongue open. First dorsal pore 12/13. Setal arrangement lumbricine, setae aa = 2.7-4.3 ab = 0.9-1.1 bc =1.4-2.5 cd = 0.15-0.16 dd on xii, aa = 3.3-3.4 ab = 1.2-1.3 bc = 1.9-2.5 cd = 0.16-0.19 dd on xxiv, a, b on viii and ix copulatory being surrounded by tumescence's. Clitellum xiii-xvii. Male genital field depressed, deeply so on xvii and xix. Male pores minute at or slightly lateral to a or b; prostatic pores minute at b; seminal grooves straight or convex between the setal arcs of xvii and xix. Female pores paired. Spermathecal pores minute, presetal on viii and ix, close to setal arcs at ab. Genital markings when present, oval, unpaired

Complimentary Contributor Copy

and median or 19/20-23/24, at aa or bb, paired on xviii at bc. Septa 4/5, 7/8-11/12 muscular, 5/6/7 absent. Gizzard single between septa 4/5 and 7/8. Intestine begins in xvii, typhlosole in xxii-xxiii to lxxxix-xcviii. Last pair of hearts in xii. Holandric. Testes and male funnels free in x and xi: seminal vesicles in ix and xii. Penial setae ornamented with circles of fine spines, tip bluntly pointed 0.55-0.73 mm long, 13-17µ diameter. Each spermatheca with a clavate ental diverticulum, duct shorter than ampulla. Copulatory setae ornamented with transverse rows of serrated ridges, tip claw-shaped, 0.51-0.82mm long, 15-23µ diameter. Genital marking glands absent.

4. General habitat: Recorded insandy loam soils.

18 *Octochaetona parva* Gates

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Indore division; Barbani: EWS137F4 (19.08.2015).
3. Diagnosis: Length: 70-80 mm, Segments: 80-88; unpigmented, Prostomium open epilobous, Setae lumbricine, Clitellum bright yellow orange, annular in 13-17, setae retained, furrows obscured. Male pores, a circular hollow from ½ 17-½ 19; probably on 18, in seminal grooves which run vertically from slight prostatic porophores on 17 and 19. Male genital field indistinct, female pores paired in anterio-ventral depressed groove on xiv. Spermathecal pores two pairs, mid-ventral in viii and ix in small co-joined pad-like papillae. Spermathecae with clavate ental diverticulum duct shorter than ampulla. Testes and male funnel in x and xi. Seminal vesicles in xi and xii. Micromero-nephridia in each segment, Genital markings unpaired on 20/21/22. Caecae and Typhlosole indistinct.
4. General habitat: Recorded in sandy loam soil.

19 *Ramiella bishambari* Stephenson

1. Origin: Native

Complimentary Contributor Copy

2. Locality and voucher specimens with collections no (s) and date (s): Sagar division; Sagar: EWS113F3 (25.07.2015).
3. Diagnosis: Length 20-38 mm, diameter 0.5-1.2 mm, 130-145 segments. Prostomium epilobic, tongue closed. First dorsal pore 6/7. Clitellum annular xiv-xvi. Male pores at or slightly lateral to a; prostatic pores minute at b, seminal grooves convex between the setal arcs of xvii and xix. Female pore paired, presetal within lines. Spermathecal pores minute on viii and ix. Genital markings when present, small, circular to oval prepaired setal on vii-ix, xvii, xx, postsetal on vii, viii,x,xi, at close to ab; unpaired and median, postsetal on xix or 19/20. Septa 4/5 slightly muscular, 5/6-12/13 muscular. Oesophagus with single gizzard in vi. Intestine begins in xv, typhlosole lamelliform. Testes and male funnels free in x and xi; seminal vesicles in xii. Penial setae ornamented with a few transverse rows of triangular teeth. Each spermatheca with a sessile oval ental diverticulum. Genital marking glands absent.
4. General habitat: Recorded in moist soil.

20 *Ramiella nainiana* Gates

1. Origin: Native
2. Locality and voucher specimens with collections no (s) and date (s): Bhopal division; Sehor: EWS131F1 (14.08.2015); Sagar division; Sagar: EWS229F1 (02.09.2015).
3. Diagnosis: Length 27-64 mm, diameter 1.4-3.4 mm, 140-162 segments. Prostomium epilobic, tongue closed. First dorsal pore 3/4 or 4/5 or in region of 5/6-8/9. Setae aa=2.8-3 ab=1.2-1.3 bc=2.3-2.7 cd=0.28 dd on xii, aa=3.3-3.7 ab=1.2 bc=2.2-2.6 cd=0.2 dd on xxiv. Clitellum annular covering xiii- xvii segments. Male pores at or slightly lateral to b; prostatic pores minute at b, seminal grooves concave between the setal arcs of xvii and xix at ab. Female pore single, median. Spermathecal pores minute, presetal, on viii and ix, at or close to b. Genital markings single, median on x, xiii, intersegmental with a single central area on 16/17-19/20, 23/24-31/32. Septa 4/5-

Complimentary Contributor Copy

11/12 muscular. Oesophagus with single gizzard in vi. Intestine begins in xv, typhlosole simple, lamelliform, xvii-xix to 1xxxvii-xciv. Testes and male funnels free, in x and xi; seminal vesicles in ix and xii. Penial setae ornamented with a few irregular spaced triangular teeth, spade-shaped. Each spermatheca with a ventrally directed digitiform ental diverticulum. Genital marking glands absent.

4. General habitat: Recorded in mineral soil in forest area.

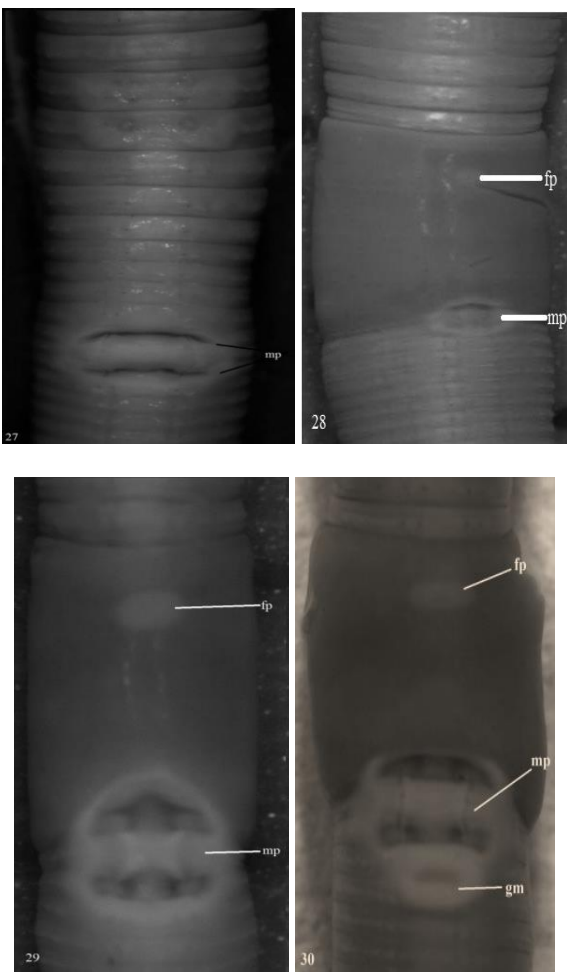

Figure 12. Genital region of *Octochaetona sp*., *O. beatrix*,*O. paliensis* and *O. parva*.

Complimentary Contributor Copy

The dichotomy in divergence allowed us to successfully conduct identification tests using the neighbor - joining tree (Figure 14). In our study the average interspecific divergence was more than 0.35%. All congeneric species pairs showed at least 25% divergence (Figure 15). Study presents the first work of earthworms of Central India with use of integrated taxonomy. The work may be helpful for DNA-based identification system for earthworms of the area and allowing for identification to be performed by anyone with access to a basic DNA sequencing laboratory. However, the molecular addition is only the tool for the identification of earthworms and can't replace the traditional morpho-anatomical species identification system especially for the earthworms. The manuscript included the digital information of genital region of 31 species belonging to 15 genera spread over four families of Oligochaete fauna from Madhya Pradesh (State located in central part of the country), India.

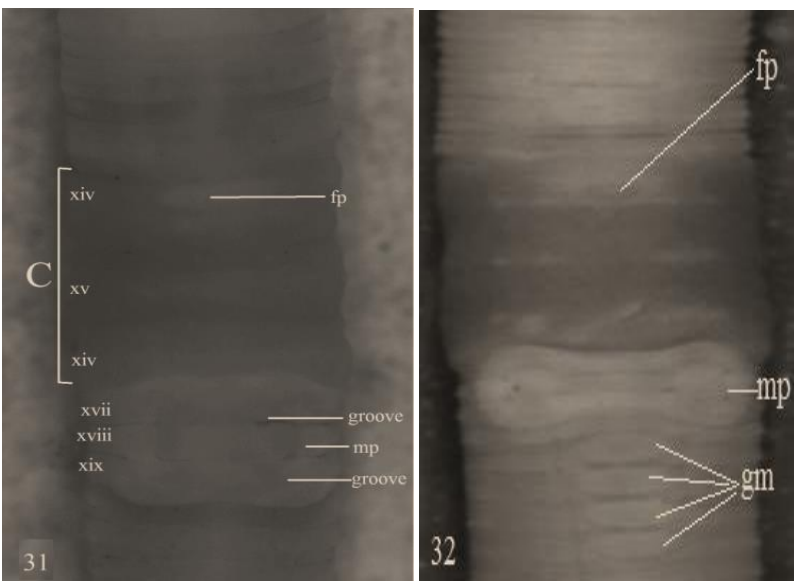

Figure 13. Genital region of *Ramiella bishambari* and *R. nainiana*.

Complimentary Contributor Copy

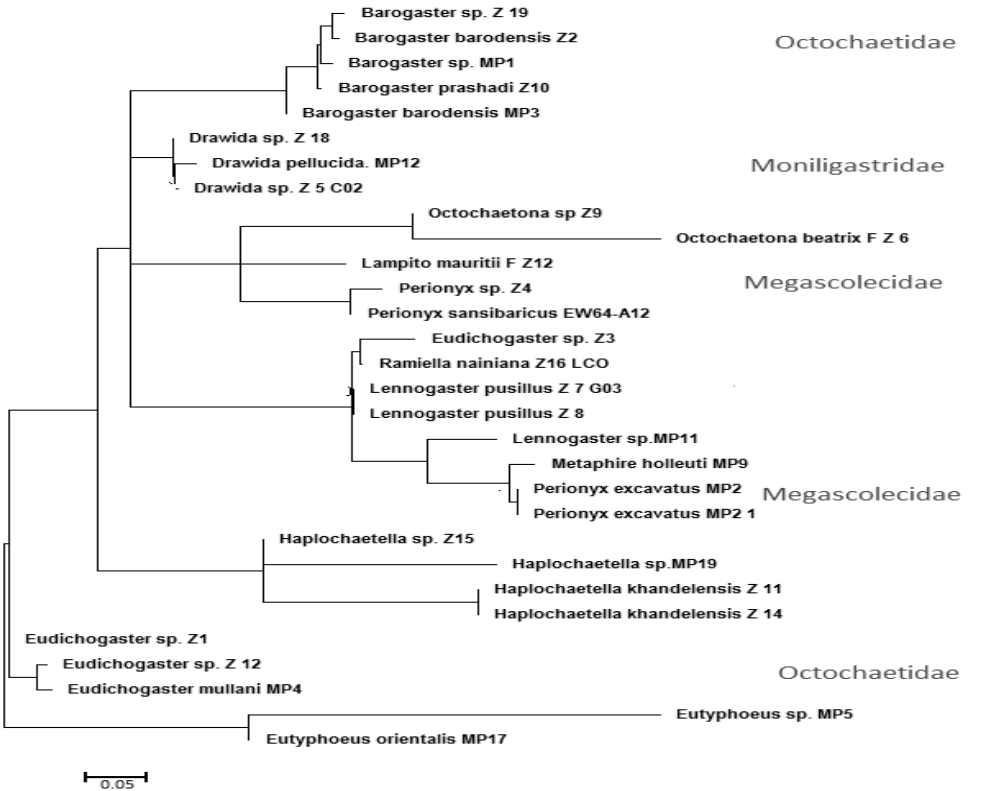

Figure 14. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei mode.

Complimentary Contributor Copy

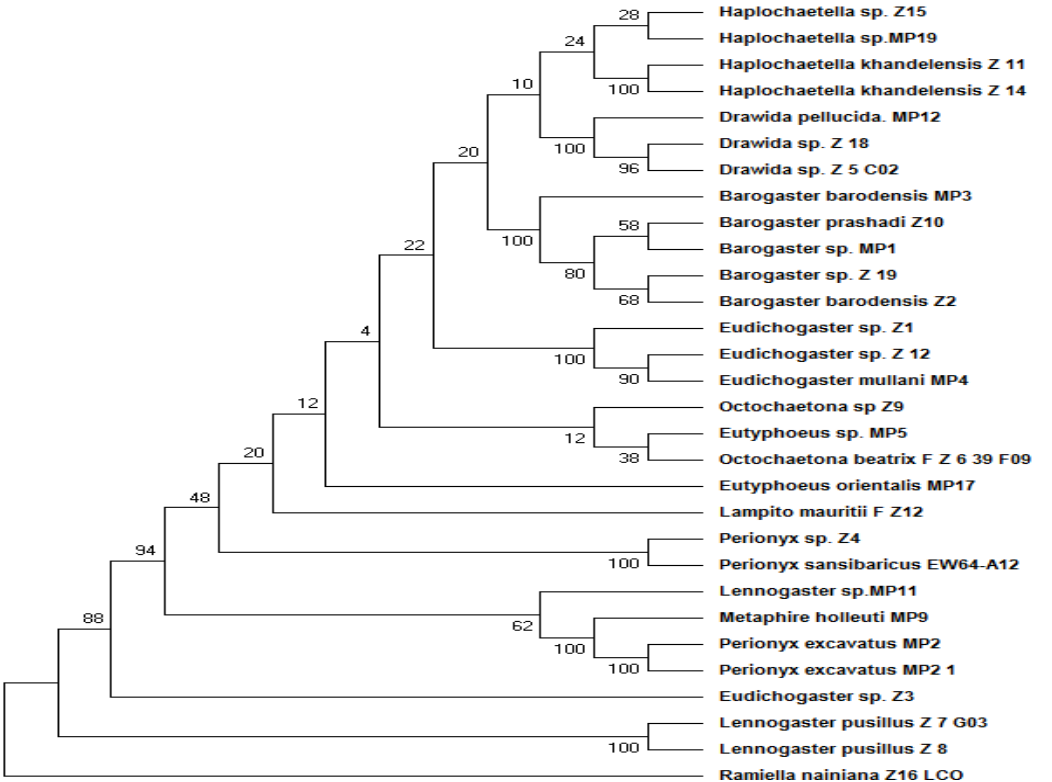

Figure 15. The most parsimonious tree with length =1158 is shown. The consistency index is (0.341105), the retention index is (0.582604), and the composite index is 0.198729 (0.198729) for all sites.

Complimentary Contributor Copy

ACKNOWLEDGMENTS

Authors acknowledge financial support of Department of Biotechnology, Ministry of Science and Technology, Government of India.

REFERENCES

Bely A. E. and Wray G. A., 2004. Molecular phylogeny of annelid worms (Annelida; Clitellata) based on cytochrome c oxidase I. *Mol. Phylogenet. Evo*. 30: 50-63.

Blakemore R. J., 2006 Checklist of 505 Earthworm species from India and adjacent the region (excluding Myanmar) compiled from various sources. http://www.ncbi.org.in/biota/fauna/.

Darwin C., 1881. *The formation of vegetable mould through the action of worms, with observations on their habitats*. Murray, London, 326 pp.

Edwards C. A. and Lofty J. R. 1977. *Biology of the Earthworms*. Chapman and Hall, London.

Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. and Biotech*. 3(5): 294-299.

Fragoso C., Brown G.G., Patrón J.C., Blanchart E., Lavelle P., Pashanasi B., Senapati B. and Kumar T.,1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of earthworms. *Applied Soil Ecology* 6:17-35.

Gates G. E., 1972. Burmese earthworms. An introduction to the systematics and biology of megadrile oligochaetes with special reference to southeast Asia. *Trans. Am. phil. Soc*., 62(7): 1-326.

Gates G. E., 1939. Indian earthworms. VI. Nelloscolex gen. nov. *Rec. Indian Mus.* 41 (I): 37-44.

Gates G. E., 1949. On some Indian ocnerodrilids. *Proc. Indian Acad. Sci*. 30(5): 279-283.

Complimentary Contributor Copy

Gates G. E., 1949. On some Indian ocnerodrilids. *Proc. Indian Acad. Sci.* (B)30(5): 279-283.

Gates G. E., 1956. Reproductive organ Polymorphism in earthworms of the Oriental Megascolecine Genus *Pheretima* Kinberg 1867. *Evolution* 10: 213-227.

Gates G. E., 1960. On Burmese earthworms of the family Megascolecidae. *Bull. Mus. comp. Zool. Harv.* 123 (6): 201-282.

Hansen, 1877. Die Tatigkeit des Regenwurms (Lumbricus terrestris L.) Fur die Fruchtbarkeit des Erdbodens [Activity of the rainworm (Lumbricus terrestris L.) For the fertility of the ground]. *Zeitschrift furWissenschaftliche Zoologies* 28: 354-364.

Hartenstein R., Edwards N. F. and Kaplan, D. L., 1979. A progress report on the potential use of earthworms in sludge management. In: *Proc. 8th Nat Sludge Conference*. Information Transfer Inc., Silver Spring, Maryland.

Julka J. M., 1988. The fauna of India and The adjacent countries.Megascolecidae: Octochaetidae (Earthworms) Haplotaxida: Lumbricina: Megascolecidae: Octochaetidae xiv. *Zoological Survey of India*, Calcutta.pp.400.

Kuhnelt W., 1950. *Bodenbiologie*.Verlag Herold, Wien.

Kushwaha T., Vishwakarma A., Paliwal Rahul, Burla Sashidhar, Yadav Shweta, 2015. A simple protocol to extract DNA from earthworm tissue for molecular studies. *Research and Reviews Journal of Zoological Science*4 (1): 33-37.

Lavelle P., 1988. Earthworm activities and the soil system. *Biol.Fertil.Soils* 6: 237-251.

Lee K.E., 1985. Earthworms: Their ecology and relationships with soils and land use. Academic Press, Sydney.

Muller P. E., 1878. Studier over Skovjord.I. Om bogemuld od bogermor paa sand og ler.*Tidsskrift for Skovbrug* [Studies of Skovjord.I. About bogemuld od bogermor of sand and clay.*Tidsskrift of Forestry*], 3: 1-124.

Complimentary Contributor Copy

Paliwal R., 2008. Annelida: Oligochaeta: 29-53. In: *Faunal Diversity of Jabalpur District, Madhya Pradesh.Zoological Survey of India*, Kolkata. pp. 29-53.

Perrier E., 1872. Recherches pour setvir a l histoire des *Lombriciens terrestres. Nouv. Arck Mus. Hist. Nat.*, Paris, 8: 5-198.

Reynolds J. W., 1994. Earthworms of the world. *Global Biodiversity*,4: 11-16.

Satchell J. E., 1967. Lumbricidae: In: A. Burges and F. Raw (eds.). *Soil Biology*, Academic Press, London. pp. 25 9-322.

Satchell J. E., 1983. Earthworm ecology in forest soil. In: J.E. Satchell (ed.). *Earthworm ecology from Darwin to vermiculture*. Chapman and Hall, London.

Senapati B. K. and Dash, M. C., 1982. Earthworm as waste conditioner. *I. E. Journal*, 11(2): 53-57.

Stephenson J., 1916. On a collection of Oligochaeta belonging to the Indian Museum. *Rec. Indian Mus.* 12: 299-354.

Stephenson J., 1926. Description of Indian Oligochaeta. *Rec. Indian Mus.*28: 249-268.

Stephenson J., 1920. On a collection of Oligochaeta from the lesser known parts of India and from eastern Persia. *Mem. Indian Mus*., 7: 191-261.

Stephenson J., 1923. *Oligochaeta. The Fauna of British India, including Ceylon and Burma*, xxiv+518 pp. Taylor and Francis, London.

Templeton R., 1844. Descrption of *Megascolex caeruleus*. *Proc.zool. Soc.Lond*., 12: 89-91.

White G., 1789. The natural history of Selborne, Benjamin White, London.

Complimentary Contributor Copy

Complimentary Contributor Copy

In: Earthworms
Editor: Clayton G. Horton
ISBN: 978-1-53612-176-6
© 2017 Nova Science Publishers, Inc.

Chapter 3

EARTHWORM POPULATION DYNAMICS: MODELING APPROACH AND CHALLENGES

Davorka K. Hackenberger*, Branimir K. Hackenberger and Željka Lončarić
Department of Biology, University of Osijek, Osijek, Croatia

ABSTRACT

Earthworms are often recognized as key organisms in soil ecosystems. Their population dynamics is influenced by many abiotic and biotic factors. However, living in the soil hinders the observation of their population dynamics. Modeling is one of the promising approaches to the research of earthworm population dynamics. Nowadays, the development of more complex and realistic models is no longer held back by the computational power as it has increased and become more available. Depending on the objective, different types of models are applied in earthworm research: convection-dispersion equation, dynamic energy budget, differential equations, individual-based model and matrix-based population models. So far, matrix models were mostly applied in ecotoxicological studies for a single species. Also, we present a preliminary investigation on the applicability of the Lefkovitch discrete

* Corresponding Author Email: davorka@biologija.unios.hr.

Complimentary Contributor Copy

model of stage structured population dynamics adapted to life cycle parameters for three earthworm ecological categories, i.e., epigeic, endogeic and anecic, to simulate earthworm population dynamics in the grassland ecosystem. In this investigation, a predation pressure is presented as an important variable which can be viewed as an other type of pressure on the earthworm population, such as pollution, environmental stress or land management, causing additional or extrinsic mortality to earthworm population.

Eventually, modeling of earthworm population dynamics within ecological monitoring could detect changes in their population structure, and therefore indicate presence of various environmental pressures. Also, modeling can reduce efforts regarding field sampling and could be used to predict consequences of climate change on earthworm populations.

Keywords: earthworms, population models, matrix models, earthworm population modeling

1. INTRODUCTION: EARTHWORMS POPULATION ECOLOGY

Many aspects of earthworm biology and ecology have been intensively researched over the several past decades. Earthworms were given this attention due to their known beneficial roles in soil processes and their sensitivity to contaminants, environmental stress and impact on soil quality. All those features label earthworms as key organisms in soil ecosystems [1].

Earthworm population dynamics, in both space and time, is influenced by multiple abiotic and biotic factors. Biotic factors include inter- and intra-specific competition, parasitism and predation. Among abiotic factors soil temperature and moisture are highlighted as the two most important factors [2]. Soil temperature and soil moisture are key factors influencing growth, survival, fecundity and activity of earthworms [3] and, indirectly, influencing earthworm habitat and food availability [4]. Moreover, soil temperature and moisture affect the majority of life cycle traits such as weight, cocoon incubation time, onset of sexual maturity, reproduction and life span. Accurate information about these traits are crucial for the

Complimentary Contributor Copy

construction of simulation models and, consequently, for a better understanding of earthworm population dynamics [5]. Life cycle traits are usually obtained from experiments in controlled conditions with a constant temperature and moisture content as the unstable, fluctuating conditions found in the environment are hard to achieve in a laboratory. However, temperature fluctuations are found to have a significant influence on the length of earthworm life stages and overall mortality and cocoon production [6].

Earthworms have both vertical and horizontal spatial dynamics. During the adverse conditions, earthworms can migrate or aestivate. The average soil depth that earthworms inhabit is species dependent, but seasonal changes are related to environmental conditions. Earthworm seasonal migration to deeper soil levels is initiated by unsuitable environmental factors in the upper soil level. Earthworms go deeper either when the temperature is too low or too high in combination with low soil moisture [3]. Some species can also become inactive during the adverse environmental conditions, mostly during the summer months. Earthworms also tend to migrate horizontally for various reasons: to avoid adverse conditions, due to intraspecific competition or to find better food source.

Abiotic environmental stress factors influence numerous aspects of earthworm life cycle and are in a close interaction with various biotic factors such as food availability, predation and competition. However, living in the soil, a complex and opaque medium, makes an in situ observation of earthworms time and labor intensive [7]. Moreover, the observation is further complicated with spatial and temporal variations in abundance and structure of earthworm populations. Consequently, there are still some gaps in the comprehension of earthworm population dynamics.

Earthworms are most often classified into ecological categories, or functional types, which are distinguished by their ecological, as well as morphological traits [8]. These categories are: epigeic, endogeic and anecic. Although, some species are difficult to assign exclusively to only one category, since most species have various life cycle traits corresponding to category traits. The number of earthworm species within

Complimentary Contributor Copy

a given community may vary from one to fifteen species, with three to six species in most communities regardless of the type of habitat or geographic position [3]. Population dynamics among earthworm from different ecological categories differs as those species have different ecological preferences [9]. The use of ecological categories according to Bouché [8] has been recommended for a more comprehensive understanding of earthworm population structure [10, 11].

2. MODELING EARTHWORM POPULATION DYNAMICS

The objective of modeling earthworm population dynamics can vary; either to observe the influence of toxic agents on earthworm population dynamics (ecotoxicological models) or the effects earthworms have on ecosystem functioning, i.e., feedback models [12]. The types of the models used for the earthworm population dynamics so far are matrix-based population models, individual-based models, dynamic energy budget models and differential equations.

Most models describing the earthworm population dynamics in natural environment are developed for tropical climate and, hence, tropical earthworm species [13, 14, 15], and only a few for a temperate climate [17, 18, 19]. Models were developed for different species and, until recently, the majority of models included only a single species. However, novel models are considering representative species of all three earthworm ecological categories – anecic, endogeic and epigeic [1, 18, 19].

As mentioned above, the earthworm population dynamics, in both space and time, is influenced by multiple abiotic and biotic factors. As soil temperature and moisture are the most important, the majority of models have incorporated those factors [17, 18, 19, 20, 21, 22, 23].

Until now, the matrix population models have been employed in earthworm population research mostly in an ecotoxicological context [24, 25, 26]. Only recently, matrix models were used in two studies with earthworm density predictions regarding soil climate [17, 18], with the lattertaking into considertion representative species for each of the three

Complimentary Contributor Copy

ecological categories. However, dynamic population models could elucidate reasons for fluctuations and differences in earthworm abundance both between seasons and locations [19] and point out possible pressures upon earthworm population.

3. MATRIX BASED MODEL WITH ECOLOGICAL CATEGORIES

3.1. Introduction

We have applied the Lefkovitch discrete model as a tool to simulate earthworm population dynamics as a function of soil temperature and soil moisture. Our main hypothesis was that the earthworm population dynamics could be adequately predicted by grouping earthworm species into three ecological categories regardless of the number of species within the group present at the location. To test the hypothesis we have adapted the Lefkovitch discrete model of stage structured population dynamics to earthworm life cycle parameters and calculated it according to a weekly time step. The life cycle parameters for each age stage and ecological category were estimated from the data available in the literature (Table 1). The traits of earthworm behavior were also included, i.e., vertical migration in the case of adverse conditions. The simulated values were then compared with the field-measured data.

After comparison of model outputs with the field-measured data it was evident that, on some locations, model outputs give significant overestimations. This was indication that some other environmental parameters are affecting populations on some of the locations. We added additional variable to the model - predation pressure. We aimed to make a step toward applicable solution for the modeling of earthworm population dynamics with increased predictive capabilities and practical values of such models for a wide range of purposes.

Complimentary Contributor Copy

Table 1. Life history parameters in relation to soil temperature

	Juvenile stage duration (w)			Cocoon incubation (w)			Cocoon production ($cwo^{-1}w^{-1}$)			Cocoon viability (%)		
T(°C)	**anec**	**endo**	**epi**	**anec**	**endo**	**epi**	**anec**	**endo**	**epi**	**anec**	**endo**	**epi**
5				33.95 [a,c,h,r,s]	30.57 [a,l,s,t,u]	23.91 [f,p,t,v]						
7.5	38.85 [a,b,c]	7.5 [a,d,e]										
9			20 [f]									
9.6				25.25 [a,c,s]	16.9 [s]							
10	25.62 [b,g]	7.86 [a]		18.74 [a,h,i,r,s,v]	18.88 [a,d,k,t,u]	26.19 [d,t,v]		0.34 [a,s,t]		70 [a]	60.85 [a,t]	
12.5	19.3 [b]											
15	17.64 [a,b,g,h,i]	12.55 [a,k,l,m]	16 [n]	12.2 [a,i,j,n,r,s,v,z]	9.23 [a,k,m,s,t,u]	15.13 [n,t,v]	0.36 [a,r,x]	0.33 [a,k,m]	1.31 [f,n,p]	77.01 [a,h,j,s,z,x]	69.57 [a,m,t]	90 [n]
16				5.99 [c]						87 [c]		
17.5	15.1 [b]											
18	13.7 [b]	13 [l]	11 [f]						1.4 [f]			
20	14.71 [a,b,g,o]	6.57 [a,k]	7.79 [f]	7.49 [a,h,i,o,r,s,v,z]	6.01 [a,d,k,m,s,t,u]	6.14 [f,t,v]	0.46 [a,o,s,z]	0.57 [a,m]	1.31 [f,p,v]	55.75 [a,o,z]	81.5 [a,m]	56.67 [f,p]
22.5	20.6 [b]			4.63 [s]	4.11 [s]	5.36 [p]						
25			10.57 [p]	9.81 [a,h,r]	4.33 [s]	4.16 [f,p]	0.52 [c]	0.34 [k,s]	1.25 [f,p,v]	41 [a]		74.5 [f,p]
26				6.01 [a,s]	13.3 [a,k,s]							

[a] Lowe CN, Butt KR. Culture techniques for soil dwelling earthworms: A review. *Pedobiologia* 2005; 49: 401–413.

