Abstract

The biological diversity in the soil is much higher than most people can imagine, and a very important group of animals living there are earthworms and other clitellate worms. It has been found that several clitellate morphospecies are in fact complexes of so called cryptic species, and the number of species in this group may be much higher than previously believed. In this thesis, I explore the species boundaries of terrestrial clitellates by combining various data sources and methods as a basis for taxonomical decisions. A widely used approach for studying cryptic species is DNA-barcoding, where a single standardised marker is used for the identification of organisms and discovering new species. For animals, the marker generally used is the mitochondrial COI gene.

In the thesis, I present four different cases, from three different clitellate families: 1) A variety of *Rhyacodrilus falciformis* was shown to be a distinct species, whereas other COI clusters were found to be part of the same species. 2) Extensive cryptic diversity was found in the genus previously known as *Cognettia*, a group that includes *C. sphagnetorum*, a well-studied model in soil biology. This taxon and *C. glandulosus* were both found to be complexes of cryptic species. The generic taxonomy of *Cognettia* was revised and the genus split into its two senior synonyms, *Euenchytraeus* and *Chamaedrilus*, and the species in the *C. sphagnetorum* complex were revised and described. 3) In the earthworm *Aporrectodea longa,* two well separated COI lineages were found to be part of the same species. 4) In the earthworm genus *Lumbricus*, the previously noted split between *L. terrestris* and *L. herculeus* was verified using nuclear data, and in the morphospecies *L. rubellus* seven cryptic species were found. The first evidence of limited hybridisation within the genus was also found, between *L. terrestris* and *L. herculeus*, as well as and between species within *L. rubellus s.lat*.

To conclude, I have shown that the species diversity among terrestrial clitellates is larger than previously known based on morphology, and that species can be robustly delimited using a combination of mitochondrial and nuclear markers, supported with morphology. I have also found that using DNA-barcoding alone will often overestimate the number of clitellate species, and caution is required when using it.

Keywords: Annelida, Clitellata, cryptic species, DNA-barcoding, Enchytraeidae, Lumbricidae, Naididae, Oligochaeta, species delimitation

Svensk sammanfattning

Mångfalden av organismer i marken är mycket större än vad de flesta kan föreställa sig. En viktig djurgrupp som lever under markytan är daggmaskar och andra gördelmaskar (Clitellata). Det har visats sig att många gördelmaskarter egentligen är komplex av så kallade kryptiska arter, dvs. arter som är så morfologiskt lika att man kan tro att de är en och samma art, och att antalet arter kan vara mycket större än vad man tidigare antagit. I den här avhandlingen utforskar jag artgränserna hos landlevande gördelmaskar, genom att kombinera olika data och metoder för att kunna svara på taxonomiska frågor. Ett vanligt angreppsätt för att studera kryptiska arter är DNA-streckkodning, där en standardiserad genetisk markör används för att artbestämma individer, men också för att avgränsa arter. För djur används vanligtvis en bit av den mitokondriella genen COI som streckkod.

I avhandlingen presenterar jag fyra fall, från tre olika gördelmaskfamiljer, där olika kombinationer av metoder och data har används. 1) en variant av *Rhyacodrilus falciformis* visade sig vara en distinkt art, medan andra COI-kluster tillhörde samma art. 2) Omfattande kryptisk diversitet hittades i gruppen tidigare känd som *Cognettia*, ett släkte som innehåller arten *C. sphagnetorum*, som används som modell inom markbiologi. Den senare visade sig vara ett komplex bestående av minst fyra arter i Norden. Släktestaxonomin för *Cognettia* reviderades och släktet delades upp i dess två seniora synonymer, *Euenchytraeus* och *Chamaedrilus*, och arterna inom *C. sphagnetorum* komplexet reviderades och beskrivs. 3) Inom den långa daggmasken, *Aporrectodea longa* finns två väl separerade COI-kluster, men dessa visade sig tillhöra samma art. 4) Inom daggmasksläktet *Lumbricus* verifierades den föreslagna uppdelningen av den stora daggmasken *L. terrestris* i två arter, *L. terrestris s.str* och *L. herculeus*, och den stora lövmasken, *L. rubellus*, visade sig bestå av sju arter. De första bevisen för hybridisering inom släktet upptäcktes, mellan *L. terrestris* och *L. herculeus* samt mellan arter inom *L. rubellus* komplexet.

Sammanfattningsvis har jag visat att artdiversiteten hos marklevande gördelmaskar är mycket större än vad man tidigare ansett baserat på morfologi, och att det är möjligt att göra robusta artavgränsningar genom att flera datakällor integreras i en kombination av analyser. Jag har även visat att DNA-streckkodning ofta överskattar antalet arter av gördelmaskar, och att resultaten från sådana analyser måste tolkas försiktigt.

Table of Contents

1. Papers included in the thesis

The thesis is based on the following publications, which are referred to by their Roman numerals.

Paper I[a]. Martinsson, S., Achurra, A., Svensson, M. & Erséus C. (2013). Integrative taxonomy of the freshwater worm *Rhyacodrilus falciformis* s.l. (Clitellata: Naididae), with the description of a new species. *Zoologica Scripta*, 42(6), 612–622. doi:10.1111/zsc.12032.

Paper II[b]**.** Martinsson, S. & Erséus, C. (2015). Cryptic diversity in the well-studied terrestrial worm *Cognettia sphagnetorum* (Clitellata: Enchytraeidae). *Pedobiologia*, 57(1), 27-35. doi:10.1016/j.pedobi.2013.09.006.

Paper III[c]**.** Martinsson, S., Rota, E. & Erséus, C. (2014). Revision of *Cognettia* (Clitellata, Enchytraeidae): re-establishment of *Chamaedrilus* and description of cryptic species in the *sphagnetorum* complex. *Systematics and Biodiversity*, 13(3), 257-277. doi:10.1080/14772000.2014.986555.

Paper IV[c]**.** Martinsson, S., Rhodén, C. & Erséus, C. (2015). Barcoding gap, but no support for cryptic speciation in the earthworm *Aporrectodea longa* (Clitellata: Lumbricidae). *Mitochondrial DNA*, 1-9. doi:10.3109/19401736.2015.1115487.

Paper V[d]**.** Martinsson, S., & Erséus, C. (2017) Cryptic speciation and limited hybridization within *Lumbricus* earthworms (Clitellata: Lumbricidae). *Molecular Phylogenetics and Evolution,* 106, 18-27. doi:10.1016/j.ympev.2016.09.011.

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2. Introduction

The estimates of the number of species on earth vary greatly from 0.5 to 100 million species, and the estimates are not converging over time (Caley *et al.*, 2014). A reasonable estimate is 5±3 million species of which about 1.5 million are described, and the remaining 0.5-6.5 million species are still awaiting description (Costello *et al.*, 2013). With the increased use of molecular methods, ranging from the early uses of protein electrophoresis and DNA hybridisation to the analysis of single genes and more recently large parts of genomes, it is now clear that many species are a complex of morphologically very similar species, so called cryptic species (Bickford *et al.*, 2007). With the possibilities to explore new sources of data, new challenges emerge and therefore, in recent years a great amount of work on how to best incorporate and use genetic data to delimit species has been published (see Sites & Marshall, 2003; Fujita *et al.*, 2012). It is also necessary to combine the new data with old knowledge and traditional methods, in order not to lose information about the taxa, and for this an integrative approach is useful (Dayrat, 2005). For this purpose several data types, e.g., morphology, genetic information and ecological data, are combined. The link to older information is retained, by insuring that taxa are being named in agreement with the International Code of Zoological Nomenclature (ICZN, 1999), and that good morphological descriptions are provided, together with the evidence from other sources. However, it is not always possible to describe taxa delimited by molecular methods alone. In many cases it is hard to determine which of the species in a complex that is the nominal taxon upon which the original description was based. If possible synonymy is involved, available old names need, as far as possible, to be tied to specific lineages, before new species can be formally described and named in the complex.

Even in such a relatively well studied area as NW Europe, soil is one habitat, where there are still many species to discover. This is due to the diversity and abundance of organisms in the soil. Therefore it is sometimes are called “the poor man’s rainforest” (Usher *et al.*, 1979; Giller, 1996). The soil in a small patch of temperate woodland can contain more than 1,000 invertebrate species (Schaefer & Schauermann, 1990), and the soil fauna has been estimated to contain 23 % of the described biodiversity (Decaëns *et al.*, 2006). Among the soil fauna, earthworms are one of the most well-known and recognised group of organisms. Together with the other major group of terrestrial annelids, the potworms (family

Enchytraeidae), they can greatly change the properties of soil and affect the soil community, and are therefore considered important ecosystem engineers (Darwin, 1881; Jouquet *et al.*, 2006). They also play important roles in the decomposition of organic matter and in nutrient cycling (Standen, 1978; Laakso & Setälä, 1999). In both these groups there are numerous species still to be described and the number of cryptic species is high (e.g. King *et al.*, 2008; Collado *et al.*, 2012; Matamoros *et al.*, 2012). We will not be able to understand the role of different terrestrial annelid species in the ecosystems if they remain unknown. It is therefore important to properly delimit and describe the soil fauna.

In this thesis I explore the species boundaries of mainly terrestrial North European clitellates. This is performed with an integrative approach where mitochondrial and nuclear gene sequences are combined and complemented with morphology in order to delimit and describe species. The molecular data are analysed using a wide variety of methods, in order to better understand the diversity of the studied groups. In my thesis, I have chosen four taxonomic groups representing different cases where I have been using varying combinations of data and methods to test species boundaries and solve taxonomical problems, in two of the cases I also describe the included species.

3. Specific aims of the thesis

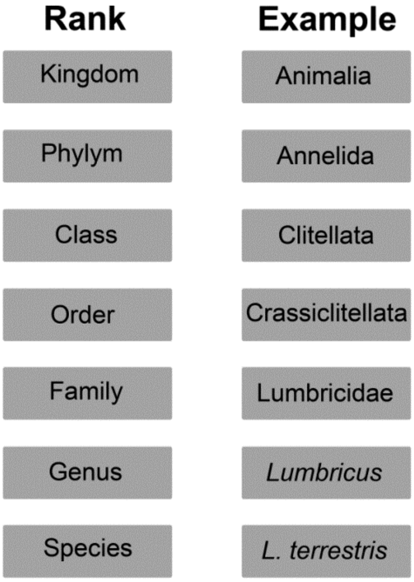
The specific aims of this thesis were:

- To test the species limits of *Rhyacodrilus falciformis* using integrative taxonomy (paper I).

- To delimit and describe the North European species in the former "*Cognettia sphagnetorum*-complex" using molecular markers, as well as revising the generic taxonomy of the species earlier placed in *Cognettia* and describe the delimited species (papers II & III).

- To test if two divergent mt-lineages within *Aporrectodea longa* constitute different species or are part of the same species (paper IV)

- To test whether cryptic mt-lineages within the earthworm-genus *Lumbricus* are different species or not, and explore possible hybridisation between cryptic species in this genus (paper V).



Rank	Example
Kingdom	Animalia
Phylym	Annelida
Class	Clitellata
Order	Crassiclitellata
Family	Lumbricidae
Genus	*Lumbricus*
Species	*L. terrestris*

Figure 1. Ranks in zoological systematics. On the right, as an example, the names for the ranks that include the earthworm *Lumbricus terrestris* are given.

4. What is a species?

Species is one of the basic units in biology, in particular in systematics and taxonomy (e.g., Cohan, 2002). However, what a species actually is and how to group individuals into species have long been debated, more so than for any other taxonomical rank. The species rank is generally believed to be more biological meaningful than the other ranks (the main ranks in Zoological systematics are shown in Fig. 1) for which there are no objective criteria (see e.g., Mayr, 1943). Thus, there are two principal questions, one about the species concept as such, i.e. what characterises the unit "species", and one about how to delimit species, i.e., which criteria are needed to be fulfilled in order to classify two organisms as the same or different species (de Queiroz, 2007).

