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Review Paper

Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy?

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ABSTRACT

Traditionally, phylogenetic relationships within Lumbricidae have been investigated by using morphological characters which show high variability between individuals. Furthermore, the lack of agreement in their ranking as diagnostic characters for taxonomic and phylogenetic purposes has led to situations in which the same species receives different names in different parts of the world or may be included in different genera depending on the classification system proposed. Although the recent use of molecular tools in metazoan phylogeny has questioned our traditional understanding of animal classification, these techniques have not been yet fully exploited in earthworm evolutionary biology.

Here we construct molecular phylogenies to contrast them with the traditional morphological classification within the Lumbricidae. We have put special emphasis on clarifying the phylogenetic relationships of those species commonly found in the European Atlantic area, trying to highlight some of the nomenclatural problems associated with certain species and to provide a better understanding of this group for earthworm ecologists. We obtained DNA sequences for two fragments of the mitochondrial COI and 16S genes for thirty-six earthworm taxa belonging to the family Lumbricidae and seven species belonging to six outgroup families according to Sims and Gerard (1999)'s terminology (Criodrilidae, Glossoscolecidae, Megascolecidae, Acanthodrilidae, Eudrilidae and Hormogastridae). We interpreted the results in the light of the available information on genital, somatic and genetic characters published over the last 100 years and performed a detailed anatomical study of certain species for which there are contradictory descriptions.

In agreement with previous results, our study concludes that the 16S rDNA and COI sequence fragments have a limited discriminatory value above the genus level. In general, the relationships suggested by 16S were more in agreement with morphology than COI but the two genes proved to be useful at the shallowest taxonomic levels. Accordingly, both *Lumbricus* and *Octolasion* genera were shown to form distinctive clades despite the distinct geographical distribution of some of their members (e.g. *Lumbricus friendi versus Lumbricus terrestris*). In contrast, in the case of the heterogeneous *Dendrobaena, Aporrectodea* and the so-called "catch-all genus *Allolobophora*", the phylogenetic resolution was very limited. In addition, both markers seem to be suitable in revealing ecological similarities within species complexes. This was particularly true in the case of the *Octolasion* species and those included in the *Ap. caliginosa* complex, which suggest that occupying different niches in the soil may act as an isolating mechanism of their populations and could lead to speciation.

Importantly, our results provide good support for removing Levinsen's *L. eiseni* from the *Lumbricus* genus, although its taxonomic position is still problematic and further studies are required, using slower markers and by including a greater number of the forms of each genus in the analyses. Similarly, the anatomical, genital and genetic characters investigated here do not allow the full clarification of the taxonomical status of *Dendrobaena/Eisenia hortensis* and *D./E. veneta*; however, in order to maintain the nomenclature stability, we propose to retain them in *Dendrobaena* until further analyses are carried out on test type specimens (e.g. paratypes or topotypic material). Furthermore, more research is also needed to solve the great heterogeneity of the genus *Dendrobaena*.

Our review also highlights the need for more collaborative work between alpha taxonomists and molecular phylogeneticists to make any meaningful progress in earthworm classification, so that the

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material included in the phylogenetic trees is properly identified and the results interpreted adequately. Furthermore, in order to avoid further taxonomical ambiguities, soil ecologists are advised to provide the most accurate identification of the species used in their studies or at least a good description of the specimens used together with the geographical location and the soil characteristics where the material was collected. Preserving some material for further taxonomic and genetic reference is also advised for amending possible mistakes.

We finally conclude that anatomical or genetic information alone cannot define any lumbricid genus and that gathering morphological, biological, physiological, ecological and genetic evidence together provides the best tool for solving the current "unequal chaos" of lumbricid taxonomy and for full elucidation of phylogenetic relationships within the Lumbricidae.

1. Introduction

The phylogenetic relationships within the well studied family of earthworms (Lumbricidae) have been described as "a taxonomic chaos unequalled among the oligochaetes" (Ljungström, 1972; p. 12). Part of the problem is the fact that the established taxonomy is mainly based on a few key morphological characters, both external (pigmentation, setal distance, location of the clitellum and tubercula pubertatis, shape of the prostomium, position of the first dorsal pore and the male pores, arrangement of papillae, etc.) and internal (musculature, number and position of seminal vesicles and spermathecae, shape of the typhlosole and nephridial bladders, etc.), which often show high variability between individuals. Finally, the preservation method employed can also alter the appearance of many of these characters, aggravating the situation.