[b] Daniel O, Kohli L, Bieri M. Weight gain and weight loss of the earthworm *Lumbricus terrestris* L. at different temperatures and body weights. *Soil Biology and Biochemistry* 1996; 28: 1235–1240.

[c] Fernández R, Novo M, Gutiérrez M, Almodóvar A, Díaz Cosín DJ. Life cycle and reproductive traits of the earthworm *Aporrectodea trapezoides* (Dugès, 1828) in laboratory cultures. *Pedobiologia* 2010; 53: 295–299.

[d] Butt KR. Reproduction and growth of the earthworm *Allolobophora chlorotica* (Savigny, 1826) in controlled environments. *Pedobiologia* 1997; 41: 369–374.

[e] Edwards CA, Bohlen PJ. *Biology and Ecology of Earthworms*, 3rd edition. Springer: London, 1995.

[f] Elvira C, Domínguez J, Mato S. The growth and reproduction of *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure mixed cultures with *Eisenia andrei*. *Applied Soil Ecology* 1997; 5: 97–103.

Complimentary Contributor Copy

[g] Berry EC, Jordan D. Temperature and soil moisture content effects on the growth of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under laboratory conditions. *Soil Biology and Biochemistry* 2001; 33: 133–136.
[h] Butt KR. The effects of temperature on the intensive production of *Lumbricus terrestris* (Oligocaheta: Lumbricidae). *Pedobiologia* 1991; 35: 257–264.
[i] Holmstrup M, Zachariassen KE. Physiology of cold hardiness in earthworms. *Comparative Biochemistry and Physiology A Physiology* 1996; 115: 91–101.
[j] Butt KR, Briones MJI. Life cycle studies of the earthworm *Lumbricus friendi* (Cognetti, 1904). *Pedobiologia* 2011; 54(Supplement): S27–S29.
[k] Gerard BM. *The biology of certain British earthworms in relation to environmental conditions*, PhD Thesis, University of London, 1960.
[l] Edwards CA, Lofty JR. *Biology of Earthworms*, Bookworm Publishing Company, Incorporated, 1976.
[m] Lowe CN, Butt KR. Life cycle traits of the parthenogenetic earthworm *Octolasion cyaneum* (Savigny, 1826), *European Journal of Soil Biology* 2008; 44: 541–544.
[n] Bindesbøl A-M, Bayley M, Damgaard C, Holmstrup M. Life-history traits and population growth rate in the laboratory of the earthworm *Dendrobaena octaedra* cultured in copper-contaminated soil. *Applied Soil Ecology* 2007; 35: 46–56.
[o] Monroy F, Aira M, Gago JÁ, Domínguez J. Life cycle of the earthworm *Octodrilus complanatus* (Oligochaeta, Lumbricidae), *Comptes Rendus Biologies* 2007; 330: 389–391.
[p] Dominguez J, Edwards CA. *Vermicomposting organic wastes: A review*, in: Shakir SH, Mikhail WZA (Eds.), Soil Zoology for Sustainable Development in The 21st Century, El Cairo, pp. 369–396.
[r] Baker GH, Whitby WA. Soil pH preferences and the influences of soil type and temperature on the survival and growth of *Aporrectodea longa* (Lumbricidae): The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. *Pedobiologia* 2003; 47: 745–753.
[s] Holmstrup M. Cocoon production of *Aporrectodea longa* Ude and *Aporrectodea rosea* Savigny (Oligochaeta; Lumbricidae) in a Danish grass field. *Soil Biology and Biochemistry* 1999; 31: 957–964.
[t] Holmstrup M, Ostergaard IK, Nielsen A, Hansen BT. The realtionship between temperature and cocoon incubation time for some Lumbricid earthworm species. *Pedobiologia* 1991; 35: 179–184.
[u] Jensen KS, Holmstrup M. Estimation of earthworm cocoon development time and its use in studies of in situ reproduction rates. *Applied Soil Ecology* 1997; 7: 73–82.
[v] Holmstrup M, Ostergaard IK, Nielsen A, Hansen BT. Note on the incubation time of earthworm cocoons at three constant temperatures. *Pedobiologia* 1996; 40: 477–478.
[z] Butt KR. Reproduction and growth of three deep-burrowing earthworms (Lumbricidae) in laboratory culture in order to assess production for soil restoration. *Biology and Fertility of Soils* 1993; 16: 135–138.
[x] Kostecka J, Butt KR. Ecology of the earthworm *Allolobophora carpathica* in field and laboratory studies. *European Journal of Soil Biology* 2001; 37: 255–258.

Complimentary Contributor Copy

3.2. Materials and Methods

3.2.1. Field Data

The field data used for the development of this model application is described in detail in Hackenberger and Hackenberger [27]. Briefly, earthworms were sampled on two occasions, in the spring and in the summer. Sampling was done at seven locations along the altitudinal transect which comprised two different climate types. At each location 49 subsamples, each 0.25 m^2, were taken arranged in a regular grid with 5.5 m intersample distance. Data were pooled from all subsamples (i.e., each location enters into the analyses as a single datum point) to avoid pseudoreplication. Earthworms were determined to a species level, counted and categorized into categories according to their morphological traits. Each species is allocated to a single ecological category proposed for earthworms (epigeic, endogeic and anecic).

3.2.2. Soil Temperature and Moisture Data

At each location, during sampling, soil samples were taken and basic physicochemical properties were determined (pH, soil texture, organic matter content, bulk density). Soil temperature was measured at three depths (surface, 10 cm and 15 cm) and air temperature and humidity were also recorded with a data logger. Additional meteorological data were obtained from the Croatian Hydrometeorological State Institute for the meteorological stations that are nearest to the sampling locations. All necessary data were implemented in the Soil Temperature and Moisture Model (STM^2) [28] in order to obtain soil moisture and soil temperature data on various soil depths during the model simulation period.

3.2.3. Model Parameterization

A mechanistic stage-based Lefkovitch matrix population model [29] was utilized to describe the earthworm population dynamics at a weekly time step. In this model individuals were divided into three stages: cocoons (*C*), juveniles (*J*) and adults (*A*) [3] (Figure 1). Earthworms were considered juveniles if they had neither tubercula pubertatis nor clitellum.

Complimentary Contributor Copy

The possibility that mature individuals lose their clitellum and cease reproduction if the environmental conditions are adverse was also taken into account [3]. This possibility was applied for endogeic and anecic earthworms, while it was omitted for epigeic species as they do not have this trait. Vertical migration was included in the case of unfavorable conditions for adult and juvenile endogeic and anecic earthworms. For all other stages the vertical migration was not allowed in the model (Table 2). As the cocoons are hard to collect in the field we have modified the setup of Pelosi et al. [17] and set an initial stock of cocoons as the number of adult earthworms at the first (spring) sampling.

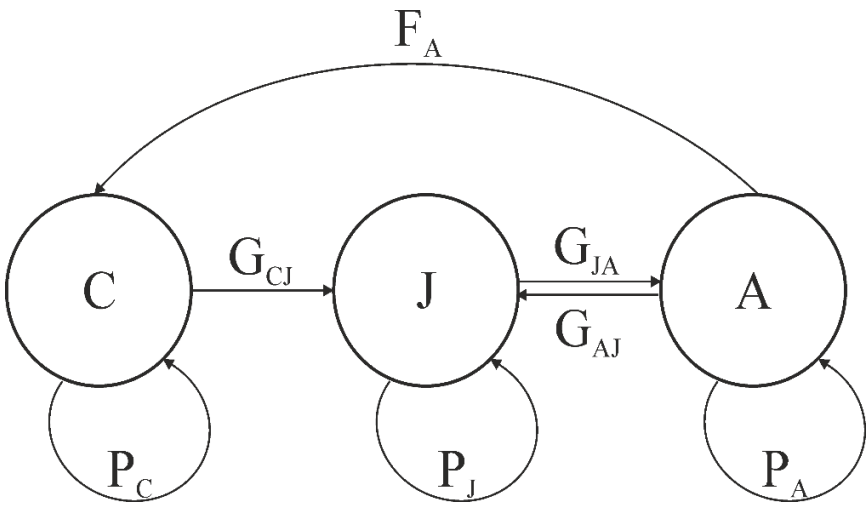

Figure 1. Graph representing the earthworm life cycle. *C* – cocoon stage, *J* – juvenile stage, *A* – adult stage, *PC* – probability of staying in the cocoon stage, *PJ* – probability of staying in the juvenile stage, *PA* – probability of staying in the adult stage, *GCJ* – probability of transition from cocoon to juvenile stage, *GJA* – probability of transition from juvenile to adult stage, *GAJ* – probability of losing reproductive characteristics, i.e., probability of returning to a juvenile stage (only for endogeic and anecic category), *FA* – Fecundity of adult individuals. Probability of staying in a stage or transition to a next stage and fecundity are calculated for the projection interval, i.e., for one week.

Complimentary Contributor Copy

As an additional factor in earthworm population regulation, a predation pressure variable (mortality caused by other factors, e.g., predation or some other adverse effect) was calculated. It was included separately for juvenile and adult stage and expressed in percentages on a weekly basis.

Initial values of a population vector for adults and juveniles are set according to the sampled number of earthworms during the spring sampling at each location. Simulations were run for a 13 week period, in a simulating period from mid-April to early July.

3.2.4. Life History Parameters

The data on life history parameters such as: duration of cocoon incubation, cocoon hatchability, duration of juvenile stage (from hatching to development of sexual characters) in relation to soil temperature and moisture content was obtained from the available literature (Table 1). All data are given as weekly values, same as the time step set for this model. Furthermore, the average soil depths, which different life stages and different species inhabit, were also obtained from the literature. Those values and values for the vertical migration are given in Table 2, together with the optimal soil temperature and moisture for a life stage and ecological category.

Table 2. Values of soil depths with vertical migration, optimal temperatures, optimal moisture and the number of hatchlings per cocoons for each ecological category and life stage used in the model

	Soil depth (cm)					*Optimal temperature (°C)*			*Optimal moisture (%)*			*Hatchling cocoon-1*
	Adult	*Vertical migration (>22°C)*	*Juvenile*	*Vertical migration (>22°C)*	*Cocoon*	*Adult*	*Juvenile*	*Cocoon*	*Adult*	*Juvenile*	*Cocoon*	
Anecic	*20*	*30*	*15*	*20*	*17.5*	*12*	*16.7*	*21.2*	*32.5*	*25*	*25*	*1*
Endogeic	*15*	*30*	*10*	*20*	*6*	*12*	*19.4*	*21.1*	*32.5*	*25*	*25*	*1*
Epigeic	*5*		*5*		*5*	*16.5*	*20*	*21.3*	*42.5*	*25*	*25*	*1.26*

Complimentary Contributor Copy

3.2.5. Life Stage Survival Rate

The survival curves for juvenile and adult stages for the three earthworm categories were modeled using the O'Neill optimum curve (1) [30] (Figure 2).

$$f(T) = hr_{\max}\left[\frac{T_{\max} - T}{T_{\max} - T_{opt}}\right]^{X} \exp\left[\frac{X(T - T_{opt})}{T_{\max} - T_{opt}}\right]$$
$$\text{with} \quad X = \frac{1}{400}W^2\left[1 + \left(1 + \frac{40}{W}\right)^{\frac{1}{2}}\right] \quad \text{and} \quad W = (Q-1)(T_{\max} - T_{opt}) \tag{1}$$

Where *Tmax* is a maximum temperature that organism can tolerate, *Topt* is an optimum temperature for development and *Q* is a form coefficient. *rmax* is maximal survival rate under the optimal temperature. The value *h* denotes a step function, which takes the value of 1 for *Tmax* > T ≥ 0°C. In all of our simulations, *h* is equal to 1. This function was chosen because it describes most accurately a nonlinear dependency between temperature and survival of earthworms as they are more tolerant to lower than to higher temperatures.

Survival of juvenile and adult stage in response to soil moisture was modeled using the three-parameter Gaussian function (2).

$$f(x) = ae^{-\frac{(x-b)^2}{2c^2}} \tag{2}$$

The parameter *a* controls the height of the curve peak, i.e., maximal survival rate under optimal moisture, *b* is the position of the center of the peak, and *c* controls the width of the "bell."

Complimentary Contributor Copy

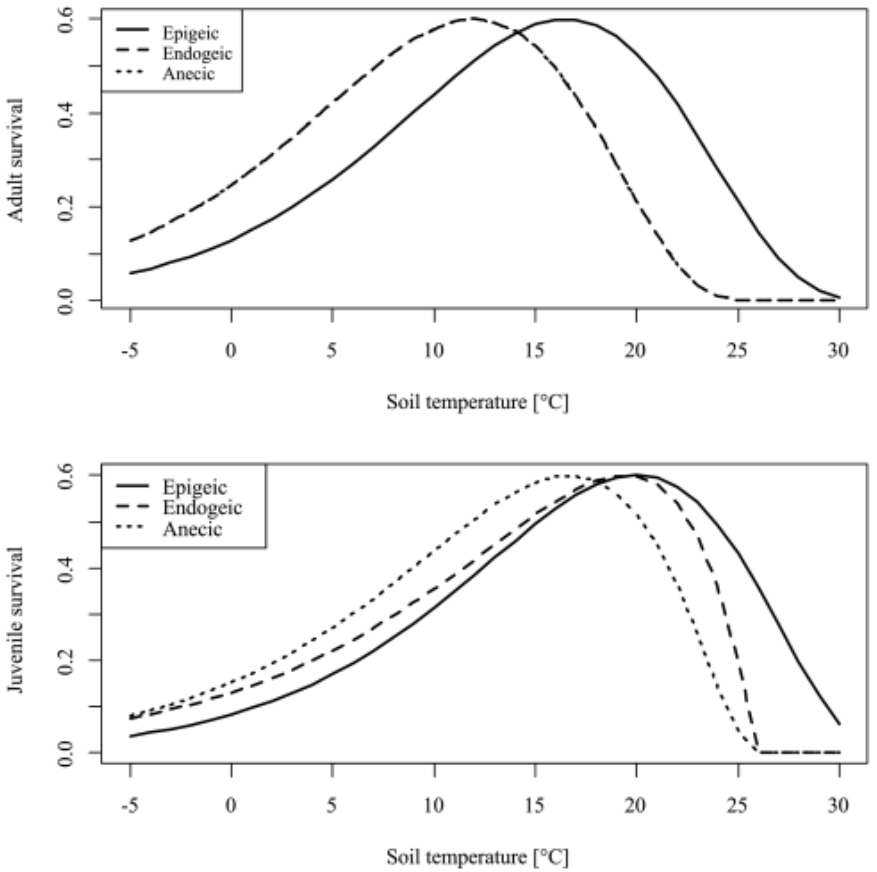

Figure 2. The survival curves for juvenile and adult stages for the three earthworm categories against the soil temperature.

Literature data on which factor, soil temperature or soil moisture, is more important for the survival of earthworms differ. Some authors state that they are equally important [4, 31], but the majority give soil temperature precedence [32]. We have set the ratio of survival dependence on temperature and moisture to 60:40. This means that for soil temperature dependent survival curves for all three earthworm categories *rmax* parameter of O'Neill function was set to 0.6, i.e., maximal survival which related to temperature was set to 60%. Accordingly, in the case of the Gaussian function soil moisture dependent parameter *a* that control height of the peak is set to 0.4 for all three earthworm categories, as it is a

Complimentary Contributor Copy

maximal assumed survival related to soil moisture. After calculating survival in response to soil temperature and soil moisture separately, the obtained values were summed to determine an overall survival value.

The survival rate of cocoons was adjusted as a constant value at 0.99 (i.e., 99%) because cocoons represent a resistant form that tolerates unfavorable conditions [3].

3.2.6. Transition Probabilities

This model parameterizes earthworm life cycle in three stages and uses projection interval of one week. Therefore, in order to construct a stage-based projection matrix it was necessary to estimate probabilities of an individual remaining in the same stage, as well as probabilities of growing into the next developmental stage during a weekly projection interval. For modeling of the transition probabilities functions proposed by Crouse et al. [33] were used: G_i – probability for individual to survive and grow to the next developmental stage (3) and P_i – probability for individual to survive and remain in the same developmental stage (4):

$$G_i(t) = \frac{s_i(t)^{d_i(t)}\left(1 - s_i(t)\right)}{1 - s_i(t)^{d_i(t)}} \tag{3}$$

$$P_i(t) = \left(\frac{1 - s_i(t)^{d_i(t)-1}}{1 - s_i(t)^{d_i(t)}}\right) s_i(t) \tag{4}$$

Where s_i is temperature and soil moisture dependent survival of stage i at time t and d_i is stage duration of the stage i as a response of soil temperature at the time t.

The duration of juvenile and cocoon stage was calculated with the available literature data (Table 1) and fitted with different functions against temperature (Figure 3), according to tolerability of ecological category. Stage durations were then calculated for a weekly interval from fitted functions. The adult stage duration of all three ecological categories was

Complimentary Contributor Copy

set as a fixed value with 38.62 weeks for epigeic species, 42.59 weeks for endogeic species and 99.52 weeks for anecic species according to literature data.

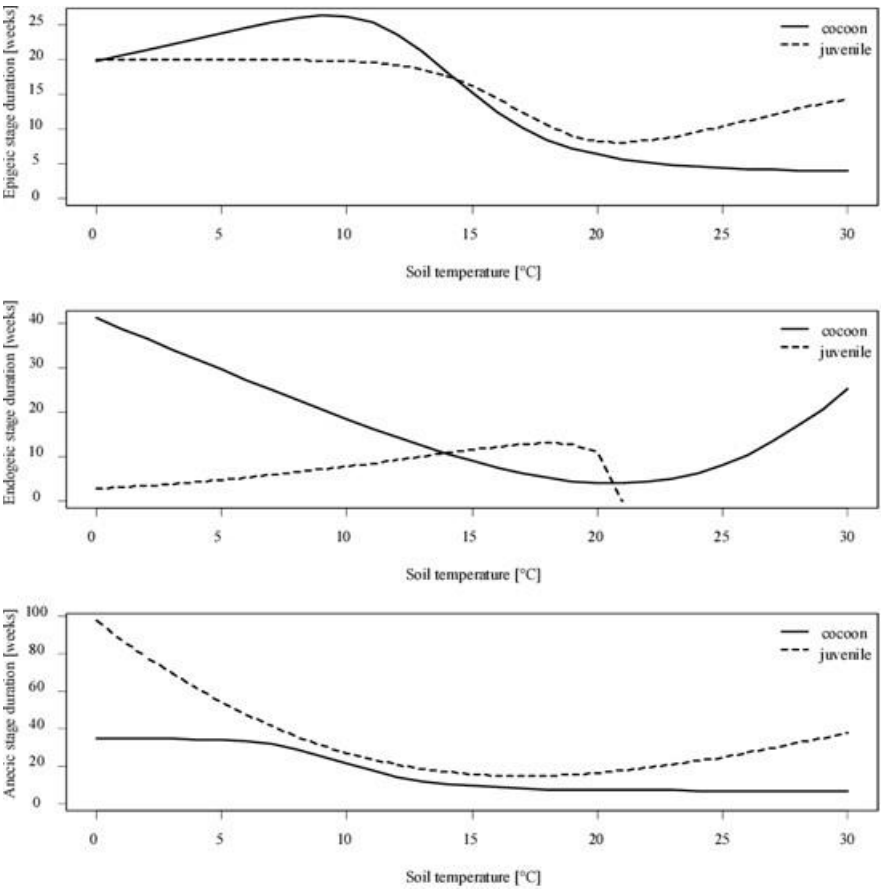

Figure 3. The duration of juvenile and cocoon stage dependent on temperature for all three categories.

3.2.7. Fecundity

To calculate the fecundity number of hatchlings per cocoon, the temperature dependent cocoon production and temperature dependent cocoon viability data were needed. Number of hatchlings per cocoon was set as a fixed value according to the available literature data (Table 1).

Complimentary Contributor Copy

Temperature dependent cocoon production was calculated using literature data and fitted with the five-parameter Brain-Cousens function [34] for epigeic and endogeic category and the O'Neill optimum curve for the anecic category. Literature data on weekly cocoon viability at various soil temperatures were fitted using the O'Neill optimum curve. All functions used, the obtained function parameters and correlation coefficients together with literature data are presented in Table 3.

Finally, the fecundity for all three earthworm categories was calculated according to the function (5):

$$fec(t) = nh * cp(t) * viab(t) \tag{5}$$

where *nh* is a number of hatchlings per cocoon, *cp* represents weekly cocoon production at the time *t* in response to soil temperature and *viab* is the cocoon viability at the time *t* in response to the soil temperature.

3.2.8. Computational Methods and Matrix Analysis

Computations, simulations, and plotting were performed using R software [35]. Experimental data were fitted using the *drc* (dose-response curve) package under an R software environment [36].

Perturbation analysis is important in quantifying the effects of changes in individual vital rates (i.e., matrix elements) to population growth. Sensitivities represent the partial derivatives of λ with respect to vital rates, and they quantify the impact of an absolute change in λ with an infinitesimal absolute change in a vital rate [37]. Elasticities on the other hand, represent the proportional sensitivities, i.e., they quantify the effects of proportional changes in vital rates on λ. The elasticity of λ with respect to matrix element a_{ij} is defined as [29]:

$$\begin{aligned} e_{ij} &= \frac{a_{ij}}{\lambda}\frac{\partial \lambda}{\partial a_{ij}} \\ &= \frac{\partial \log \lambda}{\partial \log a_{ij}} \end{aligned} \tag{6}$$

Complimentary Contributor Copy

Table 3. All functions, obtained function parameters and correlation coefficients used in the model

			Function used	General form	Function parameters					*r*
EPIGEIC EARTHWORMS	ADULTS	Stage duration	fixed value = 38.62 [days]							
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)= ((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.6	T_{max}=33	T_{opt}=16.5	Q=2.5		
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.425	c=1			
	JUVENILE	Soil temperature dependent stage durations	Cedergreen-Ritz-Streibig model	f(x,b,c,d,e,f)=d-((d-c+(fexp(-1/x)))/(1+(exp(b(log(x)-log(e)))))	b=-9.47	c=-656.895	d=19.949	e=18.271	f=-694.029	0.9970
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.6	T_{max}=33	T_{opt}=20	Q=2.5		
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.25	c=1			

Complimentary Contributor Copy

			Function used	General form	Function parameters					*r*
	COCOONS	Survival	fixed value = 0.99							
		Soil temperature dependent stage durations	five-parameter Brain-Cousens function	f(x,b,c,d,e,f)=c+((d-c+fx)/(1+exp(b(log(x)-log(e))))	b=7.150	c=3.755	d=19.795	e=14.171	f=0.825	0.9998
		Number of hatchlings per coocon	fixed value=1.26 [hatchlings/cocoon/earthworm]							
		Cocoon production	five-parameter Brain-Cousens function	f(x,b,c,d,e,f)=c+((d-c+fx)/(1+exp(b(log(x)-log(e))))	b=19.684	c=1.245	d=0.318	e=18.362	f=0.662	0.9999
		Cocoon viablility	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.9	T_{max}=30	T_{opt}=15	Q=3.1		0.859526
ENDOGEIC EARTHWORMS	ADULTS	Stage duration	fixed value = 42.59 [days]							
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.6	T_{max}=25.8	T_{opt}=12	Q=2.5		
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.325	c=1			

Complimentary Contributor Copy

Table 3. (Continued)

			Function used	General form	Function parameters					*r*
	JUVENILE	Soil temperature dependent stage durations	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=13	T_{max}=21	T_{opt}=18	Q=2.9		0.8035
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.6	T_{max}=25.8	T_{opt}=19.4	Q=2.5		
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.25	c=1			
	COCOONS	Survival	fixed value=0.99							
		Soil temperature dependent stage durations	five-parameter Brain-Cousens function	f(x,b,c,d,e,f)= c+((d-c+fx)/(1+exp(b(log(x)-log(e))))	b=4.559	c=299.360	d=41.114	e=42.938	f=-2.309	0.970193
		Number of hatchlings per coocon	fixed value=1 [hatchlings/cocoon/earthworm]							
		Cocoon production	five-parameter Brain-Cousens function	f(x,b,c,d,e,f)=c+((d-c+fx)/(1+exp(b(log(x)-log(e))))	b=-21.183	c=0.335	d=2.938	e=23.043	f=-0.093	0.999625

Complimentary Contributor Copy

			Function used	General form	Function parameters					*r*
		Cocoon viablility	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.82	T_{max}=25	T_{opt}=20	Q=2.5		0.998822
ANECIC EARTHWORMS	ADULTS	Stage duration	fixed value = 99.52							
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.6	T_{max}=25.8	T_{opt}=12	Q=2.5		
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.325	c=1			
	JUVENILE	Soil temperature dependent stage durations	five-parameter Brain-Cousens function	f(x,b,c,d,e,f)= c+((d-c+fx)/(1+exp(b(log(x)-log(e))))	a=1.886	b=321.348	c=97.412	e=32.095	f=-10.313	0.988811
		Soil temperature dependent survival	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2)(1+(1+40/W)^0.5); W=(Q-1) (T_{max}-T_{opt})	r_{max}=0.6	T_{max}=25.8	T_{opt}=16.7	Q=2.5		

Complimentary Contributor Copy

Table 3. (Continued)

			Function used	General form	Function parameters					*r*
		Soil moisture dependent survival	three-parameter Gaussian function	f(x,a,b,c)=aexp(-((x-b)^2)/c)	a=0.4	b=0.25	c=1			
	COCOONS	Survival	fixed value=0.99							
		Soil temperature dependent stage durations	four-parameter logistic function	f(x,b,c,d,e)=c+((d-c)/(1+exp(b(log(x)-log(e)))))	a=5.889	b=6.731	d=34.463	e=10.175		0.97436
		Number of hatchlings per coocon	fixed value=1 [hatchlings/cocoon/earthworm]							
		Cocoon production	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp((X(T-T_{opt}))/ (T_{max}-T_{opt})); X=((1/400)W^2) (1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.52	T_{max}=32	T_{opt}=25	Q=2.5		0.999914
		Cocoon viablility	O'Neill optimum curve	f(T, T_{max}, T_{opt}, r_{max}, Q)=((T_{max}-T_{opt})/(T_{max}-T_{opt}))^Xexp ((X(T-T_{opt}))/(T_{max}-T_{opt})); X=((1/400)W^2) (1+(1+40/W)^0.5); W=(Q-1)(T_{max}-T_{opt})	r_{max}=0.82	T_{max}=30	T_{opt}=16	Q=2.3		0.872782

[1] Richter O, Diekkrüger B, Nörtersheuser P. *Environmental Fate Modelling of Pesticides: From the Laboratory to the Field Scale*. Wiley & Sons: New York, 2008.

[2] Cedergreen N, Ritz C, Streibig JC. Improved empirical models describing hormesis. *Environmental Toxicology and Chemistry* 2005; 24: 3166–3172.

[3] Brain P, Cousens R. An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Research* 1989; 29: 93–96.

[4] Seber GAF, Wild CJ. *Nonlinear Regression*, Wiley & Sons: New York, 1989, p. 330.

Complimentary Contributor Copy

Elasticity analysis was performed to examine elasticity of population growth rate on individual parameters of Lefkovitch stage-structured matrix. Elasticities were calculated separately for each projection matrix during 13 week simulation period and for each location. Then, average elasticities were calculated for each ecological category. Matrix analyses were performed in R (version 3.3.2, R Development Core Team 2011) using the package *Popbio* [38].

3.3. Results and Discussion

3.3.1. Model Predictions

There is a lack of detailed life cycle analysis of majority of earthworm species in the available literature. The existing data were mainly obtained in the experiments with constant temperature while, for instance, daily temperature fluctuations can significantly influence the stage duration or the speed of reproduction in earthworms [39]. This can complicate the parameterization of the model, which is commonly one of the major bottlenecks in population dynamical model studies [24]. To bypass these limitations we used the ecological category approach instead of modeling dynamics of a specific earthworm species. According to Pop [11] earthworm communities commonly include one or two species of red epigeic earthworms, two to four species of endogeic unpigmented earthworms and none to two species of very large lumbricids. The results from the field experiment used for this model are in line with the above statement as the anecic category never had more than one species, epigeic 0-3 species, and endogeic four species on average per location [27]. Therefore, even though we have modeled the population dynamics according to an ecological category, the anecic category was modeled only for one species, which was also the case for the epigeic category at two locations (L2 and L4). The overall results of the model prediction were satisfactory with only a few under/over estimations of the field observed earthworm densities.

Complimentary Contributor Copy

3.3.2. Endogeic Species Simulation

Species from the endogeic category were present at all sampled locations and comprised the highest number of species. A decline in abundance was a general trend at the first five locations (L1-L5) (Figure 4). A similar trend has been observed in England at the beginning of dry or wet periods [40]. The simulated values were in accordance with the field sampling data at most locations. The predicted values underestimated field densities for the juvenile stage at locations L1 and L6 and for the adult stage at the location L6. The only overestimation was the predicted abundance of juveniles at the location L7. The under prediction of the juvenile density at the location L1 could be attributed to a partial flooding in the spring and a consequent delay of favorable conditions [27]. This delay could result in less juveniles and adults and more cocoons in the population structure at the time of the spring sampling. It has been indicated that variable cocoon incubation times could not only be a result of a varying climatic conditions but a strategy for survival as well [41]. In order to determine if the number of cocoons in the spring was a possible reason for an underestimation, we have run a simulation with an increased initial number of cocoons. The simulation with the triple initial number of cocoons gave an accurate prediction of both juvenile and adult stage, indicating a number of cocoons as a source of simulation underestimation.