4.1 Species concepts

Several species concepts (SC) have been suggested over the years (Table 1). One of the most well-known is the Biological, or Reproductive, species concept, by which a species consists of the individuals that can reproduce with each other (Wright, 1940; Mayr, 1942). Other noticeable concepts are the Ecological SC, by which closely related individuals sharing a niche or adaptive zone constitute a species (Van Valen, 1976), and the phylogenetic SC, which can be divided into several concepts. Some of the latter are: the Diagnosable SC, where a species consists of the smallest group of self-perpetuating organisms that share a unique sets of characters (Nelson & Platnick, 1981); the Monophyletic SC, where individuals are grouped into species because of evidence of monophyly, and species are the taxa recognized in a classification that are the least inclusive (Rosen, 1979; Mishler & Brandon, 1987); the Cladistic, or Hennigian, SC, where a species consists of a set of organisms existing between two speciation events, or between a speciation event and an extinction (Hennig, 1950; Ridley, 1989). However, in practice many of the species concepts are hard to use for making taxonomical decisions, and for the majority of species, what is sometimes called the Taxonomical, or Cynical, SC has been used. In this concept a species constitutes the specimens considered by a taxonomist to be members of it (Blackwelder, 1967).

In all the species concepts above there is no clear distinction between the conceptual problem of defining the species category, and the practical problem of delimitating specimens into, and defining the boundaries between species. In an attempt to separate these two problems, de Queiroz (2007) proposed a "unified species concept", whereby a separately evolving meta-population lineage is the sole requirement of a species. In this concept the other previously suggested SC are incorporated as secondary criteria for the assessment of lineage separation (species delimitation). The more secondary species criteria support a divergence, the stronger the case is for speciation. However, one piece of evidence, if properly examined, may be enough to establish lineage separation.

With the recent developments of methods for species delimitation (see 4.2) yet another species concept has been presented, the Multispecies Coalescent SC, according to which a species constitutes a branch of a species tree, and is defined by abrupt speciation and no genetic exchange after the speciation event (Aydin *et al.*, 2014). Under this SC, a species is still a separately evolving meta-population lineage, which is in agreement with the unified SC suggested by de Queiroz (2007). Under the multispecies coalescent SC, species are testable through the statistical nature of the multispecies coalescent model (Aydin *et al.*, 2014).

Table 1. List of species concepts discussed in this thesis, with their main criteria for determining species. *species concepts that belong to the group of phylogenetic species concepts

Species concept	**Main criteria for a species**
Biological	Interbreeding
Ecological	Sharing the same niche
Diagnosable*	Sharing of unique set of characters
Monophyletic*	Monophyly
Cladistic*	Set of organisms existing between speciation events
Taxonomic	Judgement by taxonomist
Unified	Separately evolving meta-population
Multispecies Coalescent	Forming a branch on a species tree

4.2 Species delimitation

Species delimitation is the process of dividing individuals into species. As noted in 4.1, traditionally there has been no real distinction between species concept and delimitation. The concept used has dictated the data needed to properly test the species boundaries. For example, if one would like to use the Biological SC and test for reproductive isolation between two populations, breeding experiments are needed, preferably over many generations, as species may hybridise and produce offspring with reduced fertility, and for a majority of species such experiments would be very hard to perform. Due to this, morphology has traditionally been used, and in the case of the biological SC, gaps in variation of morphological characters have been used to infer reproductive isolation. In fact, to date taxonomists have in reality, far too often, resorted to the “Cynical” SC when deciding about species boundaries.

Species delimitation can be divided into two steps, species discovery and species validation (Carstens *et al.*, 2013). During the species discovery phase hypotheses about species boundaries are formed, and specimens are grouped into groups/putative species. This is usually done using a single data source, e.g., morphology or DNA-barcoding. These hypotheses are then tested in the species validation phase with additional data and analyses (Carstens *et al.*, 2013). Species delimitation using sequence data has commonly been performed with a single locus and focused on genetic distances, reciprocal monophyly or diagnostic characters (Fujita *et al.*, 2012), i.e., doing the species discovery phase only, and simply accepting the groups found as species. However, a single marker is not enough for a solid well-supported delimitation, and the delimitation success increases with the number of markers (Dupuis *et al.*, 2012).

With the introduction of molecular data, the development of methods used for species delimitation has intensified (see review by Sites & Marshall, 2003). Many of the methods that use sequence data can only analyse one locus at a time, or a concatenated matrix, i.e., a matrix were several genes for each individual are pasted after each other to form one dataset, with the assumption that all loci share the same history, an assumption often known to be false (Degnan & Rosenberg, 2009). If many loci are studied, they have to be analysed independently and the results compared, and combined with other available information e.g., morphological and ecological data before a decision is made on the basis of the amount of congruence between the loci (Padial *et al.*, 2010; Schlick-Steiner *et al.*, 2010). However, there are methods to analyse several loci together in a single analysis for species delimitation, and some of these are based on the multispecies coalescent model. In this model, genes evolve inside a species phylogeny where the branches are species and the properties of the branches restrict the gene trees. One of these restrictions is that the divergence times between species have to be more recent than the coalescent times for any genes shared between them, assuming no genetic transfer after speciation (Rannala & Yang, 2003). This model can be used for statistical testing of species assignments (Fujita *et al.*, 2012; Rannala, 2015). Most of these methods require the user to assign the specimens to putative species that are then tested, usually by collapsing the species tree and joining sister species, and testing which of the assignments better fit the model (Fujita *et al.*, 2012; Rannala, 2015). In theory it is possible to assign each specimen to its own putative species, but, at least for some software, it may increase the computational time so that the analyses are not practically possible to be run (Yang & Rannala, 2014). The priors selected for the analyses are known to sometimes greatly affect the result. They should be selected carefully, and it may be wise to run several analyses varying some of the priors (Leache & Fujita, 2010; Rannala, 2015).

In this thesis, several methods for species delimitation have been used. All studies have had a DNA-barcoding approach (see 5) in the species discovery phase, where a COI dataset has been analysed using distance methods. The results from these analyses have then been validated with various methods. In paper II, gene tree congruence was used for validating the putative species given by the distance analyses, and in paper III, morphological differences were also found between most of the delimited species (paper III; Martinsson *et al.*, 2015c). In paper I, the species hypotheses were tested using gene trees combined with two statistical tests that estimate the probability that monophyletic groups are the result of random coalescence (Rosenberg, 2007; Rodrigo *et al.*, 2008), and the species boundaries were also supported by morphological data. In paper IV, we used the same molecular methods as in paper I, but complemented with haplotype networks, as well as a multi locus coalescent species delimitation analysis, and scrutiny of body size data. Finally, in paper V, haplotype networks, gene trees and multi-locus coalescent species delimitation analyses were used to delimit species.

4.3 Cryptic species

“*Evolution has no reason to facilitate our work of classification*”
Grandjean (1954, translated in James, Samuel W. & Davidson, 2012)

Cryptic species are species that are morphologically indistinguishable or so similar that they have been classified under the same species name (Bickford *et al.*, 2007). This is a common phenomenon among several organismal groups (Pfenninger & Schwenk, 2007), not the least among annelid worms (see reviews by Erséus & Gustafsson, 2009; Nygren, 2014). Due to the lack of externally discernible characters, especially in immature specimens, many species in

Clitellata, the group of annelids studied in this thesis, have proved hard to distinguish without the aid of molecular markers, and the diversity has in many cases been shown to be underestimated when based on morphology alone (e.g. Gustafsson *et al.*, 2009; Kvist *et al.*, 2010; Envall *et al.*, 2012; Matamoros *et al.*, 2012; Novo *et al.*, 2012).

Several organisms used as models organisms in biology have been found to be complexes of cryptic species. We know that such species may differ in both ecological and physiological traits. This makes it problematic to use them in experiments, etc., especially if it is not know which of the cryptic lineages is studied, making comparisons between studies less meaningful. It has been found that different species within a complex can differ significantly in, e.g., their response to pollutants (Sturmbauer *et al.*, 1999; Feckler *et al.*, 2012; Kille *et al.*, 2013; Feckler *et al.*, 2014), their susceptibility to parasite infection (Beauchamp *et al.*, 2002), their predation risk (Cothran *et al.*, 2013), and host preferences (Zhang *et al.*, 2011; Hambäck *et al.*, 2013). This is the case for clitellates, where several species are being used as models (Erséus & Gustafsson, 2009; Halanych & Borda, 2009; Römbke & Egeler, 2009), many of which are morphospecies now known to be complexes of cryptic species. In other cases species have been misidentified and either used alone under the wrong name or as part of a mixture with the species it has been identified as (e.g., Siddall *et al.*, 2007; Gustafsson *et al.*, 2009; Römbke *et al.*, 2016). This represents a major problem for the interpretation of the result from these studies and for comparisons between studies.

It is important to better define the species boundaries in taxa used as model organisms, and then to explore the possible ecological and physiological differences between the cryptic species revealed. Needless to say, in all cases the species and specimens studied should be identified by molecular methods, e.g., DNA-barcoding.

5. DNA-Barcoding

DNA-barcoding is a concept intended to facilitate the identification of organisms, by matching the sequence of a short standardized marker for identification with a reference library (Hebert *et al.*, 2003). The identification is often based on the assumption that intraspecific divergence is distinctly lower than interspecific divergence (the so-called barcoding gap) and is performed by comparing the sequence of an unidentified individual with known sequences in a database (see review by Taylor & Harris, 2012). The usefulness of DNA barcoding depends on the quality of the reference library, which needs to have sufficient samples of each species from across its range to cover both geographical and intraspecific variation (Ekrem *et al.*, 2007; Bergsten *et al.*, 2012; Kvist, 2013). A minimum sample size of 20 individuals seems to be needed to correctly estimate the genetic diversity within each species (Luo *et al.*, 2015).

The region selected as the universal barcoding region in animals is a part of the cytochrome c oxidase subunit 1 (COI) gene (Hebert *et al.*, 2003). It belongs to the mitochondrial genome, which is haploid and almost exclusively transmitted maternally, and thus reduces the effective population size (Ne) to generally one-fourth of that of nuclear markers (Birky *et al.*, 1989), increasing the genetic drift fourfold, resulting in faster lineage sorting and shorter time to monophyly (Neigel & Avise, 1986). However, in hermaphrodites, such as clitellates, the effective population size is half of that of nuclear genes, as all individuals can contribute with mitochondrial DNA to the next generation. The smaller effective population size together with the fact that mt genes normally evolve several times faster than nuclear genes (Brown *et al.*, 1979; Brown *et al.*, 1982; Lin & Danforth, 2004) has made such markers widely used in studies of recent divergence and species delimitation in several animal groups including clitellates (e.g. Heethoff *et al.*, 2004; James *et al.*, 2010; Dózsa-Farkas *et al.*, 2012; Timm *et al.*, 2013).

DNA-barcoding as a specimen identification tool has been found successful for many animal groups (Waugh, 2007). It is good for matching specimens of different life stages and/or sexes, and thereby it increases the number of individuals that can be identified to species level compared with only using morphology, where often only adults, and in cases with non-hermaphroditic animals, where often only one sex can be reliably identified (Ekrem *et al.*, 2010; Richard *et al.*, 2010; Stur & Ekrem, 2011). In studies of earthworms and other clitellates, DNA barcoding has been used to study invasive species (Porco *et al.*, 2013; Martinsson *et al.*, 2015a; Rota *et al.*, 2016), to test model organisms used in ecotoxicology (Römbke *et al.*, 2016), and together with other data, to discover cryptic species (e.g. King *et al.*, 2008; Gustafsson *et al.*, 2009; James *et al.*, 2010).

When DNA-barcoding is used for species discovery and delimitation, the barcodes are often separated into clusters, based on the existence of a barcoding-gap. These clusters are considered as possible species, or Molecular Operational Taxonomic Units (MOTUs). However, this approach often overestimates the number of species (Dasmahapatra *et al.*, 2010), and more data is always needed to confirm the result of DNA barcoding when used for species delimitation (for clitellate examples see e.g., Achurra & Erséus, 2013; papers I; IV; V).

