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Sorry *atlanticus*, you are not my type: molecular assessment splits *Zophoscolex* (Lumbricidae: Crassiclitellata) into French and Iberian genera

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Molecular phylogenetics contributes to making important advances in the challenging field of earthworm taxonomy. Use of this type of analysis has enabled clarification of the phylogenetic relationships between early-branching genera of Lumbricidae within the highly diverse Franco-Iberian realm. However, molecular phylogenetic studies of the genus *Zophoscolex* are scarce and have led to taxonomic uncertainty due to insufficient sampling and the absence of the type species, *Z. atlanticus*, from such studies. The present study investigated 11 species of *Zophoscolex* (including *Z. atlanticus*), and the phylogenetic relationships were deduced from seven molecular markers (*COI*, *COII*, 16S, tRNAs, *ND1*, 12S, 28S) by Bayesian and maximum likelihood inference. The findings show that species of *Zophoscolex* did not belong to a single clade. *Zophoscolex atlanticus* was placed in a clade with *Z. micellus*, *Z. graffi* and *Ethnodrilus zajonci*. Other species of the genus were found to belong to the genera *Cataladrilus* and *Compostelandrilus*. Finally, most of the Iberian species form a distinct clade, which was formally described as the revised genus ***Castellodrilus***. Based on these findings, *Zophoscolex* is restricted to French representatives. These results highlight the importance of incorporating type species in molecular phylogenetic analyses in order to reconcile taxonomy and systematics.

ADDITIONAL KEYWORDS: earthworms – molecular phylogenetics – systematics – taxonomy.

INTRODUCTION

Traditional morphology-based taxonomy of the earthworm (Crassiclitellata: Annelida) faces several difficulties. Earthworms have few main diagnostic characters and the ones they have are usually displayed only during short periods of the year when the earthworms are sexually mature. The variability in these characters appears to be constrained due to the limiting nature of the soil habitat (morphological stasis; Jones *et al.*, 1992), leading to the overlapping of diagnostic characters between species (Pérez-Losada *et al.*, 2009) and the widespread existence of cryptic species (King *et al.*, 2008). In addition, high intraspecific variability can be observed in the same characters (Briones *et al.*, 2009).

For all these reasons, multigene molecular phylogenetic methods are contributing greatly to clarify earthworm relationships, allowing the description of new species (Novo *et al.*, 2012; Marchán *et al.*, 2020), new genera (Domínguez *et al.*, 2018; Marchán *et al.*, 2018), the placing species in their correct genera (Csuzdi *et al.*, 2017) and providing the first robust genus-level phylogeny of the family Lumbricidae (Domínguez *et al.*, 2015). This approach is increasingly being applied to the native earthworm fauna of the Iberian Peninsula and southern France (Hormogastridae and Lumbricidae). These are areas of great evolutionary interest, because they probably

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are the centre of diversification for these families (Omodeo & Rota, 2008; Novo *et al.*, 2015) due to their high diversity, endemicity and presence of putatively early-branching genera.

For instance, multigene phylogenetic analyses focusing on lumbricid genera have provided key information on the phylogenetic relationships between *Prosellodrilus* Bouché, 1972, *Cataladrilus* Qiu & Bouché, 1998, *Postandrilus* Qiu & Bouché, 1998 and the new genera *Galiciandrilus* Domínguez *et al.*, 2017 and *Compostelandrilus* Domínguez *et al.*, 2017 (Pérez-Losada *et al.*, 2011; Domínguez *et al.*, 2018). The phylogenetic analysis carried out in the latter study revealed a close phylogenetic affinity between *Zophoscolex cyaneus* (Briones & Díaz Cosín, 1993) and *Postandrilus*, *Galiciandrilus* and *Compostelandrilus*. *Zophoscolex cyaneus* was initially described as *Eophila cyanea* and its assignment to *Zophoscolex* Qiu & Bouché, 1998 is not firmly supported.

Zophoscolex is a Franco-Iberian genus that has received little attention, despite its high diversity (22 species in four subgenera: *Aquilonibericus, Castillodrilus*, *Euibericus* and *Zophoscolex*). The only previous molecular phylogenetic analysis to include a representative of *Zophoscolex*, *Z. zhongi* Qiu & Bouché, 1998a (James & Davidson, 2012), was limited to three molecular markers (18S, 16S and 28S) and six representatives of the Lumbricidae.