The endogeic species present at the locations L6 and L7 (maritime climate) had a different trend than the endogeic species at other sampled locations. As with the location L1, an additional simulation for the location L6 with an increased initial number of cocoons was conducted. This simulation gave a more accurate prediction of juveniles, while the adults were still underestimated. A reason for this could be the model parameterization, which assumes the reproductive cessation and return to a virtually juvenile stage during adverse field conditions. As the parameterization was set according to the results for earthworm species and populations from the continental climate, perhaps the temperature limit for species and populations under submediterranean climate differs. Accordingly, it has been indicated that a response to temperature is similar between the species from the same climate [19] and that, for instance, the

Complimentary Contributor Copy

base temperature is higher for the tropical species [42]. At the location L7, the increase of the initial cocoon density gave an adequate prediction. The L6 and L7 locations are differentiated from other locations by climate. This fact could be responsible for a seemingly different population dynamics. Most probably, the cause of higher initial cocoon number at these locations is different from the location L1. At these locations favorable conditions for reproduction start earlier in the year and cocoons were already deposited in the soil during the spring sampling. This high density of cocoons in the spring time has led to an underestimation in the model by Johnston et al. [43], who hypothesize that it might be due to higher temperatures when population densities were high. However, the high cocoon density has been observed in the spring sampling [44]. In Greece, under the Mediterranean climate, full activation of ovaries and spermatogenetic activity unfolds from February to May [45], i.e., earlier than under the continental climate. Similarly, the highest number of cocoons (*A. caliginosa* – endogeic species) was observed in March in Libya [46]. Contrary, in the continental climate cocoon production peaks in late spring and early summer [47].

3.3.3. Anecic Species Simulation

Anecic species were sampled at four locations and each time a single species was present. Notable difference between the simulation and field densities was observed only at the location L1 where the simulated data underestimated the abundance of adult earthworms in the summer sampling. The first assumption was that the tolerance to the abiotic factors (soil temperature and moisture) is higher than the one set in the model, as the abundance of adults has a steeper decline from the week 8 than it should have in order to reach the field observed density (Figure 4). However, at the location L3, using the same model parameters and modeling the same species (*L. terrestris*), a decline in the abundance of adults gave an accurate prediction. The same was the case with the prediction of the anecic population at the location L6. Even though the population trend was opposite and the modeled species was different, the density predictions were acceptable. Using additional simulations, we have

Complimentary Contributor Copy

tested if the reason for the discrepancy between the field and model densities was a higher initial number of cocoons, as in the case of endogeic earthworms, or if the adult earthworms went deeper in the soil than initially parameterized during the adverse conditions. None of those simulations gave any more accurate predictions of adult density. Hence, the conclusion is that some other factor influenced the population dynamics at this location. It is known that the dynamics of earthworm populations are the result of diverse interrelated environmental and population factors. Growth rate may be influenced by many environmental factors other than temperature [19]. And, for instance, food supply and space requirements have been marked as the key factors influencing *L. terrestris* population that may regulate population dynamics [17]. In the case of this specific location the reason for the higher adult abundance in the field sampling result from the colonization of *L. terrestris* individuals from the adjacent areas that were not flooded or where the water level was lower during the early spring flooding event. The influence of flooding on the populations of *L. terrestris* described in literature is diverse. It ranges from the lack of L. terrestris individuals in the waterlogged soils [48, 49], their emergence on the surface [50] to no effects of summer flooding [51]. However, it seems that the duration of flooding and the level of water are important factors when assessing the effects on the *L. terrestris* populations. As *L. terrestris* is a large and mobile earthworm species, the scenario of an active horizontal migration from a flooded site cannot be excluded. Moreover, a quick recolonization of the site from adjacent areas can be assumed [52]. As the main colonizers, more mature and large individuals of the population are expected [53], which coincides with the increased density of the adult *L. terrestris* in the field that could not be achieved with the model prediction. This implies that sampling data collected at occasionally or regularly flooded habitats has to be interpreted carefully [54] and that special care should be given when parameterizing for the population dynamics model.

Complimentary Contributor Copy

3.3.4. Epigeic Species Simulation

Species from this category were present only on the continental slope (L1-L5) (Figure 4). The simulated densities corresponded to the field data for both juvenile and adult stage at all five locations. The maximum number of species per location was three (L1 and L3). The two most abundant epigeic species (*Dendrobena octaedra* and *Lumbricus rubellus*) sampled have a well-known life cycle, as they are a cosmopolitan species. Additionally, these species are frequently used in ecotoxicological and ecological research. The only obstacle for the simulation of the epigeic population dynamics for the present data was the fact that in the spring sampling no adult or juvenile epigeic earthworms were sampled at the location L3. Therefore, the population should be build up from the stock of cocoons. The strategy of the epigeic species is to survive adverse abiotic conditions (particularly freezing and drought) as cocoons [3] and they vanish after even a short-term winter frost (e.g., *L. rubellus*) [39]. However, some epigeic species are also among the most tolerant earthworms to freezing (e.g., *D. octaedra*) [55]. For the least a month each year, the soil at the location L3 is covered with snow, which could result in a decreased epigeic earthworm population with a spring predominance of cocoons in the population structure. However, the complete absence of epigeic individuals in the spring sampling could probably be ascribed to a very low population abundance that has been missed by sampling. Interestingly, in the summer sampling as much as three epigeic species were sampled at that location. At the locations L2 and L4, the population of both juveniles and adults declined, while at the locations L1 and L5 the difference in density remained rather small. Predicted cocoon densities at all locations were high, higher than in any other functional group. This is in accordance with the epigeic species life strategy of high cocoon production [56].

3.3.5. Predation Pressure

Apart from the abiotic parameters, the earthworm population dynamics is under the influence of various biotic factors such as predation, competition for food and space [57]. A certain degree of predation, or

Complimentary Contributor Copy

additional mortality, is expected in earthworm populations as they play a key role in many trophic chains and are a prey for many animal species (birds, mammals, reptiles, amphibians, and various invertebrates) [31, 58]. In the present model, and after the first simulation, a possibility of predation pressure was added as an additional parameter. This parameter can be adjusted separately for the juvenile and adult stage. A similar assumption of the predation pressure, but without the stage differentiation, was introduced into the model of pesticide effect assessment on the earthworm population dynamics [59]. The results showed that the mortality rate is induced by the density dependent predation, which serves as a stress buffer and has a different effect at the individual and population levels. In the present research, the percentage of predation pressure varies equally between the locations and the ecological groups (Table 3). The highest average additional mortality was found at the location L4 (28.75%), followed by the location L2 (22.5%), and the lowest at the location L1 (5.7%). The overall average additional mortality was higher for the juvenile (16.25%) than for the adult (11%) earthworms. The literature data also indicate that the predation was higher on small and medium-sized individuals [60, 61]. Across the functional groups, the highest predation pressure was calculated for epigeic earthworms (19%), while endogeic and anecic earthworms had the same predation pressure (11.14% and 11.25%, respectively). This is expected and commonly acknowledged since the epigeic species are the most exposed group of earthworms to predation and physical perturbations of soil [3]. In the available literature only a few papers identify the earthworm species that were consumed by various predators. The analyses of the diet of legless lizard *Anguis fragilis* showed that this species is not limited to feeding on epigeic species, but can consume anecic and endogeic species too [62]. On the other hand, the analysis of the stomach content of *Pluvialis apricaria* showed the highest percentage of *A. caliginosa* (endogeic species) followed by *L. rubellus* (epigeic) and *L. terrestris* (anecic) [60]. When analyzing life stages, the highest predation pressure was set for adult epigeic earthworms (20%) while for adult endogeic and anecic earthworms it was negligible (8% and 5%, respectively). Juvenile epigeic earthworms were also under the highest

Complimentary Contributor Copy

predation pressure (18%) followed by anecic (17.5%) and endogeic (14.29%) juvenile earthworms. The Monte Carlo simulations revealed that earthworms are being predated in proportion to their densities in the field with little evidence of prey choice [63].

Quantitative data on the number of consumed earthworms, the effect of predation on population or the significance of earthworms as a component of diet for their predators is generally lacking. Previous research indicated a negative [60] or negligible [64] influence of predation, particularly birds, on earthworm populations. The study of golden plover (*P. apricaria*) showed that the earthworm abundance declined for 50% in comparison with the predation protected areas after 22 days [60]. Similar results were obtained by Barnard and Thompson [65] who noticed a decrease of earthworm population of 46% and 71% in the exposed fields after three months, and consequent increase of 11% and 14% in the protected fields. Furthermore, the microcosmic experiment on the predation of Chilopoda showed a mortality increase of 31% - 64% during the 56 days experiment [61]. However, it seems that the predation does not have a long-term influence on the earthworm population dynamics, even though short-term period of intensive feeding of their predators exists, which reduces the earthworm abundance [3]. Accordingly, the study conducted in the colder time of the year (October to May), showed no significant difference in the abundance and distribution of earthworms in predated and predation-free fields [61]. Additionally, as the second sampling was conducted during a summer month, the possibility of underestimation of earthworm abundance due to the inactivity should be taken into account. Namely, the percentage of the adult individuals of endogeic *A. caliginosa* species in the aestivation can vary from 5% to 25% [66]. This refers only to an endogeic and anecic category and mostly to adult individuals. Even though we have discussed it in the view of predation pressure, the additional mortality parameter is not exclusively related to predation but also to other sources of stress to which earthworm population could be exposed and should be considered in that way, or considered as a discrepancy from “ideal” conditions.

Complimentary Contributor Copy

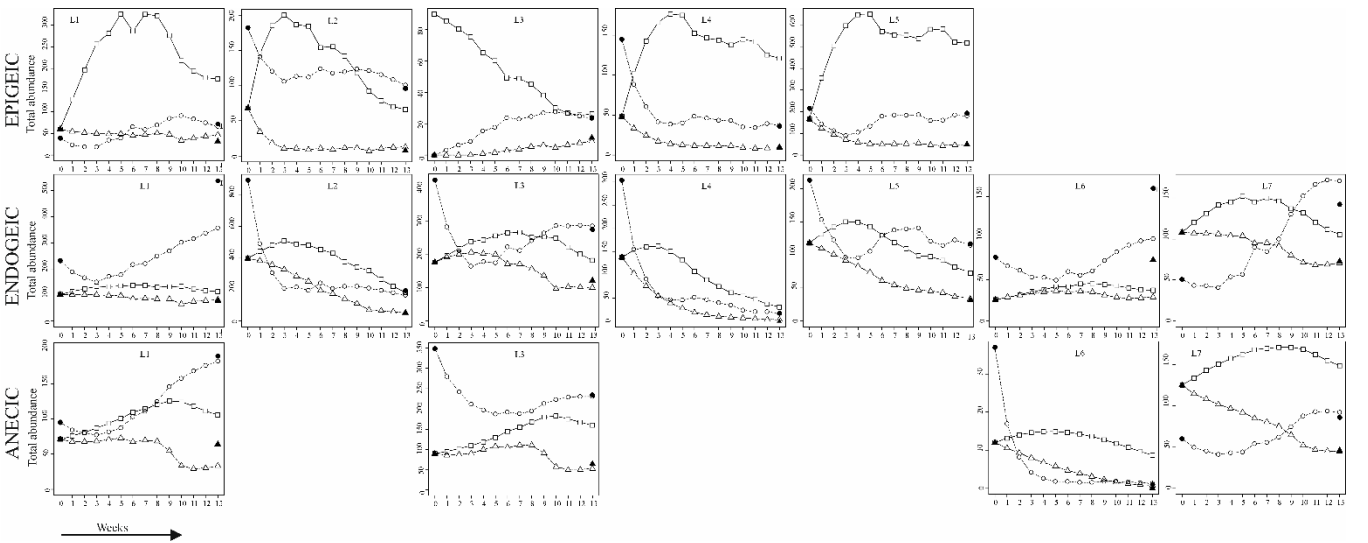

Figure 4. Population dynamics predictions for each location and earthworm ecological category during the 13 weeks of simulation. Legend: (▲) - field density of adults, (•) - field density of juveniles, (▵--▵--▵) - predicted density of adults, (o·····o·····o)- predicted densities of juveniles, (▫—▫—▫)- predicted density of cocoons.

Complimentary Contributor Copy

3.3.6. Elasticity Analysis

The elasticities were consistent during the simulation period and between locations for a specific earthworm category. Average elasticities for a 13 week period at all locations are shown in Figure 5. For all earthworm ecological categories, the highest value of elasticity has matrix parameter *PA* - probability of an adult remaining in the adult stage during projection interval (i.e., survival of adults). The second highest value of elasticities for anecic and endogeic earthworms has parameter *PJ* - probability that juvenile will remain in the juvenile stage during projection interval (i.e., survival of juveniles), while for epigeic earthworms the second highest value of elasticity has cocoon survival. The proportional changes in those matrix elements will result in the highest changes in the population growth rate.

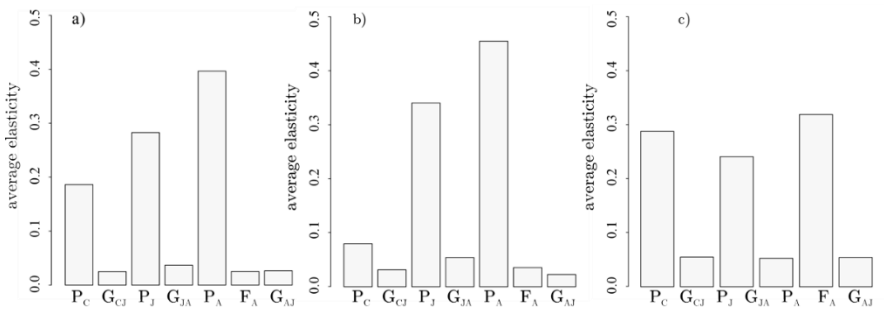

Figure 5. The average elasticity of a population growth rate to changes in vital rates: a) Anecic earthworms, b) Endogeic earthworms, c) Epigeic earthworms. (P_C – probability of remaining in the cocoon stage, P_J – probability of remaining in the juvenile stage, P_A – probability of remaining in the adult stage, G_{CJ} – probability of transition from cocoon to juvenile stage, G_{JA} – probability of transition from juvenile to adult stage, G_{AJ} – probability of losing reproductive characteristics, i.e., probability of returning to a juvenile stage, F_A – fecundity of adult individuals).

Our results are consistent with the results obtained by Svendsen et al . [41] who identified the adult survival as the most important parameter influencing population dynamics of *L. terrestris* and the results obtained by Klok et al . [25] who found that population dynamics of *L. rubellus* is most sensitive to adult and juvenile survival. Pelosi et al. [17] also found that

Complimentary Contributor Copy

population growth rate of *L. terrestris* is very sensitive to juvenile survival parameters.

4. CHALLENGES AND FUTURE DIRECTIONS

In this chapter we have presented an application of the mechanistic model to simulate the population dynamics of earthworms grouped into ecological categories in relation to soil climate (temperature and moisture) and predation pressure. This model could be suitable for the application in various aspects of environmental management. The inclusion of population modeling in ecological monitoring of earthworms would indicate changes in the population structure and changes in various pressures on earthworm populations. These various pressures could be explained by, for example, the predation pressure parameter as in this simulation. Such a parameter offers an explanation for variance between laboratory and field data. In order to differentiate sources of pressure the predation pressure parameters should be further elaborated in future research. This study confirmed the lack of many aspects on earthworm ecology and lack of data on life cycles of many species as well. Additionally, a problem with cocoon density should be addressed in the future research as it was the most possible reasons for majority of over and underestimations in our model.

Today, an ecological modelling is a well-established discipline. It is recognized that the understanding of complex phenomena requires modelling and that formal approaches can, to a considerable extent, capture the quantitative and qualitative understanding we have of biological systems and their environmental interactions [67].

Although, there is a fair amount of a papers with statistical well processed data about physiological and ecological properties of different earthworm species there is a lack of papers with earthworm population dynamics models and spatial simulations. It is already shown that different types of modelling can enable different viewpoints to a same topic. Moreover, such an approach expands the primer of research, broadening strongly reduced initial model or relations. There are several possible ways

Complimentary Contributor Copy

of future usage and development of earthworm life cycle models and simulations. Most probably the aim of earthworm population simulations will be focused on a usage of simulations for planning and implementation of ecological and ecotoxicological monitoring and for a usage of simulations for chemical testing on earthworms *in silico*.

Planning of ecological or ecotoxicological monitoring should start with more or less simple model or simulation of most important parts of the system to be monitored. Successfully planned monitoring involves enough preliminary data, which enables the possibility to construct a simple model or simulation. On the one hand such a model should be used for the control of the results of monitoring, and on the other hand, the results of monitoring should be used for model calibration and for required modifications of model. In that way both, the results of monitoring and models or simulations, become more reliable over time.

Thanks to a modern computer technology, among others to multicore processors (CPU) and graphical processing units (GPU), many "old fashion" modeling techniques become useful and attractive again. As a result of parallel computing techniques and multicores CPUs and GPUs with thousands of cores discrete modelling implementations with many recursions and several hundreds of parameters are now possible and are not time consuming as they were in near history.

A special challenge in a future ecological research on earthworms could be construction and usage of combination of several different model types as well as a connection of models with databases and with online data received during a monitoring. Such modeling, which includes a set of interdependent science-based components that together form the basis for constructing an appropriate modeling system, is called an integrated modeling [68].

Additionally, the usage of databases with sufficient amount of data could enable application of artificial neural networks for finding the relationships between parameters.

Complimentary Contributor Copy

REFERENCES

[1] Palm J, van Schaik NLMB, Schröder B. Modelling distribution patterns of anecic, epigeic and endogeic earthworms at catchment-scale in agro-ecosystems. *Pedobiologia* 2013; 56(1): 23–31.

[2] Lowe CN, Butt KR. Culture techniques for soil dwelling earthworms: A review. *Pedobiologia* 2005; 49: 401-413.

[3] Edwards CA, Bohlen PJ. *Biology and Ecology of Earthworms*. 3rd edition. London: Springer; 1995. 426 p.

[4] Curry JP. Factors affecting the abundance of earthworms in soils. In: *Earthworm ecology*. CRC Press; 2004. p. 401–24.

[5] Daniel O. Population dynamics of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) in a meadow. *Soil Biology and Biochemistry* 1992; 24(12): 1425–1431.

[6] Uvarov AV. Responses of an earthworm species to constant and diurnally fluctuating temperature regimes in laboratory microcosms. *European Journal of Soil Biology*. 1995; 31: 111–118.

[7] Pavao-Zuckerman MA. *Soil Ecology*. In: Jorgensen, SE, editor. Encyclopedia of Ecology. Amsterdam: Elsevier; 2008. p. 3277–3283.

[8] Bouché MB. Strategies lombriciennes. *Ecological Bulletin* 1977; 25: 122–132.

[9] Rožen A. Internal regulation of reproduction seasonality in earthworm *Dendrobaena octaedra* (Savigny, 1826) (Lumbricidae, Oligochaeta). *Soil Biology and Biochemistry* 2006; 38: 180-182.

[10] Grossi J-L, Brun J-J. Effect of climate and plant succession on lumbricid populations in the French Alps. *Soil Biology and Biochemistr*y 1997; 29(3–4): 329–333.

[11] Pop VV. Earthworm-vegetation-soil relationships in the Romanian Carpathians. *Soil Biology and Biochemistry* 1997; 29(3–4): 223–229.

[12] Schneider A-K, Schröder B. Perspectives in modelling earthworm dynamics and their feedbacks with abiotic soil properties. *Applied Soil Ecology* 2012; 58: 29–36.

Complimentary Contributor Copy

[13] Barot S, Rossi J-P, Lavelle P. Self-organization in a simple consumer–resource system, the example of earthworms. *Soil Biology and Biochemistry* 2007; 39(9): 2230–2240.

[14] Blanchart E, Marilleau N, Chotte J-L, Drogoul A, Perrier E, Cambier C. SWORM: an agent-based model to simulate the effect of earthworms on soil structure. *European Journal of Soil Science* 2009; 60(1): 13–21.

[15] Martin S, Lavelle P. A simulation model of vertical movements of an earthworm population (*Millsonia anomala* Omodeo, Megascolecidae) in an african savanna (Lamto, Ivory Coast). *Soil Biology and Biochemistry* 1992; 24(12): 1419–1424.

[16] Marinissen JCY, Bosch F van den. Colonization of new habitats by earthworms. *Oecologia* 1992; 91(3): 371–376.

[17] Pelosi C, Bertrand M, Makowski D, Roger-Estrade J. WORMDYN: A model of *Lumbricus terrestris* population dynamics in agricultural fields. *Ecological Modelling* 2008; 218(3–4): 219–234.

[18] Vorpahl P, Moenickes S, Richter O. Modelling of spatio-temporal population dynamics of earthworms under wetland conditions—An integrated approach. *Ecological Modelling* 2009; 220(24): 3647–3657.

[19] Moreau-Valancogne P, Bertrand M, Holmstrup M, Roger-Estrade J. Integration of thermal time and hydrotime models to describe the development and growth of temperate earthworms. *Soil Biology and Biochemistry* 2013; 63: 50–60.

[20] Daniel O, Kohli L, Bieri M. Weight gain and weight loss of the earthworm *Lumbricus terrestris* L. at different temperatures and body weights. *Soil Biology and Biochemistry* 1996; 1235-1240.

[21] Jensen KS, Homstrup M. Estimation of earthworm cocoon development time and its use in studies of *in situ* reproduction rates. *Applied Soil Ecology* 1997; 7: 73-82.

[22] Jager T, Reinecke SA, Reinecke AJ. Using process-based modelling to analyse earthworm life cycles. *Soil Biology and Biochemistry* 2006; 38: 1-6.

Complimentary Contributor Copy

[23] Hobbelen PHF, van Gestel CAM. Using dynamic energy budget modeling to predict the influence of temperature and food density on the effect of Cu on earthworm mediated litter consumption. *Ecological Modelling* 2007; 202: 373-384.

[24] Klok C, de Roos AM. Population Level Consequences of Toxicological Influences on Individual Growth and Reproduction in *Lumbricus rubellus* (Lumbricidae, Oligochaeta). *Ecotoxicology and environmental safety* 1996; 33(2): 118–127.

[25] Klok C, De Roos A, Marinissen JC, Baveco HM, Ma W-C. Assessing the effects of abiotic environmental stress on population growth in *Lumbricus rubellus* (Lumbricidae, Oligochaeta). *Soil Biology and Biochemistry* 1997; 29(3): 287–293.

[26] Jager T, Klok C. Extrapolating toxic effects on individuals to the population level: the role of dynamic energy budgets. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2010; 365(1557): 3531–3540.

[27] Hackenberger DK, Hackenberger BK. Earthworm community structure in grassland habitats differentiated by climate type during two consecutive seasons. *European Journal of Soil Biology* 2014; 61: 27–34.

[28] Spokas K, Forcella F. Software Tools for Weed Seed Germination Modeling. *Weed Science* 2009; 57(2): 216–227.

[29] Caswell H. *Matrix Population Models*. 2nd edition. Sunderland, Mass: Sinauer Associates; 2000. 710 p.

[30] Richter O, Diekkrüger B, Nörtersheuser P. *Environmental Fate Modelling of Pesticides: From the Laboratory to the Field Scale*. John Wiley & Sons; 2008. 297 p.

[31] Lee K-LE. *Earthworms: Their Ecology and Relationships With Soils and Land Use*. Sydney ; Orlando: Academic Press; 1986. 411 p.

[32] Presley ML, McElroy TC, Diehl WJ. Soil moisture and temperature interact to affect growth, survivorship, fecundity, and fitness in the earthworm *Eisenia fetida*. *Comparative Biochemistry and Physiology Part A: Physiology* 1996; 114(4): 319–26.

Complimentary Contributor Copy

[33] Crouse DT, Crowder LB, Caswell H. A Stage-Based Population Model for Loggerhead Sea Turtles and Implications for Conservation. *Ecology* 1987; 68(5): 1412.

[34] Brain P, Cousens R. An Equation to Describe Dose Responses where there is Stimulation of Growth at Low Dose. *Weed Research* 1989; 29: 93–96.

[35] R Development Core Team. R: A language and Environment for Statistical Computing, R Foundation for Statistical Computing. Vienna, Austria; 2014.

[36] Ritz C, Streibig JC. Bioassay Analysis Using R. *Journal of Statistical Software* 2005; 12(5).

[37] Caswell H. A general formula for the sensitivity of population growth rate to changes in life history parameters. *Theoretical Population Biology* 1978; 14: 215-230.

[38] Stubben, CJ, Milligan BG. Estimating and Analyzing Demographic Models Using the *popbio* Package in R. *Journal of Statistical Software* 2007; 22:11.

[39] Uvarov AV, Tiunov AV, Scheu S. Effects of seasonal and diurnal temperature fluctuations on population dynamics of two epigeic earthworm species in forest soil. *Soil Biology and Biochemistry* 2011; 43(3): 559–570.

[40] Eggleton P, Inward K, Smith J, Jones DT, Sherlock E. A six year study of earthworm (Lumbricidae) populations in pasture woodland in southern England shows their responses to soil temperature and soil moisture. *Soil Biology and Biochemistry* 2009: 41(9): 1857–1865.

[41] Svendsen TS, Hansen PE, Sommer C, Martinussen T, Grønvold J, Holter P. Life history characteristics of *Lumbricus terrestris* and effects of the veterinary antiparasitic compounds ivermectin and fenbendazole. *Soil Biology and Biochemistry* 2005; 37(5): 927–936.

[42] Trudgill DL, Honek A, Li D, Van Straalen NM. Thermal time – concepts and utility. *Annals of Applied Biology* 2005; 146(1): 1–14.

Complimentary Contributor Copy

[43] Johnston ASA, Hodson ME, Thorbek P, Alvarez T, Sibly RM. An energy budget agent-based model of earthworm populations and its application to study the effects of pesticides. *Ecological Modelling* 2014; 280: 5–17.

[44] Monroy F, Aira M, Gago JA, Dominguez J. Life cycle of the earthworm *Octodrilus complanatus* (Oligochaeta; Lumbricidae). *Comptes Rendus Biologies* 2007; 330: 369-396.

[45] Panidis S. Biology and reproduction of *Octodrilus complanatus* (Duges, 1828) (Annelida: Oligocaheta: Lumbricidae). PhD Thesis: Aristotle University of Thessaloniki; 1987.

[46] Nair A, Bennour SA. Cocoons and hatchlings of *Aporrectodea caliginosa* (Savigny 1826) (Oligochaeta: Lumbricidae) in Benghazi, Libya. *Journal of Arid Environments* 1998; 40(4): 459-466.

[47] Gerard BM. Factors affecting earthworms in pastures. *Journal of Animal Ecology* 1967; 36: 235–252.

[48] Beylich A, Graefe U. Annelid coenoses of wetlands representing different decomposer communities. In: Broll PDG, Merbach PDW, Pfeiffer PDE-M, editors. *Wetlands in Central Europe*. Springer Berlin Heidelberg; 2002: p. 1–10.

[49] Keplin B, Broll G. Earthworm coenoses in wet grassland of Northwest Germany. Effects of restoration management on a Histosol and a Gleysol. In: *Wetlands in Central Europe*. Springer Verlag; 2002. p. 11–34.

[50] Zorn MI, Van Gestel CAM, Eijsackers H. Species-specific earthworm population responses in relation to flooding dynamics in a Dutch floodplain soil. *Pedobiologia* 2005; 49(3): 189–98.

[51] Pižl V. Earthworm succession in abandoned fields - A comparison of deductive and sequential approaches to study. *Pedobiologia* 1999; 43(6): 705–712.

[52] Plum N. Terrestrial invertebrates in flooded grassland: A literature review. *Wetlands* 2005; 25(3): 721–737.

Complimentary Contributor Copy

[53] Nuutinen V, Butt KR, Jauhiainen L. Field margins and management affect settlement and spread of an introduced dew-worm (*Lumbricus terrestris* L.) population. *Pedobiologia* 2011; 54, Supplement: S167–72.

[54] Plum NM, Filser J. Floods and drought: Response of earthworms and potworms (Oligochaeta: Lumbricidae, Enchytraeidae) to hydrological extremes in wet grassland. *Pedobiologia* 2005; 49(5): 443–453.

[55] Holmstrup M, Overgaard J, Bindesbøl A-M, Pertoldi C, Bayley M. Adaptations to overwintering in the earthworm *Dendrobaena octaedra*: Genetic differences in glucose mobilisation and freeze tolerance. *Soil Biology and Biochemistry* 2007; 39(10): 2640–2650.

[56] Evans AC, Guild WJM. Studies on the Relationships Between Earthworms and Soil Fertility. *Annals of Applied Biology* 1948; 35(4): 471–484.

[57] Klok C. Effects of earthworm density on growth, development, and reproduction in *Lumbricus rubellus* (Hoffm.) and possible consequences for the intrinsic rate of population increase. *Soil Biology and Biochemistr*y 2007; 39(9): 2401–2407.

[58] Baubet E, Bonenfant C, Brandt S. Diet of the wild boar in the French Alps. *Galemys* 2004; 16: 99–111.

[59] Baveco JM, Roos AMD. Assessing the Impact of Pesticides on Lumbricid Populations: An Individual Based Modelling Approach. *Journal of Applied Ecology* 1996; 33(6): 1451–1468.

[60] Bengston S-A, Nilsson A, Nordstrom S, Rundgren S. Effects of bird predation on lumbricid populations. *Oikos* 1976; 27: 9–12.

[61] Judas M. Predator-pressure on earthworms: field experiments in a beechwood. *Pedobiologia* 1989; 33: 339–354.

[62] Brown DS, Jarman SN, Symondson WOC. Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology Resources* 2012; 12(2): 259–266.

[63] King RA, Vaughan IP, Bell JR, Bohan DA, Symondson WOC. Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology* 2010; 19(8): 1721–1732.

Complimentary Contributor Copy

[64] Cuendet G. Etude du comportement alimentaire des Mouettes rieuses (*Larus ridibundus*) et de leur influence sur les populations de vers de terre. *Ornithologischer Beobachter* 1977; (2): 87–8.

[65] Barnard CI, Thompson BA. *Gulls and plovers. The ecology of mixed-species feeding groups*. London: Croom-Helm; 1985.

[66] Fernández R, Novo M, Gutiérrez M, Almodóvar A, Díaz Cosín DJ. Life cycle and reproductive traits of the earthworm *Aporrectodea trapezoides* (Dugès, 1828) in laboratory cultures. *Pedobiologi*a 2010; 53(5): 295–299.