For the calculation of pairwise genetic distances, the use of the K2P model (Kimura, 1980) became the standard in barcoding studies (Collins *et al.*, 2012; Srivathsan & Meier, 2012). However the use of this model was never well justified; when model testing is performed on COI datasets, K2P is rarely found to be the best fitting model (Collins *et al.*, 2012; Srivathsan & Meier, 2012; Barley & Thomson, 2016). When using a model to “correct” the genetic distances, i.e., to try to account for multiple substitutions at a single site, the genetic distances are generally greater than for uncorrected distances, and the differences are greater on longer distances (Fregin *et al.*, 2012). This will widen the barcoding-gap, as the longer interspecific distances will be increased more than the shorter intraspecific distances, which will increase the number of MOTU:s identified (Barley & Thomson, 2016). It has been suggested to either use uncorrected distances (Collins *et al.*, 2012; Srivathsan & Meier, 2012), or the best fitting model in each case (Fregin *et al.*, 2012; Barley & Thomson, 2016) when calculating pairwise genetic distances in barcoding studies. In this thesis uncorrected genetic distances were used in papers I, II, IV and V, and also corrected distances based on the model selected by model testing in papers I and II.

It should also be noted that COI alone is a poor option for phylogeny estimation and the resulting phylogeny often shows striking differences in the relationships between species compared to other gene trees and multi-locus phylogenies (e.g. Nylander *et al.*, 1999; Goto & Kimura, 2001; Martinsson *et al.*, 2011; Klinth *et* *al.*, 2016), therefore caution is needed when interpreting trees based on COI alone.

6. Phylogeny estimation

A phylogeny is a representation of evolutionary relationships between organisms, usually depicted as a tree. Phylogenies can be estimated based on various data sources, e.g., morphology, ecology and genetic information, as long as the traits considered are heritable. There are three main methods for phylogeny estimations: parsimony, maximum likelihood and Bayesian inference. A parsimony analysis tries to find the tree that requires the least numbers of character changes, i.e., it tries to find the shortest tree. One of the main assumptions of parsimony analyses is that character changes are unlikely and rare, and therefore that the shortest tree is a good approximation of the true relationships between the studies organisms (e.g. Quicke, 1993). However, under conditions where changes are not uncommon, parsimony performs less well, and it is sensitive to a phenomena called 'long branch attraction' (see review by Bergsten, 2005), where long branches, i.e., branches on which many character changes are being erroneously inferred to be closely related as some of the changes by chance, will result in the same character states. Under maximum likelihood the goal is to find the tree that makes the data the most probable given the model. In the case of gene data, the model is that of sequence evolution, i.e., it models the frequencies of the bases, and the rate for the various substitutions. As an example, the simplest model, the Jukes-Cantor model, assumes equal base frequencies and equal substitution rates for all substitutions (Jukes & Cantor, 1969). Bayesian inference of phylogeny is closely allied to maximum likelihood methods, but the optimal hypothesis is the one that maximises the posterior probability, which is the maximum likelihood times the prior probability of the hypothesis (Holder & Lewis, 2003). The prior probability is given as a distribution, and reflects the researcher's belief, or prior knowledge about that parameter. Priors can be more or less informative; the estimation of parameters with weaker priors will be more influenced by the data than parameters with stronger priors (Holder & Lewis, 2003).

There are several methods for combining datasets for phylogeny estimation, traditionally the most common has been to concatenate the datasets, i.e., to paste the sequences from each gene, one after each other to create one larger dataset. This works if the datasets are largely overlapping in taxon sampling. However, one major problem with this approach is that it assumes that all genes included share the same history, something that in most cases is not true. On the contrary, gene trees may often differ significantly. One way of overcoming this problem is to use the multispecies coalescent model (Fig. 2) when estimating a species tree. This model is an extension of the coalescent theory, which describes the expected distribution of times for the merging or coalescence of lineages when finding their common ancestor, moving backwards in time (Kuhner, 2009). The multispecies coalescent model is an extension of this theory, developed for analysing several genes simultaneously when estimating the phylogeny of a group of species (Rannala & Yang, 2003). It is based on the fact that the divergence times between species have to be more recent than the coalescent times for any genes shared between them, assuming no genetic transfer after speciation, and it is more able to handle incongruence between the gene trees (Degnan & Rosenberg, 2009).

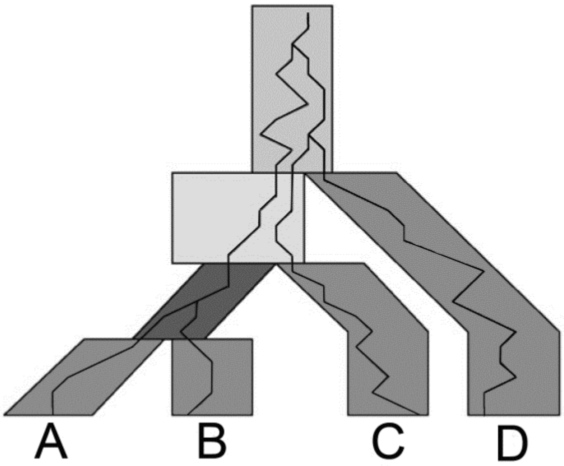


Figure 2. The Multispecies Coalescent model. The boxes are species, and the width represents the population size and the height the time, going from past (top) to present (bottom). The coalescence of gene lineages (the black line running within the species tree) of different species must be more ancient than the speciation event separating these species. Modified from Degnan and Rosenberg (2009).

All phylogenies estimated in this thesis are based on gene sequence data. Single gene trees have all been estimated using a Bayesian approach, either with traditional Bayesian inference (papers II and V) or coalescent genealogy sampling (papers I and IV). Multi-loci, multi-species trees have been estimated with the multispecies coalescent (papers II and V), and in one case using Maximum Likelihood on a concatenated dataset (paper I).

7. Study organisms

> *"Worms have played a more important part in the history of the world than most persons would at first suppose"*
> (Darwin, 1881 p. 305)

In this thesis, terrestrial and semi-terrestrial worms of Clitellata, a class within the phylum Annelida, were used as models. Annelida includes the mainly marine polychaetes as well as earthworms and other oligochaetes and leeches, the two latter comprise Clitellata.

Clitellates (Fig. 3) are bilaterally symmetrical segmented worms with a spacious coelom. Unlike the polychaetes they lack parapodia, and their prostomium, a (normally short) body compartment anterior to the mouth segment, lacks appendages. The chaetae are relatively few (lacking in leeches and a few oligochaetes), and in most cases situated in four bundles per segment, starting from segment II. They are exclusively functional hermaphrodites (except for a few asexual species), i.e., they have both male and female reproductive organs mature at the same time. The gonads are restricted to a few segments, with the male gonads anterior to the female ones. With few exceptions they possess spermathecae, which receive sperm at copulation. Further, sexually mature worms possess a clitellum, which secretes the substance forming the cocoon that the eggs are laid in, as well as nutrients to the embryo (see, e.g., Borradaile & Potts, 1958 for more ditails). The shape, position and number of gonads are of fundamental importance for the classification. The morphology of the male duct varies both between and within families, and the position and shape of the spermathecae are also important for the classification of clitellates (Brinkhurst & Jamieson, 1971).

In this thesis I have studied terrestrial and semi-terrestrial representatives from the families Naididae, Enchytraeidae and Lumbricidae.

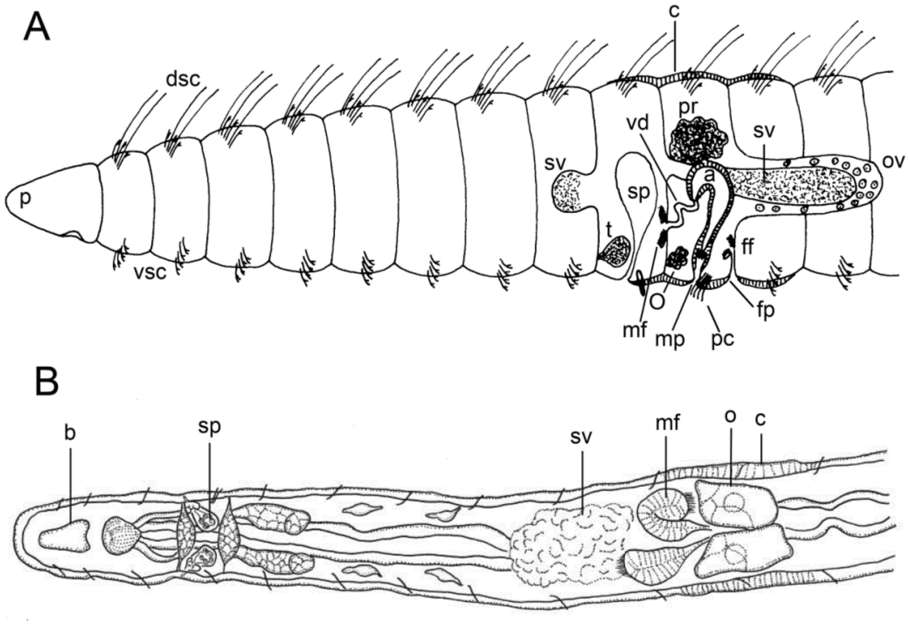


Figure 3. Clitellate morphology. **A.** anterior part of a Naididae (subfamily Tubificinae) lateral view, showing among other things the genital region. Modified from Caramelo and Martínez-Ansemil (2012). **B.** Anterior part of a *Marionina* (Enchytraeidae), dorsal view. Modified from Torii (2012). The male funnel, vas deferens, atria and maple pore are part of the male duct. Abbreviations: a = atrium, b = brain, c = clitellum, dsc = dorsal somatic chaeta, ff = female funnel, fp = female pore, mf = male funnel, mp = male pore, o = ovary, ov = ovisac, p = prostomium, pr = prostate, sp = spermathecae, sv = seminal vesicle, t = testis, vd = vas deferens, vsc = ventral somatic chaeta.

7.1. Phylogeny and classification of Clitellata, with focus on studied taxa

The phylum Annelida (segmented worms) has traditionally been divided into three classes, the Polychaeta (bristle worms), Oligochaeta, and Hirudinea (including Acanthobdellida, Branchiobdellida (crayfish worms), and Euhirudinea/Hirudinida (leeches)) (e.g. Sawyer, 1986; Hickman *et al.*, 2003). However, it has been shown that both Polychaeta and Oligochaeta are paraphyletic, i.e., both Oligochaeta, and Hirudinea are nested within Polychaeta, and Hirudinea is nested within Oligochaeta (e.g. Siddall *et al.*, 2001; Erséus & Källersjö, 2004; Rousset *et al.*, 2007; Struck *et al.*, 2007; Struck *et al.*, 2011; Parry *et al.*, 2016). The lineage including Oligochaeta and Hirudinea is often referred to as Clitellata, as referred to in this thesis. Phylogenetic studies have also shown that the former phyla Pogonophora, Echiura and Sipuncula, as well as the two enigmatic genera *Diurodrilus* and *Lobatocerebrum* are all part of Annelida (e.g. McHugh, 1997; Struck *et al.*, 2007; Laumer *et al.*, 2015; Struck *et al.*, 2015).