Furthermore, in certain occasions, the lack of agreement in their ranking as diagnostic characters for taxonomic and phylogenetic purposes has led to situations in which the same species receives different names in different parts of the world and may be included in different genera depending on the classification system proposed. For example, three different generic names are given to one of the most widely distributed earthworm species *Aporrectodea caliginosa*, i.e. *Aporrectodea* Örley, 1855, *Allolobophora* Eisen, 1873 and *Nicodrilus* Bouché, 1972. This "*Allolobophoran* heterogeneity" was recognised a long time ago (Michaelsen, 1900; Friend, 1910; Ljungström, 1972; Gates, 1980) and, in this particular case, this taxonomic chaos could have been prevented if Eisen (1873) had not failed to designate a type specimen. As a result, 83 years later Omodeo (1956) chose *Allolobophora chlorotica* as the type species of *Allolobophora*, which shows some anatomical differences from the rest of the species already included, and consequently, they were given a new generic name *Aporrectodea*.

This "classical" phylogenetic classification of Oligochaeta was established on the basis of the belief that the genital system is much more conservative and resistant to evolutionary change than the somatic system (Michaelsen, 1900; Stephenson, 1930). However, a series of contributions appearing after 1940 proposed that somatic characters, such as pigmentation, setal arrangement, type of prostomium, parts of the excretory system (nephridial vesicles and the nephropores and their locations), circulatory system, structure of the musculature and of the digestive system (the calciferous gland and its associated sacs, shape of the typhlosole and number of atyphlosolate segments in the intestine), helped to clarify heterogeneous genera (Pop, 1941; Omodeo, 1956; Sims, 1966; Gates, 1962, 1970, 1972, 1978a; Perel, 1976; Csuzdi, 1984).

Although the recent use of molecular tools in metazoan phylogeny has questioned our traditional understanding of animal classification (e.g. Dunn et al., 2008), these techniques have not yet been fully exploited in earthworm systematics. Most efforts have been placed in resolving the basal diversification of the phylum Annelida and thus, molecular data have confirmed the monophyly of the Clitellata (Moon et al., 1996; Kim et al., 1996; McHugh, 1997, 2000; Winnepenninckx et al., 1998; Kojima, 1998; Martin et al., 2000; Erséus et al., 2000; Martin, 2001; Siddall et al., 2001; Struck et al., 2002) as well as several of its constituent taxa, namely Hirudinida (leeches), Branchiobdellida and Acanthobdellida (Siddall et al., 2001) and the validity of Crassiclitellata (= oligochaetous clitellates with a multilayered clitellum), a taxon proposed by Jamieson in 1988 (Jamieson et al., 2002; Erséus and Källersjö, 2004) and which includes among others the earthworm family Lumbricidae.

Many families remain to be analysed at the molecular level (Jamieson and Ferraguti, 2006) and in the case of Lumbricidae only a few attempts have been made in order to clarify the taxonomical position of certain species. For example, Pop et al. (2003) analysed 18 species using 18S, 16S and COI sequences, suggesting that the 18S rDNA sequences have discriminatory value mostly at higher taxonomic levels because they are highly conserved, whereas the 16S and COI fragments seem to have complementary taxonomic value at the genus and species level. Furthermore, a later study (Pop et al., 2007) including 23 taxa confirmed that the COI gene gives highly variable information and sometimes in disagreement with that of the 16S, but still suitable for revealing phylogenetic relatedness in closely-related species or in species complexes.

The conclusions emerging from these studies partly confirmed the morphology-based taxonomical arrangements and for example, it is now widely accepted that *Criodrilus lacuum* is a primitive species falling outside the family Lumbricidae (Blakemore, 2007a), which shares the semi-aquatic habitats as certain lumbricid species (e.g. *Eiseniella tetraedra*), and therefore supports the hypothesis of an aquatic origin for the Clitellata (Erséus and Källersjö, 2004). Furthermore, mitochondrial and DNA sequences have also confirmed the validity of the genera *Octodrilus* and *Octolasion* since they support the same phylogenetic conclusions derived from morphological and anatomical characters (Csuzdi et al., 2005; Pop et al., 2008a). In contrast, the heterogeneity of the genus *Dendrobaena* remains unresolved (Cech, 2005). Interestingly, although there seems to be some support for *Aporrectodea* to be distinct from *Allolobophora*, its resolution proved to be very limited, whereas other genera previously believed to be homogeneous such as *Eisenia* and *Lumbricus* were less supported (Pop et al., 2007).

Attempting to establish the phylogenetic relationships among all members of the family Lumbricidae would have been a major task as it would have required a complete inventory. Unfortunately, many geographical areas have not been sampled and their earthworm fauna is totally unknown. For these reasons, in this study, we have put special emphasis on those species commonly found in the European Atlantic area, trying to highlight some of the nomenclatural problems associated with certain species and to provide a better understanding of this group for earthworm ecologists. The area not only has a long history of earthworm taxonomy, but also its earthworm communities include a high number of invasive species which have successfully extended their geographical boundaries as a result of historical, economic and social factors as well as climatic and land use changes. Because most earthworm ecology papers published in the literature