[67] Breckling B, Jopp F, Reuter H. *Historical Background of Ecological Modelling and Its Importance for Modern Ecology*. In: Modelling Complex Ecological Dynamics; Jopp F, Reuter H, Breckling B eds, Springer, 2011; p. 29-40.

[68] Laniak FG, Olchin G, Goodall J, Voinov A, Hill M, Glynn P, Whelan G, Geller G, Quinn N, Blind M, Peckham S, Reaney S, Gaber N, Kennedy R, Hughes A. Integrated environmental modeling: A vision and roadmap for the future. *Environmental Modelling and Software* 2013; 39: 3-23.

Complimentary Contributor Copy

In: Earthworms
Editor: Clayton G. Horton
ISBN: 978-1-53612-176-6
© 2017 Nova Science Publishers, Inc.

Chapter 4

EARTHWORMS AS ECOSYSTEM ENGINEERS: A REVIEW

Renée-Claire Le Bayon[1,*], Géraldine Bullinger-Weber[2], Andreas Schomburg[1], Pascal Turberg[3], Rodolphe Schlaepfer[3] and Claire Guenat[3]

[1]Functional Ecology Laboratory, Biological Institute, University of Neuchâtel, Switzerland
[2]iTEC - Institut des Technologies de l'Environnement Construit - Haute école d'ingénierie et d'architecture, Fribourg, Switzerland
[3]Laboratory ECOS & WSL, École Polytechnique Fédérale de Lausanne (EPFL), School of Architecture, Civil and Environmental Engineering, Lausanne, Switzerland

ABSTRACT

The concept of ecosystem engineering has emerged decades ago and highlights the direct or indirect modulation of available resources by organisms through their biological activities. Ecosystem engineers create biogenic structures (aggregates, burrows) that may serve as habitats for

* Corresponding author Email: claire.lebayon@unine.ch, Phone: +41 (0) 32 718 23 65.

Complimentary Contributor Copy

other species than themselves. This chapter aims at overviewing the key role played by earthworms as ecosystem engineers through their bioturbation activities involving soil mixing as well as their influence on the decomposition and mineralization of litter by breaking down organic matter, and their influence on the gas and water exchange or nutrient transfer in the soil. Focus is made on the engineering processes and especially the formation of biogenic structures in relation to soil structure (burrows, casts) in the framework of soil function interactions, particularly in the drilosphere. Special attention is paid to soil aggregates' fabric and new tools that may help to discriminate their origin. Finally, management and ecosystem engineer's future challenges will be highlighted regarding soil ecosystem services in the context of ecosystem restoration.

Keywords: earthworms, bioturbation, hotspots, drilosphere, ecosystem services

1. INTRODUCTION

The role of plants and animals in ecosystem functioning has been long recognized, and naturalists and biologists have been conscious that organisms modulate the physical and chemical processes occurring in ecosystems. As these non-trophic relationships between biota and their environment did not fit in the usual categories of ecological interactions, the term "ecosystem engineering" was chosen and validated by most of scientists (Jones et al., 1994; Wright et al., 2004; Cuddington et al., 2007; Berke, 2010). In terrestrial ecosystems, soil biodiversity promotes multiple functions simultaneously within which ecosystem engineers are the main drivers. Soil macro-invertebrates play a key role in soil organic matter transformations and nutrient dynamics at different spatial and temporal scales. This chapter aims at overviewing the key role endorsed by earthworms through their bioturbation activities in the context of soil functions and ecosystem services.

Complimentary Contributor Copy

2. SOIL ORGANISMS AS ECOSYSTEM ENGINEERS AND KEYSTONE SPECIES

2.1. The Concept of Ecosystem Engineers Applied to Soil

In their first article on the topic, Jones et al., (1994) defined ecosystem engineers as "*organisms that directly or indirectly modulate the availability of resources (other than themselves) to other species by causing physical state changes in biotic or abiotic materials. In so doing, they modify, maintain and/or create habitats*". Such organisms are involved in the modification, maintenance, creation and/or destruction of habitats (Jones et al., 1997a, 1997b; Wright et al., 2004) independently from their origin (animal, plant, microorganisms) and their location (aquatic, terrestrial ecosystems). Ecosystem engineers play hence key roles in the production and evolution of habitats both for themselves and other organisms and controlling their activities through physical and biochemical processes. As a consequence, ecosystem engineers typically enhance environmental heterogeneity, thereby increasing niche opportunities and the diversity of the community at the landscape level (Jones et al., 1997a, 1997b; Wright et al., 2002).

Going deeper in the engineering concept, Jones et al., (1997a, 1997b, 2010) distinguished two kinds of ecosystem engineers, i.e., allogenic and autogenic engineers. On one hand, allogenic engineers modify the environment by mechanically changing living or non-living materials from one physical state to another. On the other hand, autogenic engineers modify the environment by modifying themselves. Several ecosystem engineers may be both autogenic and allogenic engineers; this is notably the case of trees being at the same time a habitat for birds (branches, trunk cavities) and an allogenic engineer digging burrows and developing its roots network (Jones et al., 1997a, 1997b).

Focusing on soil, invertebrates are key components of soil functions and properties, and their occurrence as ecosystem engineers with regards to their bioturbation activities were originally recognized by the Greek

Complimentary Contributor Copy

philosopher Aristote who considered earthworms as "*the intestines of the earth*". Later, Shaler (1892) and Darwin (1881) also pointed out that soils are structured and created by earthworms and other invertebrates. The representatives of macrofauna namely ants, termites, and earthworms, are probably the most important soil ecosystem engineers through their bioturbation activity, i.e., the formation of biogenic structures. These organisms act as allogenic engineers and construct different types of structures such as nests, mounds, casts and burrows, etc. Some of them act deep in the soil, sometimes several meters down (Lee, 1985; Edwards and Bohlen, 1996; Lavelle et al., 1997, 2016; Coleman et al., 2004; Shipitalo and Le Bayon, 2004; Bardgett, 2005; Jouquet et al., 2006; Blouin et al., 2013; Havlicek and Mitchell, 2014).

Recently, Jones et al., (2010) pointed out various changes in ecosystems resulting from engineers' activities. This new approach includes not only the structural changes (dams, burrows, nests, etc.) but also chemical abiotic changes (concentration of organic matter, fluxes of nutrients, gases, energy, etc.), these latter causing biotic changes (habitat for other organisms, enzyme activities in biogenic structures, etc.) and finally an engineer feedback (niches, protection from predation, food supply, etc.). This notion of feedback and interdependency of processes in engineering systems seems indeed essential to get an overview of the global functioning of the ecosystem. Along the same lines, functional groups of ecosystem engineers were proposed by Berke (2010) who classified these organisms as followed: i) structural engineers, ii) bioturbators, iii) light engineers and iv) chemical engineers. What is especially interesting in this outlook is that an ecosystem engineer may belong to several functional groups. Consequently, this classification is not mutually exclusive given that many engineers affect ecosystems though multiple pathways simultaneously (Berke, 2010).

This new approach of Jones et al., (2010) is also applicable to soil-engineers that are responsible of physical modifications but includes also biological and biochemical processes. Soil ecosystem engineers are indeed responsible of the creation of biogenic structures through their bioturbation activities. From vertebrates to invertebrates passing through plant roots,

Complimentary Contributor Copy

mixing and remoulding soil material and organic matter lead to the formation and the emergence of new areas of biological and functional properties. Platt et al., (2016) reviewed the impacts of soil disturbing vertebrates resulting in physical structures that lead to the creation of nutrient-rich patches being sometimes implied in global processes such as soil erosion. Plants are also included in ecosystem engineering because they create a gradient of soil water content around their root system (Guttierez and Jones, 2006). Through their selective feeding and the choice of the building material, soil macrofauna creates most of the time gradients of organic and mineral particles, leading to concentration of nutrients in their living space (Edwards and Bohlen, 1996; Frouz et al., 2003; Edwards, 2004; Bardgett, 2005; Véle et al., 2011; Jouquet et al., 2014).

In a review, Jouquet et al., (2006) went further in the definition of soil ecosystem engineers and, focusing on their behaviour, these authors discriminated extended phenotype engineers from accidental engineers. The first ones concentrate their activities on the building of biogenic structures in order to maintain optimal conditions for their growth, while the second ones expend energy in moving through the soil to be as close as possible to their optimal environment. According to Jouquet et al., (2006), termites and ants would be extended phenotype engineers, with impacts on ecosystem functioning and feedback loops affecting themselves; earthworms as for them can be considered as both extended phenotype or accidental engineers depending on the species and the ecological category.

Generally, most organisms, including the ones living in soil, can be considered as ecosystem engineers to some degree; hence, this concept applies broadly to many organisms in a wide range of habitats (Berke, 2010). Indeed, it is difficult to imagine a life strategy that does not in some way lead to a degree of modification of the abiotic environment. Given the ubiquity of ecosystem engineering, some researchers have argued that if all organisms are ecosystem engineers, the concept cannot be considered as useful (Reichman and Seabloom, 2002a, 2002b). Moreover, one of the most commonly asked questions about the ecosystem engineering concept is some variant of "*how do ecosystem engineers differ from keystone species*?" (Wright and Jones, 2006).

Complimentary Contributor Copy

2.2. Are Ecosystem Soil Engineers Keystone Species?

Although many of the similarities and differences between ecosystem engineers and keystone species were discussed in the original papers (Jones et al., 1994, 1997a, 1997b), the topic seems to be a perennial one in conferences, seminars, workshops, and other expert meetings (Wright and Jones, 2006).

The term "*keystone species*" was first mentioned by Paine (1966) after several studies examining the interaction strengths of food webs in rocky intertidal ecosystems. More common recent definitions of keystone species usually focus on species, mostly of high trophic status, whose activities exert a disproportionately influence on the patterns of species occurrence, distribution, and density in a community relative to their abundance and biomass (Paine, 1969; Power et al., 1996; Nunez and Dimarco, 2012). Thus, when the density of a keystone species falls below some threshold, the species diversity in the area may decrease, triggering ecological chain reactions and ending with degraded or simplified ecosystems. As a consequence, a keystone species' disappearance would start a domino effect and affect other species that rely on it for survival, and would be then vulnerable to potential disappearance and extinction. Paine (1969) coined the term "*trophic cascade"* to describe the cascade down the trophic ladder (or food web) to the lowest level, often reducing habitat complexity and species diversity. While some keystone species have large effects on communities and ecosystems through ecosystem engineering, others have their effects through trophic interactions or other processes, such as pollination (Wright and Jones, 2006).

In the ecosystem engineering concept, the organism's abundance or density is neither taken into account nor trophic interactions in the form of provision or consumption of tissue (Jones et al., 1994, 1997a, 1997b). Ecosystem engineers directly or indirectly modulate the availability of resources (other than themselves) to other species by causing physical state changes in biotic or abiotic materials. Reichman and Seabloom (2002b) reinforced this idea suggesting that the term "*ecosystem engineering*" should be restricted to cases in which the physical modification of the

Complimentary Contributor Copy

environment is "*large relative to purely physical processes operating in the system*". To some extent, the key difference between the ecosystem engineering concept and the keystone species concept seems to be that the former is process focused, while the latter is outcome focused (Jones et al., 1997a; Wilby, 2002).

As noted above, all organisms modify the environment to some extent, and they cannot all be keystone species. Some species are however described as both keystone species and ecosystem engineers. This is the case for corals or reef-forming molluscs whose activities create suitable habitats for their own use as well as for many other species that otherwise might not be able to utilize the local habitats (Mills et al., 1993; Gutiérrez et al., 2003; Haemig, 2012). Ecological controversies between engineering and keystone species concepts are still in debate and understanding their interactions and potential cross-referencing is especially important for sustainability of ecosystem processes and the functioning of ecosystems at the global scale.

3. HOTSPOTS, HOTSPHERES AND HOT MOMENTS IN SOIL

Soils are the most heterogeneous system over several space-time scales, especially areas of activity as pointed out by Beare et al. (1995). According to these authors, such *"hotspots" of intense biological activity also called "hotspheres" may* represent more than 90% of the total biological activity concentrated in less than 10% of the total soil volume. Five hotspheres, most of them reliable to biological processes, were thus defined by Beare et al., (1995): i) the rhizosphere in the vicinity of plant roots, ii) the detritusphere located in the first organic layers, iii) the aggregatusphere encompassing soil aggregates, iv) the porosphere focused on voids and finally the v) the drilosphere under the influence of earthworms (see sections 4.2 and 4.3).

Complimentary Contributor Copy

Whereas the engineering effects of earthworms are studied across many systems because their abundance is quantified, research on termites and ants as engineers has focused mainly on a few sites. The consideration of soil macrofauna as soil engineers led to the fact that the main spheres representing functional domains in soil as defined by Beare et al., (1995) were completed by two other ones: the myrmecosphere under the influence of ants, and the termitosphere relative to termites' activity (Brown et al., 2000; Lavelle and Spain, 2001; Lavelle et al., 2006). While termites are mainly key soil organisms in tropical and subtropical soils (Jouquet et al., 2016), ants are well known in temperate forest (Frouz et al., 2003; Cherix et al., 2006; Véle et al., 2011; King et al., 2013) and earthworms in natural or semi-natural ecosystems, grasslands, urban or agricultural soils (Edwards, 2004; Shipitalo and Le Bayon, 2004; Blouin et al., 2013; Amossé et al., 2015, 2016; Lavelle et al., 2016).

Recently, Kuzyakov and Blagodatskaya (2015) defined microbial hotspots as small soil volumes with much faster process rates and much more intensive interactions compared to the average soil conditions. In this study, the microbial hotspots, resulting from inputs of labile organics by plants that are highly variable over short periods of time, are called the "*hot moments*". Irrespective of their volume, usually between 1% and 5 %, or even 0.2 % going deeper in the subsoil, such microbial hotspots are mainly responsible for the ecologically relevant processes in soil. Kuzyakov and Blagodatskaya (2015) also underlined that these hotspots and hot moments could be extended and may occur in the rhizosphere or detritusphere, with a high impact on the soil at a larger-scale.

Future research is hence needed for a better approach of soil dynamics in space and time due to its extremely high heterogeneity of biological activities. Challenges are especially strong in understanding the nature of interactions, for example between soil fauna, plants, microbes and the characteristics and role of hotspots of activity and hot moment events.

Complimentary Contributor Copy

4. EARTHWORMS: ONE OF THE MOST EFFICIENT SOIL ENGINEERS

4.1. Ecology and Habits

Earthworms constitute the largest terrestrial faunal biomass occurring worldwide and usually preferring moist habitats of moderate temperature (Lee, 1985; Edwards and Bohlen, 1996; Coleman et al., 2004; Bardgett, 2005). Earthworm populations contribute to approximately 40-90% of soil macrofaunal biomass and 8% of total soil biomass in many ecosystems (Sinha et al., 2013). Their spatial distribution is widely heterogeneous and earthworms develop different reproductive strategies (r and K) in order to exploit more effectively their edaphoclimatic environment depending on several factors, i.e., plant cover and soil properties (soil texture, soil organic matter content) as well as internal population processes, i.e., reproduction rates and dispersal mode (Lee, 1985; Edwards and Bohlen, 1996; Edwards, 2004; Bullinger-Weber et al., 2012).

At a local scale, the spatial distribution of earthworm populations is usually observed in patches relative to location of food resources either due to surface litterfall or in the vicinity of living roots that release high amounts of carbon compared to the surrounding soil. Based on their behaviour, ecological niches and feeding ecology, earthworms are generally split into three main ecological categories (Bouché, 1977; Edwards and Bohlen, 1996; Edwards, 2004; Figure 1): i) epigeics inhabit and feed in the upper organic soil layers, and produce organic castings but rarely ingest soil particles, ii) endogeics are geophageous species that live in the upper organo-mineral soil layers and construct horizontal burrows, consuming mineral soil materials with soil organic matter in various degrees of decomposition and, iii) anecics take advantages of both the surface litter as a source of food they drag into their galleries, and the deepest mineral soil as a refuge in which they dig burrows; they usually produce surface-casts.

Complimentary Contributor Copy

Figure 1. Adult earthworms of the three ecological categories: a) the endogeic *Aporrectodea caliginosa*, b) the epigeic *Lumbricus rubellus*, c) the anecic *Aporrectodea longa* - Bars represent 1 cm.

At the landscape scale, species composition of the earthworm communities varies according to latitude and altitude and human management as well as abundance, biomass, density, seasonal activity (Lavelle and Spain, 2001; Salomé et al., 2011; Bullinger-Weber et al., 2012; Fournier et al., 2015; Mariotte et al., 2016). Hackenberger and Hackenberger (2014) thus demonstrated that earthworm species composition, the ratio of ecological categories (epigeics, endogeics and anecics) and juvenile : adult ratio changed along a transect of varying climate types and elevations. Moreover, as earthworm populations are regulated in a density-dependent manner, inter- and intra-specific interactions may strongly affect species response and thereby structure and functioning of lumbricid communities (Uvarov, 2009) with further consequence on soil processes.

Still there is scope of earthworm study on the basis of temperature regime of soil. Earthworm bioturbation activity depends on both soil temperature (Whalen et al., 2004; Uvarov et al., 2011) and moisture (Edwards and Bohlen, 1996; Kanianska et al., 2016). Until now, most of the studies on earthworm communities have been conducted on mature soils and describe interactions between biota and structure in cultivated and anthropogenic soils (Jégou et al., 1998; Davidson and Grieve, 2006a,

Complimentary Contributor Copy

2006b; Le Bayon and Binet, 2006; Amossé et al., 2016; Cunha et al., 2016). Moreover, much of the research relies on laboratory-based mesocosm or microcosms studies (Jégou et al., 2000; Whalen et al., 2004; Perreault and Whalen, 2006; Uvarov et al., 2011; Capowiez et al., 2014, 2015; Amossé et al., 2015, 2016).

An innovative study was recently set up being at the interface between laboratory experiment and natural conditions. Potvin and Lilleskov (2017) used an experimental rhizotron over 7 years to observe non-destructively *insitu* dynamics of earthworm activity. In a pine and a hardwood sites, they showed that both activity and vertical distribution of introduced earthworms is closely linked to earthworm species and soil temperature variations among seasons. As a result, the anecic *Lumbricus terrestris* typically remains active through the winter, whereas the endogeic *Aporrectodea caliginosa* is more likely to enter an aestivation period. However, activity of all earthworms decreased substantially in summer (July-August) when soil temperature was at its highest and soil moisture at its lowest for the year (Potvin and Lilleskov, 2017).

For the moment, little research on earthworm activities exists in natural environments. In floodplains where sediment deposits regularly occur relative to flood events (Bullinger-Weber and Gobat, 2006), water stable macro-aggregates due to earthworm activities have been observed even in these young soils and in pioneer stages of topsoil formation under willow forests (Guenat et al., 1999; Bullinger-Weber et al., 2007). Changes in earthworm communities tend to reflect a gradient in alluvial dynamics (Zorn et al., 2005; Schütz et al., 2008; Salomé et al., 2011; Bullinger-Weber et al., 2012; Le Bayon et al., 2013; Fournier et al., 2012, 2015). Due to their high and constant moisture conditions, Fluvisols seem to create the most suitable conditions for earthworm abundance and biomass (Salomé et al., 2011; Kanianska et al., 2016).

Complimentary Contributor Copy

4.2. The Drilosphere: A Hotsphere of Energy and Activities

The drilosphere is the part of soil which is influenced by earthworm activities usually in association with microflora and is considered as a functional domain (Lavelle et al., 1997; Lavelle, 2002; Blouin et al., 2013; Johnson-Maynard and Strawn, 2016). It encompasses earthworm burrows, surface-casts and casts produced into the soil profile, but also the earthworm gut content as well as symbionts (Ojha and Devkota, 2014). Hence, the drilosphere includes a high degree of relationships between the microorganisms (fungi, actinomycetes, bacteria), micro-, meso-, and macro invertebrates (protozoa, mites, springtails, millipedes, isopods, nematodes). This association is especially effective with the anecics (Brown, 1995; Anderson and Bohlen, 1998; Maraun et al., 1999) which manage the biological creation of burrows and casts where subordinated organisms inhabit. As a general rule, anecics and endogeics are the most concerned in favouring the drilosphere formation due to their ecological behaviour and their selective ingestion of organic and mineral particles (Curry and Schmidt, 2007). Anecic species built in soil a permanent burrow system (about 2 to 3 m depth) which is an important network of root growth activity and microbial dispersal (Ehlers et al., 1983). On their side, endogeic species are mostly located around the rhizospheric region of plant roots enriched in microorganisms relative to exudates (Rovira et al., 1987; Robertson et al., 1994; Hirth et al., 1998).

As a consequence, soil biogeochemical properties within the drilosphere differ from those of bulk soil, similar to the better known rhizosphere that is created by plant roots (Lipiec et al., 2015). Linking drilosphere and rhizosphere functioning is then obvious. Earthworms enhance plant growth and plant quality (van Groenigen et al., 2014) that is closely linked to drilosphere-rhizosphere interactions. A large part of the influences that ecosystem engineers, and especially earthworms, have on nutrient cycling and plant growth is due to the activities of microorganisms and other small organisms (fine roots, invertebrates) selectively activated within their functional domains.

Complimentary Contributor Copy

4.3. Processes in the Drilosphere

4.3.1. Earthworm Mucus and Gut

Earthworms produce polysaccharide compounds named "mucus" that is secreted at the body surface and in the forepart of their gut (Lee, 1985; Chapuis-Lardy et al., 2011). The daily cutaneous mucus production by a common temperate endogeic species was estimated from 0.2% to 0.5% of the C content of the worm (Scheu, 1991). Mucus may comprise from 4% to more than 30% of the dry matter content in the anterior gut (Trigo et al., 1999) but the posterior half gut of the earthworm is lacking of mucus: the question of reabsorption or recycling of this enriched C-source is still raised.

It was recently showed that *Aporrectodea caliginosa* mucus enhances the mineralization and humification of plant residues through the activation of microorganisms (Bityutskii et al., 2012). As a result, earthworm digestion leads to the mineralization of 5% to 20% of organic matter on average during the very short period (from <1h to 1 day) of a gut transit (Lavelle and Spain, 2001). It reportedly takes 2 to 24h for soil to pass through the digestive tract of lumbricid earthworms (Barley, 1959; Piearce 1972; Bolton and Phillipson, 1976). Moreover, the amount of soil contained in earthworm intestinal tract may be remarkable. In one square meter of temperate soil (2000 individuals m^{-2}), approximately 1L of soil was estimated to be stored within the gut of the earthworm population (Drake and Horn, 2007). Around 4 to 10% of the soil passes annually through earthworms' gut that is the equivalent to several hundred tonnes of dry soil (Lavelle et al., 2016).

The earthworm gut has been described as a "mutualistic digestive system" (Barois and Lavelle, 1986; Brown and Doube, 2004; Edwards, 2004) in which the exoenzymes produced by ingested microorganisms enhance the degradation of complex organic matter during their passage through the gut and thus enhance the capacity of the worm to assimilate nutrients. Le Bayon and Binet (2006) demonstrated that some earthworm species may have also their own enzymes, independently from the microorganisms present in the ingested soil; this is notably the case for

Complimentary Contributor Copy

alcaline phosphatases implied in organic phosphorous mineralization. Moreover, Horn et al. (2003) and Drake and Horn (2007) highlighted that the earthworm gut is a mobile anoxic microzone in aerated soils and is rich in readily degradable organic compounds, many of which appear to be microbial fermentation products produced by ingested microbes during gut passage. These authors highlighted also that this mutualistic digestive system of the earthworm is important to metabolic events linked to denitrification in the gut, and especially the emission of nitrogenous gases by the bacteria in the earthworm intestines capable of growth under anoxic conditions.

As a result, digestion processes occurring in the earthworm intestinal tract are closely linked to biogeochemical cycles implying in the soil organic matter decomposition, i.e., among others carbon, nitrogen and phosphorous. Earthworms can be thus classified as chemical engineers through the addition of mucus enriched in carbon, nitrogen and phosphorus during the gut transit (Martin et al., 1987; Schrader and Zhang, 1997; McInerney et al., 2001; Le Bayon and Binet, 2006).

4.3.2. Earthworm Casts

Earthworm acts thus as a living bioreactor that transforms and remoulds organic and mineral particles (Brown et al., 2000). Depending on ecological categories, earthworm gut transit time probably affects in a first step the degree of microaggregates disruption before being remoulded in larger aggregates (Six et al., 2002, 2004). According to Lee (1985), earthworms can process up to 25% of the Ah horizon in 1 year and thus can be important aggregate-forming agents through the production of casts within the soil and onto the surface (often called surface-casts or middens when particularly enriched in organic matter debris). Other authors also found that water-stable biogenic structures, i.e., organo-mineral aggregates, are found in the soil colonized by earthworms (Schrader and Zhang, 1997; Bossuyt et al., 2005; Jouquet et al., 2009). Most of the studies focused on surface-casts whose amount of production may reach a mean of 40 ton ha^{-1} yr^{-1} (see the review of Feller et al., 2003). In a maize plot, Le Bayon and Binet (1999) calculated that surface-casts production was 34 kg yr^{-1} kg^{-1}

Complimentary Contributor Copy

earthworms with the anecic *Lumbricus terrestris* and the endogeic *Aporrectodea caliginosa* as dominant species of the community. In grassland in Luxembourg, a total amount of 195.6 ton ha^{-1} casts was observed, 58% from endogeic earthworms and 42% from anecics (Zangerlé et al., 2016b). Discriminating surface-casts from casts observed into the soil profile, they represent 44.4 ton ha^{-1} and 151.2 ton ha^{-1}, respectively (Zangerlé et al., 2016b). Endogeic species were also showed to deposit more casts underground (95% according to Zorn et al., 2008).

The selective ingestion of organic and mineral particles (Curry and Schmidt, 2007) leads to high variations of casts' components. Shipitalo and Protz (1988) noted that casts of *Lumbricus rubellus*, an epigeic species, contained less sand than those of *Lumbricus terrestris* (anecic species), and all these casts had low sand content compared to the non-ingested soil. However, when the initial sand content of the soil is low (for instance less than 4%), small differences in the texture between the casts of *Lumbricus terrestris* and *Aporrectodea caliginosa* and the non-ingested soil are reported (Schrader and Zhang, 1997). This contradictory result highlights the need of sand particles to favour the comminution of organic material in the gizzard of earthworms by crushing and grinding actions (Brown et al., 2000). Furthermore, mixing organic and mineral particles imply mechanical action but also the co-occurrence of gut microflora that originates either from the earthworm itself or from the ingested soil.

Earthworms are thus key promoters of soil larger aggregates. The earthworm casts, fortified with mucilaginous secretion, help in aggregate formation and stability (Shipitalo and Protz, 1988; Marinissen and Dexter, 1990) which is the foundation for soil structure formation. Due to the selective ingestion of organic material, casts have higher concentration of nutrients and soil organic matter than the surrounding soil. Moreover, enzyme activities and microbial stimulation are enhanced in these biogenic aggregates due to the addition of mucus and water during the gut transit (Shipitalo and Le Bayon, 2004; Chapuis-Lardy et al., 2011; Blouin et al., 2013). These newly formed habitats are enriched in organic matter and nutrients and act as hotspots of biological activity.

Complimentary Contributor Copy

Furthermore, being major bioturbators in terrestrial ecosystems, earthworms largely contribute to the formation of stable microaggregates within macroaggregates leading to a crumb soil structure that helps the protection of organic carbon (Bossuyt et al., 2005) and the physical stabilization of organic matter (Angst et al., 2017). In pioneer environments such as floodplains with regular sediment deposits, epigeic earthworms (mainly *Lumbricus rubellus*) and enchytraeids are the first engineers involved in soil structure at short-term and then, if the texture is favourable that is mostly silt-dominated, anecic and endogeic earthworms may then colonize the different soil layers improving physical and nutrient conditions and creating long-term stable aggregates (Bullinger-Weber et al., 2007).

Future research should focus on belowground faeces, which may represent large amounts of casting activities as well as on co-construction of stable macroaggregates by several ecosystem engineers, as for instance plant roots and earthworms (Zangerlé et al., 2011, 2016a, 2016b). New research using Rock Eval pyrolysis focusing on organic matter signature may be a great tool to discriminate aggregates relative to the engineer (Schomburg et al., unpublished data). To date, differences are significant but however on a really small scales due to the fact that organic matter signature from aggregates from field samples is marked by the organic matter decay level. Both signatures from plants and earthworms are not strong enough to prevail over the background signature from bulk organic matter, however this new approach appears promising for the future.

4.3.3. Earthworm Burrows

Earthworm burrows function as soil macropores and earthworms enhance porosity as they move through the soil, thus decreasing soil density (Johnson-Maynard et al., 2007) hence improving soil aeration (Knight et al., 1992), infiltration (Stockdill, 1966) and water-holding capacity of soils especially in casts and burrow-linings. The number of burrows, their length, diameter, and above all their connectivity are essential to ensure an efficient hydraulic conductivity (Ehlers, 1975; Johnson-Maynard et. al., 2002; Pérès et al., 2010). The burrow systems of

Complimentary Contributor Copy

earthworms are greatly depending on site-specific factors such as soil texture, temperature, water content, topography, as well as the ecological categories of earthworms (Kretzschmar, 1982; Capowiez et al., 1998; Bastardie et al., 2005; Perreault and Whalen, 2006). Some species such as *Lumbricus terrestris* make deep into the soil permanent burrows in which they can live several years (Lee, 1985; Edwards and Bohlen, 1996; Lavelle and Spain, 2001). Estimates of the number of burrows in temperate region soils range from 100 to 800 m^{-2} (Lavelle, 1988). Most burrows are located between 30 and 40 cm deep in the soil and their length, diameter and branching depend on earthworm species (Kretzschmar, 1982).