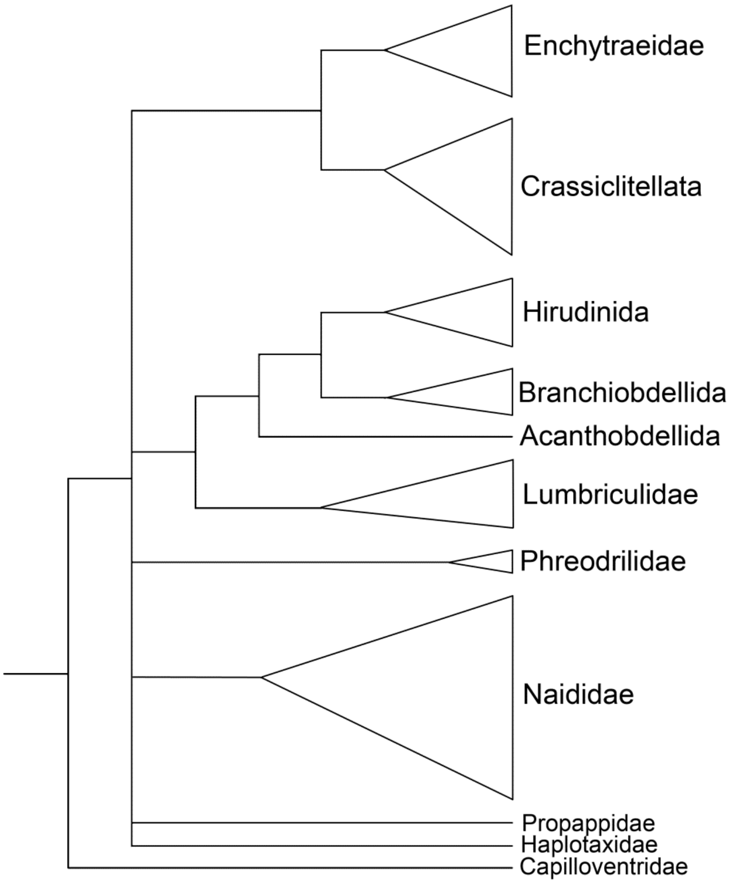


Figure 4. Phylogeny of Clitellata, modified from Erséus and Källersjö (2004)

The phylogeny of Clitellata (Fig. 4) is still poorly resolved (Erséus & Källersjö, 2004), but the monophyly of both Clitellata and Hirudinea is well supported, based on morphological characters as well as molecular studies (Martin, 2001; Siddall *et al.*, 2001; Erséus & Källersjö, 2004; Marotta *et al.*, 2008; Struck *et al.*, 2011). Additionally, a close relationship between Hirudinea and the oligochaetous family Lumbriculidae is supported by several studies (Siddall *et al.*, 2001; Erséus & Källersjö, 2004; Kaygorodova & Sherbakov, 2006; Marotta *et al.*, 2008). There is also support for a monophyletic Crassiclitellata, a group characterised by a multi-layered clitellum, as well as for Metagynophora (Crassiclitellata + Moniligastridae) (=”Megadrili”) that includes the groups usually referred to as earthworms (Jamieson, 1988; Jamieson *et al.*, 2002; James & Davidson, 2012). The family Enchytraeidae may be sister to the earthworms

(Erséus & Källersjö, 2004), and if this holds true, these two groups would form a mainly terrestrial clade consisting of a large majority of the terrestrial annelid species. Hopefully, a large ongoing phylogenomic study will resolve at least parts of the clitellate tree (C. Erséus *et al.* in prep.)

Naididae has, in several studies, been found nested within each Tubificidae (e.g. Erséus, 1990; Erséus *et al.*, 2002; Sjölin *et al.*, 2005; Christensen & Theisen, 2009), and these two are therefore now considered as one family, Naididae, by many authors (see Erséus *et al.*, 2008) so also in this thesis. The former Tubificidae was divided into five subfamilies, Rhyacodrilinae, Phallodrilinae, Limnodriloidinae, Telmatodrilinae, and Tubificinae (Erséus, 1990), whereof Rhyacodrilinae and Phallodrilinae are non-monophyletic (Sjölin *et al.*, 2005). The former Naididae *s.str.* was divided into two subfamilies, Naidinae and Pristininae (Lastočkin, 1924; Bely & Wray, 2004), both have been found monophyletic (Bely & Wray, 2004), but do not seem to be sister-groups, i.e., Naididae s.str is not monophyletic (Erséus *et al.*, 2002; Sjölin *et al.*, 2005).

The relationships within the family Enchytraeidae are rather well resolved and most genera seem to be monophyletic (Erséus *et al.*, 2010; Martinsson *et al.*, in press), with only a few problematic non-monophyletic genera, especially the genus *Marionina,* which is in great need of revision (Rota *et al.*, 2008). The largely marine littoral genus *Lumbricillus* is also non-monophyletic (Erséus *et al.*, 2010; Klinth *et al.*, 2016; Martinsson *et al.*, in press). The taxa in focus in papers II and III are species in the genus now called *Chamaedrilus* (see 7.4) are found in a clade together with *Stercutus* and *Euenchytraeus* (Martinsson *et al.*, in press). *Chamaedrilus* together with *Euenchytraeus* was earlier regarded as the genus *Cognettia* (see 7.4)

Among the earthworms, the family Lumbricidae, the only earthworm family native to northern and central Europe and the family studied in paper IV and V, is the sister-group to the Mediterranean family Hormogastridae (Novo *et al.*, 2011; James & Davidson, 2012; Novo *et al.*, 2016). The intrafamilial relationships of Lumbricidae are complex and many genera are non-monophyletic and in great need of revision (Dominguez *et al.*, 2015).

7.2. *Rhyacodrilus falciformis*

Rhyacodrilus falciformis Bretscher, 1901 (Clitellata: Naididae: Rhyacodrilinae) is the focus species in paper I. It is the type species in the genus *Rhyacodrilus* Bretscher 1901, a genus with about 40 described species (Achurra & Rodriguez, 2016), placed in the subfamily Rhyacodrilinae. This subfamily constitutes a diverse group of estuarine or freshwater naidids. The family Naididae includes over 800 described species (Sjölin *et al.*, 2005) with a worldwide distribution. It is mainly found in various marine and freshwater habitats. Only a few species are found also in wet soils and similar semi-terrestrial habitats, which confirms that the border between aquatic and terrestrials habitats is rather indistinct (Timm, 2012). *Rhyacodrilus falciformis* is one of the few species that are found in both freshwater bodies, as well as in wet soil, and it appears to be often associated with ground water (Timm *et al.*, 1996; Erséus *et al.*, 2005; Dumnicka, 2006; Achurra & Rodriguez, 2008). It may therefore be regarded as a stygophilic species (Giani *et al.*, 2011). The species is widespread in the Holarctic region, and it can be identified by the sickle-shaped penial chaetae, which have given the species its name (*falx* Latin for sickle or scythe). These chaetae are located in close proximity to the male pores in sexually mature specimens. A variety of *R. falciformis*, var. '*pigueti*', with straight penial chaetae, was described by Juget (1967) from Lake Leman on the border between France and Switzerland. However, if a variety of a species was published after 1960, it has no nomenclatorial status (ICZN 1999: §15.2). In paper I, we test if this variety constitutes a separate species, or if it is a part of *R. falciformis*.

7.3. *Cognettia*/*Chamaedrilus*

In papers II and III the taxa in focus constitute a species complex, the *C. sphagnetorum*-complex, which when I started my work was placed in the genus *Cognettia* Nielsen & Christensen, 1959. However, in paper III the species in this complex was transferred to *Chamaedrilus* Friend, 1913. These genera are placed in the family Enchytraeidae, which includes about 700 described species (Schmelz & Collado, 2015). Enchytraeids typically populate terrestrial soils and seashore sands, but they can be found in a broad range of other habitats, from deep sea sediments (Rota & Erséus, 2003), geothermal springs in lake profundals (Rota & Manconi, 2004), to glaciers (Moore, 1899; Roman Dial *et al.*, 2012), and phytotelms (water bodies held by plants) in cloud forests (Schmelz *et al.*, 2015b).

Enchytraeids have single-pointed chaetae, placed in four bundles per segment starting from II. The dorsolateral bundles are placed more laterally than in most other clitellate families. Chaetae are completely missing in some groups, e.g., the genus *Achaeta*. The male reproductive organs are normally placed in segments XI and XII, with the testis and sperm funnel in XI and the male pore and copulatory organ in XII. The female reproductive organs are partly found in segment XII, where they consist of ovaries and female funnel. The clitellum is also found in this segment, often extending to adjacent segments. The spermathecal pores are situated in the intersegmental furrow of 4/5 and the spermathecae are either confined to V or extend backwards several segments. In species that mainly reproduce by fragmentation, the sexual organs are usually shifted forward a few segments (diagnosis for the family taken from Brinkhurst & Jamieson, 1971; Schmelz & Collado, 2010).

The morphospecies *Cognettia sphagnetorum* (Vejdovský, 1878) is mainly found in the litter layer and is a strong indicator for acidity (Graefe & Schmelz, 1999). In acidic habitats, e.g., boreal coniferous forests, bogs and heathlands, it is often the dominating enchytraeid, and can be one of the dominating invertebrates in terms of biomass. It is therefore considered a keystone species in these habitats (Nurminen, 1967; Huhta & Koskenniemi, 1975; Coulson & Whittaker, 1978; Laakso & Setälä, 1999). Due to this, it is used as a model in soil biology, to study the effects of, and response to, climate change (e.g. Briones *et al.*, 1997; Haimi *et al.*, 2005; Maraldo *et al.*, 2008; Bataillon *et al.*, 2016), soil pollutants (Salminen & Haimi, 2001; Haimi *et al.*, 2006), forestry (Lundkvist, 1983) and soil processes, such as nutrient mineralization and availability (e.g. Standen, 1978; Abrahamsen, 1990; Mira *et al.*, 2002; Maraldo *et al.*, 2011). Further, it has been shown to play a key role in the decomposition of organic matter and in nutrient cycling (Standen, 1978; Laakso & Setälä, 1999). However, in paper II, *C. sphagnetorum* was found to consist of at least four species in N. Europe, and these are described in paper III. This finding makes it hard to use and compare the old studies on this taxon, as it is difficult to identify which of the cryptic species were studied in which paper.

The taxonomic history of the group of species that used to be referred to as *Cognettia* is complex. The history is given in detail in paper III, but is summarised here. The genus was established by Nielsen and Christensen (1959). The type species of *Cognettia*, *Pachydrilus sphagnetorum* Vejdovský, 1878, was originally described from a *Sphagnum* bog in SW Poland. Friend (1913) described a new species and genus, *Chamaedrilus chlorophilus* Friend, 1913, from England, which he regarded as close to two species later placed in *Cognettia*, viz., *Marionina sphagnetorum* and *M. glandulosa.* Later Friend (1919) transferred these two species to *Chamaedrilus*, this paper, however, seems to have been overlooked by most later authors. Both Delphy (1921) and Černosvitov (1937b) regarded *Ch. chlorophilus* as synonymous to *Pachydrilus sphagnetorum*. When establishing *Cognettia,* Nielsen and Christensen (1959) did not mention *Chamaedrilus chlorophilus*, but when they redescribed the type species of *Cognettia*, *C. sphagnetorum*, Nielsen and Christensen (1959) mentioned the frequent occurrence of a morphological variant that is identical with *Ch. chlorophilus*, thus, Nielsen and Christensen (1959) treated *C. sphagnetorum* and *Ch. chlorophilus* as one and the same species (Rota *et al.*, 2015). Under the assumption that Friend's *Chamaedrilus chlorophilus* is closely related to *C. sphagnetorum*, Schmelz and Collado (2010) correctly pointed out that *Chamaedrilus* is a senior synonym to *Cognettia*. However, Schmelz and Collado (2010) also suggested that an even older genus name, *Euenchytraeus* Bretscher, 1906 had been established for a species likely to belong in the *Cognettia* assemblage. *Euenchytraeus* was erected for a Swiss alpine species, *Eu. bisetosus* Bretscher, 1906, with nephridia at septum 2/3, which is an unusual character for enchytraeids. *Euenchytraeus* was later regarded as a part of *Marionina* by (Černosvitov, 1937a), and the genus and species seem to have fallen by the wayside until Schmelz and Collado (2010) synonymised *E. bisetosus* with *Cognettia clarae* Bauer, 1993, a species also with nephridia at septum 2/3. A third species bearing head nephridia, the Siberian *C. piperi* Christensen and Dózsa-Farkas, 1999, has also been described.

