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The disjunct distribution of relict earthworm genera clarifies the early historical biogeography of the Lumbricidae (Crassicitellata, Annelida)

Running title: Disjunct distribution of lumbricid genera

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Keywords

Systematics; Molecular Phylogenetics; Time-calibrated phylogeny; Ecological Niche Modelling; Earthworms

Abstract

The historical biogeography of the Lumbricidae, the main Palearctic earthworm family, may be linked to the paleogeography of their putative ancestral range in Iberia-Southeastern France-Corsica-Sardinia. Although molecular phylogenetics has recently been used to analyze most endemic genera in this area, the genus *Kritodrilus* has not yet been studied. The generotype *Kritodrilus calarensis* and other closely related species inhabit Southeastern France. However, other species from the Iberian Peninsula (e.g. *Kr. osellai*) have been assigned to *Kritodrilus*, the same for the Vindobonic (Hungarian-Austrian-Czech-Slovakian) *Dendrobaena mrazeki*. In order to unravel the paleobiogeographic, evolutionary and taxonomic significance of *Kritodrilus*, the aforementioned species (and three potential relatives) were sequenced for seven molecular markers. Molecular phylogenetic inference recovered a clade of French *Kritodrilus* (including *Et. setusmontanus*) closely related to the Northwestern Iberian *Galiciandrilus* and *Compostelandrilus*, the latter being a sister clade to the Vindobonic species (described as a new genus). Time-calibrated phylogenetic inference estimated that these three clades diverged around 55Ma. Ecological Niche Models for these clades were mainly influenced by the same environmental variables, but showed non-overlapping highly suitable areas (except for *Kritodrilus*). Ancestral area reconstruction inferred the origin of the Lumbricidae in Corsica-Sardinia and the Iberian Peninsula, with eventual expansion eastwards towards France, Central Europe and the Carpatobalkan area. For *Kritodrilus* and its relatives, this expansion may have been contemporaneous with the Paleocene-Eocene Thermal Maximum. Later climatic changes may explain the strikingly disjunct ranges. This work highlights the evolutionary and biogeographic significance of these isolated earthworm lineages and the importance of their preservation.

Introduction

Earthworms (Annelida, Megadrili) are an ancient animal group, originating around the Permo-Triassic boundary (about 250 mya) (Marchán et al. 2017, but see Erséus et al. 2020 for a slightly younger estimate) and with the major clades predating or splitting during the break-up of Pangea (about 175 mya) (Anderson et al. 2017). Due to the apparent correlation between paleogeographic and cladogenetic events in this animal group (Fernández et al. 2013), the origin and diversification of earthworm families is a promising research topic in the fields of zoology, biogeography and geology.

The native earthworm families in the Palearctic region are Criodrilidae Vejdovsky, 1884 Hormogastridae, Michaelsen, 1900 and Lumbricidae Rafinesque-Schmaltz, 1815, with the latter being the most diverse and widely distributed (44 genera and around 670 species: Blakemore, 2008). Considering the large number of endemic species and genera, these sister taxa are believed to have originated and diversified in Southwestern Europe representing the Franco-Iberian earthworm domain (Csuzdi and Zicsi 2003). Novo et al. (2015) inferred an origin of the family Hormogastridae in Southeastern France using molecular phylogenetic inference and performing ancestral area reconstruction analysis. Thus, it is to be expected that Lumbricidae would share this ancestral area. The presence of several endemic lumbricid genera (*Ethnodrilus* Bouché 1972, *Kritodrilus* Bouché 1972, *Avelona* Qiu & Bouché 1998) and species groups (*Zophoscolex* (*Zophoscolex*) Qiu & Bouché 1998, *Allolobophora* (*Gatesona*) *chaetophora*, *Helodrilus* (*Acystodrilus*) Qiu & Bouché 1998) supports that hypothesis.

The most complete and up-to-date molecular phylogeny of Lumbricidae (Domínguez et al. 2015) includes representatives of most of the genera, helping to untangle the controversial systematics of the family. However, *Ethnodrilus*, *Kritodrilus*, *Zophoscolex* and *Avelona* were among the few genera not included in the analysis. Jiménez et al. (2021) has recently recovered the genus *Zophoscolex* Qiu & Bouché 1998 as an early-branching, French endemic genus included in a large clade of Franco-Iberian genera (together with *Ethnodrilus*), adding more evidence regarding the importance of this region in the early evolution of the Lumbricidae. *Avelona* has been found to be closely related to *Allolobophora* (*Gatesona*) and *Helodrilus* (*Acystodrilus*), which together form a clade endemic to the Massif Central (Marchán et al. unpublished).