Another positive feedback of earthworm burrows is that soil organic matter concentrates in burrows mainly due to the translocation of detrital resources from the soil surface by earthworms, especially by the anecic *Lumbricus terrestris*. Burrow-linings are thus enriched in organic matter compared to the non-ingested soil (Le Bayon and Milleret, 2009). Moreover, burrow walls are regularly smeared with mucus as earthworms move up and downs several times a day. Earthworm epidermal mucus is enriched in water but also in carbon and nitrogen as previously highlighted, and it serves as a lubricant improving the displacement of the worms inside the soil. These specific conditions of resource enrichment lead to the presence of specific and active communities of microflora and microfauna in burrow walls (Tiunov and Scheu, 1999; Jégou et al., 2000; Savin et al., 2004) and enzyme activities are thus enhanced over the 2-3 mm thickness all along the linings (Le Bayon and Binet, 2006). So, as for casts, burrows are hotspots of bioavaiblable nutrients that could directly be absorbed by plant roots (Milleret et al., 2010; Hoang et al., 2016). In this way, Cameron et al., (2014) showed that the roots of *Achillea millefolium* preferentially occupy earthworm burrows (*Lumbricus terrestris*) where nutrient availability was presumably high due to earthworm excreta. In addition, from a physical point of view, earthworm burrows provide interconnected channels that make easier the penetration of roots into the soil without losing much energy. Burrows may also serve as a regeneration niche for plants in grassland ecosystems (Milcu et al., 2006).

Complimentary Contributor Copy

New techniques that are more and more sophisticated and reliable have emerged these last twenty years to study deeper the size and three-dimensional orientation of earthworm burrows. The non-destructive 3D X-ray computed tomography is very useful for evaluating the spatial pattern of earthworm burrows (Jégou et al., 1999; Capowiez et al., 2014, 2015; Amossé et al., 2015, 2016). This method also helps to follow the duration of earthworm burrows over time. For example, a microcosm experiment, Francis et al., (2001) reported that the endogeic *Aporrectodea caliginosa* creates more temporary burrows than the epigeic *Lumbricus rubellus. These worms tend indeed to refill their burrows after several days through the emission of casts. However,* the anecic *Lumbricus terrestris* seems to intensively re-use their continuous, vertical burrows (Francis et al., 2001; Nuutinen, 2011; Grigoropoulou and Butt, 2012). In natural conditions, Bastardie et al., (2005) extracted soil cores from a permanent pasture and found two different burrow forms: i) one very short and disconnected burrows with small diameters that were probably built by endogeic species like *Aporrectodea caliginosa* and *Octolasion tyrtaeum lacteum*, and ii) long and continuous burrows with large diameters built by the anecics *Lumbricus terrestris* and *Aporrectodea giardi*. The volume of soil occupied by the two types of burrows also varied temporally, and this fact was attributed to differences in burrowing activities and longevity of burrows created by the earthworm species at their study site (Bastardie et al., 2005). Such research on the persistence or not of burrows in soils is crucial for a better understanding of soil structuring processes and nutrient fluxes.

Researchers who want to understand the temporal dynamics of burrowing, to better estimate water and chemical transport through these macropores or to predict nutrient transformations and trophic relationships in the drilosphere, need to acquire detailed information on how earthworm burrowing fluctuates with changing soil conditions. Moreover, one might keep in mind that the burrowing activity could be underestimated due to the fact that the high frequency monitoring of this activity is unfrequently carried out (Turberg, personal communication). X-ray tomography could in principle provide this high frequency monitoring which should be used in

Complimentary Contributor Copy

the future. Another research perspective would be to draw dynamic functional relationships between the burrowing activity of earthworm and some soil factors (soil texture, nutrient content, composition (e.g., toxicity), temperature, humidity, aeration, etc.). Moreover, using the X-ray tomography to evaluate the burrowing effect on the soil density is challenging, not only linked to the voids created by the burrows themselves but also linked to the soil aggregates and to the earthworm casts. Finally, the 3D X-ray tomography is mainly applicable for earthworm burrows created under artificial conditions in the lab. Application for field samples is challenging and current research is ongoing especially on freeze coring as a new promising technique allowing the observation of intact galleries (Schomburg et al., unpublished data).

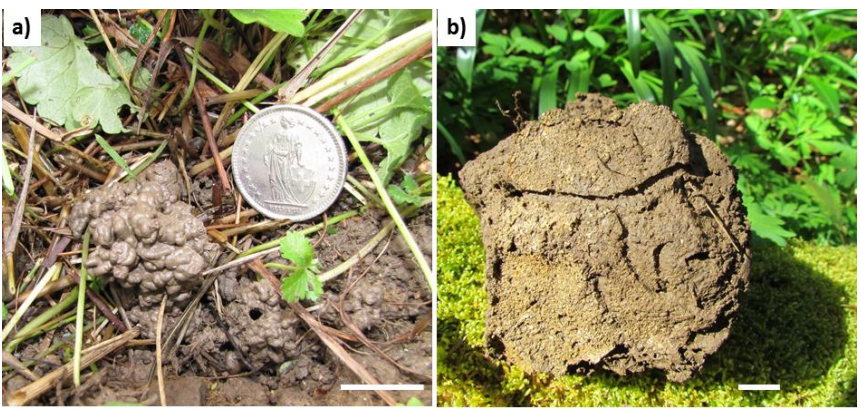

Figure 2. Biogenic structures originated from earthworm activities: a) fresh earthworm surface-casts produced by anecic earthworms; the burrow hole on the right gives an idea of the earthworm diameter; b) earthworm burrow from an anecic species - Bars represent 2 cm.

4.4. The Drilosphere Involved in Engineering Processes

As defined before, ecosystem engineers change biotic or abiotic materials in their environment thereby creating or modifying habitats and hence controlling availability of resources to other species (Jones et al., 1994, 1997a, 1997b; Lavelle et al., 1997; Blouin et al., 2013). However, an

Complimentary Contributor Copy

interesting aspect is that these authors explicitly exclude trophic interactions from ecosystem engineering in the form of provision or consumption of tissue. Dissimilatory and assimilatory processes are also excluded (ex: assimilation and/or decomposition of organic compounds). But earthworm defecation clearly involves assimilation and dissimilation suggesting that, following strictly the definition, casts but also faeces from termites, ants, etc. would be excluded from engineering processes despite the fact that they all play a role in constructing the physical matrix of an ecosystem. So, according to Berke (2010), defining ecosystem engineering as independent or irrespective of assimilation and dissimilation can lead to contradictory interpretation. Berke (2010) suggest then that when assimilatory and dissimilatory processes alter the availability of non-tissue resources, they should be included under the umbrella of ecosystem engineering. We totally agree with this point of view and we also suggest that engineering processes also occur directly in the intestinal tract of earthworms, these latter acting as alive bioreactors.

So, based on the functional classification of ecosystem engineers proposed by Berke (2010), earthworms can be considered as i) structural engineers; ii) chemical engineers; iii) bioturbators. As previously described (sections 4.3.2 and 4.3.3), the structural function refers to both aggregates and burrows formation and the chemical one to earthworm involvement in nutrient cycling. Earthworms are also recognized as one of the three top bioturbators in soils, together with ants and termites (Jouquet et al., 2006). As a result, soils are homogenised at the soil profile scale. By contrast, the hotspots resulting from earthworm activities, where nutrient availability and microbial activity are higher compared to the soil matrix, contribute to increase the spatial heterogeneity of soils (Eisenhauer et al., 2008). The bioturbation has also an impact on the biological composition of soil. Earthworms are known to impact soil seed banks (Forey et al., 2011), mainly by dispersing and feeding on seeds (Eisenhauer et al., 2010). Indeed, earthworms play a role in seed transport and translocation into deep soil layers, by accelerating or decelerating seed germination and seedling establishment (Clause et al., 2015). Eisenhauer et al. (2010) found that the selective ingestion of seeds depends on several variables:

Complimentary Contributor Copy

earthworm body size, species specific habits and mode of digestion (e.g., depending on gut enzyme activities and gizzard contraction (Eisenhauer et al., 2010). In a context of global warming, plants that are highly reliant on earthworms for propagation will either benefit or suffer from climate change impact on these organisms.

In addition to their chemical role (section 4.3.1), earthworms are also structural allogenic engineers and bioturbators (Berke, 2010) belonging to burrowing and excavating organisms. By changing the initial composition of the ingested soil, the biogenic structures as newly-formed aggregates and galleries have undoubtedly many positive roles in soil structure formation (Mackay and Kladivko, 1985; Ketterings et al., 1997).

5. EARTHWORMS ARE INVOLVED IN SOIL ECOSYSTEM SERVICES

5.1. Ecosystem Services

Ecosystems services are defined as benefits that people can obtain from environmental resources provided by ecosystems such as clean air, water, food and materials. Human health and wellbeing is at the central core of the concept of ecosystem services being divided in four main categories according to the Millennium Ecosystem Assessment (2005): i) supporting services, essential for the production of all other ecosystem services including soil formation, photosynthesis, primary production, nutrient cycling and water cycling, etc., ii) provisioning services grouping products obtained from ecosystems, including food, fibre, fuel, genetic resources, biochemicals, fresh water, etc., iii) regulating services obtained from the regulation of ecosystem processes, including regulation of air quality, climate, water, erosion, pest, pollination, etc., and finally iv) cultural services that are non-material benefits being for instance spiritual enrichment, cognitive development, reflection, recreation and aesthetic experiences.

Complimentary Contributor Copy

Through their activities, soil ecosystem engineers and especially earthworms hence participate actively to ecosystem services (Fonte and Six, 2010; Blouin et al., 2013; Bertrand et al., 2015; Dewi and Senge, 2015; Petrosillo and Zurlini, 2016). Ecosystems services were estimated to account to up to US $33 trillion dollars per year of human capital equivalent and soil biota contributes to approximately 38% of this amount (Skubala, 2013).

The main contributions of earthworms are related to both supporting and regulation services, however, many things still need to be discovered in this domain. According to Adhikari and Hartemink (2016), only a few studies have linked soil properties to ecosystems services and their interactions, and these are mostly influenced by its use and management. These authors point out that most studies have focused on provisioning and regulating ecosystem services relating to soil physico-chemical and biological properties. Future research projects should focus on exploring functional diversity of soil biota and the spatial aspects of soil properties to the lowest level of ecosystem services (water purification, gene pool, climate regulation).

5.2. Supporting Services Provided by Earthworms

Earthworms participate to soil formation via mixing the organic and mineral components, and their bioturbation activities contribute to the homogenisation of soils while the hotspots from the drilosphere increase the spatial heterogeneity of soils (Eisenhauer et al., 2008; Lavelle et al., 2016). They enhance the availability of nutrients for plant growth and fertilize the soil leading to an increase in yield productivity. On average, earthworm presence in agroecosystems leads to a 25% increase in crop yield and a 23% increase in aboveground biomass (van Groenigen et al., 2014). The magnitude of these effects depends on presence of crop residue onto the soil surface, earthworm density and ecological types as well as rate of fertilization. So, earthworms contribute indirectly to ecosystem primary production influencing soil nutrients levels, regulating and

Complimentary Contributor Copy

controlling soil nutrient repartition, concentration and ionic forms (Wurst et al., 2005; Eisenhauer and Scheu, 2008).

Recently, Kim et al. (2017) pointed out the close interactions between earthworm engineering processes, nutrients cycles and plant growth. Their study in agricultural fields in New Zealand highlights that some combination of earthworm-mediated soil aeration, modification of moisture conditions in the rhizosphere and drilosphere, and comminution of soil organic matter may drastically modify microbial communities (root nodulation and dehydrogenase activity are enhanced in the presence of earthworms) and thus could significantly impact the nitrogen cycle. This may have also consequences on the composition of plant communities (Wurst et al., 2005; Eisenhauer et al., 2008; Kim et al., 2017).

Moreover, these services related to plant growth depend not only on earthworm activities but also their biomass, abundance and turnover rates. As showed by Arnone and Zaller (2014), at a low earthworms density (37 individuals m^{-2}), plants produced more deep roots to compensate the lower nutrient availability in shallow soils resulting in a reduction of casting activities. At the opposite, at high earthworm densities (114 and 169 individuals m^{-2}), large amounts of casts and greater nutrient availability compensated for physical disruption of roots by worms. How earthworms affect plant growth and densities of aboveground and belowground organisms strongly depends on whether earthworms are alive or dead (Kos et al., 2016). Using cadavers of earthworms incorporated in soil samples, these authors demonstrated that the nutrients from the decomposing invertebrates may be rapidly mineralized by microorganisms then being available for uptake by the plants.

Specific affinities seem to exist between earthworms and plants, not only at the community or population scale, but also at the species level (Zangerlé et al., 2011). Earthworms and mycorrhizal fungi, especially arbuscular (AMF), are commonly co-occurring and interacting. These interactions depend on earthworm and plant species and are, generally speaking, beneficial for soil ecosystems as they result in an increased soil aggregate stability (Milleret et al., 2009, 2010; Kohler-Milleret et al., 2013), a dispersal of non-pathogenic fungal spores and an increase of AMF

Complimentary Contributor Copy

colonization of plant roots by 140% (Zaller et al., 2013; Trouvé et al., 2014).

5.3. Regulation Services Provided by Earthworms

One of the most regulation services earthworms are involved in is probably water regulation. Because water infiltration is closely related to soil structure maintenance, macropores and aggregates originating from earthworms are of course implied. As a result, burrows morphological characteristics (diameter, length, branching aspects, etc.) strongly depend on the ecological group of earthworms but also on their sexual maturity. For instance, juveniles of *Lumbricus terrestris* seem to have a more intensive digging activity than adults (Lee, 1985) probably due to intra-specific competition (Grigoropoulou et al., 2008, 2009).

Perennial occupation of burrows of anecic earthworms is well-known (Nuutinen, 2011; Grigoropoulou and Butt, 2012), and those of *Lumbricus terrestris* may persist for at least 7 years in northern forests (Potvin and Lilleskov, 2017). When burrows persist such a long time in the soil, they function as preferential soil water flow paths (Shipitalo et al., 2000; Capowiez et al., 2015) increasing the infiltration rate and consequently decreasing soil erosion, sometimes up to 50% (Sharpley et al., 1979; Le Bayon et al., 2002; Shuster et al., 2002; Mariani et al., 2007). Concomitant to vertical burrows, the horizontal burrowing in the topsoil increases overall porosity and drainage, and reinforces the connectivity of the overall network (Pérès et al., 2010). Contradictory data were nevertheless obtained in the tropics that highlight again the importance of earthworm types involved in the processes. Blanchart (1992) and Blanchart et al. (1997) thus discovered that compacting species of endogeic earthworms enhance soil bulk density by producing large globular casts whereas fine granular casts emitted by decompacting species increase soil porosity. Consequently, decompacting species increase water infiltration thereby reducing runoff while compacting species decrease water infiltration (Blanchart et al., 1999).

Complimentary Contributor Copy

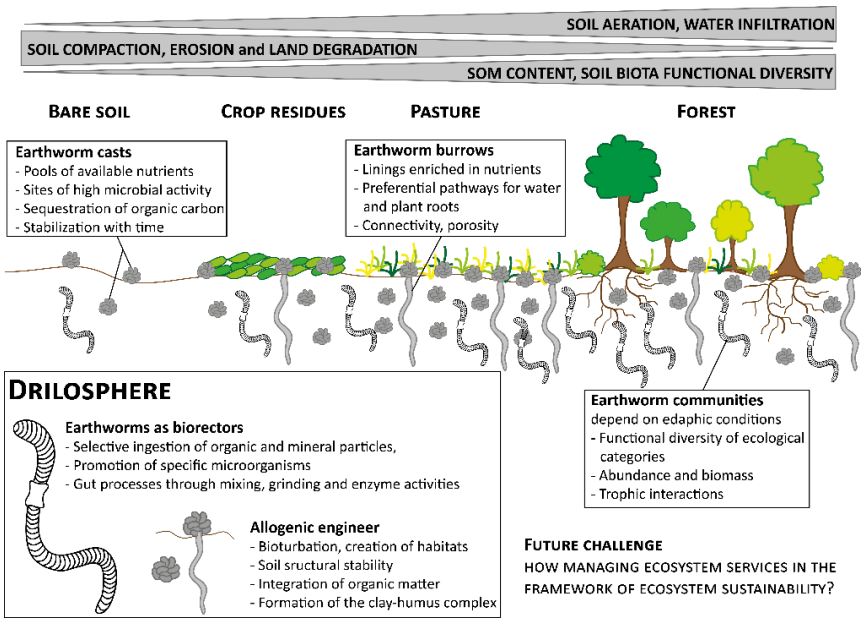

Figure 3. Conceptual scheme highlighting the role of earthworms as ecosystem engineers and their impact on ecosystem functions and services. SOM : Soil Organic Matter.

Burrows may also act as preferential microsites for nutrient and pesticide adsorption and retention, with further consequences on leaching (Edwards et al., 1990; Shuster et al., 2003; Binet et al., 2006). This may also happen with casts. The storage and stabilization of organic matter in earthworm aggregates are processes of regulation of carbon fluxes at the ecosystem scale. Exposed to rainfall events, surface-casts may be impacted by raindrops, and the fine soil particles they contain are then easily detached and transported during rainfall events (Blanchart et al., 1999; Le Bayon et al., 2002; Mariani et al., 2007). As a consequence, surface-casts, especially fresh-egested ones, may contribute to soil erosion up to 15 ton ha^{-1} yr^{-1} in agroecosystems (Le Bayon and Binet, 1999, 2001). Casts are also zones of nutrient accumulation (Le Bayon and Milleret, 2009; Jouquet et al., 2012; Blouin et al., 2013) and atrazine sorption and persistence (Binet et al., 2006) that may contribute to nutrient losses and groundwater table pollution.

Complimentary Contributor Copy

Other regulation service earthworms may modulate is carbon recycling which is of particular importance in the context of greenhouse emissions. The effects of earthworms on net carbon sequestration at the ecosystem scale are difficult to quantify and estimate. A recent meta-analysis suggested that earthworms may increase CO_2 emissions by 33% but do not affect soil organic carbon stocks (Lubbers et al., 2013). However, according to Zhang et al., (2013), these emissions largely depend on earthworm ecological categories and may be partially offset or even overcompensated from plant uptake or by carbon sequestration in biogenic structures.

6. ECOSYSTEM ENGINEER'S FUTURE CHALLENGES

Ecosystems engineers are involved in the maintenance of ecosystem health and the provision and enhancement of ecological services, thus contributing to the proactive sustainability of ecosystems integrating human society with the natural environment, for the benefit of both (Wright and Jones, 2006; Stokes et al., 2012). Many hard tasks remain to do in this framework, notably regarding earthworms' implication and usefulness in the context of climate change, aboveground-belowground interactions and restoration of ecosystem services.

6.1. Climate Change May Cause Changes in Above- Below Ground Interactions

Soils remain relatively unknown despite decades of extensive research and are often described as "complex adaptive systems" showing very sophisticated levels of self-organization (Coleman, 2011; Wall et al., 2012; Lavelle et al., 2016). One of the main challenges for the future is to better understand how soil biodiversity is implied in the context of ecosystem development and functioning. The complex interactions among organisms, structures, and processes in soils that make soils dynamics are difficult to

Complimentary Contributor Copy

describe and predict (Lavelle et al., 2016). The framework proposed by Jones et al., (2010) might be helpful and applicable to approach in a better way the complex engineer communities and/or networks (i.e., multiple co-acting engineers with a variety of structural, abiotic and biotic effects and feedbacks).

At a global scale, climate change induces migration of species, and soil-dwelling invertebrates may cause severe above- and belowground consequences on native ecosystems (Hiltpold et al., 2017). Regarding earthworms communities, both the ratio of ecological categories and the proportion of adults and juveniles may change along transects of varying climate types and elevations (Hackenberger and Hackenberger, 2014). Potvin and Lilleskov (2017) demonstrated that endogeic earthworms are more likely than anecic earthworms to adjust activity states in response to climate change mediated shifts in soil moisture and temperature. Mariotte et al. (2016) showed in grassland how reduced summer rainfall can influence plant functional groups, with cascading effects on earthworms. Focusing on bioturbation activities, the annual production of surface-casts may be changed due to elevated atmospheric CO_2 (Zaller and Arnone, 1997) and it is well-known that earthworm activity is modulated by soil temperature and moisture (see section 4.1).

Moreover, in regions with high amounts of native species (i.e., Australia, New Zealand), the invasion of peregrine earthworms may largely impact the functioning of natural and agroecosystems (Baker et al., 2006; Uvarov, 2009). On the opposite, in areas devoid of earthworms, lumbricid invasions may cause substantial changes in ecosystem structure and functioning (Frehlich et al., 2006). In the same way, exotic earthworms may be drivers of "savannification" of the forest through increasing soil bulk density, decreasing nitrogen availability and removing the organic layer, thus inhibiting the establishment of tree seedlings (Frehlich et al., 2006). Changes in plant communities may thus be induced by earthworm invasive species as vectors of seeds (Forey et al., 2011). Eisenhauer et al., (2008) also highlight the intimate relationship between earthworms and plant diversity.

Complimentary Contributor Copy

Questions still remain over the role of soil biodiversity in climate change mitigation and adaptation strategies. As earthworms emerge as important biological invaders with largely unknown consequences for native soil food webs and functions, it appears essential to better understand how it works. There is dearth information on this topic and this makes it difficult to develop new models explaining the structuring of soil communities in the framework of climate change (Bardgett and van der Putten, 2014). Nishijima et al., (2016) proposed as an issue to link engineering processes to resources type (allochthonous *versus* autochthonous resources). According to their model, integrating trophic and physical biotic interactions, with consideration for resource types and the direction and strength of engineering feedbacks, would be important for fully understanding the dynamics and structure of communities involving ecosystem engineers. This would allow linkages between population biology, landscape and community ecology (Wright et al., 2004), and between physiology and ecosystems (Caraco et al., 2006).

6.2. Restoration of Ecosystem Services by Earthworms

Earthworms are involved in restoring ecosystem services via direct and indirect mechanisms (Wall et al., 2012; Jouquet et al., 2014). This is especially true in the situations when soil is degraded or land is under rehabilitation after mining. Jouquet et al., (2014) reviewed the management of earthworm and termite activity for the restoration of ecosystems using methods to promote soil engineer activity, either directly through field inoculation and/or stimulation or indirectly through the utilization of vermicompost. Another review on the potential of earthworms to restore ecosystem services showed that earthworms may accelerate soil restoration, improve primary production and facilitate the restoration of a functional ecosystem in mining areas (Boyer and Wratten, 2010).

One of the most famous precursors for the inoculation of earthworms is undoubtedly Butt (1999, 2008, 2011) and Butt et al., (1995) from United Kingdom with their innovative Earthworm Inoculation Unit (EIU)

Complimentary Contributor Copy

technique being devised to link cultivation of selected species with direct soil introduction. This EIU have demonstrated superior outcomes compared with more traditional earthworm inoculation (Butt, 2011). In Switzerland, Hasinger et al., (2015) also inoculated earthworms on an agricultural field devoid of organic layers. This field was used over several years as a storage place for gravels for the construction of a motorway. Improving conditions as best as possible for earthworm survival (compost, mulch, etc.), Hasinger et al., (2015) observed that the inoculated earthworms survived and that colonization by neighbouring individuals coming from adjacent cultures occurred. After one year, the seed and straw productivity were of 80 % and 75% of the accepted norms, respectively.

These encouraging results highlight the importance of such approach in ecosystem restoration also for management practices in the context of demographic human population growth. Rey et al., (2015) proposed a framework allowing an overview of management practices and ecosystem services in order to integrate ecological engineering and ecological intensification linking thus management practices to ecosystem services. In the same way, Bender et al., (2016) proposed "soil ecological engineering" as an approach combining management practices enhancing overall biological diversity in human land-use systems with targeted manipulations of soil biota to deliver specific desired functions in order to provide food security while minimizing negative environmental impacts. Such models are essential to ensure human wellbeing with respect to ecosystem sustainability in the future.

REFERENCES

Adhikari, K., Hartemink, A. E. (2016). Linking soils to ecosystem services-A global review. *Geoderma*, 262: 101-111.

Amossé, J., Le Bayon, R.-C., Gobat, J.-M. (2015). Are urban soils similar to natural soils of river valleys? *Journal of Soils and Sediments*, 15: 1716-1724.

Complimentary Contributor Copy

Amossé, J., Dozsa-Farkas, K., Boros, G., Rochat, G., Sandoz, G., Fournier, B., Mitchell, E., Le Bayon, R.-C. (2016). Patterns of earthworm, enchytraeid and nematode diversity and community structure in urban soils of different ages. *European Journal of Soil Biology*, 73: 46-78.

Anderson, O. R., Bohlen, P. J. (1998). Abundance and diversity of Gymnamoehae associated with earthworm (*Lumbricus terrestris*) middens in a northeastern U.S. forest. *Soil Biology and Biochemistry*, 30: 1213-1216.

Angst, S., Mueller, C. W., Cajthaml, T., Angst, G., Lhotáková, Z., Bartuska, M., Spaldonová, A., Frouz, J. (2017). Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter. *Geoderma*, 289: 29-35.

Arnone, J. A. I., Zaller, J. G. (2014). Earthworm effects on native grassland root system dynamics under natural and increased rainfall. *Frontiers in Plant Science*, 5, 152: 1-8.

Baker, G. H., Brown, G., Butt, K., Curry, J. P., Scullion, J. (2006). Introduced earthworms in agricultural and reclaimed land: their ecology and influences on soil properties, plant production and other soil biota. *Biological Invasions*, 8: 1301-1316.

Bardgett, R. D. (2005). The Biology of Soil - A community and ecosystem approach. Oxford University Press, Oxford and New York. 256 pp.

Bardgett, R. D., van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, 515: 505-511.

Barley, K. P. (1959). The influence of earthworms on soil fertility. II. Consumption of soil and organic matter by the earthworm *Allolobophora caliginosa* (Savigny). *Australian Journal of Agriculture Research*, 10: 179-185.

Barois, I., Lavelle, P. (1986). Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Soil Biology and Biochemistry*, 18: 539-41.

Complimentary Contributor Copy

Bastardie, F., Capowiez, Y., Cluzeau, D. (2005). 3D characterisation of earthworm burrow systems in natural soil cores collected from a 12-year-old pasture. *Applied Soil Ecology*, 30: 34-46.

Beare, M. H., Coleman, D. C., Crossley, D. A., Hendrix, P. F., Odum, E. P. (1995). A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant and Soil*, 170: 5-22.

Bender, F. S., Wagg, C., van der Heijden, M.G.A. (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology and Evolution* 2077, 31: 440-452.

Berke, S.K. (2010). Functional groups of ecosystem engineers: a proposed classification with comments on current issues. *Integrative and Comparative Biology*, 50: 147-157.

Bertrand M., Barot, S., Blouin, E., Whalen, J., de Oliveira, T., Roger-Estrade, J. (2015). Earthworm services for cropping systems. A review. *Agronomy for Sustainable Development*, 35: 553-567.

Binet, F., Kersanté, A., Munier-Lamy, C., Le Bayon, R.-C., Belgy, M., Shipitalo, M. (2006). Lumbricid macrofauna alter atrazine mineralization and sorption in a silt loam soil. *Soil Biology and Biochemistry*, 38: 1255-1263.

Bityutskii, N. P., Maiorov, E. I., Orlova, N. E. (2012). The priming effects induced by earthworm mucus on mineralization and humification of plant residues. *European Journal of Soil Biology*, 50: 1-6.

Blanchart, E. (1992). Restoration by earthworms (Megascolecidae) of the macroaggregate structure of a destructured savanna soil under field conditions. *Soil Biology and Biochemistry*, 24: 1587-1594.

Blanchart, E., Lavelle, P., Braudeau, E., Le Bissonnais, Y., Valentin, C. (1997). Regulation of soil structure by geophagous earthworm activities in humid savannas of Côte d'Ivoire. *Soil Biology and Biochemistry*, 29: 431-439.

Blanchart, E., Albrecht, A., Alegre, J., Duboisset, A., Villenave, C., Pashanasi, B., Lavelle, P., Brussaard, L. (1999). Effects of earthworms on soil structure and physical properties. In: Lavelle, P., Brussard, L., Hendrix, J. (Eds). Earthworm Management in Tropical Agrogroecosystems. London: CAB International. pp. 149-172.

Complimentary Contributor Copy

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G, Brussard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., Brun, J.-J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 64: 161-182.

Bolton, P. J., Phillipson, J. (1976). Burrowing, feeding, egestion, and energy budgets of *Allolobophora rosea* (Savigny) (Lumbricidae), *Oecologia*, 23: 225-245.

Bossuyt, H., Six, J., Hendrix, P. F. (2005). Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology and Biochemistry*, 37: 251-258.

Bouché, M. B. (1977). Stratégies lombriciennes. In: Lohm, U., Person, T. (Eds). Soil Organisms as Components of Ecosystems. Ecological Bulletins n°25, Swedish Natural Science Research Council, Stockholm: pp.122-132. [Strategies of earthworms. In: Lohm, U., Person, T. (Eds). Soil Organisms as Components of Ecosystems. Ecological Bulletins n°25, Swedish Natural Science Research Council, Stockholm: pp.122-132.].

Boyer, S., Wratten, S. D. (2010). The potential of earthworms to restore ecosystem services after opencast mining. *Basic and Applied Ecology*, 11: 196-203.

Brown, G. G. (1995). How do earthworms affect microfloral and faunal community diversity? *Plant and Soil*, 170: 209-231.

Brown, G. G., Barois, I., Lavelle, P. (2000). Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *European Journal of Soil Biology*, 36:177-198.

Brown, G. G., Doube, B. M. (2004). Functional interactions between earthworms, microorganisms, organic matter, and plants. In: Edwards C.A. (Ed). Earthworm Ecology. Boca Raton, FL: CRC Press. 2nd ed. pp. 213-239.

Bullinger-Weber, G., Gobat, J.-M. (2006). Identification of facies models in alluvial soil formation: The case of a Swiss alpine floodplain. *Geomorphology*, 74: 181-195.