In paper III of this thesis we revise the generic taxonomy of the species previoulsy placed in *Cognettia*, and formally synonomise the genus with *Chamaedrilus*, and also transfer *C. piperi* and *C. clarae* to *Euenchytraus*. After our revision of *Cognettia*, and synonomisation of it with *Chamaedrilus*, a case have been submitted to the International Commision for Zoological Nomenclature (the Commision) (Schmelz *et al.*, 2015a) asking the commision to give *Cognettia* preceedence over *Chaemedrilus* and/or *Euenchytraeus* when they are considered synonyms, we have commented on the case argueing against this proposal (Rota *et al.*, 2015). The main argument for giving preceedence to *Cognettia* is a well-known name and that it is a widely used in the fields of soil biology, ecology, and ecotoxicology (Schmelz *et al.*, 2015a). However, the great majority of research has been on two species, *C. sphagnetorum* and *C. glandulosa*, both which has been shown to be complexes of cryptic species, and one cannot tell which single taxonomic unit was the object of each ecological study, or where and when a mixture of species was involved, therefore we argue against this propocal (Rota *et al.*, 2015).

7.4. Lumbricidae

In papers IV and V, the earthworm family Lumbricidae is in focus, in paper IV *Aporrectodea longa* is (Ude, 1885) the study organisms, and paper V treats five species in the genus *Lumbricus* L. 1758. Lumbricidae includes about 300 valid species (Dominguez *et al.*, 2015) placed in more than 40 genera (Csuzdi, 2012). A large majority of the species are terrestrial, but a few are amphibious. The classification of genera in the family is mainly based on the morphology of the calciferous glands and nephridia (Sims & Gerard, 1985), but, as noted in 7.2, many genera are non-monophyletic and in great need of revision.

The genus *Aporrectodea* Örley, 1885 includes about 50 valid species (Csuzdi, 2012), whereof one, *A. longa*, is the focus in paper IV. This species is widespread in northern and central Europe and introduced to North America and Australasia (Sims & Gerard, 1985). The genus *Aporrectodea* is highly polyphyletic and includes at least five clades scattered across the lumbricid tree (Dominguez *et al.*, 2015). The lineages that includes the type species, *A. trapezoides* (Dugès, 1828), also includes *A. longa*, (Dominguez *et al.*, 2015). In addition, according to Dominguez *et al.* (2015), *Lumbricus* L., 1758 is the sister to *Aporrectodea s.str.*, *Lumbricus* was found monophyletic, but only represented by two species, the genus was also found to be monophyletic in paper V where five described species were included, with a limited sample of out-groups. The genus *Lumbricus* consists of 14 valid taxa (13 species and one subspecies) (James *et al.*, 2010; Csuzdi, 2012). All species are native to Europe. Some (e.g., *L. terrestris* L., 1758 and *L. rubellus* Hoffmeister, 1843) are widespread and found in a large part of Europe, and have also been introduced to other continents (e.g. N. America and Australasia), whereas others (e.g. *L. badensis* Michaelsen, 1907 and *L. klarae* Zicsi & Csuzdi, 1999) have a very restricted distribution. Five species are found in Scandinavia, viz., *L. castaneus* (Savigny, 1826), *L. festivus* (Savigny, 1826), *L. herculeus* (Savigny, 1826), *L. rubellus* and *L. terrestris*. The alpine *L. meliboeus* Rosa, 1884 was also reported from N. Sweden (Piguet, 1919), but has not been found in Scandinavia since that questionable record (Julin, 1949; Stöp-Bowitz, 1969; Pižl, 1994; Milutinović *et al.*, 2013), and the species should not be included in the Swedish checklist. *Lumbricus herculeus* is a cryptic lineage recently recognised as separate from *L. terrestris* using DNA-barcoding and statistical differences in body size (James *et al.*, 2010), and many well separated mt-lineages are known for *L. rubellus* (e.g. Donnelly *et al.*, 2013; Sechi, 2013; Giska *et al.*, 2015).

Lumbricid earthworms often account for a large part of the invertebrate biomass in temperate soils (Edwards, 2004), and in some habitats, e.g., pastures and grasslands, they can be found in well over a metric ton per hectare (Paoletti, 1999). Several species are used as models in ecology, toxicology and physiology (Halanych & Borda, 2009; Römbke & Egeler, 2009; Fründ *et al.*, 2010). Earthworms can be classified into three functional groups: litter layer dwellers (epigeics), that live in and feeds on litter, mainly leaf litter; mineral soil dwellers (endogeics), that live in and feed on soil; and vertical burrowers (anecics) that lives in borrows in the soil, but mainly feeds on soil and litter collected at night from the surface (Bouché, 1977), but further subdivisioning of these categories is possible (Lavelle, 1981). These functional groups also differ in their reproduction strategies, epigeic species generally produce many cocoons (up to more than 100 cocoon/year), endogeic species produce an intermediate number of cocoon, whereas anecic species produce few cocoons (<10/year) (Paoletti, 1999). Of the species studied in this thesis, *Aporrectodea longa, Lumbricus terrestris* and *L. herculeus* are anecic, whereas *L. rubellus*, *L. castaneus* and *L. festivus* are epigeic.

8. Main results

Of the five papers included in the thesis four had the direct aim to test species boundaries (papers I, II, IV and V), whereas in paper III, the species delimited in paper II were formally described. Three papers (I, II and V) found support for taxonomical splitting among nominal species, whereas one found support for not splitting a species (paper IV). In the thesis three new species are described and two more redescribed. In all four species delimitation papers, deep intraspecies divergence was found in the barcoding gene COI, with 4.6-14.1 % uncorrected p-distances, which shows the limitation of analysing COI alone for species delimitation. Such a single gene approach would have resulted in oversplitting of the species in every paper.

Using an integrative approach, combining three genetic markers and morphology I found that despite the existence of seven barcoding clusters in the naidid *Rhyacodrilus falciformis* it only consists of two species. The clusters were tested using gene-trees combined with statistical tests of the distinctness, and evidence from morphology. One of the two species delimited is *R. falciformis s.str*. and the other is the previously recognised variety '*pigueti'*, which is described as a new species *R. pigueti* Achurra & Martinsson, 2013 (paper I)

By comparing the gene-trees of four genetic markers, as well as the results of clustering methods, I have shown that the number of species in the enchytraeid genus *Cognettia* in N Europe is twice that previously believed (from four to eight species) (paper II). Further, the genus has been revised and split into its two senior synonyms, *Euenchytraeus* and *Chamaedrilus*, with all the N European species now placed in *Chamaedrilus*. Two species*, Chamaedrilus chalupskyi* Martinsson, Rota & Erséus, 2015 and *Ch. pseudosphagnetorum* Martinsson, Rota & Erséus, 2015 have been described as new to science and two more, *Ch. chlorophilus* and *Ch. sphagnetorum*, have been redescribed, including a lectotype designation for the former, and a neotype designation for the latter (paper III).

By combining distance methods, parsimony networks and gene-trees, as well as testing the statistical distinctness of two divergent mitochondrial lineages, and also applying a multilocus species delimitation analysis based on the multispecies coalescence model, I showed that the two mitochondrial lineages within the earthworm *Aporrectodea longa* belong to the same species (paper IV)

In the earthworm genus *Lumbricus*, I found support for the proposed split between *L. terrestris* and *L. herculeus*, and support for existence of at least seven cryptic species within the morphotaxon *L. rubellus*. This was achieved by analysing COI and a nuclear marker (H3) with a combination of parsimony networks and gene-trees as well as multilocus species delimitation analyses. I also found the first evidence for hybridisation between species of *Lumbricus* (paper V).

9. Conclusions

In this thesis, I have shown that the diversity of terrestrial clitellates in Europe is much higher than expected from morphological studies alone, and that the combination of mitochondrial and nuclear markers, supplemented with morphology, can be used to delimit clitellate species using various methods. I have also shown that that DNA-barcoding will overestimate the number of species if used alone, and that it can only be used reliably for specimen identification after species have been robustly delimited using additional data, and even then it is problematic as hybridisation may occur.

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11. References

Abrahamsen, G. (1990). Influence of *Cognettia sphagnetorum* (Oligochaeta, Enchytraeidae) on nitrogen mineralization in homogenized mor humus. *Biology and Fertility of Soils*, 9, 159-162.

Achurra, A., & Erséus, C. (2013). DNA barcoding and species delimitation: the *Stylodrilus heringianus* case (Annelida : Clitellata : Lumbriculidae). *Invertebrate Systematics*, 27, 118-128.

Achurra, A., & Rodriguez, P. (2008). Biodiversity of groundwater oligochaetes from a karst unit in northern Iberian Peninsula: ranking subterranean sites for conservation management. *Hydrobiologia*, 605, 159-171.

Achurra, A., & Rodriguez, P. (2016). Syntopy in subterranean fauna: Trophic specialisation in two new species of *Rhyacodrilus* Bretscher, 1901 (Annelida, Clitellata, Rhyacodrilinae). *Zoologischer Anzeiger*, 261, 1-11.

Aydin, Z., Marcussen, T., Ertekin, A. S., & Oxelman, B. (2014). Marginal likelihood estimate comparisons to obtain optimal species delimitations in *Silene* sect. Cryptoneurae (Caryophyllaceae). *PLoS ONE*, 9, e106990.

Barley, A. J., & Thomson, R. C. (2016). Assessing the performance of DNA barcoding using posterior predictive simulations. *Molecular Ecology*, 25, 1944-1957.

Bataillon, T., Galtier, N., Bernard, A., Cryer, N., Faivre, N., Santoni, S., Severac, D., Mikkelsen, T. N., Larsen, K. S., Beier, C., Sorensen, J. G., Holmstrup, M., & Ehlers, B. K. (2016). A replicated climate change field experiment reveals rapid evolutionary response in an ecologically important soil invertebrate. *Global Change Biology*, 22, 2370-2379.

Bauer, R. (1993). *Cognettia clarae* n. sp. - eine neue Enchytraeiden-Art aus einem österreichischen Fichtenwald (Oligochaeta; Enchytraeidae). *Linzer Biologische Beiträge*, 25, 685-689.

Beauchamp, K. A., Gay, M., Kelley, G. O., El-Matbouli, M., Kathman, R. D., Nehring, R. B., & Hedrick, R. P. (2002). Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*. *Diseases of Aquatic Organisms*, 51, 113-121.

Bely, A. E., & Wray, G. A. (2004). Molecular phylogeny of naidid worms (Annelida: Clitellata) based on cytochrome oxidase I. *Molecular Phylogenetics and Evolution*, 30, 50-63.

Bergsten, J. (2005). A review of long-branch attraction. *Cladistics*, 21, 163-193.

Bergsten, J., Bilton, D. T., Fujisawa, T., Elliott, M., Monaghan, M. T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G. N., Ribera, I., Nilsson, A. N., Barraclough, T. G., & Vogler, A. P. (2012). The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, 61, 851-869.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, 22, 148-155.

Birky, C. W., Jr., Fuerst, P., & Maruyama, T. (1989). Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics*, 121, 613-627.

Blackwelder, R. E. (1967). *Taxonomy: a text and reference book*. New York: Wiley.

Borradaile, L. A., & Potts, F. A. (1958). *The Invertebrata: A manual for the use of students*. London: Cambridge University press.

Bouché, M. B. (1977). Strategies lombriciennes. *Ecological Bulletins*, 25, 122-132.

Bretscher, K. (1906). Über ein neues Enchytraeiden Genus. *Zoologischer Anzeiger*, 29, 672-674.

Brinkhurst, R. O., & Jamieson, B. G. M. (1971). *Aquatic Oligochaeta of the world*. Edinburgh: Oliver and Boyd.

Briones, M. J. I., Ineson, P., & Piearce, T. G. (1997). Effects of climate change on soil fauna; Responses of enchytraeids, Diptera larvae and tardigrades in a transplant experiment. *Applied Soil Ecology*, 6, 117-134.

Brown, W. M., George, M., Jr., & Wilson, A. C. (1979). Rapid evolution of animal mitochondrial DNA. *PNAS*, 76, 1967-1971.