Furthermore, no currently published phylogenetic work has included the species *Zophoscolex atlanticus* (Bouché, 1972), which was designated by Qiu & Bouché (1998) as the type species of the genus *Zophoscolex* (thus being the name-bearer according to ICZN). Thus, only its inclusion in a phylogenetic context will enable the name *Zophoscolex* to be assigned to a clade. The inclusion of type species for genera of interest in molecular phylogenetics studies is of the utmost importance for a reconciliation between taxonomy and evolutionary relationships, and is slowly being adopted in earthworm systematics (de Sosa *et al.*, 2019). By including *Z. atlanticus* and ten additional species of the genus in a phylogenetic analysis, the aims of the present study were as follows:

1. To resolve the phylogenetic relationships between the genus *Zophoscolex* and the other members of the early-branching genera of Lumbricidae.
2. To check the validity of the genus *Zophoscolex* as monophyletic or to demonstrate its artificial status as a non-monophyletic taxon encompassing phylogenetically unrelated species.
3. To analyse the phylogenetic relationship of the type species of *Zophoscolex* to other species of the genus.

MATERIAL AND METHODS

SAMPLING AND MORPHOLOGICAL STUDY

Two sampling surveys were carried out, in April and November 2019, throughout the northern Iberian Peninsula (Castilla y León and the Basque Country) and south-western France, with the aim of capturing species of the genus *Zophoscolex*. The locations where the type species were originally found were also visited (Qiu & Bouché, 1998a) (Table 1). Individuals were collected by digging and hand-sorting, and were washed with distilled water, fixed in 96% ethanol and maintained at –20 °C in the laboratory. Ethanol-fixed specimens of *Zophoscolex* from the collection of the Faculty of Biological Sciences of the Complutense University of Madrid (UCMLT Collection) were also included in the study.

The morphology of the specimens was examined under a binocular stereomicroscope following Qiu & Bouché (1998a). The external characters considered were weight, length, number of segments, position of the clitellum, position of the pubertal tubercles, position of the first dorsal pore, position of spermathecal pores and distance between chaetae. In order to ensure reliable identification, several specimens of each species were dissected and their main internal characters, such as the shape of nephridial vesicles, shape and position of calciferous (Morren's) glands, number and position of seminal vesicles and spermathecae, were studied following Qiu & Bouché (1998a).

MOLECULAR ANALYSIS

Samples were obtained from the body wall of two individual specimens of each of the following species: *Z. alavanensis* Qiu & Bouché, 1998, *Z. atlanticus* (Bouché, 1969), *Z. chitae* (Díaz Cosín *et al.*, 1988), *Z. graffi* (Bouché, 1972), *Z. hongae* Qiu & Bouché, 1998, *Z. micellus* (Bouché, 1972), *Z. opisthoporus* Qiu & Bouché, 1998 and *Z. zhongi* Qiu y Bouché, 1998, and from one specimen (no more were available) each of *Z. ibericus* (Trigo *et al.*, 1988), *Z. eurytrichos* Qiu & Bouché, 1998 and *Z. pulvinus* Qiu & Bouché, 1998. In total, 23 representatives of 11 species of the four subgenera of *Zophoscolex* were included, providing a comprehensive representation of the diversity of the genus. Unpublished sequences for *Ethnodrilus zajonci* Bouché, 1972 (a putative close relative of the genus) were provided by the authors.

Genomic DNA was extracted and isolated using the DNeasy Blood & Tissue Kit (QIAGEN). Different regions of the mitochondrial genes 16S rRNA, tRNAs for Leu and Ala, 12S rRNA, NADH dehydrogenase subunit 1 (*ND1*), cytochrome *c* oxidase subunit II (*COII*) and cytochrome *c* oxidase subunit I (*COI*) and the

Table 1. Species of the genus *Zophoscolex* studied, sampling locations and Genbank accession numbers for the sequenced molecular markers