Complimentary Contributor Copy

Bullinger-Weber, G., Le Bayon, R.-C., Guenat, C., Gobat, J.-M. (2007). Influence of some physicochemical and biological parameters on soil structure formation in alluvial soils. *European Journal of Soil Biology*, 43: 57-70.

Bullinger-Weber, G., Guenat, C., Salomé, C., Gobat, J.-M., Le Bayon, R.-C. (2012). Impact of flood deposits on earthworm communities in alder forests from a subalpine floodplain (Kandersteg, Switzerland). *European Journal of Soil Biology*, 49: 5-11.

Butt, K. R., Frederickson, J., Morris, R.M. (1995). An earthworm cultivation and soil inoculation technique for land restoration. *Ecological Engineering*, 4: 1-9.

Butt, K. R. (1999). Inoculation of earthworms into reclaimed soils: the UK experience. *Land Degradation and Development*, 10: 565-575.

Butt, K. R. (2008). Earthworms in soil restoration: lessons learned from United Kingdom case studies of land reclamation. *Restoration Ecology*, 16: 637-641.

Butt, K. R. (2011). The Earthworm Inoculation Unit Technique: development and use in soil improvement over two decades. In: Karaca, A. (Ed). Biology of Earthworms, Soil Biology. Springer Verlag, Heidelberg. pp. 87-106.

Cameron, E. K., Cahill, J. F., Bayne, E. M. (2014). Root foraging influences plant growth responses to earthworm foraging. *PLoS One*, 9: e108873.

Capowiez, Y., Pierret, A., Daniel, O., Monestiez, P., Kretzschmar, A. (1998). 3D skeleton reconstructions of natural earthworm burrow systems using CAT scan-images of soil cores. *Biology and Fertility of Soils*, 27: 51-59.

Capowiez, Y., Bottinelli, N., Jouquet, P. (2014). Quantitative estimates of burrow construction and destruction, by anecic and endogeic earthworms in repacked soil cores. *Applied Soil Ecolology*, 74: 46-50.

Capowiez, Y., Bottinelli, N., Sammartino, S., Michel, E., Jouquet, P. (2015). Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae). *Biology and Fertility of Soils*, 51: 869-877.

Complimentary Contributor Copy

Caraco, N., Cole, J., Findlay, S., Wigand, C. (2006). Vascular plants as engineers of oxygen in aquatic systems. *BioScience*, 56: 219-225.

Chapuis-Lardy, L., Le Bayon, R.-C., Brossard, M., López-Hernández, D., Blanchart, E. (2011). Role of Soil Macrofauna in Phosphorus Cycling. In: Bünemann, E., Oberson, A., Frossard, E. (Eds). Phosphorus in Action 26. Springer, Berlin Heidelberg. pp. 199-213.

Cherix, D., Freitag, A., Maeder, A. (2006). Fourmis des bois du parc du jurassien vaudois. *Parc Jurassien Vaudois et Musée de Zoologie*, Lausanne. 120 pp. [Forest ants from the Jura Parc, canton of Vaud. *Jura Parc of the Canton of Vaud and Museum of Zoology*, Lausanne. 120 pp.]

Clause, J., Barot, S., Forey, E. (2015). Effects of cast properties and passage through the earthworm gut on seed germination and seedling growth. *Applied Soil Ecology*, 96: 108-113.

Coleman, D. C., Crossley, D. A., Hendrix, P. F. (2004). Fundamentals of Soil Ecology, 2nd Edition. Academic Press, San Diego, CA. 386 pp.

Coleman, D .C. (2011). Understanding soil processes: one of the last frontiers in biological and ecological research. *Australasian Plant Pathology*, 40: 207–214.

Cuddington, K., Byers, J., Wilson, W., Hastings, A. (2007). Ecosystem Engineers, Plants to Protists. 1st Ed. Academic Press, London. 432 pp.

Cunha, L., Brown, G. G., Stanton, D. W. G., Da Silva, E., Hansel, F. A., Jorge, G., McKey, D., Vidal-Torrado, P., Macedo, R.S., Velasquez, E., James, S.W., Lavelle, P., Kille, P. (2016). Soil animals and pedogenesis: the role of earthworms in anthropogenic soils. *Soil Science*, 181: 110-125.

Curry, J., Schmidt, O. (2007). The feeding ecology of earthworms- a review. *Pedobiologia*, 50: 463-477.

Darwin, C. (1881). The formation of vegetable mould, through the action of worms, with observations on their habits. London: John Murray. 326 pp.

Davidson, D., Grieve, I.C. (2006a). Relationships between biodiversity and soil structure and function: Evidence from laboratory and field experiments. *Applied Soil Ecology*, 33: 176-185.

Complimentary Contributor Copy

Davidson, D., Grieve, I. C. (2006b). The influence of soil fauna on soil structural attributes under a limed and untreated upland grassland. *Land Degradation and Development*, 17: 393- 400.

Dewi, D. S., Senge, M. (2015). Earthworm diversity and ecosystem services under threat. *Reviews in Agricultural Science*, 3: 25-35.

Drake, H. L., Horn, M. A. (2007). As the worm turns: the earthworm gut as a transient habitat for soil microbial biomes. *Annual Review of Microbiology*, 61: 169-89.

Edwards, C. A., Bohlen, P. J. (1996). The Biology and Ecology of Earthworms. 3rd ed. Chapman and Hall, London. 426 pp.

Edwards, C.A. (2004). Earthworm Ecology, 2nd ed. CRC Press LLC, St. Lucie Press, Boca Raton. 456 pp.

Edwards, W., Shipitalo, M., Owens, L., Norton, L. (1990). Effect of *Lumbricus terrestris* L. burrows on hydrology of continuous no-till corn fields. *Geoderma*, 46: 73- 84.

Ehlers, W. (1975). Observations on earthworm channels and infiltration on tilled and untilled loess soil. *Soil Science*, 119: 242-247.

Ehlers, W., Kopke, U., Hesse, F., Bohm, W. (1983). Penetration resistance and root growth of oats in tilled and untilled loess soil. *Soil Tillage and Research*, 3: 261-275.

Eisenhauer, N., Milcu, A., Sabais, A. C. W., Scheu, S. (2008). Animal ecosystem engineers modulate the diversity invasibility relationships. *Plos One*, 3: e3489.

Eisenhauer, N., Scheu, S. (2008). Earthworms as the drivers of the competition between grasses and legumes. *Soil Biology and Biochemistry*, 40: 2650-2659.

Eisenhauer, N., Butenschoen, O., Radsick, S., Scheu, S. (2010). Earthworms as seedling predators: Importance of seeds and seedlings for earthworm nutrition. *Soil Biology and Biochemistry*, 42: 1245-1252.

Feller, C., Brown, G. G., Blanchart, E., Deleporte, P., Chernyanskii, S.S. (2003). Charles Darwin, earthworms and the natural sciences: various lessons from past to future. *Agriculture, Ecosystems and Environment*, 99: 29-49.

Complimentary Contributor Copy

Fonte, S. J., Six, J. (2010). Earthworms and litter management contributions to ecosystem services in a tropical agroforestry system. *Ecological Applications*, 20: 1061-1073.

Forey, E., Barot, S., Decaëns, T., Langlois, E., Laossi, K. R., Margerie, P., Scheu, S., Eisenhauer, N. (2011). Importance of earthworm-seed interactions for the composition and structure of plant communities: A review. *Acta Oecologica-International Journal of Ecology*, 37: 594-603.

Fournier, B., Samaritani, E., Shrestha, J., Mitchell, E., Le Bayon, R.-C. (2012). Community ecology of earthworms in a restored floodplain and potential as bioindicators of river restoration. *Applied Soil Ecology*, 59: 87-95.

Fournier, B., Gillet, F., Le Bayon, R.-C., Mitchell, E., Moretti, M. (2015). Functional responses of multi-taxa communities to disturbance and stress gradients in a restored floodplain. *Journal of Applied Ecology*, 52: 1364-1373.

Francis, G. S., Tabley, F. J., Butler, R. C., Fraser, P. M. (2001). The burrowing characteristics of three common earthworm species. *Australian Journal of Soil Research*, 39: 1453-1465.

Frelich, L. E., Hale, C. M., Scheu, S., Holdsworth, A. R., Heneghan, L., Bohlen, P.J., Reich, P.B. (2006). Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions*, 8: 1235-1245.

Frouz J., Holec, M., Kalcík, J. (2003). The effect of *Lasius niger* (Hymenoptera, Formicidae) ant nest on selected soil chemical properties. *Pedobiologia,* 47: 205–212.

Grigoropoulou, N., Butt, K. R., Lowe, C. N. (2008). Effects of adult *Lumbricus terrestris* on cocoons and hatchlings in Evans' boxes. *Pedobiologia*, 51: 343-349.

Grigoropoulou, N., Butt, K. R., Lowe, C. N. (2009). Interactions of juvenile *Lumbricus terrestris* with adults and their burrow systems in a two-dimensional microcosm. *Pesquisa Agropecuária Brasileira,* 44: 964-968.

Complimentary Contributor Copy

Grigoropoulou, N., Butt, K. R. (2012). Assessment of burrow re-use by *Lumbricus terrestris* L. through field experimentation. *Zeszyty Naukowe*, 15: 43-51.

Guenat, C., Bureau, F., Weber, G., Toutain, F. (1999). Initial stages of soil formation in a riparian zone: importance of biological agents and lithogenic inheritance in the development of the soil structure. *European Journal of Soil Biology*, 35: 153-161.

Gutiérrez, J. L., Jones, C. G., Strayer, D. L., Iribarne, O. O. (2003). Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos*, 101: 79-90.

Gutiérrez, J. L., Jones, C.G. (2006). Physical ecosystem engineers as agents of biogeochemical heterogeneity. *BioScience*, 56: 227–36.Hackenberger, D.K., Hackenberger, B.K. (2014). Earthworm community structure in grassland habitats differentiated by climate type during two consecutive seasons. *European Journal of Soil Biology*, 61: 27-34.

Haemig, P. D. (2012). Ecosystem Engineers: wildlife that create, modify and maintain habitats. ECOLOGY.INFO #12, http://www.ecology.info/ecosystem-engineers.htm.

Hasinger, G., Kohler, R., Didier, S., Le Bayon, R.-C. (2015). Influence de l'introduction d'une population de vers de terre sur un sol reconstitué dépourvu d'horizon A. *VBB-Bulletin-BSA*, 16: 29-35. [Influence of earthworm introduction on a reclaimed soil devoid of A-horizon. *VBB-Bulletin-BSA*, 16: 29-35.]

Havlicek, E., Mitchell, E. D. (2014). Soils Supporting Biodiversity. In "Interactions in Soil: Promoting Plant Growth". In: Dighton, J., Krumins, J.A. (Eds). Series Biodiversity, Community and Ecosystems, Volume 1. pp. 27-58.

Hiltpold, I., Johnson, S. N., Le Bayon, R.-C., Nielsen, U. N. (2017). Climate Change in the Underworld: Impacts for Soil-Dwelling Invertebrates. In: Johnson, S. N., Jones, T. H. (Eds). Global Climate Change and Terrestrial Invertebrates. Wiley-Blackwell (an imprint of John Wiley & Sons Ltd) (Verlag). pp. 201-228.

Complimentary Contributor Copy

Hirth, J. R., McKenzie, B. M., Tisdall, J. M. (1998). Roots of perennial ryegrass (*Lolium perenne*) influence the burrowing of the endogeic earthworm *Aporrectodea rosea*. *Soil Biology and Biochemistry*, 14: 2181-2183.

Hoang, D. T. T., Razavi, B. S., Kuzyakov, Y., Blagodatskaya, E. (2016). Earthworm burrows: Kinetics and spatial distribution of enzymes of C-, N- and P- cycles. *Soil Biology and Biochemistry,* 99: 94-103.

Horn, M. A., Schramm, A., Drake, H. L. (2003). The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms. *Applied Environmental Microbiology*, 69: 1662-1669.

Jégou, D., Cluzeau, D., Balesdent, J., Tréhen, P. (1998). Effects of four ecological categories of earthworms on carbon transfer in soil. *Applied Soil Ecology*, 9: 249–255.

Jégou, D., Hallaire, V., Cluzeau, D., Tréhen, P. (1999). Characterization of the burrow system of the earthworm *Lumbricus terrestris* and *Aporrectodea giardi* using X-ray computer tomography and image analysis. *Biology and Fertility of Soils*, 29: 314-318.

Jégou, D., Cluzeau, D., Hallaire, V., Balesdent, J., Tréhen, P. (2000). Burrowing activity of the earthworms *Lumbricus terrestris* and *Aporrectodea giardi* and consequences on C transfers in soil. *European Journal of Soil Biology*, 36: 27-34.

Johnson-Maynard, J. L., Graham, R. C., Wu, L., Shouse, P. J., (2002). Modification of soil structural and hydraulic properties after 50 years of imposed chaparral and pine vegetation. *Geoderma*, 110: 227-240.

Johnson-Maynard, J. L., Umiker, K. J., Guy, S.O. (2007). Earthworm dynamics and soil physical properties in the first three years of no-till management. *Soil and Tillage Research*, 94: 338-345.

Johnson-Maynard, J., Strawn, D. G. (2016). Linking physical and biogeochemical properties and processes in the drilosphere. *Soil Science*, 181: 126–132.

Jones, C. G., Lawton J.H., Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, 69: 373-386.

Complimentary Contributor Copy

Jones, C. G., Lawton, J. H., Shachak, M. (1997a). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, 78: 1946-1957.

Jones, C. G., Lawton, J. H., Shachak, M. (1997b). Ecosystem engineering by organisms: Why semantics matters. *Trends in Ecology and Evolution*, 12: 275-275.

Jones, C. G., Guttierez, J. L., Byers, J. E., Crooks, J. A., Lambrinos, J. G., Talley, T.S. (2010). A framework for understanding physical ecosystem engineering by organisms. *Oikos*, 119: 1862-1869.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., Lepage, M. (2006). Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. *Applied Soil Ecology*, 32: 153-164.

Jouquet, P., Zangerlé, A., Rumpel, C., Brunet, D., Bottinelli, N., Tran, Duc, T. (2009). Relevance and limitations of biogenic and physicogenic classification: a comparison of approaches for differentiating the origin of soil aggregates. *European Journal of Soil Science*, 60: 1117-1125.

Jouquet, P., Janeau, J. L., Pisano, A., Tran Sy, H., Orange, D., Nguyet Minh, L. T., Valentin, C. (2012). Soil engineers influence water runoff, soil detachment and the transfer of nutrients in a tropical steep slope fallow. *Applied Soil Ecology*, 61:161–168.

Jouquet, P., Blanchart, E., Capowiez, Y. (2014). Utilization of earthworms and termites for the restoration ecosystem functioning. *Applied Soil Ecology*, 73: 34-40.

Jouquet, P., Bottinelli, N., Shanbhag, R.R., Bourguignon, T., Traoré, S., Abbasi, S.A. (2016). Termites: the neglected soil engineers of tropical soils. *Soil Science*, 181: 157-165.

Kanianska, R., Jadudová, J., Makovníková, J., Kizeková, M. (2016). Assessment of relationships between earthworms and soil abiotic and biotic factors as a tool in sustainable agricultural. *Sustainability*, 8: 1-14.

Complimentary Contributor Copy

Ketterings, Q. M., Blair, J. M., Marinissen, J. C. Y. (1997). Effects of earthworm on soil aggregate stability and carbon and nitrogen storage in a legume cover crop agroecosystem. *Soil Biology and Biochemistry*, 29: 401-408.

Kim, Y. N., Robinson, B., Lee, K. A., Boyer, S., Dickinson, N. (2017). Interactions between earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a former agricultural soil. *Applied Soil Ecology*, 110: 79-87.

King, J. R., Warren, R. J., Bradford, M. A. (2013). Social insects dominate eastern US temperate hardwood forest macroinvertebrate communities in warmer regions. *PLoS One*, 8: e75843.

Knight, D., Elliott, P. W., Anderson, J. M., Scholefield, D. (1992). The role of earthworms in managed, permanent pastures in Devon. Engl. *Soil Biology and Biochemistry*, 24: 1511-1517.

Kohler-Milleret, R., Le Bayon, R.-C., Chenu, C., Gobat, J.-M., Boivin, P. (2013). Impact of two root systems, earthworms and mycorrhizae on the physical properties of an unstable silt loam Luvisol and plant production. *Plant and Soil*, 370: 251-265.

Kos, M., Jing, J., Keesmaat, I., Declerck, S. A. J., Wagenaar, R., Bezemer, T.M.B. (2016). After-life effects: Living and dead invertebrates differentially affect plants and their associated above- and belowground multitrophic communities. *Oikos*, volume in press. DOI: 10.1111/oik.03734.

Kretzschmar, A. (1982). The burrow system of earthwormsin grassland – seasonal variations of field observations. *Revue d'Ecologie et de Biologie du Sol*, 19: 579-591.

Kuzyakov, Y., Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept and review. *Soil Biology and Biochemistry*, 83: 184-199.

Lavelle, P. (1988). Earthworm activities and the soil system. *Biology and Fertility of Soils*, 6: 237- 251.

Complimentary Contributor Copy

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O. W., Dhillion, S. (1997). Soil function in a changing world: the role of invertebrate ecosystem engineers. *European Journal of Soil Biology*, 33: 159-193.

Lavelle, P., Spain, A. V. (2001). Soil Ecology. Kluwer Academic Publishers, Springer Science. Dordrecht, The Netherlands. 654 pp.

Lavelle, P. (2002). Functional domains in soils. *Ecological Research*, 17: 441-450.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P. (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Biology*, 42: S3–S15.

Lavelle, P., Spain, A., Blouin, M., Brown, G., Decaëns, T., Grimaldi, M., Jiménez, J.J., McKey, D., Mathieu, J., Velasquez, E., Zangerlé, A. (2016). Ecosystem engineers in a self-organized soil: A review of concepts and future research questions. *Soil Science*, 181: 91-109.

Le Bayon, R.-C., Binet, F. (1999). Rainfall effects on erosion of earthworm casts and phosphorus transfer by water runoff. *Biology and Fertility of Soils*, 30: 7-13.

Le Bayon, R.-C., Binet, F. (2001). Earthworm surface casts affect soil erosion by runoff water and phosphorus transfer in a temperate maize crop. *Pedobiologia*, 45: 430-442.

Le Bayon, R.-C., Moreau, S., Gascuel-Odoux, C., Binet, F. (2002). Annual variations in earthworm surface-casting activity and soil transport by water runoff under a temperate maize agroecosystem. *Geoderma*, 106: 121-135.

Le Bayon, R.-C., Binet, F. (2006). Earthworms change the distribution and availability of phosphorous in organic substrates. *Soil Biology and Biochemistry*, 38: 235-246.

Le Bayon, R.-C., Milleret, R. (2009). Effects of earthworms on phosphorus dynamics – a review. *Dynamic Soil Dynamic Plant*, 3: 21-27.

Complimentary Contributor Copy

Le Bayon, R.-C., Bullinger-Weber, G., Gobat, J.-M., Guenat, C. (2013). Earthworm communities as indicators for evaluating floodplain restoration success. In: Alcantara E.H. (Ed). Environmental Management, Restoration and Ecological Implications. NOVA Science Publisher. Environmental Research Advances, New York. pp. 47-68.

Lee, K. E. (1985). Earthworms: Their Ecology and Relationships with Soil and Land Use. Academic Press, Australia.

Lipiec, J., Brzezinska, M., Turski, M., Szarlip, P., Frac, M. (2015). Wettability and biogeochemical properties of the drilosphere and casts of endogeic earthworms in pear orchard. *Soil and Tillage Research*, 145: 55-61.

Lubbers, I. M., van Groenigen, K. J., Fonte, S. J., Six, J., Brussaard, L., van Groenigen, J.W. (2013). Greenhouse gas emissions from soils increased by earthworms. *Nature Climate Change*, 3: 187–194.

Mackay, A. D., Kladivko, E. J. (1985). Earthworm and rate of breakdown of soybean and maize residues in soil. *Soil Biology and Biochemistry*, 17: 851-857.

Maraun, M., Alphei, J., Bonkowski, M., Buryn, R., Migge, S., Peter, M., Schaefer, M., Scheu, S. (1999). Middens of the earthworm *Lumbricus terrestris* (Lumbricidae): microhabitants for micro, and meso-fauna in forest soil. *Pedobiologia*, 43: 276-287.

Mariani, L., Jimenez, J.J., Asakawa, N., Decaëns, T. (2007). What happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in Neotropical savannahs. *Soil Biology and Biochemistry*, 39: 757-767.

Marinissen, J. C. Y., Dexter, A. R. (1990). Mechanisms of stabilization of earthworm casts and artificial casts. *Biology and Fertility of Soils*, 9: 163–167.

Mariotte, P., Le Bayon, R.-C., Eisenhauer, N., Guenat, C., Buttler, A. (2016). Subordinate plant species moderate drought effects on earthworm communities in grasslands. *Soil Biology and Biochemistry*, 96: 119-127.

Complimentary Contributor Copy

Martin, A., Cortez, J., Barois, I., Lavelle, P. (1987). Les mucus intestinaux de ver de terre, moteur de leurs interactions avec la microflore. *Revue d'Ecologie et de Biologie du Sol*, 24: 549-558. [Intestinal mucus of earthworms drives interactions with microflora. *Revue d'Ecologie et de Biologie du Sol*, 24: 549-558.]

McInerney, M., Little, D. J., Bolger, T. (2001). Effect of earthworm cast formation on the stabilization of organic matter in fine soil fractions. *European Journal of Soil Biology*, 37: 251-254.

Milcu, A., Schumacher, J., Scheu, S. (2006). Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogeneity. *Functional Ecology*, 20: 261-268.

Millennium Ecosystem Assessment (2005). Found at the web site: http://www.millenniumassessment.org [Accessed on April 2017].

Milleret, R., Le Bayon, R.-C., Lamy, F., Gobat, J.-M., Boivin, P. (2009). Impact of root, mycorrhiza and earthworm on soil physical properties as assessed by shrinkage analysis. *Journal of Hydrology*, 373: 499-507.

Milleret, R., Le Bayon, R.-C., Tarnawski, S., Boivin, P., Gobat, J.-M. (2010). Earthworm, mycorrhiza and root interactions: their effects on some chemical, physical and biological soil properties. *Bulletin de la Société Suisse de Pédologie*, 30: 81-83.

Mills, L. S., Soulé, M. E., Doak, D. F. (1993). The keystone-species concept in ecology and conservation. *BioScience*, 43: 219-224.

Nishijima, S., Takimoto, G., Miyashita, T. (2016). Autochthonous or allochthonous resources determine the characteristic population dynamics of ecosystem engineers and their impacts. *Theorical Ecology*, 9: 117-127.

Nunez, N. A., Dimarco, R. D. (2012). Keystone species. In: Pardy, B., Nagle, J. C., Schmitz, O. J., Craig, R. K., Smith, W. K. (Eds). The berkyshire encyclopedia of sustainability: Ecosystem management and sustainability. Berkshire Publishing Group, Volume 5. pp 226-230.

Nuutinen, V. (2011). The meek shall inherit the burrow: feedback in earthworm soil modification. In: Karaca, A. (Ed). Biology of earthworms. Springer, Berlin. pp. 123-140.

Complimentary Contributor Copy

Ojha, B., Devkota, D. (2014). Earthworms: 'Soil and Ecosystem Engineers'-a Review. *World Journal of Agricultural Research*, 2: 257-260.

Paine, R. T. (1966). Food web complexity and species diversity. *American Naturalist*, 100: 65-75.

Paine, R. T. (1969). *A note on trophic complexity and community stability. The American Naturalist*, 103: 91-93.

Pérès, G., Bellido, A., Curmi, P., Marmonier, P., Cluzeau, D. (2010). Relationships between earthworm communities and burrow numbers under different land-use systems. *Pedobiologia*, 54: 37-44.

Perreault, J. M., Whalen, J. K. (2006). Earthworm burrowing in laboratory microcosms as influenced by soil temperature and moisture. *Pedobiologia*, 50: 397-403.

Petrosillo, I., Zurlini, G. (2016). The important role of ecological engineers in providing ecosystem services at landscape level. *Animal Conservation*, 19: 500-501.

Piearce, T. G. (1972). The calcium relations of selected Lumbricidae. *Journal of Animal Ecology*, 41: 167-187.

Platt, B. F., Kolb, D. J., Kunhardt, C. G., Milo, S. P., New, L. G. (2016). Burrowing through the literature: the impact of soil-disturbing vertebrates on physical and chemical properties of soil. *Soil Science*, 181: 175-191.

Potvin L. R., Lilleskov, E. A. (2017). Introduced earthworm species exhibited unique patterns of seasonal activity and vertical distribution, and *Lumbricus terrestris* burrows remained usable for at least 7 years in hardwood and pine stands. *Biology and Fertility of Soils*, 53: 187-198.

Power, M. E., Tilman, D., Estes, J. A., Menge, B. A., Bond, W. J., Mills, L. S., Gretchen, D., Castilla, J. C., Lubchenco, J., Paine, R. T. (1996). Challenges in the quest for keystones. *BioScience*, 46: 609-620.

Reichman, O. J., Seabloom, E. W. (2002a). The role of pocket gophers as subterranean ecosystem engineers. *Trends in Ecology and Evolution*, 17: 44-49.

Complimentary Contributor Copy

Reichman, O. J., Seabloom, E. W. (2002b). Ecosystem engineering: A trivialized concept? *Trends in Ecology and Evolution*, 17: 308.

Rey, F., Cécillon, L., Cordonnier, T., Jaunatre, R., Loucougaray, G. (2015). Integrating ecological engineering and ecological intensification from management practices to ecosystem services into a generic framework: a review. *Agronomy for Sustainable Development*, 35: 1335-1345.

Robertson, L.N., Raford, B.J., Bridge, B., McGarry, D., Blakemore, R.J., Sabag M. (1994). Tropical earthworms under cropping in Queensland. In: Pankhurst, C.E. (Ed). Soil Biota: Management in sustainable farming system, CSIRO. East Melbourne, Australia. pp: 33-34.

Rovira, A.D., Smettem, K.R.J., Lee, K.E. (1987). Effect of rotation and conservation tillage on earthworms in red-brown earth under wheat. *Australian Journal of Agricultural Research*, 38: 829-834.

Salomé, C., Guenat, C., Bullinger-Weber, G., Gobat, J.-M., Le Bayon, R.-C. (2011). Earthworm communities in alluvial forests: influence of altitude, vegetation stages and soil parameters. *Pedobiologia*, 54: 89-98.

Savin, M. C., Görres, J. H., Amador, J. A. (2004). Microbial and microfaunal community dynamics in artificial and *Lumbricus terrestris* (L.) burrows. *Soil Science Society of America Journal*, 68: 116-124.

Scheu, S. (1991). Mucus excretion and carbon turnover of endogeic earthworms. *Biology and Fertility of Soils*, 12: 217-220.

Schrader, S., Zhang, H. (1997). Earthworm casting: stabilization or destabilization of soil structure? *Soil Biology and Biochemistry*, 29: 469-475.

Schütz, K., Nagel, P., Dill, A., Scheu, S. (2008). Structure and functioning of earthworm communities in woodland flooding systems used for drinking water production. *Applied Soil Ecology*, 39: 342-351.

Shaler, N.S. (1892). The origin and nature of soils. In: Twelfth Annual Report of the Director, 1890–1891. Washington, DC: Dep. Interior, US Geol. Surv./US GPO. pp. 213-345

Complimentary Contributor Copy

Sharpley, A. N., Syers, J. K. (1976). Potential role of earthworm casts for the phosphorus enrichment of runoff waters. *Soil Biology and Biochemistry*, 8: 341-346.

Sharpley, A. N., Syers, J. K., Springett, J. A. (1979). Effect of surface-casting earthworms on the transport of phosphorus and nitrogen in surface runoff from pasture. *Soil Biology and Biochemistry*, 11: 459-462.

Shipitalo, M. J., Protz, R. (1988). Factors influencing the dispersability of clay in worm casts. *Soil Science Society of American Journal*, 52: 764-769.

Shipitalo, M. J., Dick, W. A., Edwards, W. M. (2000). Conservation tillage and macropore factors that affect water movement and the fate of chemicals. *Soil and Tillage Research*, 53: 167–183.

Shipitalo, M., Le Bayon, R.-C. (2004). Quantifying the effects of earthworms on soil aggregation and porosity. Earthworm Ecology (ed C.A. Edward), pp. 183-200. CRC Press LLC, Boca Raton.

Shuster, W. D., McDonald, L. P., McCartney, D. A., Parmele, R. W., Studer, N. S., Stinner, B.R (2002). Nitrogen source and earthworm abundance affected runoff volume and nutrient loss in a tilled-corn-agroecosystem. *Biology and Fertility of Soils*, 35: 320-327.

Shuster, W. D., Shipitalo, M. J., Subler, S., Aref, S., McCoy, E. L. (2003). Earthworm additions affect leachate production and nitrogen losses in typical Midwestern agroecosystems. *Journal of Environmental Quality*, 32: 2132-2139.

Sinha, M. P., Srivastava, R., Gupta, D. K. (2013). Earthworm biodiversity of Jharkhand: Taxonomic description. *The Bioscan*, 8: 293-310.

Six, J., Conant, R.T., Paul, E. A., Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C saturation of soils. *Plant and Soil*, 241: 155-176.

Six, J., Bossuyt, H., De Gryze, S., Denef, K. (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*,79: 7-31.

Skubała, P. (2013). Biodiversity and ecosystem services in soil under threat. *Journal of Pollution Effects and Control*, 1: e101.

Complimentary Contributor Copy

Stockdill, S. M. J. (1966). The effect of earthworms on pastures. *Proceedings of the New Zealand Ecological Society* 13: 68-75.

Stokes, A., Barot S., Lata, J.-T., Lacroix, G., Jones, C.G., Mitschf, W.J. (2012). Ecological engineering: From concepts to applications. *Ecological Engineering*, 45: 1-4.

Tiunov, A. V., Scheu S. (1999). Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae). *Soil Biology and Biochemistry,* 31: 2039-2048.