Brown, W. M., Prager, E. M., Wang, A., & Wilson, A. C. (1982). Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution*, 18, 225-239.

Buchholz, R. (1863). Beiträge zur Anatomie der Gattung *Enchytraeus*, nebst Angabe der um Königsberg vorkommenden Formen derselben. . *Schriften der königlichen physikalisch-ökonomischen Gesellschaft zu Königsberg* 3, 93-132.

Caley, M. J., Fisher, R., & Mengersen, K. (2014). Global species richness estimates have not converged. *Trends in Ecology & Evolution*, 29, 187-188.

Caramelo, C., & Martínez-Ansemil, E. 2012. Microscopic anatomy of aquatic oligochaetes (Annelida, Clitellata): a zoological perspective. In A. Méndez-Vilas (Ed) *Current microscopy contributions to advances in science and technology* pp. 21-27. Badajoz: Formatex.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, 22, 4369-4383.

Černosvitov, L. (1937a). System der Enchytraeiden. *Bulletin de l'Association Russe pour les recherches scientifiques à Prague (section des sciences naturelles et matématiques)*, 5, 263-295.

Černosvitov, L. (1937b). Zur Kenntnis der Enchytraeiden. III. Revision der Friendschen Enchytraeiden-Typen. *Zoologische Anzeiger*, 117, 191-205.

Christensen, B., & Dózsa-Farkas, K. (1999). The enchytraeid fauna of the Siberian tundra (Oligochaeta, Enchytraeidae). *The Royal Danish Academy of Sciences and Letters, Biologiske Skrifter*, 52, 1-37.

Christensen, B., & Theisen, B. F. (2009). Phylogenetic status of the family Naididae (Oligochaeta, Annelida) as inferred from DNA analyses. *Journal of Zoological Systematics and Evolutionary Research*, 36, 169-172.

Cohan, F. M. (2002). What are bacterial species? *Annual Review of Microbiology*, 56, 457-487.

Collado, R., Hass-Cordes, E., & Schmelz, R. M. (2012). Microtaxonomy of fragmenting *Enchytraeus* species using molecular markers, with a comment on species complexes in enchytraeids. *Turkish Journal of Zoology*, 36, 85-94.

Collins, R. A., Boykin, L. M., Cruickshank, R. H., & Armstrong, K. F. (2012). Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. *Methods in Ecology and Evolution*, 3, 457-465.

Costello, M. J., May, R. M., & Stork, N. E. (2013). Can we name Earth's species before they go extinct? *Science*, 339, 413-416.

Cothran, R. D., Henderson, K. A., Schmidenberg, D., & Relyea, R. A. (2013). Phenotypically similar but ecologically distinct: differences in competitive ability and predation risk among amphipods. *Oikos*, 122, 1429-1440.

Coulson, J. C., & Whittaker, J. B. 1978. Ecology of moorland animals. In O. W. Heal & D. F. Perkins (Eds) *Production ecology of British moors and montane grasslands* pp. 52-93. Berlin: Springer-Verlag.

Csuzdi, C. (2012). Earthworm species, a searchable database. *Opuscula Zoologica (Budapest)*, 43, 97-99.

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

Dasmahapatra, K. K., Elias, M., Hill, R. I., Hoffman, J. I., & Mallet, J. (2010). Mitochondrial DNA barcoding detects some species that are real, and some that are not. *Molecular Ecology Resources*, 10, 264-273.

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, 85, 407-415.

de Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, 56, 879-886.

Decaëns, T., Jiménez, J. J., Gioia, C., Measey, G. J., & Lavelle, P. (2006). The values of soil animals for conservation biology. *European Journal of Soil Biology*, 42, S23-S38.

Degnan, J. H., & Rosenberg, N. A. (2009). Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution*, 24, 332-340.

Delphy, J. (1921). *Études sur l'organisation et le développement des Lombriciens limicoles thalassophiles*. Valognes: Pillu-Roland.

Dominguez, J., Aira, M., Breinholt, J. W., Stojanovic, M., James, S. W., & Perez-Losada, M. (2015). Underground evolution: new roots for the old tree of lumbricid earthworms. *Molecular Phylogenetics and Evolution*, 83, 7-19.

Donnelly, R. K., Harper, G. L., Morgan, A. J., Orozco-Terwengel, P., Pinto-Juma, G. A., & Bruford, M. W. (2013). Nuclear DNA recapitulates the cryptic mitochondrial lineages of *Lumbricus rubellus* and suggests the existence of cryptic species in an ecotoxological soil sentinel. *Biological Journal of the Linnean Society*, 110, 780-795.

Dózsa-Farkas, K., Porco, D., & Boros, G. (2012). Are *Bryodrilus parvus* Nurminen, 1970 and *Bryodrilus librus* (Nielsen and Christensen, 1959) (Annelida: Enchytraeidae) really different species? A revision based on DNA barcodes and morphological data. *Zootaxa*, 3276, 38-50.

Dugès, A. (1828). Recherche sur la circulation, la respiration, et la reproduction des Annélides sétigéres abranches. *Annales des Sciences Naturelles Paris*, 15, 284-336.

Dumnicka, E. (2006). Composition and abundance of oligochaetes (Annelida : Oligochaeta) in springs of Krakow-Czestochowa upland (Southern poland): Effect of spring encasing and environmental factors. *Polish Journal of Ecology*, 54, 231-242.

Dupuis, J. R., Roe, A. D., & Sperling, F. A. (2012). Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. *Molecular Ecology*, 21, 4422-4436.

Edwards, C. A. (2004). *Earthworm ecology*. Boca Ratón: CRC Press.

Ekrem, T., Stur, E., & Hebert, P. D. N. (2010). Females do count: documenting Chironomidae (Diptera) species diversity using DNA barcoding. *Organisms Diversity & Evolution*, 10, 397-408.

Ekrem, T., Willassen, E., & Stur, E. (2007). A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution*, 43, 530-542.

Envall, I., Gustavsson, L. M., & Erseus, C. (2012). Genetic and chaetal variation in *Nais* worms (Annelida, Clitellata, Naididae). *Zoological Journal of the Linnean Society*, 165, 495-520.

Erséus, C. (1990). Cladistic analysis of the subfamilies within the Tubificidae (Oligochaeta). *Zoologica Scripta*, 19, 57-63.

Erséus, C., & Gustafsson, D. 2009. Cryptic speciation in clitellate model organisms. In D. H. Shain (Ed) *Annelids in modern biology* pp. 31-46. Hoboken, New Jersey: John Wiley & Sons, Inc.

Erséus, C., & Källersjö, M. (2004). 18S rDNA phylogeny of Clitellata (Annelida). *Zoologica Scripta*, 33, 187-196.

Erséus, C., Källersjö, M., Ekman, M., & Hovmöller, R. (2002). 18S rDNA phylogeny of the Tubificidae (Clitellata) and its constituent taxa: dismissal of the Naididae. *Molecular Phylogenetics and Evolution*, 22, 414-422.

Erséus, C., Rota, E., Matamoros, L., & De Wit, P. (2010). Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution*, 57, 849-858.

Erséus, C., Rota, E., Timm, T., Grimm, R., Healy, B., & Lundberg, S. (2005). Riverine and riparian clitellates of three drainages in southern Sweden. *Annales de Limnologie - International Journal of Limnology*, 41, 183-194.

Erséus, C., Wetzel, M. J., & Gustavsson, L. (2008). ICZN rules—a farewell to Tubificidae (Annelida, Clitellata). *Zootaxa*, 1744, 66-68.

Feckler, A., Thielsch, A., Schwenk, K., Schulz, R., & Bundschuh, M. (2012). Differences in the sensitivity among cryptic lineages of the *Gammarus fossarum* complex. *Science of the Total Environment*, 439, 158-164.

Feckler, A., Zubrod, J. P., Thielsch, A., Schwenk, K., Schulz, R., Bundschuh, M., & Frid, C. (2014). Cryptic species diversity: an overlooked factor in environmental management? *Journal of Applied Ecology*, 51, 958-967.

Fregin, S., Haase, M., Olsson, U., & Alstrom, P. (2012). Pitfalls in comparisons of genetic distances: a case study of the avian family Acrocephalidae. *Molecular Phylogenetics and Evolution*, 62, 319-328.

Friend, H. (1913). British enchytraeids. V. Species new to science. *Journal of the Royal Microscopical Society*, 1913, 255-271.

Friend, H. (1919). A new British enchytræid worm. *Nature*, 104, 174.

Fründ, H.-C., Butt, K., Capowiez, Y., Eisenhauer, N., Emmerling, C., Ernst, G., Potthoff, M., Schädler, M., & Schrader, S. (2010). Using earthworms as model organisms in the laboratory: Recommendations for experimental implementations. *Pedobiologia*, 53, 119-125.

Fujita, M. K., Leache, A. D., Burbrink, F. T., McGuire, J. A., & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution*, 27, 480-488.

Giani, N., Sambugar, B., Martínez-Ansemil, E., Martin, P., & Schmelz, R. M. (2011). The groundwater oligochaetes (Annelida, Clitellata) of Slovenia. *Subterranean Biology*, 9, 85.

Giller, P. S. (1996). The diversity of soil communities, the 'poor man's tropical rainforest'. *Biodiversity and Conservation*, 5, 135-168.

Giska, I., Sechi, P., & Babik, W. (2015). Deeply divergent sympatric mitochondrial lineages of the earthworm *Lumbricus rubellus* are not reproductively isolated. *BMC Evolutionary Biology*, 15, 217.

Goto, S. G., & Kimura, M. T. (2001). Phylogenetic utility of mitochondrial COI and nuclear Gpdh genes in Drosophila. *Molecular Phylogenetics and Evolution*, 18, 404-422.

Graefe, U., & Schmelz, R. M. (1999). Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. *Newsletter on Enchytraeidae*, 6, 59-67.

Grandjean, F. (1954). Essai de classification des Oribates (Acariens). *Bulletin de la Societe Zoologique de France*, 78.

Gustafsson, D. R., Price, D. A., & Erséus, C. (2009). Genetic variation in the popular lab worm *Lumbriculus variegatus* (Annelida: Clitellata: Lumbriculidae) reveals cryptic speciation. *Molecular Phylogenetics and Evolution*, 51, 182-189.

Haimi, J., Knott, K. E., Selonen, S., & Laurikainen, M. (2006). Has long-term metal exposure induced changes in life history traits and genetic diversity of the enchytraeid worm *Cognettia sphagnetorum* (Vejd.)? *Environmental Pollution*, 140, 463-470.

Haimi, J., Laamanen, J., Penttinen, R., Raty, M., Koponen, S., Kellomaki, S., & Niemela, P. (2005). Impacts of elevated CO2 and temperature on the soil fauna of boreal forests. *Applied Soil Ecology*, 30, 104-112.

Halanych, K. M., & Borda, E. 2009. Developing models for Lophotrochozoan and annelid Biology. In D. H. Shain (Ed) *Annelids in modern biology* pp. 1-12. Hoboken, New Jersey: John Wiley & Sons.

Hambäck, P. A., Weingartner, E., Ericson, L., Fors, L., Cassel-Lundhagen, A., Stenberg, J. A., & Bergsten, J. (2013). Bayesian species delimitation reveals generalist and specialist parasitic wasps on *Galerucella* beetles (Chrysomelidae): sorting by herbivore or plant host. *BMC Evolutionary Biology*, 13, 92.

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society Biological Sciences Series B*, 270, 313-321.

Heethoff, M., Etzold, K., & Scheu, S. (2004). Mitochondrial COII sequences indicate that the parthenogenetic earthworm *Octolasion tyrtaeum* (Savigny 1826) constitutes of two lineages differing in body size and genotype. *Pedobiologia*, 48, 9-13.

Hennig, W. (1950). *Grundzüge einer Theorie der phylogenetischen Systematik*. Berlin: Deutscher Zentralverlag.

Hickman, C., P., Roberts, L. S., & Larson, A. (2003). *Animal diversity*. New York: McGraw-Hill.