Species	Subgenus	Sampling location	Coordinates	*COI*	*COII*	16S	tRNAs	*ND1*	12S	28S
Z. ibericus	*Euibericus*	Quinta do Reconço (Portugal)	43.779567° -1.070836°	MW394357	MW411209	MW391787	MW411232,42	MW411220	MW393206	MW405920
Z. chitae	*Euibericus*	Guijo de Granadilla (Cáceres, Spain)	40.193753° -6.163317°	MW394350	MW411202	MW391780	MW411225,35	MW411213	MW393199	MW405913
Z. alavanensis	*Aquiloniberricus*	Azazeta (Álava, Spain)	42.776309° -2.496491°	MW394352	MW411204	MW391782	MW411227,37	MW411215	MW393201	MW405915
Z.atlanticus	*Zophoscolex*	Dax (Landes, France)	43.779567° -1.070836°	MW394353	MW411205	MW391783	MW411228,38	MW411216	MW393202	MW405916
Z. pulvinus	*Euibericus*	Los Altos (Burgos, Spain)	42.788173° -3.620787°	MW394356	MW411208	MW391786	MW411231,41	MW411219	MW393205	MW405919
Z. opisthoporus	*Castellodrilus*	Hortigüela (Burgos, Spain)	42.064848° -3.414508°	MW394355	MW411207	MW391785	MW411230,40	MW411218	MW393204	MW405918
Z. eurytrichos	*Castellodrilus*	Covarrubias (Burgos, Spain)	42.061583° -3.521030°	MW394354	MW411206	MW391784	MW411229,39	MW411217	MW393203	MW405917
Z. hongae	*Aquiloniberricus*	San Martín de Gomacín (Navarra, Spain)	42.707805° -1.803889°	MW394351	MW411203	MW391781	MW411226,36	MW411214	MW393200	MW405914
Z. micellus	*Zophoscolex*	Anterrieux (Cantal, France)	44.836314° 3.028972°	MW394359	MW411211	MW391789	-	MW411222	-	MW405922
Z. graffi	*Zophoscolex*	Treban (Allier, France)	44.093631° 2.341437°	MW394358	MW411210	MW391788	-	MW411221	-	MW405921
Z. zhongi	*Zophoscolex*	Sant Joan de les Abadesses (Gerona, Spain)	42.236779° 2.288965°	-	MW411212	MW391790	MW411233,43	MW411223	-	MW405923

nuclear gene 28S rRNA were amplified by polymerase chain reaction (PCR) with the primers described by Pérez-Losada *et al.* (2005, 2009), and under the same conditions outlined in Pérez-Losada *et al.* (2011) and Domínguez *et al.* (2015). The amplified PCR products were purified using the Multiscreen PCRμ96 purification kit (Millipore) and bidirectionally sequenced using an Applied Biosystems (ABI) 377XL type automatic sequencer in STAB Vida (Portugal). Sequences obtained in this work are available on Genbank with accession numbers 394357-411241.

Sequences were aligned with MAFFT v.7 (Katoh & Standley, 2013) by using the default parameters. The sequences of the seven molecular markers were then concatenated in BioEdit v.7.0.9 (Hall, 1999), to obtain a single sequence of 4739 base pairs for each of the specimens.

Reference sequences belonging to the families Lumbricidae, Hormogastridae and Criodrilidae (the latter two were chosen as outgroups) and determined by Domínguez *et al.* (2015, 2018), Pérez-Losada *et al.* (2015), Csuzdi *et al.* (2017), Bozorgi *et al.* (2019) and de Sosa *et al.* (2019) were retrieved from GenBank. Bayesian inference and maximum likelihood phylogenetic approaches were implemented with MrBayes v.3.2.6 (Ronquist *et al.*, 2012) and RAxML-NG (Kozlov *et al.*, 2019), respectively, both through the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010).

The optimal evolutionary model for each gene was selected using JModelTest v.2.1.3 (Darriba *et al.*, 2012), following the Akaike information criterion (AIC; Akaike, 1973) and the Bayesian information criterion (BIC; Schwarz, 1978) (which agreed in all cases). GTR+I+G was chosen as the optimal evolutionary model for the *COI*, 28S and *ND1* markers; GTR+G was chosen for 12S; and the HKY+G+I model was selected for *COII*, 16S and tRNAs. These models were implemented in both the Bayesian and maximum likelihood analyses.

For Bayesian inference, the number of generations was set at 50 000 000. Two independent parallel analyses were initiated and 20% of the trees obtained were discarded. The other trees were combined to find the maximum posterior probability and to estimate the phylogeny.

The maximum likelihood analysis was performed with ten different starting trees, and support of resulting topology was estimated using 1000 rapid bootstrap replicates.

A time-calibrated phylogenetic inference was estimated using relative dating. To generate a suitable starting tree, the maximum likelihood tree was converted into an ultrametric tree by non-parametric rate smoothing (NPRS) using the function chronopl in the R package *ape* v5.2.