Trigo, D., Barois, I., Garvin, M.H., Huerta, E., Irisson, S., Lavelle, P. (1999). Mutualism between earthworms and soil microflora. *Pedobiologia*, 43: 866-873.

Trouvé, R., Drapela, T., Frank, T., Hadacek, F., Zaller, J.G. (2014). Herbivory of an invasive slug in a model grassland community can be affected by earthworms and mycorrhizal fungi. *Biology and Fertility of Soils*, 50: 13-23.

Uvarov, A. V. (2009). Inter- and intraspecific interactions in lumbricid earthworms: Their role for earthworm performance and ecosystem functioning. *Pedobiologia*, 53: 1-27.

Uvarov, A. V., Tiunov, A.V., Scheu, S. (2011). Effects of seasonal and diurnal temperature fluctuations on population dynamics of two epigeic earthworm species in forest soil. *Soil Biology and Biochemistry*, 43: 559-570.

van Groenigen, J. W., Lubbers, I. M., Vos, H. M. J., Brown, G. G., De Deyn, G. B., van Groenigen, K. J. (2014). Earthworms increase plant production: a meta-analysis. *Scientific Reports* 4, 6365: 1-7.

Véle, A., Holuša, J., Frouz, J., Konvička, O. (2011). Local and landscape drivers of ant and carabid beetle communities during spruce forest succession. *European Journal of Soil Biology*, 47: 349–356.

Whalen, J. K., Sampedro, L., Waheed, T. (2004). Quantifying surface and subsurface cast production by earthworms under controlled laboratory conditions. *Biology and Fertility of Soils*, 39: 287-291.

Wall, D. H., Bardgett, R. D., Behan-Pelletier, V., Herrick, J. E., Jones, T. H, Six, J., Strong, D. R., van der Putten, W.H. (2012). Soil Ecology and Ecosystem Services. OUP Oxford. 424 pp.

Complimentary Contributor Copy

Wilby, A. (2002). Ecosystem engineering: A trivialized concept? *Trends in Ecology and Evolution*, 17: 307.

Wright, J. P., Jones, C. G., Flecker, A. S. (2002). An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia*, 132: 96-101.

Wright, J. P., Gurney, W. S. C., Jones, C. G. (2004). Patch dynamics in a landscape modified by ecosystem engineers. *Oikos*, 105: 336–348.

Wright, J. P., Jones, C.G. (2006). The concept of organisms as ecosystem engineers ten years on: Progress, limitations, and challenges. *BioScience*, 56: 203-209.

Wurst, S., Langel, R., Scheu, S. (2005). Do endogeic earthworms change plant competition? A microcosm study. *Plant and Soil*, 271: 123–130.

Zaller, J. G., Arnone, J. A. (1997). Activity of surface-casting earthworms in a calcareous grassland under elevated atmospheric CO_2. *Oecologia*, 111: 249-254.

Zaller, J. G., Wechselberger, K. F., Gorfer, M., Hann, P., Frank, T., Wanek, W., Drapela, T. (2013). Subsurface earthworm casts can be important soil microsites specifically influencing the growth of grassland plants. *Biology and Fertility of Soils*, 49: 1097-1107.

Zangerlé, A., Pando, A., Lavelle, P. (2011). Do earthworms and roots cooperate to build soil macroaggregates? A microcosm experiment. *Geoderma*, 167-168: 303-309.

Zangerlé, A., Hissler, C., McKey, D., Lavelle, P. (2016a). Using near infrared spectroscopy (NIRS) to identify the contribution of earthworms to soil macroaggregation in field conditions. *Applied Soil Ecology*, 104: 138-147.

Zangerlé, A., Hissler, C, Van Schaik, L., McKey, D. (2016b). Identification of earthworm burrow origins by near infrared spectroscopy: Combining results from field sites and laboratory microcosms. *Soil and Tillage Research*, 155: 280-288.

Complimentary Contributor Copy

Zhang W., Hendrix, P. F., Dame, L. E., Burke, R. A., Wu, J., Neher, D. A., Li, J., Shao, Y., Fu, S. (2013). Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization. *Nature Communications* 4 (2576). doi:10.1038/ ncomms3576.

Zorn, M. I., Van Gestel, C. A. M., Eijsackers, H. (2005). Species-specific earthworm population responses in relation to flooding dynamics in a Dutch floodplain soil. *Pedobiologia*, 49: 189-198.

Zorn, M. I., Van Gestel, C. A. M., Morrien, E., Wagenaar, M., Eijsackers, H. (2008). Flooding responses of three earthworm species, *Allolobophora chlorotica*, *Aporrectodea caliginosa* and *Lumbricus rubellus*, in a laboratory-controlled environment. *Soil Biology and Biochemistry*, 40: 587-593.

Complimentary Contributor Copy

Complimentary Contributor Copy

In: Earthworms
Editor: Clayton G. Horton
ISBN: 978-1-53612-176-6
© 2017 Nova Science Publishers, Inc.

Chapter 5

EARTHWORMS AS A SUITABLE ORGANISM FOR SOIL POLLUTION MONITORING: POSSIBILITIES AND LIMITATIONS

Mirna Velki and Sandra Ečimović*
Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

ABSTRACT

Considering the importance of the soil in the food production and constant input of diverse chemicals (from industrial activities, urban waste, agrochemicals) that present serious threat to soil quality, there is a growing concern regarding the soil pollution and the adverse effects on soil ecosystems. Earthworms are naturally in contact with the solid and aqueous soil phases, and are continuously exposed to chemicals present in their soil environment. Due to their sensitive reactions towards environmental influences, and their importance in the structure and proper functioning of the soil ecosystems, earthworms are often used as test organism in soil ecotoxicological studies. The toxicity and adverse effects of soil pollutants can be assessed by applying various bioassays.

* Corresponding author E-mail: sandra@biologija.unios.hr.

Complimentary Contributor Copy

Combination of different assays and measured endpoints increases the predictive power of negative influences and risks. However, even though the possibilities are diverse, there are also limitations in these studies that have to be considered. In this chapter an overview of recent research regarding the assessment of various pollutants on earthworms is given with emphasis on the possible improvement of the investigation in soil pollution monitoring using these organisms.

Keywords: ecotoxicology, earthworms, soil ecosystems, soil pollution, monitoring

INTRODUCTION

Due to the increasing concern regarding the soil pollution caused by wide variety ways, such as industrial activities, urban waste, intensive use of biocides and fertilizers in agriculture and atmospheric deposition, there is an increasing interest in the scientific community and international agencies for soil pollution monitoring and assessment. Soil pollution has serious consequences for the environment, causing decrease in soil fertility, alteration of soil structure, disturbance of the balance between flora and fauna in the soil, contamination of the crops and groundwater, and pose a significant threat for living organisms (Bezchlebova et al., 2007; Asharf et al., 2014). In addition, healthy soil is the basis for sustainable and healthy food production. Soils supply the essential nutrients, water, oxygen and root support that food-producing plants need to grow (FAO, 2015). The most frequently found chemicals in soil are heavy metals, pesticides, petroleum hydrocarbons, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and different solvents (Lanno et al., 2004; Lionetto et al., 2012).

To assess the effects of pollution, cost effective and reliable methods are the major prerequisite. The traditional methods to assess the soil pollution do not provide indication of deleterious effects of pollutants to the organisms. Namely, these methods are based on the analysis of the

Complimentary Contributor Copy

concentrations of pollutants in the soil and comparison with specific threshold values. This provides good service in support of policy, mainly in relation to regulation of hazardous chemicals, but does not give realistic insight into the biological effects. Additionally, the traditional methods neglect several essential aspects such as bioavailability, interactive effects of pollutants (synergism, potentiation and antagonisms) and low concentrations of increasingly complex mixtures of pollutants in the soil. In the last two decades of the twentieth century attention was given to subcellular responses to pollutant exposure. Hence, new biological approaches to soil pollution assessments and monitoring, such as the measurement of biochemical, histological and cellular responses of organisms have become of major importance for the assessment the quality of this environmental compartment (Rao et al., 2003; Hackenberger at al., 2015). In addition, these approaches are potentially very useful as prognostic and diagnostic early warning tests and offer the potential of specificity and sensitivity.

Soil invertebrates may serve as good sentinel organisms of soil pollution assessment because they are in direct contact with soil, and are indirectly exposed through the food chain (Spurgeon and Hopkin, 1996; Rao et al., 2003; Cotter-Howells et al., 2005; Nahmani, 2007). Among terrestrial invertebrates, earthworms are priority test organisms for assessing soil pollution because of their high biomass and characteristics. Namely, earthworms represent a major component of the soil biomass (>80%) and, due to their favorable contribution on the soil structure and fertility, play an important role in the functioning of soil ecosystems. Their burrowing and feeding activities contribute to improve soil structure, aeration, and increased water infiltration. In addition, by their activity in the soil, earthworms help to increase soil fertility and formation of an organic matter layer in top soils (Edwards and Bohlen, 1996; Lukkari et al., 2006). Moreover, they have the capacity to accumulate and concentrate large quantities of inorganic and organic pollutants (Rao et al., 2003), so these are good reasons why earthworms have been used extensively in ecotoxicological studies (Cortet et al., 1999; Lanno et al., 2004). There are two main routes leading to exposure of earthworms to soil

Complimentary Contributor Copy

pollutants. One of them is alimentary tract, by ingesting large amounts of soil (Morgan et al., 2004), and second is the earthworm skin which is extremely permeable to water and it represent significant route for pollutant uptake (Saxe et al., 2001; Jager et al., 2003; Vijver et al., 2003).

Several earthworm species (*Eisenia fetida* and *Eisenia andrei*) are preferred as test organisms for toxicity testing (OECD, 1984). *E. fetida* and *E. andrei* are epigeic earthworms that live in the soil surfaces, forming no permanent burrows (Spurgeon et al., 2002), so the ecological relevance of these species has been questioned (Sanchez-Hernandez, 2006), as well as their suitability as bioindicator organisms when pollution occur at soil depths. Despite this, these two species have been considered as a suitable model species due to the standardization of acute and chronic ecotoxicological assays. They are easily maintained laboratory species, they have relatively short reproductive cycle and are readily available commercially or can be bred easily in the laboratory (OEHHA, 2009).

In the following section methods and endpoints suited for assessing chemical pollution in soil environment will be presented. Additionally, a summary of recent research regarding the assessment of various pollutants on earthworms will be given with the emphasis on the possible improvements of the investigation in soil pollution monitoring using these organisms.

TOXICITY TESTING WITH EARTHWORMS

Studies on the effects of different pollutants on earthworms can be conducted either under the laboratory conditions or in the field. Intermediates between these are also possible in terms of partial simulation of environmental condition in the laboratory my using the microcosmic and mesocosmic systems. Laboratory tests have an important role in the environmental risk assessment (ERA) process based on the ecotoxicological knowledge obtained on earthworms as test organisms (Kula and Larink, 1996; Van Gestel, 1992; Spurgeon et al., 2002). There are numerous factors affecting the pollutant toxicity in the nature that

Complimentary Contributor Copy

cannot be replicated in the laboratory. The primary goals of the laboratory tests have been the assessment of potential toxicity of new chemicals to be introduced into the environment, and assessment the risk associated with potential hazards of chemicals from historically contaminated soils (testing of environmental samples).

Several standardized tests prescribed by the Organization for Economic Corporation and Development (OECD) for assessing the toxicity of contaminated soil to earthworms exist (OECD 1984, 2004). The acute earthworm toxicity test (14 days) only measures lethality (OECD, 1984), therefore it may be insufficient for predicting long-term population fitness following chronic exposure to contaminated soil (Whitfield et al. 2011; Bagul, 2016). Chronic test (OECD, 2004), based on the inhibition of earthworm reproduction, provides a more ecologically relevant sub-lethal endpoint than lethality, but it requires a longer exposure period for assessment (8 weeks) (Handy et al., 2003). This study includes the observation of unusual behavior and morphology, the counting and weighing of the adult worms after the four primary weeks and the number of juveniles hatched at the end of the second 4-week period (OECD, 2004). The initial acute paper contact toxicity test (OECD, 1984) is easy to perform and gives high repeatability and the possibility of direct comparison of results (Edwards and Bohlen, 1992; Zhang et al., 2009). Although, this method excludes the impact and contribution of soil components and other environmental variables. The acute artificial soil test (OECD, 1984) is more eco-relevant and representative of natural exposure of earthworms to pollutants. In general, soil toxicity tests with earthworms use a standard soil type, OECD artificial soil or the LUFA 2.2 standard soil. However, a more environmental realistic approach is to perform laboratory toxicity tests with field soils. Extrapolation of the laboratory-based OECD earthworm toxicity tests to contaminated field sites is difficult. However, both of these test are best as an initial screening study either for comparing relative toxicities and/or selecting/ranking for further testing (Sanchez-Hernandez, J.C., 2006). Terrestrial avoidance behavior test is one more test for assessing effects of soil contamination (ISO, 2008). It is rapid sublethal screening method for evaluating the habitat

Complimentary Contributor Copy

function of soils and the influence of contaminants and chemicals on earthworm behavior.

As mentioned earlier, studies on the effects of different pollutants on earthworms could be also conducted under simulated field conditions. They can be carried out in microcosmic or mesocosmic systems. Soil microcosm experiments are carried out indoors in laboratory scale, under stable ambient conditions (Burrows and Edwards, 2002), while mesocosm experiments are carried out as an outdoor system in field scale and are structurally and functionally closer to the real environmental scenarios (Svendsen and Weeks, 1997b; Spurgeon et al., 2005b). These tests could be conducted with the single species or with multiple species based on the concept of a simple food chain using at least three species from different trophic level (Lioneto et al., 2012). Microcosm and mesocosm experiments enable the exposure of earthworm species from all ecological categories under conditions close to the environmental conditions, the simultaneous monitoring of changes at the different levels of biological organization and the assessment of environmental sensitivities, and therefore provides the ability to obtain more environmentally relevant and robust data (Velki et al., 2014). Only several studies employed microcosmic experiments in investigation of the impact of pesticides on the earthworms. Santos et al., (2011) microcosm experiment employed to study the effects of insecticide dimethoate, herbicide glyphosate, and acaricide spirodiclophen on the earthworm *E. andrei.* The impact of fungicide carbendazim on biomass of *Lumbricus rubellus* using microcosm was investigated by Burrows and Edwards (2002). Microcosmic system was also applied for research of metal effects (Wu et al., 2012), PAHs (Bogomolov et al., 1996), and volatile organic compounds (An, 2005) on *Apporrectodea caliginosa*. Velki et al., (2014) in their research investigated the effects of three commonly used insecticides (organophosphates dimethoate and pirimiphos-methyl, and pyrethroid deltamethrin) on the activities of molecular biomarkers using three different ecological categories of earthworms (epigeic *E. andrei* and *L. terrestris*, endogeic *Octolasion lacteum* and anecic *L. terrestris*) exposed in a microcosmic system. Mesocosmic experiments were employed by Svendsen and Weeks (1997b)

Complimentary Contributor Copy

and Spurgeon et al., (2005b) to study the effects of Cu and Cd on the earthworm *L. rubellus.*

All these tests have some advantages and also limitations. The main advantages of the laboratory tests are the controlled and stable conditions that enable good repeatability of the results. Specifically, soil temperature, moisture, pH, etc., are controlled during the study which eliminates the effects of the fluctuating conditions (and subsequent stress) on the measured endpoint. In addition, these tests are of low cost, rapid and simple which makes their application desirable (at least for the preliminary screening of the effect). On the other hand, there are limitations of these tests, such as the lack of ecological meaning and the impact of fluctuating environmental variables (which is always present in the environmental conditions) is not assessed (Sanchez-Hernandez, 2006). In the case of field studies the advantages are following: realistic exposure conditions, influence of environmental variables on earthworm sensitivity to pollutants can be assessed, earthworms show integrated responses to accumulation and toxic effects. However, the implementation of these studies is complex and often the possible arising issues cannot be timely predicted. Some of limitations of field studies tests include: difficulties in sampling, time consuming procedures, tolerance or resistance to pollution could have developed, relative complexity of result interpretation (physico-chemical and biological stressors present in the natural environment). Therefore, the microcosmic and mesocosmic systems may present the optimal compromise between "simple but ecologically irrelevant" laboratory tests and "complex and unpredictable but ecologically relevant" field studies.

ENDPOINTS IN EARTHWORM ECOTOXICOLOGY

The choice of an optimal endpoint for testing toxicants may depend on several factors, including sensitivity, accuracy, repeatability, simplicity and cost. Sensitive endpoints permit detection of lower levels of toxicants, may allow the use of shorter testing intervals, or may provide insight into the mechanism of action of toxicants (Jiang et al., 2016). The most common

Complimentary Contributor Copy

and most basic endpoint measured in toxicity testing is lethality (LC_{50}, LD_{50}). Its insufficient sensitivity only enables it to reflect acute toxicity (Jiang et al., 2016). Other common endpoints include sublethal and behavioral endpoints, reproductive success, functional endpoints, immune response, tissue measures (OEHHA, 2009).

Due to the many limitations of the classical approach to environmental toxicology, there is a growing interest in ecotoxicological investigations for increasing the knowledge on molecular and cellular responses of earthworms to pollutants as biomarkers of pollutant exposure and effect, to be used in soil monitoring and assessment programs (Scott-Fordsmand and Weeks, 2000; Beliaeff and Burgeott, 2002; Lukkari et al., 2004b; Sanchez-Hernandez, J.C., 2006). Biomarkers are useful tools for estimating the exposure level to pollutants and sublethal effects of pollutants on organisms before the damage becomes irreversible (Martin-Diaz et al., 2004; Rodriguez-Castellanos and Sanchez-Hernandez, 2007). Such diagnostic and prognostic early warning tools offer the potential of specificity, sensitivity and are traditionally defined as molecular, biochemical, cellular and physiological alterations caused by external stressors (Hugget et al., 1992; Peakall and Shugart, 1990; Kurelec, 1998). Additionally, the use of biomarkers can provide insights into the possible mode of action/mechanism of toxicity (Ankley et al., 2010) and have defined an adverse outcome pathway (AOP) as a conceptual framework for summarizing existing knowledge about linkages between a direct, molecular-level initiating event and an adverse outcome at a level of biological organization relevant to ecological risk assessment (Stepić et al., 2013). The concept of "adverse outcome pathways (AOP)" links mechanistic responses on the cellular level with whole organism population, community and potentially ecosystem effects and services (Lionetto et al., 2012). A broad group of potential biomarkers of toxic compounds were described for earthworms, including biomarkers from the molecular to the organismal level. The markers included esterase's activity, antioxidant defenses, biotransformation enzymes, metal-binding proteins, DNA alterations and genotoxicity biomarkers, lysosomal integrity, sperm quality, and immunological, neurological, histological, and

Complimentary Contributor Copy

behavioral responses. There are several common ecological endpoints serving in identifying soil pollution, e.g., changes in abundance, biomass or species richness of natural populations, which provide an indication of pollutant exposure only (Nahmani and Lavelle 2002; Sanchez-Hernandez, 2006).

The analysis of esterase's activities (cholinesterase (AChE) and carboxylesterase (CES)) are the most commonly applied biomarker of earthworms exposure to the organophosphate and carbamate compounds (Rao and Kavitha, 2004; Reinecke and Reinecke, 2007a, 2007b; Rodriguez-Castellanos and Sanchez-Hernandez, 2007). Several other studies have shown that AChE inhibition can be caused by other compounds such as PAHs (Payne et al., 1996), heavy metals (Labrot et al., 1996), and herbicides (Moraes et al., 2007). CES are also play an important role in pesticide detoxification (Maxwell, 1992; Chanda et al., 1997).

Besides the esterase's activities, the antioxidant defense system includes many molecular biomarkers that have been widely used in the soil contamination research (Labrot et al., 1996; Ribera et al., 2001; Łaszczyca et al., 2004; Antunes et al., 2008; Schreck et al., 2008; Stepić et al., 2013; Velki and Hackenberger, 2012, 2013; Velki and Ečimović, 2016). Among the oxidative stress markers, there are several antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR)) and one non-enzymatic antioxidant (reduced glutathione (GSH)) as well as lipid peroxidation (LPO), an indicator of oxidative damage (Calisi et al., 2014).

One of the most important enzyme systems involved in biotransformation processes is the cytochrome P450-dependent monooxygenase or mixed-function oxidase (MFO) system (e.g., CYP1A) (Livingstone, 1990; Stenersen, 1992; Nebert and Gonzalez, 1987). The MFO system is involved in phase I of biotransformation processes, wherein catalyzes the degradation of both endogenous and exogenous lipophilic substrates to polar water-soluble products which are more easily excreted. The properties and role of the MFO is well-documented for aquatic animals than for the terrestrial (Stenersen, 1983; Galgani and Payne, 1991). Its activity increases, apparently to enhance the degradation

Complimentary Contributor Copy

and clearance of offending compounds. This suggest that the activity of the MFO system in organisms exposed to contaminated areas might be a measure of the degree of chemical contamination (Galgani and Payne, 1991). In earthworms (*Lumbricus terrestris*), there is good evidence of presence this enzyme systems, but no studies have attempted to establish a dose response relationship between chemical dose and the P-450 level (Berghout et al., 1989, 1991; Eason et al., 1998; Lee, 1998). Biotransformation enzyme glutathione-*S*-transferase (GST) belongs to a phase II family of detoxifying enzymes and it is involved in biotransformation and detoxification of various xenobiotic chemicals, including pesticides (Booth et al., 1998; Jin-Clark et al., 2002). GST enzymes have important role in biotransformation as catalysts for the conjugation of various electrophilic compounds with tripeptide glutathione (Saint-Denis et al., 1996; Stegeman et al., 1992). This biomarker has been widely used in toxicity studies with earthworms (Stenersen, 1983; Booth et al., 1998; Xiao et al., 2006; Aly and Schröder, 2008).

Metallothioneins and other metal-binding proteins that are involved in regulation and detoxication of trace metals was used as biomarker of metal polluted soil in few studies with earthworms. Studies demonstrated a significant induction of metallothionein proteins in different earthworm species such as *L. rubellus*, *E.*, and *E. andrei* exposed to cadmium (Brulle et al., 2007; Ndayibagira et al., 2007; Calisi et al., 2009;) or in *Lumbricus mauritii* exposed to Pb and Zn contaminated soil (Liang et al., 2009) and in *L. terrestris* exposed to cadmium, copper, and mercury (Calisi et al., 2011, 2013). Novel techniques including several differential and subtractive approaches as well as fully quantitative PCR were applied to identify metal responsive genes (Höckner et al., 2015). The analysis of gene expression changes is a powerful tool, for detection the existence of a stress within a population and for analyzing mechanistically the response to a stress (Brulle et al., 2007).

Histopathological examination is a useful tool and has long been used for the assessment of exposure to stressors (Mayers and Fournie, 2002; Eseigbe et al., 2013; Stanley and Joy, 2014). Even though the histopathological approach is qualitative, it has been shown to be a reliable

Complimentary Contributor Copy

tool for evaluating the effects of environmental contamination at the tissue and organ level and represents an integration of cumulative effects of biochemical and physiological changes to the organism.

In the recent years, the use of the immune system as a sublethal biomarker for wide range of chemicals has been of increasing interest (Fitzpatrick et al., 1992; Goven et al., 1994; Peakall, 1994). In earthworms, coelomocytes as constitutive elements of coelomic fluid are predominantly responsible for immune responses. Like mammalian leukocytes, they are sensitive to foreign material (Goven et al., 1993). Earthworm coelomic fluid is particularly interesting from a toxicological point of view, because it is responsible for pollutant disposition and tissue distribution to the whole organism. Its cells (coelomocytes) are involved in the internal defense system and any impairment of coelomocyte functioning can compromise the health of the entire organism (Reinhart and Dollahon, 2003; Calisi et al., 2014). Since the coelomocytes can be obtained relatively easily, and nondestructively, by extrusion, physiological fluid seems to be very interesting for the development of novel nondestructive pollution biomarkers (Engelmann et al., 2004).

Important indicator for evaluation of deleterious effect and genotoxic effects of many pollutants in soil is the DNA damage and genotoxicity biomarkers. DNA damage may result in inappropriate gene expression, genotoxic and mutagenic effects. Two most extensively used methods for the detection of genotoxicity potential of chemicals in the environment are: the single cell gel electrophoresis assay (known as the Comet assay) and micronucleus test. Previous studies have shown that the Comet assay is rapid, effective and sensitive method for analyzing and quantifying DNA damage levels in the coelomocytes of earthworms exposed to genotoxic compounds, both *in vivo* and *in vitro* conditions (Singh et al., 1988; Verschaeve and Gilles, 1995; Salagovic et al., 1996; Reinecke and Reinecke, 2004; Espinosa-Reyes; Liu et al., 2010; Klobučar et al., 2011; Zhang et al., 2014). Micronucleus assay has emerged as one of the preferred methods for assessing chromosome damage (chromosome loss and chromosome breakage) accumulated during lifespan of the cells (Ali and Naaz, 2013).

Complimentary Contributor Copy

The measurement of gene/protein expression and metabolite levels (toxicogenomic studies) are very useful methods for detection of environmental stress in the study of long-term exposure impacts (Calzolai et al., 2007). During the last decade the implementation of metabolomics techniques for the assessment of soil contaminations is on the rise. Metabolomics has potential as a sensitive and rapid technique that can elucidate the relationship between metabolite levels and an external stressor, such as contaminant exposure, nutritional deficit or a disease. The development of metabolomics has provided the tools for the determination of multiple biomarkers across different levels biological organization, and therefore a better assessment of the ecological consequences of contamination (Hernandez-Soriano and Jimenez-Lopez, 2014).

Same as in the case of the laboratory test and field studies, each biomarker also has certain advantages and disadvantages. The ideal biomarker, a biological endpoint that would be specific, sensitive and cover all aspects of possible adverse effects of pollutants, simply does not exist. Therefore, the application of a battery of biomarkers is necessary for effective evaluation of the effects of pollutant exposure and assessment of the environmental stress (Beliaeff and Burgeot, 2002; Aarab et al., 2004).

POSSIBLE IMPROVEMENT OF MONITORING SOIL POLLUTION USING EARTHWORMS

Toxic substances can cause effects at different levels of biological organization, from molecular to ecosystem levels. The effects at low biological levels occur early, are of high specificity but are not ecologically relevant. On the other hand, changes at high biological levels have high ecological relevance but are specific and occur after long period when it is too late to intervene in the environment. Therefore, a combination of these is certainly necessary for gaining the proper insight into the long-term pollutant effects. As a first screening testing, endpoints at lower levels should be assessed. It is know that the effects at higher level are preceded

Complimentary Contributor Copy

by the changed at lower levels (but the changes at lower levels do not necessarily lead to changes at higher levels). Then if the changes are observed, endpoints at higher organizational levels should be assessed.

As already mentioned, the conditions under which organisms are exposed to pollutant will surely have influence on the final toxic effects. Simulated field studies offer the opportunity to study the effects of pollutants on the biomarker responses in the organisms under the influence of multiple environmental variables (for review see Sanchez-Hernandez, 2006). This is of great importance since the bioavailability and the toxic kinetics of the pollutant will inevitable change during the changing temperatures and moisture conditions of soil or habitat in general (Jannsen and Bergema, 1991; Bruus Pedersen et al., 1997; van Gestel, 1997). However, for the pollutants for which no previous data on toxicity is available, it would be recommended to first test using the laboratory tests in order to assess if the pollutant affects the model organisms and to determine the range of concentrations that should be tested under environmentally relevant conditions. Namely, field studies are quite expensive, and from a practical point of view, it is impossible to test all chemicals in the field. Laboratory tests are, therefore, required to gain insight into the potential risk of chemicals for ecosystems and can be used as a pre-screening methods.

Besides the measurement of the responses in the model organisms, biomarker responses can also be measured in field-sampled organisms (Aamond et al., 2007; Denoyelle et al., 2007). Using native earthworm populations for biomarker analysis integrates the bioavailability of pollutants, exposure pathways and temporal aspect of exposure (Spurgeon et al., 2002; Sanchez-Hernandez, 2006). This is a significant aspect since it is important to assess the harmfulness of polluted soils site-specifically in order to characterize possible risks to the functioning of the ecosystem. So earthworms can be sampled in the polluted areas and the measurement of (molecular) biomarkers can be conducted in order to evaluate if the pollution affected the earthworms and to check the possible mechanisms of action. The possible limitation of such studies is the need for a reference site. Namely, in order to be able to determine if the pollutant significantly

Complimentary Contributor Copy

affected some parameter, the same has to be compared to the response from the unpolluted (Spurgeon and Hopkin, 1994; Vandecasteele et al., 2004) site. This can be sometimes impossible to fulfill due to the practical reasons and also the possible influence of the differences in the conditions between the experimental and reference site has to be taken into account in the interpretation of the obtained results.

Another aspect is the investigation of the factors that can cause changes in earthworm responses. Additional studies with different species of earthworm, including different endpoints, temperature regimes and soil types, are required. Research should be extended to ecologically relevant species of earthworms. In fact, it is well known that the sensitivity and tolerance of different species may vary after exposure to various chemical pollutants (Roberts and Dorough, 1984). Investigations assessing the sensitivities of multiple earthworm species have showed that *E. andrei* and *E. foetida*, commonly used model species, are less sensitive compared to other tested earthworm species (e.g., Ma and Bodt, 1993; De Silva et al., 2010; Velki and Hackenberger, 2012; Velki and Hackenberger, 2013). Moreover, also the other soil fauna species (e.g., springtails) should be included in order to get a comprehensive knowledge on the malfunction in the soil biological processes due to soil pollutants. So, there is a need to acquire more knowledge on the chemical nature, mode of action, and means of degradation of pollutants in soil, as well as the susceptibility of different species used as model organisms to investigated pollutant. Knowing the possible contributions of different factors to the toxicity of certain pollutant, as well as understanding the differences in the species-specific sensitivities, will enable more precise prediction of the pollutant effects to the organisms in the environment.