Hoffmeister, W. (1843). Beitrag zor Kenntnis deutscher Landanneliden. *Archiv für Naturgeschichte*, 91, 183-198.

Holder, M., & Lewis, P. O. (2003). Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics*, 4, 275-284.

Huhta, V., & Koskenniemi, A. (1975). Numbers, biomass and community respiration of soil invertebrates in spruce forests at two latitudes in Finland. *Annales Zoologici Fennici*, 12, 164-182.

ICZN (1999). *International code of zoological nomenclature*. London: The International Trust for Zoological Nomenclature.

James, S. W., & Davidson, S. K. (2012). Molecular phylogeny of earthworms (Annelida: Crassiclitellata) based on 28S, 18S and 16S gene sequences. *Invertebrate Systematics*, 26, 213.

James, S. W., Porco, D., Decaens, 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.

Jamieson, B. G. M. (1988). On the phylogeny and higher classification of the Oligochaeta. *Cladistics*, 4, 367-401.

Jamieson, B. G. M., Tillier, S., Tillier, A., Justine, J.-L., Ling, E., James, S., McDonald, K., & Hugall, A. F. (2002). Phylogeny of the Megascolecidae and Crassiclitellata (Annelida, Oligochaeta): combined versus partitioned analysis using nuclear (28S) and mitochondrial (12S, 16S) rDNA. *Zoosystema*, 24 707-734.

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.

Juget, J. (1967). Quelques données nouvelles sur les oligochètes du léman: composition et origine du peuplement. *Annales de Limnologie*, 3, 217-229.

Jukes, T. H., & Cantor, C. R. 1969. Evolution of protein molecules. In H. N. Munro (Ed) *Mammalian protein metabolism* pp. 21-132. New York: Academic Press.

Julin, E. (1949). De svenska daggmaskarterna. *Arkiv för Zoologi*, 42, 1-58.

Kaygorodova, I. A., & Sherbakov, D. Y. (2006). Molecular phylogenetic study of the systematic position of Baikalian oligochaetes in Clitellata. *Russian Journal of Genetics*, 42, 1390-1397.

Kille, P., Andre, J., Anderson, C., Ang, H. N., Bruford, M. W., Bundy, J. G., Donnelly, R., Hodson, M. E., Juma, G., Lahive, E., Morgan, A. J., Stürzenbaum, S. R., & Spurgeon, D. J. (2013). DNA sequence variation and methylation in an arsenic tolerant earthworm population. *Soil Biology and Biochemistry*, 57, 524-532.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.

King, R. A., Tibble, A. L., & Symondson, W. O. (2008). Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology*, 17, 4684-4698.

Klinth, M. J., Martinsson, S., & Erséus, C. (2016). Phylogeny and species delimitation of North European *Lumbricillus* (Clitellata, Enchytraeidae). *Zoologica Scripta*, early online, 15 pp.

Kuhner, M. K. (2009). Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution*, 24, 86-93.

Kvist, S. (2013). Barcoding in the dark? A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. *Molecular Phylogenetics and Evolution*, 69, 39-45.

Kvist, S., Sarkar, I. N., & Erséus, C. (2010). Genetic variation and phylogeny of the cosmopolitan marine genus *Tubificoides* (Annelida: Clitellata: Naididae: Tubificinae). *Molecular Phylogenetics and Evolution*, 57, 687-702.

Laakso, J., & Setälä, H. (1999). Sensitivity of primary production to changes in the architecture of belowground food webs. *Oikos*, 87, 57-64.

Lastočkin, D. A. (1924). New and rare Copepoda and Oligochaeta from Central Russia. *Izvestija Rossijskogo gidrologičeskogo instituta*, 9, 1-22.

Laumer, C. E., Bekkouche, N., Kerbl, A., Goetz, F., Neves, R. C., Sorensen, M. V., Kristensen, R. M., Hejnol, A., Dunn, C. W., Giribet, G., & Worsaae, K. (2015). Spiralian phylogeny informs the evolution of microscopic lineages. *Current Biology*, 25, 2000-2006.

Lavelle, P. (1981). Stratégies de reproduction chez les vers de terre. *Acta Oecologica*, 2, 117-133.

Leache, A. D., & Fujita, M. K. (2010). Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings of the Royal Society Biological Sciences Series B*, 277, 3071-3077.

Lin, C.-P., & Danforth, B. N. (2004). How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. *Molecular Phylogenetics and Evolution*, 30, 686-702.

Linnaeus, C. (1758). *Systema Naturae (10th edition)*. Holmiae: Salvi.

Lundkvist, H. (1983). Effects of clear-cutting on the enchytraeids in a Scots Pine forest soil in Central Sweden. *Journal of Applied Ecology*, 20, 873-885.

Luo, A., Lan, H., Ling, C., Zhang, A., Shi, L., Ho, S. Y., & Zhu, C. (2015). A simulation study of sample size for DNA barcoding. *Ecol Evol*, 5, 5869-5879.

Maraldo, K., Christensen, B., & Holmstrup, M. (2011). The excretion of ammonium by enchytraeids (*Cognettia sphagnetorum*). *Soil Biology & Biochemistry*, 43, 991-996.

Maraldo, K., Schmidt, I. K., Beier, C., & Holmstrup, M. (2008). Can field populations of the enchytraeid, *Cognettia sphagnetorum*, adapt to increased drought stress? *Soil Biology and Biochemistry*, 40, 1765-1771.

Marotta, R., Ferraguti, M., Erséus, C., & Gustavsson, L. M. (2008). Combined-data phylogenetics and character evolution of Clitellata (Annelida) using 18S rDNA and morphology. *Zoological Journal of the Linnean Society*, 154, 1-26.

Martin, P. (2001). On the origin of the Hirudinea and the demise of the Oligochaeta. *Proceedings of the Royal Society Biological Sciences Series B*, 268, 1089-1098.

Martinsson, S., Achurra, A., Svensson, M., & Erséus, C. (2013). Integrative taxonomy of the freshwater worm *Rhyacodrilus falciformis* s.l. (Clitellata: Naididae), with the description of a new species. *Zoologica Scripta*, 42, 612–622.

Martinsson, S., Cui, Y., Martin, P. J., Pinder, A., Quinlan, K., Wetzel, M. J., & Erséus, C. (2015a). DNA-barcoding of invasive European earthworms (Clitellata: Lumbricidae) in south-western Australia. *Biological Invasions*, 17, 2527-2532.

Martinsson, S., Dózsa-Farkas, K., Rota, E., & Erséus, C. (in press). Placing the forgotten: On the positions of *Euenchytraeus* and *Chamaedrilus* in an updated enchytraeid phylogeny (Clitellata: Enchytraeidae). *Invertebrate Systematics*.

Martinsson, S., & Erséus, C. (2017). Cryptic speciation and limited hybridization within *Lumbricus* earthworms (Clitellata: Lumbricidae). *Molecular Phylogenetics and Evolution*, 106, 18-27.

Martinsson, S., Kjaerandsen, J., & Sundberg, P. (2011). Towards a molecular phylogeny of the fungus gnat genus *Boletina* (Diptera: Mycetophilidae). *Zoologica Scripta*, 40, 272-281.

Martinsson, S., Rhodén, C., & Erséus, C. (2015b). Barcoding gap, but no support for cryptic speciation in the earthworm *Aporrectodea longa* (Clitellata: Lumbricidae). *Mitochondrial DNA*, 1-9.

Martinsson, S., Rota, E., & Erséus, C. (2015c). On the identity of *Chamaedrilus glandulosus* (Michaelsen, 1888) (Clitellata, Enchytraeidae), with the description of a new species. *Zookeys*, 501, 1-14.

Matamoros, L., Rota, E., & Erséus, C. (2012). Cryptic diversity among the achaetous *Marionina* (Annelida, Clitellata, Enchytraeidae). *Systematics and Biodiversity*, 10, 509-525.

Mayr, E. (1942). *Systematics and the origin of species from the viewpoint of a zoologist*. New York: Columbia University Press.

Mayr, E. (1943). Criteria of subspecies, species and genera in ornithology. *Annals of the New York Academy of Sciences*, 44, 133-139.

McHugh, D. (1997). Molecular evidence that echiurans and pogonophorans are derived annelids. *PNAS*, 94, 8006-8009.

Michaelsen, W. (1907). Zur Kenntnis der deutschen Lumbriciden fauna. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten*, 24, 189-193.

Milutinović, T., Tsekova, R., Milanović, J., & Stojanović, M. (2013). Distribution, biogeographical significance and status of *Lumbricus meliboeus* Rosa, 1884 (Oligochaeta, Lumbricidae) at the European scale: first findings in Serbia and in Bulgaria. *North-Western Journal of Zoology*, 9, 63-69.

Mira, L., Katja, I., & Heikki, S. (2002). The significance of *Cognettia sphagnetorum* (Enchytreaidae) on nitrogen availability and plant growth in wood ash-treated humus soil. *Plant and Soil*, 246, 31-39.

Mishler, B. D., & Brandon, R. N. (1987). Individuality, pluralism, and the Phylogenetic Species Concept. *Biology and Philosophy*, 2, 397-414.

Moore, J. P. (1899). A snow-inhabiting enchytræid (*Mesenchytræus solifugus* Emery) collected by Mr. Henry G. Bryant on the Malaspina Glacier, Alaska. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 51, 125-144.

Neigel, J. E., & Avise, J. C. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of specialtion. In E. Nevo & S. Karlin (Eds) *Evolutionary processes and theory* pp. 515-534. New York: Academic Press.

Nelson, G., & Platnick, N. I. (1981). *Systematics and biogeography*. New York: Columbia University Press.

Nielsen, C. O., & Christensen, B. (1959). The Enchytraeidae. Critical revision and taxonomy of European species. *Natura Jutlandica*, 8-9, 1-160.

Novo, M., Almodovar, A., Fernandez, R., Giribet, G., & Diaz Cosin, D. J. (2011). Understanding the biogeography of a group of earthworms in the Mediterranean basin--the phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta). *Molecular Phylogenetics and Evolution*, 61, 125-135.

Novo, M., Almodovar, A., Fernandez, R., Trigo, D., Diaz-Cosin, D. J., & Giribet, G. (2012). Appearances can be deceptive: different diversification patterns within a group of Mediterranean earthworms (Oligochaeta, Hormogastridae). *Molecular Ecology*, 21, 3776-3793.

Novo, M., Fernández, R., Andrade, S. C. S., Marchán, D. F., Cunha, L., & Díaz Cosín, D. J. (2016). Phylogenomic analyses of a Mediterranean earthworm family (Annelida: Hormogastridae). *Molecular Phylogenetics and Evolution*, 94, 473-478.

Nurminen, M. (1967). Ecology of enchytraeids (Oligochaeta) in Finnish coniferous forest soil *Annales Zoologici Fennici*, 4, 147-157.

Nygren, A. (2014). Cryptic polychaete diversity: a review. *Zoologica Scripta*, 43, 172-183.

Nylander, J. A. A., Erseus, C., & Kallersjo, M. (1999). A test of monophyly of the gutless Phallodrilinae (Oligochaeta, Tubificidae) and the use of a 573-bp region of the mitochondrial cytochrome oxidase I gene in analysis of annelid phylogeny. *Zoologica Scripta*, 28, 305-313.

Örley, L. (1885). A palaearktikus övben élő Terrikoláknak revíziója és elterjedése. *Értekezések a Természettudományok Körébõl*, 15, 1-34.

Ørsted, A. (1844). *De regionibus marinis*. Copenhagen: Dissertatio Inauguralis.

Padial, J. M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.

Paoletti, M. G. (1999). The role of earthworms for assessment of sustainability and as bioindicators. *Agriculture, Ecosystems & Environment*, 74, 137-155.

Parry, L. A., Edgecombe, G. D., Eibye-Jacobsen, D., & Vinther, J. (2016). The impact of fossil data on annelid phylogeny inferred from discrete morphological characters. *Proceedings of the Royal Society Biological Sciences Series B*, 283.