The final ultrametric tree was generated with BEAST v.1.10 (Suchard *et al.*, 2018). Each partition was trimmed with GBlocks (Castresana, 2000) under the less stringent parameters, and the best-fitting evolutionary models (shown above) were specified. A normal distribution of mean = 1 and SD = 0.05 was implemented for the root of the tree as a relative calibration. A Yule diversification model and an uncorrelated lognormal relaxed clock were specified. Three parallel runs were performed, each of which included 50 million generations, sampling every 5000th generation. Tree and log files were combined in LogCombiner v.1.10 (Suchard *et al.*, 2018) by resampling at lower frequency (15 000) and results were visualized in TRACER v. 1.7.1 (Rambaut *et al.*, 2018). The final tree was generated by TreeAnnotator v.1.10 (Suchard *et al.*, 2018) with a burn-in of 2000.

RESULTS

Morphological characters

All studied specimens matched the original descriptions by Bouché (1972) and Qiu & Bouché (1998a).

Phylogenetic inferences

Bayesian inference (Fig. 1; Supporting Information, Fig. S1) and maximum likelihood inference of the phylogenetic tree provided consistent topologies. The genus *Zophoscolex* was not recovered as monophyletic. Instead, the studied representatives were scattered within a larger clade comprising *Cataladrilus*, *Compostelandrilus*, *Ethnodrilus*, *Postandrilus* and *Prosellodrilus*.

Zophoscolex atlanticus (the type species of the genus) was recovered within a strongly supported clade including *Z. micellus* and *Z. graffi* but also *Ethnodrilus zajonci* as its closest relative. Representatives of *Prosellodrilus* and *Cataladrilus* were recovered as a sister-clade.

Zophoscolex zhongi was recovered within *Cataladrilus*, with *Ca. edwarsi* as a sister-taxon. *Zophoscolex cyaneus* was included within an independent clade comprising *Postandrilus*, *Galiciandrilus* and *Compostelandrilus*, with the latter as a sister-taxon.

The other Iberian *Zophoscolex* representatives (*Z. alavanensis*, *Z. chitae*, *Z. eurytrichos*, *Z. hongae*, *Z. ibericus*, *Z. opisthoporus* and *Z. pulvinus*) resolved in a well-supported clade (monophyly, sister-taxa of equal rank; Lin *et al.*, 2013). Internal relationships were well resolved, with *Z. opisthoporus* and *Z. eurytrichos* as a sister-clade to (*Z. chitae–Z. ibericus–Z. pulvinus)* and (*Z. hongae–Z. alavanensis)*.