Even though earthworm biomarkers have become increasingly relevant for the evaluation of pollutant effects on soil organisms, some aspects need to be further evaluated. In order to confirm the usefulness of biomarker approach under environmental conditions it is necessary to increase the number of investigations of native earthworm populations for assessment of polluted soils. Finally, even though the standardization of procedures for such studies is not possible due to different characteristics of each

Complimentary Contributor Copy

environmental site, it is certainly advisable to prescribe the guidelines on the selection of biomarkers, chosen species and applied experimental design in order to obtain results that can be (at least to some extent) comparable.

CONCLUSION

Chemical characterization of the soil does not provide specific biological information about potential hazards to soil organisms. In addition, the utilization of individual endpoints can lead to unprecise assumptions regarding the effects at higher levels of biological organization. Therefore, for the proper integration of biomonitoring strategies, it is necessary to apply a suite of ecotoxicological tools which would consist of conducting the preliminary pre-screening laboratory tests and would be (depending of the obtained results) followed by tests carried out under environmentally relevant conditions. The low biological levels specific biomarkers need to be complemented with ecologically more relevant high biological level endpoints in order to be able to accurately predict the long-term pollutant effects. Investigations involving native earthworm populations for assessment of polluted soils should be significantly increase.

REFERENCES

Aamodt, S., Konestabo, H. S., Sverdrup, L. E., Gudbrandsen, M., Reinecke, S. A., Reinecke, A. J., Stenersen, J. (2007). Recovery of cholinesterase activity in the earthworm *Eisenia fetida* Savigny following exposure to chlorpyrifos. *Environmental Toxicology and Chemistry*. 26, 9, 1963-1967.

Complimentary Contributor Copy

Aarab, N., Champeau, O., Mora, P., Daubeze, M., Garrigues, P., Narbonne, J.-F. (2004). Scoring approach based on fish biomarkers applied to French river monitoring. *Biomarkers*. 9, 258-270.

Ali, A. and Naaz, I. (2013). Earthworm biomarkers: The new tools of environmental impact assessment. *Bioscience Biotechnology Research Communications*. 6, 2, 163-169.

Aly, M. A. and Schröder, P. (2008). Effect of herbicides on glutathione S-transferase in the earthworm, *Eisenia fetida*. *Environmental Science and Pollution Research.* 15, 143-149.

An, Y.J. (2005) Assessing soil ecotoxicity of methyl tert-buthyl ether using earthworm bioassay; closed soil microcosm test for volatile organic compounds. *Environmental Pollution.* 134, 181-186.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., Mount, D. R., Nichols, J. W., Russom, C. L., Schmieder, P. K., Serrrano, J. A., Tietge, J. E., Villeneuve, D .L. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry*. 29, 730-741.

Antunes, S. C., Castro, B. B., Nunes, B., Pereira, R., Gonçalves, F. (2008). *In situ* bioassay with *Eisenia andrei* to assess soil toxicity in an abandoned uranium mine. *Ecotoxicology and Environmental Safety*. 71,620-631.

Asharf, M. A., Maah, M. J., Yusoff, I. (2014). Soil Contamination, Risk Assessment and Remediation. *INTECH. Chapter 1*, http://dx.doi.org/10. 5772/57287.

Bagul, P. K., More, B. C., Patole, S. S. (2016). Sub Lethal Effects of Cypermethrin and Oxyfluorfen on Stress Enzyme Activities of Earthworm Species, *Eisenia foetida* Savigny, 1826. *International Journal of Innovative research in Science Engineering and Technology*. 5, 12.

Beliaeff, B., Burgeott, T. (2002). Integrated biomarker response: a useful tool for ecological risk assessment. *Environmental Toxicology and Chemistry*. 21, 6, 1316-1322.

Complimentary Contributor Copy

Berghout, A., Büld, J., Wenzel, E. (1989). Isolation and partial purification of cytochrome P-450 from the gut of the earthworm *Lumbricus terrestris*. *Biological Chemistry*. Hoppe-Seyler. 370, 614.

Berghout, A. G. R. V., Wenzel, J., Buld, J., Netter, K. J. (1991). Isolation, partial purification, and characterization of the cytochrome P-450-dependent monooxygenase system from the midgut of the earthworm *Lumbricus terrestris. Comparative Biochemistry and Physiology.* 100C, 389-396.

Bezchlebova, J., Cernohlavkova, J., Sochova, I.J.L., Kobeticovaa K., Hofman, J. (2007). Effects of toxaphene on soil organisms. *Ecotoxicology and Environmental Safety*. 68, 3, 326-334.

Bogomolov, D. M., Chen, S.-K., Parmelee, R. W., Subler, S. Edwards, C. A. (1996). An ecosystem approach to soil toxicity testing: A study of copper contamination in laboratory soil microcosms. *Applied Soil Ecology*. 4, 95-105.

Booth, L. H., Heppelthwaite, V., Eason, C. T. (1998). Cholinesterase and glutathione S-transferase in the earthworm *Apporectodea caliginosa* as biomarkers of organophosphate exposure. In: *Proceedings of the New Zealand Plant Protection Conference*. 51, 138-142.

Brulle, F., Mitta, G., Leroux, R., Lemière, S., Leprêtre, A., Vandenbulcke, F. (2007). The strong induction of metallothionein gene following cadmium exposure transiently affects the expression of many genes in *Eisenia fetida*: a trade-off mechanism? *Comparative Biochemistry and Physiology*. 144C, 4, 334-341.

Bruus Pedersen, M., Axelsen, J. A., Strandberg, B., Jensen, J., Attrill, M. J. (1999). The impact of a copper gradient on a microarthropod field community. *Ecotoxicology*. 8, 467-483.

Burrows, L. A. and Edwards, C. A. (2002). The use of integrated soil microcosms to predict effect of pesticides on soil ecosystems. *European Journal of Soil Biology*. 38, 3-4, 245-249.

Complimentary Contributor Copy

Calisi, A., Lionetto, M. G., De Lorenzis, E., Leomanni, A., Schettino, T. (2014). Metallothionein Induction in the Coelomic Fluid of the Earthworm *Lumbricus terrestris* following Heavy Metal Exposure: A short Report. *BioMed Research International*. Hindawi Publishing Corporation. ID 109386. http://dx.doi.rg/10.1155/2014/109386.

Calisi, A., Lionetto, M. G., Schettino, T. (2009). Pollutant-induced alterations of granulocyte morphology in the earthworm *Eisenia foetida*. *Ecotoxicology and Environmental Safety*. 72, 5, 1369-1377.

Calisi, A., Lionetto, M. G., Schettino, T. (2011). Biomarker response in the earthworm *Lumbricus terrestris* exposed to chemical pollutants. *Science of the Total Environment*. 409, 20, 4456-4464.

Calisi, A., Zaccarelli, N., Lionetto, M. G., Schettino, T. (2013). Integrated biomarker analysis in the earthworm, *Lumbricus terrestris*: application to the monitoring of soil heavy metal pollution. *Chemosphere.* 90, 2637-2644.

Calzolai, L., Ansorge, W., Calabrese, E., Denslow, N., Part, P., Lettieri, T. (2007). Transcriptomics and proteomics. Applications to ecotoxicology. *Comparative Biochemistry and Physiology. Part D.* 2, 245-249.

Chanda, S. M., Mortesen, S. R., Moser, V. C., Padilla, S. (1997). Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: an *in vitro* and *in vivo* comparison. *Fundamental Applied Toxicology.* 38, 148-157.

Cortet, J., Vauflery, A.G.D., Balaguer, N.P., Gomot, L., Texier, Ch., Cluzeau, D. (1999). The use of invertebrate soil fauna in monitoring pollutant effects. *European Journal of Soil Biology.* 35, 115-134.

Cotter-Howells, J., Charnock, J. M., Winters, C., Kille, P., Fry, J. C., Mortan, A. J. (2005). Metal compartmentation and speciation in a soil sentinel: The earthworm, *Dendrodrilus rubidus. Environmental Science and Technology.* 39, 7731-7740.

De Silva, P. M. C. S., Pathiratne, A., van Gestel, C. A. M. (2010). Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*. *Apply Soil Ecology*. 44, 56-60.

Complimentary Contributor Copy

Denoyelle, R., Rault, M., Mazzia, C., Mascle, O., Capowiez, Y. (2007) Cholinesterase activity as a biomarker of pesticide exposure in *Allobophora chlorotica* earthworms living in apple orchards under different management strategies. *Environmental Toxicology and Chemistry*. 26, 12, 2644-2649.

Eason, C. T., Booth, L. H., Brennan, S., Ataria, J. (1998). Cytochrome P450 activity in three earthworm species. In: Sheppard, S., Bambridge, J., Holmstrup, M., Posthuma, L. (Eds.) *Advances in Earthworm Ecotoxicology*. SETAC Press, Pensacola, FL. 191-198.

Edwards, C. A. and Bohlen, P. J. (1992). The effects of toxic chemical on earthworms. *Reviews of Environmental Contamination and Toxicology*. 125, 23-99.

Edwards, C. A. and Bohlen, P. J. (1996). *Biology and Ecology of Earthworms*. Third ed. Chapman and Hall, Boca Raton, FL, US. 85-86.

Engelmann, P., Molnár, L. Pálinkás, L., Cooper, E. L., Németh, P. (2004). Earthworm leukocyte populations specifically harbor lysosomal enzymes that may respond to bacterial challenge. *Cell and Tissue Research.* 316, 3, 391-401.

Eseigbe, F. J., Doherty, V. F., Sogbanmu, T. O., Otitoloju, A. A. (2012). Histopathology alterations and lipid peroxidation as biomarkers of hydrocarbon-induced stress in earthworm, *Eudrilus eugeniae*. *Environmental Monitorin Assessment.* 185, 2189-2196.

Espinosa-Reyes, G., Ilizaliturri, C.A., Gonzalez-Mille, D.J., Costilla, R., Diaz-Barriga, F., Carmen Cuevas, M.D. (2010). DNA damage in earthworms (*Eisenia* spp.) as an indicator of environmental stress sin the industrial zone of Coatzacoalcos, Veracruz, Mexico. *Journal of Environmental Science Health*. 4A, 49-55.

FAO (2015). Food and Agriculture Organization of the United Nations. *International Year of Soils*, 2015, Rome, Italy.

Fitzpatrick, L. C., Sassani, R., Venables, B. J., Cooper, E. L. (1993). Cellular biomarkers for measuring toxicity of xenobiotics: Effects of polychlorinated biphenyls on earthworm *Lumbricus terrestris* coelomocytes. *Environmental Toxicology and Chemistry.* 12, 863-870.

Complimentary Contributor Copy

Galgani, F. and Payne, J. F. (1991). Biological effects of contaminants: Microplate method for measurement of ethoxyresorufin-O-deethylase (EROD) in fish. *Techniques in Marine Environmental Sciences.* ISSN 0903-2606.

Goven, A. L., Chen, S. C., Fitzpatrick, L. C., Venables, B. J. (1994). Lysosyme activity in the earthworm (*Lumbricus terrestris*) coelomic fluid and coelomocytes: enzyme assay for immunotoxicity of xenobiotics. *Environmental Toxicology and Chemistry.* 13, 607-613.

Hackenberger, B. K., Velki, M., Lončarić, Ž., Hackenberger, D.K, Ečimović, S. (2015). Effect of different river flow rates on biomarker responses in common carp (*Cyprinus carpio*). *Ecotoxicology and Environmental Safety*. 112C, 153-160.

Handy, R. D., Galloway, T. S., Deplegde, M. H. (2003). A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. *Ecotoxicology*. 12, 1-4, 331-343.

Hernandez-Soriano, M. C. and Jimenez-Lopez, J.C. (2014). Metabolomics for soil assessment. In: *Environmental Risk Assessment of Soil Contamination,* Maria C. Hernandez Soriano (Ed.) InTech-Open Access. ISBN 978-953-51-1235-8.

Höckner, M., Dallinger, R., Sturzenbaum, S.R. (2015). Metallothionein gene activation in the earthworm (*Lumbricus rubellus*). *Biochemical and Biophisical Research Communication*. 460, 3, 537-542.

Hugget, R. J., Kimerly, R. A., Mehrle, P. M., Bergman, H. L. (1992). *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress*. Lewis Publishers: Chelsea, MI, US, 1992.

ISO (2008). Soil quality - Avoidance test for determining the quality of soils and effects of chemicals on behaviour - Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). ISO 17512:1:2008.

Jager, T., Fleuren, R. H. L. J., Hogendoorn, E. A., de Korte, G. (2003). Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia andrei* (Oligochaeta). *Environmental Science and Technology*. 37, 15, 3399-3404.

Complimentary Contributor Copy

Jansen, P. M. and Bergema, F. (1991). The effect of temperature on cadmium kinetics and oxygen consumption in soil arthropods. *Journal of Applied Ecology*. 10, 1493-1501.

Jiang, Y., Chen, J., Wu, Y., Wang, Q., Li, H. (2016). Sublethal toxicity endpoints of heavy metals to the nematode *Chaenorhabditis elegans. PLOS ONE*. http://dx.doi.org/10.1371/journal.pone.0148014.

Jin-Clark, Y., Lydy, M. J., Zhu, K. Y. (2002). Effects of atrazine and cyanazine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera: Chironomidae). *Environmental Toxicology and Chemistry*. 21, 598-603.

Klobučar, G. I., Štambuk, A., Šrut, M., Husnjak, I., Merkaš, M., Traven, L. (2011). *Aporrectodea caliginosa*, a suitable earthworm species for field based genotoxicity assessment? *Environmental Pollution.* 159, 841-849.

Kula, H., Larink, O., 1996. Development and standardization of test methods for the prediction of sublethal effects of chemicals on earthworms. *Soil Biology and Biochemistry*. 29, 635-639.

Kurelec, B. (1998). Biomarkers and the ecological risk assessment paradigm. In: *Modern aspects in monitoring of environmental pollution in the sea*, Ed: E. Werner, G. Müller. Akademie gemeinnutziger Wissenschaften zu Efurt.

Labrot, F., Ribera, D., Saint-Denis, M., Narbonne, J.F. (1996). *In vitro* and *in vivo* studies of potential biomarkers of lead and uranium contamination: lipid peroxidation, acetylcholinesterase, catalase and glutathione peroxidase activities in three non-mammalian species. *Biomarkers*. 1, 21-28.

Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N. (2004). The bioavailability of chemicals in soil for earthworms. *Ecotoxicology and Environmental Safety*. 57, 1, 39-47.

Łaszcyca, P., Augustyniak, M., Babczynska, A., Bednarska, K., Kafel, A., Migula, P., Wilczek, G., Witas, I. (2004). Profiles of enzymatic activity in earthworms from zinc, lead and cadmium polluted areas near Olkusz (Poland). *Environment International*. 30, 901-910.

Complimentary Contributor Copy

Lee, R.F. (1998). Annelid cytochrome P-450. *Comparative Biochemistry and Physiology*. 121C, 173-179.

Liang, S.-H., Jeng, Y.-P., Chiu, Y.-W., Chen, J.-H., Shieh, B.-S., Chen, C.-Y., Chen, C.-C. (2009). Cloning, expression, and characterization of cadmium-induced metallothionein-2 from the earthworms *Metaphire posthuma* and *Polypheretima elongata*. *Comparative Biochemistry and Physiology.* 149C, 3, 349-357.

Lionetto, M.G., Calisi, A, Trifone S. (2012). Earthworm Biomarkers as Tools for Soil Pollution Assessment. In: *Earth and Planetary Sciences, Soil Science,* "*Soil health and Land Use Management*," book edited by Maria C. Hernandez-Soriano. ISBN 978-953-307-614-0, 2012. Chapter 16, DOI: 10.5772/28265, pp. 307-614.

Liu, Y., Zhou, Q., Xie, X., Lin, D., Dong, L. (2010). Oxidative stress and DNA damage in the earthworm *Eisenia fetida* induced by toluene, ethylbenzene and xylene. *Ecotoxicology.* 19, 1551-1559.

Livingstone, D. R. (1990). Cytochrome P-450 and oxidative metabolism in invertebrates. *Biochemical Society Transactions.* 18, 15-19.

Lukkari, T., Taavitsainen, M., Väisänen, A., Haimi, J. (2004b). Effects of heavy metals on earthworms along contamination gradients in organic rich soil. *Ecotoxicology and Environmental Safety*. 59, 3, 340-348.

Lukkari, T., Teno, S., Vaisanen, A., Haimi, J. (2006). Effects of earthworms on decomposition and metal availability in contaminated soil: microcosm studies of populations with different exposure histories. *Soil Biology and Biochemistry*. 38, 2, 359-370.

Ma, W. C. and Bodt, J. (1993). Differences in toxicity of the insecticide chlorpyrifos to six species of earthworms (Oligochaeta, Lumbricidae) in standardized soil tests. *Bulletin of Envronmental Contamination and Toxicology*. 50, 864-870.

Martin-Diaz, M. L., Blasco, J., Sales, D., Del Valls, T. A. (2004). Biomarkers as tools to assess sediment quality. Laboratory and field surveys. *Trends in Analytical Chemistry.* 23, 807-818.

Maxwell, D. M. (1992). The specificity of carboxylesterase protection against the toxicity of organophosphorus compounds. *Toxicology Applied Pharmacology.* 114, 306-312.

Complimentary Contributor Copy

Mayers, M. S. and Fournie, J. W. (2002). Histopathological biomarkers as integrators of anthropogenic and environmental stressors. In: S. Marshall Adams (Ed.), *Chapter 2, Bioindicators of Stress in Aquatic Organisms*. Wiley and Sons, Boca Ration. FL. 221-287.

Moraes, B. S., Loro, V.L., Glusczak, L., Pretto, A., Menzes, C., Marchezan, E., Machado, S.O. (2007). Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (Leporinus obtusidens). *Chemosphere*. 68, 1597-1601.

Morgan, A. J., Stürzenbaum, S. R., Winters, C., Grime, G. W., Abd Aziz, N. A., Kille, P. (2004). Differential metallothionein expression in earthworm (*Lumbricus rubellus*) tissues. *Ecotoxicology and Environmental Safety*. 57, 11-19.

Nahmani, J. and Lavelle (2002). Effects of heavy metal pollution on soil macrofauna in a Grassland of Nothern France. *European Journal of Soil Biology*. 38, 297-300.

Nahmani, J., Hodson, M. E., Black, S. (2007). Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal polluted soils. *Environmental Pollution.* 49, 44-58.

Ndayibagira, A., Sunahara, G. I., Robidoux, P. Y. (2007). Rapid isocratic HPLC quantification of metallothionein-like proteins as biomarkers for cadmium exposure in the earthworm *Eisenia andrei. Soil biology and Biochemistry*. 39, 1, 194-201.

Nebert, W. and Gonzalez, F. J. (1987). P450 genes: structure, evolution, and regulation. *Annual Review in Biochemistry.* 56, 945-993.D.

OECD (1984). Guideline for testing of chemicals: Earthworm acute toxicity test. No 207, Paris, France, 1984.

OECD (2004). Guideline for testing of chemicals: Earthworm reproduction test, No 222, Paris, France, 2004.

OEHHA (2009). Soil toxicity and bioassessment test methods for ecological risk assessment. Toxicity test methods for soil microorganisms, terrestrial plants, terrestrial invertebrates and terrestrial vertebrates. *Integrated Risk Assessment Branch, Office of*

Complimentary Contributor Copy

Environmental Health Hazard Assessment, California Environmental Protection Agency. 2009, 54.

Payne, J. H., Mathieu, A., Melvin, W., Fancey, L. L. (1996). Acetylcholinesterase, an old biomarker with a new future) Field trials in association with two urban rivers and a paper mill in Newfoundland. *Marine Pollution Bulletin.* 32, 225-231.

Peakal, D. B. (1994). The role of biomarkers in environmental assessment. *Ecotoxicology*. DOI: 10.1007/BF00117080.

Peakall, D. B. and Shugart, L. R. (1990). Biomarkers: Research and Application in the Assessment of Environmental Health. *NATO ASI Series H*. Springer-Verlag, Heidelberg. 68.

Rao, J. V. and Kavitha, P. (2004). Toxicity of azodrin on the morphology and acetylcholinesterase activity of the earthworm *Eisenia foetida*. *Environmental Research*. 96, 323-327.

Rao, J. V., Pavan, Y. S., Mad Avendra, S. S. (2003). Toxic effects of chlorpyrifos on morphology and acetylcholinesterase activity in the earthworm, *Eisenia fetida. Ecotoxicology and Environmental Safety.* 54, 3, 296-301.

Reinecke, S. A. and Reinecke, A. J. (2004). The comet assay as biomarker of heavy metal genotoxicity in earthworms. *Archives of Environmental Contamination and Toxicity.* 46, 208-215.

Reinecke, S. A. and Reinecke, A .J. (2007a). The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. *Ecotoxicology and Environmental Safety.* 66, 244-251.

Reinecke, S. A. and Reinecke, A. J. (2007b). Biomarker response and biomass change of earthworms exposed to chlorpyrifos in microcosms. *Ecotoxicology and Environmental Safety.* 66, 92-101.

Reinhart, M. and Dollahon, N. (2003). Responses of coelomocytes from *Lumbricus terrestris* to native and non-native eukaryotic parasites. *Pedobiologia.* 47, 5-6, 710-716.

Complimentary Contributor Copy

Ribera, D., Narbonne, J. F., Arnaud, C., Saint-Denis, M. (2001). Biochemical response of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil, effects of carbaryl. *Soil Biology and Biochemistry*. 33, 1123-1130.

Roberts, B. L. and Dorough, H. W. (1984). Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environmental Toxicology and Chemistry*. 3, 1, 67-78.

Rodriguez-Castellanos, L., Sanchez-Hernandez, C. (2007). Earthworm biomarkers of pesticide contamination: current status and perspectives. *Journal of Pesticide Science*. 32, 360-371.

Saint-Denis, M., Labrot, F., Narbonne, J.F., Ribera, D. (1996). Glutathione, glutathione-related enzymes, and catalase activities in the earthworm *Eisenia fetida Andrei. Arcives of Environmental Contamination Toxicology.* 35, 602-614.

Salagovic, J., Gilles, J., Verschaeve, L., Kalina, I. (1996). The comet assay for the detection of genotoxic damage in the earthworms: a promising tool for assessing the biological hazards of polluted sites. *Folia Biologica.* 42, 17-21.

Sanchez-Hernandez, J. C. (2006). Earthworm Biomarkers in Ecological Risk Assessment. *Reviews of Environmental Contamination Toxicology*. 188, 85-126.

Santos, M. J .G., Ferreira, V., Soares, A. M. V. M., Loureiro, S. (2011) Evaluation of the combined effects of dimethoate and spirodiclofen on plants and earthworms in a designed microcosm experiment. *Applied Soil Ecology.* 48, 294-300.

Saxe, J. K., Impellitteri, C. A., Peijnenburg, W. J. G. M., Allen, H. E. (2001). A novel model describing heavy metal concentrations in the earthworm *Eisenia andrei. Environmental Science and Technology.* 35, 4522-4529.

Schreck, E., Geret, F., Gontier, L., Trilhou, M. (2008). Neurotoxic effect and metabolic responses induced by a mixture of six pesticide sin earthworm *Aporrectodea caliginosa nocturna*. *Chemosphere.* 71, 1832-1839.

Complimentary Contributor Copy

Scott-Fordsmand, J.J. and Weeks, J.M. (2000). Biomarkers in Earthworms. *Reviews of Envronmental Contamination Toxicology*. 165: 117-159.

Singh, N. P., McCoy, M. T., Tice, R. R., Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research.* 175, 184-191.

Spurgeon, D. J. and Hopkin, S. P. (1994). Extrapolation of the laboratory-based OECD earthworm toxicity test to metal-contaminated field sites. *Ecotoxicology*. 4, 190-205.

Spurgeon, D. J. and Hopkin, S. P. (1996). Effects of metal-contaminated soils on the growth, sexual development, and early cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. *Science of Total Environment.* 187, 167-183.

Spurgeon, D. J., Svendsen, C., Lister, Lj, Hankard, P. K. Kille, P. (2005b). Earthworm responses to Cd and Cu under fluctuating environmental conditions: a comparison with results from laboratory exposures. *Environmental Pollution.* 136, 443-452.

Spurgeon, J. D., Weeks, J. M., Van Gestel, C. A. M. (2002). A summary of eleven years progress in earthworm ecotoxicology. *Pedobiologia*. 47, 588-606.

Stanley, O. N. and Joy, O. A. (2014). Histopathological effects of glyphosate and its toxicity to the earthworm *Nsukkadrilus mbae*. *Britich Biotechnology Journal.* 4, 2, 149-163.

Stegeman, J. J., Brouwer, M., Richard, T. D. G., Foerlin, L., Fowler, B. A., Sanders, B. M., van Veld, P. A. (1992). Molecular responses to environmental contamination: Enzyme and protein systems as indicators of chemical exposure and effects. In: *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress.* Hugett, R. J., Kimerly, R. A., Mehrle, P. M., Jr., Bergman, H. L., Eds. Lewis Publishers: Chelsea, MI, US, 1992. pp. 235-335.

Stenersen, J. (1983). Detoxication of xenobiotics by earthworms. *Comparative Biochemistry and Phisiology.* 78c, 2, 249-252.

Complimentary Contributor Copy

Stenersen, J. (1992). Uptake and metabolism of xenobiotics by earthworms. In: Grieg Smith, P. W., Becker, H., Edwards, P. J., Heimbach, F. (Eds.) *Ecotoxicology of Earthworms. Intercept.* Hants, UK. 129-138.

Stepić, S., Hackenberger, B. K., Velki, M., Hackenberger, D. K., Lončarić, Ž. (2013). Potentiation effect of metolachlor on toxicity of organochlorine and organophosphate insecticides in earthworm *Eisenia andrei*. *Bulletin of Environmental Contamination Toxicology.* 91, 1, 55-61.

Svendsen, C. and Weeks, J. M. (1997b). Relevance and applicability of a simple earthworm biomarker of copper exposure. II. Validation and applicability under field conditions in a mesocosm experiment with *Lumbricus rubellus*. *Ecotoxicology and Environmental Safety*. 36, 80-88.

Vandecasteele, B., Symyn, J., Quataert, P., Muys, B., Tack, F. M. G. (2004). Earthworm biomass as additional information for risk assessment of heavy metal biomagnification: a case study for dredged sediment-derived soils and polluted floodplain soils. *Environmental Pollution*. 129, 3, 363-375.

Van Gestel, C. A. M. (1992). Validation of earthworm toxicity test by comparison with field studies: a review of benomyl, carbedazim, carbofuran, and carbaryl. *Ecotoxicology and Environmental Safety*. 23, 221-236.

Van Gestel, C. A. M. (1997). Scientific basis for extrapolating results from soil ecotoxicity tests to field conditions and the use of bioassays. In: Van Straalen NM, Løkke H. (Eds.). *Ecological Risk Assessment of Contaminants in Soil*. Chapman and Hall, London, 25-50.

Velki, M. and Ečimović, S. (2016). Important Issues in Ecotoxicological Investigations Using Earthworms. *Reviews of Environmental Contamination and Toxicology*. DOI 10.1007/398_2016_4.

Velki, M. and Hackenberger, B. K. (2013). Biomarker responses in earthworm *Eisenia andrei* exposed to pirimiphos-methyl and deltamethrin using different toxicity tests. *Chemosphere*. 90, 1216-1226.

Complimentary Contributor Copy

Velki, M. and Hackenberger, B. K. (2013). Inhibition and recovery of molecular biomarkers of earthworm *Eisenia andrei* after exposure to organophosphate dimethoate. *Soil Biology and Biochemistry*. 57, 100-108.

Velki, M. and Hackenberger, B. K. H. (2012). Species-specific differences in biomarker responses in two ecologically different earthworms exposed to the insecticide dimethoate. *Comparative Biochemistry and Physiology*. 156C, 104-112.

Velki, M., Hackenberger, B. K., Lončarić, Ž., Hackenberger, D. K. (2014) Application of microcosmic system for assessment of insecticide effects on biomarker responses in ecologically different earthworm species. *Ecotoxicology and Environmental Safety*. 104, 110-119.

Verschaeve, L. and Giles, J. (1995). Single cell gel electrophoresis assay in the earthworm for the detection of genotoxic compounds in soil. *Bulletin of Environmental Contamination Toxicology*. 54, 112-119.

Vijver, M. G., Vink, J. P. M., Miermans, C. J. H., Van Gastel, C .A. M. (2003). Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metal sin earthworms. *Soil Biology and Biochemistry*. 35, 1, 125-132.

Whitfield, C. J., Watmough, S.A., Aherne, J. (2011). Evaluation of elemental depletion weathering rate estimation methods on acid-sensitive soils of noorth-eastern Alberta, Canada. *Geoderma*. 166, 1, 189-197.

Wu, S., Zhang, H., Zhao, S., Wang, J., Li, H. Chen, J. (2012) Biomarker responses of earthworms (*Eisenia fetida*) exposure to phenanthrene and pyrene both singly and combined in microcosms. *Chemosphere*. 87, 285-293.

Xiao, N.W., Song, Y., Ge, F., Liu, X.-H., Ou-Yang, Z.-Y (2006). Biomarkers response of the earthworm *Eisenia fetida* to acetochlor exposure in OECD. *Chemosphere*. 65, 907-912.

Zhang, L., Ji, F., Li, M., Cui, Y., Wu, B. (2014). Short-term effects of dechlorane plus on the earthworm *Eisenia fetida* determined by a systems biology approach. *Journal of Hazardous Materials*. 273, 239-246.

Complimentary Contributor Copy

INDEX

Complimentary Contributor Copy

C

Complimentary Contributor Copy

D

E

Complimentary Contributor Copy

F

Complimentary Contributor Copy

G

H

I

Complimentary Contributor Copy

J

K

L

M

Complimentary Contributor Copy

N

Complimentary Contributor Copy

O

P

Complimentary Contributor Copy

Q

R

S

Complimentary Contributor Copy

T

Complimentary Contributor Copy

U

V

W

Z

Complimentary Contributor Copy