Pfenninger, M., & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7, 121.

Piguet, E. (1919). Oligochètes communs aux Hautes Alpes suisses et scandinaves. *Revue Suisse de Zoologie*, 27, 1-17.

Pižl, V. (1994). Supplementary records of earthworms (Lumbricidae) in Czech Republic. *Acta Societatis Zoologicae Biohemicae*, 58, 205-211.

Porco, D., Decaëns, T., Deharveng, L., James, S., Skarżyński, D., Erséus, C., Butt, K., Richard, B., & Hebert, P. N. (2013). Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America. *Biological Invasions*, 15, 899-910.

Quicke, D. L. J. (1993). *Principles and techniques of contemporary taxonomy*. Glasgow: Blackie Academic & Professional.

Rannala, B. (2015). The art and science of species delimitation. *Current Zoology*, 61, 846-853.

Rannala, B., & Yang, Z. H. (2003). Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics*, 164, 1645-1656.

Richard, B., Decaens, T., Rougerie, R., James, S. W., Porco, D., & Hebert, P. D. (2010). Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. *Molecular Ecology Resources*, 10, 606-614.

Ridley, M. (1989). The cladistic solution to the species problem. *Biology & Philosophy*, 4, 1-16.

Rodrigo, A., Bertels, F., Heled, J., Noder, R., Shearman, H., & Tsai, P. (2008). The perils of plenty: what are we going to do with all these genes? *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 363, 3893-3902.

Roman Dial, C., Dial, R. J., Saunders, R., Lang, S. A., Lee, B., Wimberger, P., Dinapoli, M. S., Egiazarov, A. S., Gipple, S. L., Maghirang, M. R., Swartley-McArdle, D. J., Yudkovitz, S. R., & Shain, D. H. (2012). Historical biogeography of the North American glacier ice worm, *Mesenchytraeus solifugus* (Annelida: Oligochaeta: Enchytraeidae). *Molecular Phylogenetics and Evolution*, 63, 577-584.

Rosen, D. E. (1979). Fishes from the uplands and intermontane basins of Guatemala: Revisionary studies and comparative geography. *Bulletin of the American Museum of Natural History*, 162, 267-376.

Rosenberg, N. A. (2007). Statistical tests for taxonomic distinctiveness from observations of monophyly. *Evolution*, 61, 317-323.

Rota, E., & Erséus, C. (2003). New records of *Grania* (Clitellata, Enchytraeidae) in the Northeast Atlantic, from Tromsö to the Canary Islands, with descriptions of seven new species. *Sarsia*, 88, 210-243.

Rota, E., & Manconi, R. (2004). Taxonomy and ecology of sponge-associate *Marionina spp*. (Clitellata: Enchytraeidae) from the Horomatangi geothermal system of Lake Taupo, New Zealand. *International Review of Hydrobiology*, 89, 58-67.

Rota, E., Martinsson, S., Bartoli, M., Beylich, A., Graefe, U., Laini, A., Wetzel, M. J., & Erséus, C. (2016). Mitochondrial evidence supports a Nearctic origin for the spreading limicolous earthworm *Sparganophilus tamesis* Benham, 1892 (Clitellata, Sparganophilidae). *Contributions to Zoology*, 85, 113-119.

Rota, E., Martinsson, S., & Erséus, C. (2015). Comment on the proposed precedence of *Cognettia* Nielsen & Christensen, 1959 over *Euenchytraeus* Bretscher, 1906 and *Chamaedrilus* Friend, 1913 (Annelida, Oligochaeta, ENCHYTRAEIDAE) (Case 3689; see BZN 72: 186–192). *Bulletin of Zoological Nomenclature*, 72, 303-307.

Rota, E., Matamoros, L., & Erséus, C. (2008). In search of *Marionina* (Clitellata, Enchytraeidae): A taxonomic history of the genus and re-description of the type species *Pachydrilus georgianus* Michaelsen, 1888. *Italian Journal of Zoology*, 75, 417-436.

Roule, L. (1888). Sur la structure histologique d’un oligochaete marin appartenant a` un genre nouveau. *Comptes rendus hebdomadaires des Se´ances de l’Acade´mie des Sciences*, 116, 308-310.

Rousset, V., Pleijel, F., Rouse, G. W., Erséus, C., & Siddall, M. E. (2007). A molecular phylogeny of annelids. *Cladistics*, 23, 41-63.

Römbke, J., Aira, M., Backeljau, T., Breugelmans, K., Domínguez, J., Funke, E., Graf, N., Hajibabaei, M., Pérez-Losada, M., Porto, P. G., Schmelz, R. M., Vierna, J., Vizcaíno, A., & Pfenninger, M. (2016). DNA barcoding of earthworms (*Eisenia fetida/andrei* complex) from 28 ecotoxicological test laboratories. *Applied Soil Ecology*, 104, 3-11.

Römbke, J., & Egeler, P. 2009. Oligochaete worms for ecotoxicological assessment of soils and sediments. In D. H. Shain (Ed) *Annelids in modern biology* pp. 228-241. Hoboken: Wiley-Blackwell.

Salminen, J., & Haimi, J. (2001). Life history and spatial distribution of the enchytraeid worm *Cognettia sphagnetorum* (Oligochaeta) in metal-polluted soil: Below-ground sink-source population dynamics? *Environmental Toxicology and Chemistry*, 20, 1993-1999.

Sawyer, R. T. (1986). *Leech bology and behaviour*. Oxford: Clarendon Press.

Schaefer, M., & Schauermann, J. (1990). The soil fauna of beech forests - comparison between a mull and a moder soil. *Pedobiologia*, 34, 299-314.

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. H. (2010). Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology*, 55, 421-438.

Schmelz, R. M., & Collado, R. (2010). A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). *Soil Organisms*, 82, 1-176.

Schmelz, R. M., & Collado, R. (2015). Checklist of taxa of Enchytraeidae (Oligochaeta): an update. *Soil Organisms*, 87, 149-153.

Schmelz, R. M., Collado, R., & Römbke, J. (2015a). Case 3689: *Cognettia* Nielsen & Christensen, 1959 (Annelida, Oligochaeta, ENCHYTRAEIDAE): proposed precedence over *Euenchytraeus* Bretscher, 1906 and *Chamaedrilus* Friend, 1913. *Bulletin of Zoological Nomenclature*, 72, 186-192.

Schmelz, R. M., Jocque, M., & Collado, R. (2015b). Microdrile Oligochaeta in bromeliad pools of a Honduran cloud forest. *Zootaxa*, 3947, 508-526.

Sechi, P. 2013. An evolutionary history of the peregrine epigeic earthworm *Lumbricus rubellus Cardiff School of Biosciences* (p. 193). Unpublished: Cardiff University

Siddall, M. E., Apakupakul, K., Burreson, E. M., Coates, K. A., Erseus, C., Gelder, S. R., Kallersjo, M., & Trapido-Rosenthal, H. (2001). Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Molecular Phylogenetics and Evolution*, 21, 346-351.

Siddall, M. E., Trontelj, P., Utevsky, S. Y., Nkamany, M., & Macdonald, K. S. (2007). Diverse molecular data demonstrate that commercially available medicinal leeches are not *Hirudo medicinalis*. *Proceedings of the Royal Society Biological Sciences Series B*, 274, 1481-1487.

Sims, R. W., & Gerard, B. M. (1985). *Earthworms: Keys and notes for the identification and study of the species*. London: Brill.

Sites, J. W., & Marshall, J. C. (2003). Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology & Evolution*, 18, 462-470.

Sjölin, E., Erséus, C., & Källersjö, M. (2005). Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution*, 35, 431-441.

Srivathsan, A., & Meier, R. (2012). On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics*, 28, 190-194.

Standen, V. (1978). The influence of soil fauna on decomposition by micro-organisms in blanket bog Litter. *Journal of Animal Ecology*, 47, 25-38.

Struck, T. H., Golombek, A., Weigert, A., Franke, F. A., Westheide, W., Purschke, G., Bleidorn, C., & Halanych, K. M. (2015). The evolution of annelids reveals two adaptive routes to the interstitial realm. *Current Biology*, 25, 1993-1999.

Struck, T. H., Paul, C., Hill, N., Hartmann, S., Hosel, C., Kube, M., Lieb, B., Meyer, A., Tiedemann, R., Purschke, G., & Bleidorn, C. (2011). Phylogenomic analyses unravel annelid evolution. *Nature*, 471, 95-98.

Struck, T. H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., & Halanych, K. M. (2007). Annelid phylogeny and the status of Sipuncula and Echiura. *BMC Evolutionary Biology*, 7, 57.

Stur, E., & Ekrem, T. (2011). Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa*, 2743, 27-39.

Sturmbauer, C., Opadiya, G. B., Niederstätter, H., Riedmann, A., & Dallinger, R. (1999). Mitochondrial DNA reveals cryptic oligochaete species differing in cadmium resistance. *Molecular Biology and Evolution*, 16, 967-974.

Stöp-Bowitz, C. (1969). A contribution to our knowledge of the systematics and zoogeography of Norwegian earthworms (Annelida Oligochaeta: Lumbricidae). *Nytt Magasin for Zoologi*, 17, 169-280.

Taylor, H. R., & Harris, W. E. (2012). An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. *Molecular Ecology Resources*, 12, 377-388.

Timm, T. (2012). Life forms in Oligochaeta: a literature review. *Zoology in the Middle East*, 58, 71-82.

Timm, T., Arslan, N., Rüzgar, M., Martinsson, S., & Erséus, C. (2013). Oligochaeta (Annelida) of the profundal of Lake Hazar (Turkey), with description of *Potamothrix alatus hazaricus* n. ssp. *Zootaxa*, 3716, 144-156.

Timm, T., Erséus, C., & Lundberg, S. (1996). New and unusual records of freshwater Oligochaeta from the Scandinavian peninsula. *Nordic Journal of Freshwater Reasearch*, 72, 15-29.

Torii, T. (2012). New records of semiaquatic species *Marionina* (Clitellata, Enchytraeidae) from Japan, with a description of *Marionina biwaensis* sp. nov. *Turkish Journal of Zoology*, 36, 15-24.

Ude, H. (1885). Über die Rückenporen der terricolen Oligochaeten, nebst Beiträgen zur Histologie des Leibesschlauches und zur Systematik der Lumbriciden. *Zeitschrift für wissenschaftliche Zoologie*, 43, 87-143.

Usher, M. B., Davis, P., Harris, J., & Longstaff, B. 1979. A profusion of species? Approaches towards understanding the dynamics of the populations of microarthropods in decomposer communities. In R. M. Anderson, B. D. Turner & L. R. Taylor (Eds) *Population dynamics* pp. 359-384. Oxford: Blackwell Scientific Publications.

Van Valen, L. (1976). Ecological species, multispecies, and oaks. *Taxon*, 25, 233-239.

Waugh, J. (2007). DNA barcoding in animal species: progress, potential and pitfalls. *Bioessays*, 29, 188-197.

Vejdovský, F. (1878). Zur Anatomie und Systematik der Enchytraeiden. *Sitzungsberichte der Königlich Böhmischen Gesellschaft der Wissenschaften*, 1877, 294-304.

Wright, S. 1940. The statistical consequences of Mendelian heredity in relation to speciation. In J. Huxley (Ed) *The new systematics* pp. 161-183. London: Oxford University Press.

Yang, Z., & Rannala, B. (2014). Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution*, 31, 3125-3135.

Zhang, Y.-Z., Si, S.-l., Zheng, J.-T., Li, H.-L., Fang, Y., Zhu, C.-D., & Vogler, A. P. (2011). DNA barcoding of endoparasitoid wasps in the genus *Anicetus* reveals high levels of host specificity (Hymenoptera: Encyrtidae). *Biological Control*, 58, 182-191.

Zicsi, A., & Csuzdi, C. (1999). Further contribution to the earthworm fauna (Oligoschaeta: Lumbricidae) of France, with description of five new species and one subspecies. *Revue Suisse de Zoologie Volume Hors Serie*, 106, 983-1004.