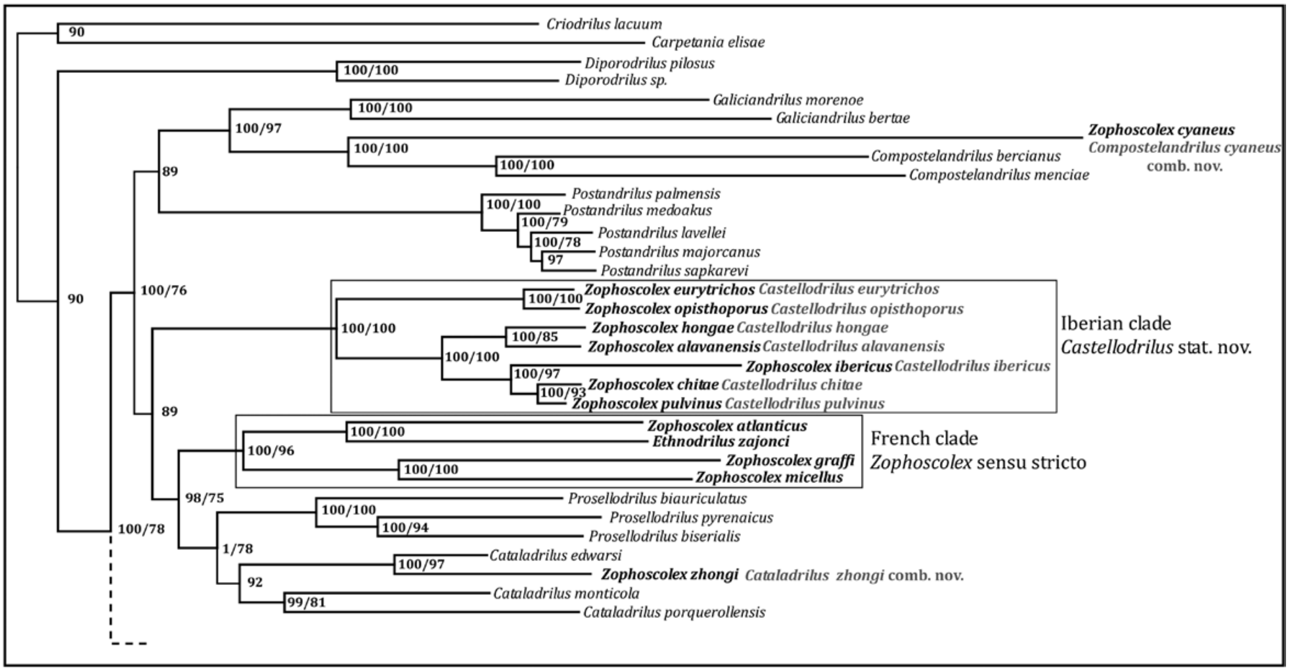

Figure 1. Detail of the clades including the *Zophoscolex* species under study by Bayesian phylogenetic analysis of the concatenated sequence of molecular markers *COI*–*COII*–16S–tRNAs–*ND1*–12S–28S. Focus species of are shown in bold. Proposed taxonomic changes are shown in grey. Posterior probability support values and bootstrap support values (from maximum likelihood analysis) higher than 70 are shown beside the corresponding nodes. The complete phylogenetic tree is shown in Supporting Information, Figure S1.

The time-calibrated phylogenetic tree (Supporting Information, Fig. S2) estimated a relative root age for the Iberian *Zophoscolex* clade that was similar to other well-established genera, while the clade including *Z. atlanticus*, *Z. micellus*, *Z. graffi* and *Ethnodrilus* showed a relative age significantly older than other lumbricid genus-level clades. The estimated root age for the clade including *Zophoscolex cyaneus* and *Compostelandrilus* was within the interval of genus-level clade root ages.

Systematics

The genus-level clade of Iberian earthworms formerly assigned to *Zophoscolex*, but unrelated to *Zophoscolex atlanticus*, is proposed as a revised genus, together with a diagnosis and list of the species included. An updated list of the species included within a re-defined *Zophoscolex* is also provided.

Phylum Annelida Lamarck, 1802

Class Oligochaeta Grube, 1850/Clitellata Michaelsen, 1919

Order Megadrili Benham, 1890/Haplotaxida Michaelsen, 1900

Family Lumbricidae Rafinesque-Schmaltz, 1815

Genus ***Castellodrilus*** (Qui & Bouché, 1998), **stat nov**

Zophoscolex (*Castellodrilus*) Qiu & Bouché, 1998: 189.

Zophoscolex (*Aquiloniberricus*) Qiu & Bouché, 1998: 189. synon. nov.

Zophoscolex (*Euibericus*) Qiu & Bouché, 1998: 189. synon. nov.

Type species: Zophoscolex (*Castellodrilus*) *opisthoporus* Qiu & Bouché, 1998 by original designation.

Zoobank registration: urn:lsid:zoobank.org:act:C2661B3C-B353-4285-A0AF-E460722378B2

Species included: ***Castellodrilus alavanensis*** (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus anamariae***? (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus chitae*** (Diaz Cosín, Mato & Trigo, 1988) **comb. nov.**, ***Castellodrilus eurythrichos*** (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus hongae*** (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus ibericus*** (Trigo *et al.*, 1988) **comb. nov.**, ***Castellodrilus joffrei***? (Qiu & Bouché, 1998) **comb.**

nov., ***Castellodrilus lopezi***? (Bouché, 1979) **comb. nov.**, ***Castellodrilus navarrensis***? (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus opisthoporus*** (Qiu & Bouché, 1998) **comb. nov.**, ***Castellodrilus pulvinus*** (Qiu & Bouché, 1998) **comb. nov.** and ***Castellodrilus vasconensis***? (Bouché, 1979) **comb. nov.**

Diagnosis: Small- to intermediate-sized (38–138 mm) Lumbricidae, with fewer than 260 segments. Prostomium epylobous. Closely paired (separate in two species) setae. First dorsal pore in segments 6/7–12/13 (rarely in 19/20, 20/21). Nephridial pores aligned. Male pores in segments ½ 15 with poorly or well-developed porophore. Spermathecal pores simple, in 9/10, 10/11. Clitellum starts in a relatively posterior to significantly posterior position (between segments 32 and 43). Tubercula pubertatis start in a relatively posterior to significantly posterior position (usually between segments 34 and 55). Calciferous gland in segments 11–15 with dilations in 11(rarely in 13). Typhlosole bifid or multifid. Two or three pairs of seminal vesicles in segments (9), 11 and 12. Nephridial vesicles inverted U- or J-shape in anterior segments (generally before 10), usually inverted V-shape between 11 and 14, digitoid with ampulla from segment 15. Strongly developed *ab* chaetophores between segments 10 and 14, sometimes forming a glandular area in segments 10–13.

Differential diagnosis: Species of *Castellodrilus* stat. nov. can be distinguished from the closely related genera *Cataladrilus*, *Ethnodrilus*, *Prosellodrilus* and *Zophoscolex* by the backward displaced clitellum (starting in segments 32–43 vs. 22–29, 21–22, 19–21 and 22–24, respectively) and tubercula pubertatis (starting in segments 34–55 vs. 27–33, 23–30, 19–25 and 30–34, respectively). The shape of the nephridial vesicles (inverted U- or J-shape in anterior segments, generally before 10, usually inverted V-shape between 11 and 14, digitoid with ampulla from segment) differentiate *Castellodrilus* from *Cataladrilus* and several species of *Prosellodrilus.* Type and position of spermathecae (simple, in 9/10, 10/11) separate *Castellodrilus* from some species of *Zophoscolex* (*Z. micellus*: 9/10, 10/11 double; *Z. graffi*: 9/10, 10/11 double or multiple; *Z. aragonensis*: 9/10, 10/11 double or multiple; *Z. albacetensis*: 9/10, 10/11 simple or double) and from all species of *Prosellodrilus* [(12/13), 13/14, 14/15].

Remarks: *Zophoscolex* (*Castellodrilus*) is here elevated to genus status as it was found to be phylogenetically unrelated to the type species of *Zophoscolex*, *Z. atlanticus*. The same reasoning was applied to *Zophoscolex* (*Aquilonibericus*) and *Zophoscolex* (*Euibericus*), which were considered synonymous to the former. None of the three names has objective priority due to being published simultaneously, thus as first revisers we chose *Castellodrilus* as the senior synonym. This is justified because *Zophoscolex* (*Castellodrilus*) *opisthoporus* (the type species of this subgenus) was included in the molecular phylogenetic analyses, unlike *Zophoscolex* (*Aquilonibericus*) *navarrensis* and *Zophoscolex* (*Euibericus*) *joffrei.*

Several species placed in *Zophoscolex* by Qiu & Bouché (1998a, b) could not be included in this work. Thus, the species included in the same subgenera (*Aquilonibericus*, *Castellodrilus* and *Euibericus*) as the studied representatives, are provisionally included within *Castellodrilus* at the genus level until further molecular phylogenetic analyses featuring these can be performed. *Zophoscolex* (*Aquilonibericus*) *microprodromos* and *Zophoscolex* (*Aquilonibericus*) *aragonensis* have been provisionally transferred to *Zophoscolex* due to the closer morphological affinity.

GENUS *ZOPHOSCOLEX* QUI & BOUCHÉ, 1998

Type species: *Zophoscolex atlanticus* Bouché, 1972.

Species included: *Zophoscolex albacetensis*? Perez Onteniente & Rodriguez Babio, 2010, *Zophoscolex andorranensis*? Qiu & Bouché, 1998, ***Zophoscolex aragonensis***? (Qiu & Bouché, 1998) **comb. nov.**, *Zophoscolex atlanticus* Bouché, 1972, *Zophoscolex byanensis*? Qiu & Bouché, 1998, *Zophoscolex diazi*? Qiu & Bouché, 1998, *Zophoscolex graffi* Bouché, 1972, *Zophoscolex micellus* Bouché, 1972, ***Zophoscolex microprodromos***? (Qiu & Bouché, 1998) **comb. nov.**, *Zophoscolex zicsianus*? Szederjesi & Csuzdi, 2016.

Remarks: Species included by Qiu & Bouché (1998b) within the subgenus *Zophoscolex* (*Zophoscolex*) are retained within *Zophoscolex*, except *Zophoscolex zhongi*, which was recovered in a different clade by phylogenetic analyses. *Zophoscolex andorranensis*, *Z. byanensis* and *Z. diazi* were not included in the phylogenetic analyses, hence their inclusion is provisional. *Zophoscolex aragonensis* and *Z. microprodromos* are transferred from *Zophoscolex* (*Aquilonibericus*) to *Zophoscolex* pending molecular phylogenetic assessment. The species *Z. albacetensis* and *Z. zicsianus* are also included as they were not originally assigned to any subgenera; their systematic placement must be confirmed by phylogenetic analyses.

DISCUSSION

Type species in integrative systematics

It is now widely acknowledged that taxonomy must reflect the evolutionary relationships of an animal group (Wiley & Lieberman, 2011). Thus, taxa higher than species level (e.g. genus) must comply with the requirement of monophyly. While some earthworm genera are highly homogeneous and do not appear to suffer from this problem (*Eisenia* Michaelsen, 1900, *Lumbricus* Linnaeus, 1758 and *Octodrilus* Omodeo, 1956), others are more complex, including several (more or less) related evolutionary lineages. For example, Csuzdi *et al.* (2017) clarified the phylogenetic relationships within the 'systematic wastebasket' of *Bimastos* Moore, 1893 and allegedly related genera *Allolobophoridella* Mrsic, 1990, *Dendrobaena* Eisen, 1873, *Dendrodrilus* Omodeo, 1956, *Eisenoides* Gates, 1969, *Healyella* Omodeo & Rota, 1989 and *Spermophorodrilus* Bouché, 1975, by including their type species in a comprehensive molecular analysis. This enabled *Allolobophoridella* and *Dendrodrilus* to be established as junior synonyms of *Bimastos*, and ruled out a close relationship between the former and *Healyella* and *Spermophorodrilus*. A more explicit effort to include the type species of an earthworm genus in order to solve its conflictive status was performed by de Sosa *et al.* (2019). *Eophila* Rosa, 1893 is another example of a 'catch-all' genus that included phylogenetically unrelated species based on a lax morphological diagnosis. The inclusion of the type species *Eophila tellinii* (Rosa, 1888) enabled a more taxonomically and biogeographically restricted genus to be established.

In this study, the inclusion of the type species *Zophoscolex atlanticus* revealed a taxonomic paradox: most of the species originally assigned to *Zophoscolex* belong to at least three other genera.

Systematic implications

According to the molecular phylogenetic analysis, *Zophoscolex* should be restricted to the species recovered in the same clade as *Z. atlanticus*. This would include *Z. graffi* and *Z. micellus* and, surprisingly, also *Ethnodrilus zajonci* (type species of *Ethnodrilus*). This species (as well as other representatives of the genus) closely resembles *Z. atlanticus* (in the clitellum position, position of first dorsal pore, number and position of spermathecae and shape of nephridial bladders), but there are some morphological differences (chaetal arrangement, calciferous glands and number of seminal vesicles). Thus, it would be premature to suggest synonymizing both genera until the other species of *Ethnodrilus* (*Et. aveli* Bouché, 1972, *Et. gatesi* Bouché, 1972 and *Et. lydiae* Bouché, 1972) are included in molecular phylogenetic analyses. Interestingly, an eventual synonymizing of *Zophoscolex* and *Ethnodrilus* would result in the disappearance of *Zophoscolex*, because *Ethnodrilus* has taxonomic priority.

The close phylogenetic relationship between *Zophoscolex cyaneus* and *Compostelandrilus* supports its inclusion in this north-western Iberian genus as ***Compostelandrilus cyaneus*** (Briones & Diaz Cosín, 1993) **comb. nov**. Alternatively, as already suggested by Domínguez *et al.* (2018), the species may constitute a genus by itself if new species with a closer affinity to *Compostelandrilus cyaneus* than to the other *Compostelandrilus* were discovered. Until further sampling efforts are focused on the area located between the ranges of these species, the more conservative option is preferred.

The inclusion of *Z. zhongi* within *Cataladrilus as* ***Cataladrilus zhongi*** (Qiu & Bouché, 1998) **comb. nov.**, close to *Cat.edwarsi* Qiu & Bouché, 1998, appears surprising due to the morphological differences (number and position of spermathecae, shape of nephridial bladders). Nevertheless, *Cat. zhongi* resembles species of *Cataladrilus* in the approximate position and extent of clitellum and tubercula pubertatis, structure of calciferous glands, general body shape and mode of life. Some species of *Zophoscolex* not included in this study and morphologically close to *Z. zhongi* (*Z. andorranensis*, *Z. byanensis* and *Z. diazi*) share overlapping ranges with the known distribution of *Cataladrilus*. A more comprehensive study incorporating these species and more representatives of *Cataladrilus* is necessary to elucidate the phylogenetic relationships in this north-eastern Iberian complex.

The revised genus *Castellodrilus* includes seven of the species studied here (*Castellodrilus alavanensis*, *Cas. chitae*, *Cas. eurythricos*, *Cas. hongae*, *Cas. ibericus*, *Cas. opisthoporus* and *Cas. pulvinus*) and tentatively includes another five species according to their close morphological affinity. These species were divided by Qiu & Bouché (1998b) into three different subgenera: *Euibericus*, *Aquiloniberícus* and *Castillodrilus*. While the internal relationships within *Castellodrilus* revealed by the phylogenetic analyses mirrored this division, the validity of each subgenus cannot be confirmed in the absence of a wider sampling of their representatives. Thus, the three subgenera were synonymized until further evidence is explored.

Biogeographic and evolutionary implications

The genus *Zophoscolex*, as defined by Qiu & Bouché (1998a, 1998b), was characterized by a relatively wide distribution, extending across southern France through the Iberian Peninsula reaching Portugal. The division supported by the present findings shows a different biogeographic scenario: *Zophoscolex* proper is restricted to France, while *Castellodrilus* appears to be endemic to the Iberian Peninsula. Remarkably, *Zophoscolex* appears to be more closely related to *Prosellodrilus* and *Cataladrilus* (which may include some species formerly described as *Zophoscolex*) than to *Castellodrilus*.

Prosellodrilus and *Cataladrilus* share a north-eastern Iberian and southern French distribution, overlapping with that of *Zophoscolex*. Hence, a primitive Pyrenean chain (emergent land since around 55 million years ago; Vacherat *et al.*, 2017) is a possible centre of origin of this clade. According to the topology revealed by the phylogenetic trees, *Castellodrilus* would be an earlier-branching clade, implying that the origin of the clade, including the aforementioned genera, may be located in the western half of the Iberian Peninsula. This area corresponds to the Iberian Massif, an ancient terrain that originated in the Variscan Orogeny and with a stable geological history since the Palaeozoic (Ribeiro *et al.*, 1990). The terrain was isolated from the eastern terrains of the Iberian Peninsula during different stages of the Mesozoic (Gil *et al.*, 2006), finally becoming connected by emerged land in the Late Cretaceous. This hypothesis could be supported by the current range of the sister clade, comprising *Compostelandrilus*, *Galiciandrilus* and *Postandrilus*. The former two clades inhabit the north-western Iberian Peninsula, while the latter is restricted to the Balearic Islands. This is suggestive of an origin in the Iberian Massif and a later colonization of the eastern Iberian Peninsula. However, a putatively early-branching Lumbricidae genus, *Kritodrilus* Dumnicka, 1983, remains to be examined by molecular phylogenetics. Although this genus includes species endemic to south-eastern France (including its type species, *Kritodrilus calarensis* Tetry, 1944), some south-eastern Iberian species have been tentatively assigned to *Kritodrilus* (Omodeo & Rota, 1989) and, surprisingly, also a Czechoslovakian species (*Kritodrilus mrazeki* Cernosvitov, 1935). The phylogenetic placement of these species will add an important piece to the biogeographic and evolutionary puzzle of early-branching Lumbricidae in the Western Mediterranean.

CONCLUSIONS

Inclusion of the type species of the genus *Zophoscolex* (*Z. atlanticus*) in molecular phylogenetic analysis, together with ten other species belonging to the genus, allows delimitation of a monophyletic *Zophoscolex* restricted to France and erection of the Iberian genus *Castellodrilus*. Several species formerly assigned to *Zophoscolex* were provisionally assigned to *Castellodrilus* on the basis of morphological similarity: further sampling and molecular analysis are required to confirm their phylogenetic affinities. Inclusion of these early-branching lumbricids in an explicit phylogenetic context provides insights into the origin and diversification of this family in the Franco-Iberian domain.

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SUPPORTING INFORMATION

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Figure S1. Complete phylogenetic tree obtained from the Bayesian phylogenetic analysis of the concatenated sequence of molecular markers *COI*–*COII*–16S–tRNAs–*ND1*–12S–28S. The posterior probability values are shown besides the corresponding nodes.

Figure S2. Ultrametric tree obtained by relative dating (root age = 1) through a BEAST phylogenetic analysis of the concatenated sequence of molecular markers *COI*–*COII*–16S–tRNAs–*ND1*–12S–28S. Singleton species and poorly resolved genera were not represented.