Thus, *Kritodrilus* remains a missing piece in the phylogenetic and historical biogeographical puzzle of the Lumbricidae. *Kritodrilus calarensis* Tetry, 1944 (generotype), *K. micrurus* Zicsi and Csuzdi, 1999 and *K. tetryae* Zicsi and Csuzdi, 1999 inhabit Provence-Alpes-Côte d'Azur and Auvergne-Rhône-Alpes (Southeastern France). *Ethnodrilus setusmontanus* Qiu and Bouché, 1998 was described in a location 85 km to the west of the aforementioned range.

Although assigned to *Ethnodrilus*, there is remarkable disjunction and morphological disparity between this and the other species of the genus. Thus, *E. setusmontanus* appears closer to *Kritodrilus* in both morphology and distribution.

Some Iberian lumbricids have also been attributed to *Kritodrilus* (*Dendrobaena osellai* Zicsi, 1970, *Dendrobaena ruffoi* Zicsi, 1970, *Dendrobaena pseudorrosea* Moreno, 1983) by Omodeo & Rota (1989), but this assignment has been criticized by other authors (Qiu and Bouché 1998, Blakemore 2008). If these lumbricids were actually members of the genus *Kritodrilus*, they would be expected to be included within the same clade as *Kritodrilus calarensis* (and the other French species) in a molecular phylogenetics study.

Allolobophora hrabei Cernosvitov, 1935 and *Dendrobaena mrazeki* Cernosvitov, 1935 are Vindobonic (Hungarian-Austrian-Czech-Slovakian) endemisms with a unique xerophilic-steppe habitat preference and geographically restricted range. Although both species appear to be closely related owing to their morphological characters, their phylogenetic position is unclear, with *D. mrazeki* eventually placed within *Kritodrilus* (Omodeo and Rota 1989) and *A. hrabei* possibly being related to *Zophoscolex* (Csuzdi and Zicsi 2003). Even though their current range is far removed from the aforementioned Franco-Iberian genera, such morphology-based links could be explained by these species being a relic of a formerly widely distributed lumbricid lineage encompassing these territories.

In this study, we explored the paleobiogeographic, evolutionary and taxonomic significance of *Kritodrilus* and potentially related taxa *Allolobophora hrabei*, *Kritodrilus mrazeki* and *Kritodrilus osellai* through their inclusion in a molecular phylogenetic context, together with the previously studied Franco-Iberian early-branching genera. Ecological Niche Modelling was used to investigate which environmental variables explain the restricted distribution of these taxa. Time-calibrated phylogenetic inference and ancestral area reconstruction provide a spatio-temporal framework for the diversification of early-branching Lumbricidae, shedding some light on the historical biogeography of this ancient group.

Materials and methods

Specimens and sampling

For the present study, 22 specimens of 6 species of Lumbricidae were studied morphologically, and one specimen of each species was analyzed through molecular phylogenetics (Table 1). Additionally, 110 specimens of 110 species (108 lumbricids and 2 outgroup taxa) were included as sequences recovered from Genbank (publications of origin shown in Appendix 1). Specimens of the species *Kritodrilus calarensis*, *Kritodrilus micrurus* and *Ethnodrilus setusmontanus* were

collected in a sampling survey carried out in Southeastern France between October and November 2019. Specimens of *Allolobophora hrabei* and *Kritodrilus mrazeki* were collected in Várbalog, NE Hungary and Podyjí National Park, Czech Republic, respectively. Specimens assigned to *Kritodrilus osellai* were available in the UCMLT collection of the Universidad Complutense de Madrid. The locations where the samples were found are listed in Table 2.

Earthworms were collected by digging and hand-sorting or by the diluted formaldehyde method (Raw 1959), rinsed with water and fixed in 96% ethanol. Sampling and handling of specimens followed ethical considerations and conformed to Directive 2010/63/EU. Species identification was conducted using the set of external and internal morphological characters used by Qiu and Bouché (1998).

Table 1

Table 2

DNA isolation and sequencing

After morphological identification, one representative specimen of each species was chosen for further analysis. Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) from ventral integument samples of approximately 5 x 5 mm. Regions of the genes for the nuclear 28S rRNA (*28S*) and mitochondrial 16S rRNA (*16S*), tRNAs Ala, Leu and Ser (*tRNAs*), 12S rRNA (*12S*), and regions of the mitochondrial genes *NADH dehydrogenase subunit 1* (*ND1*), *cytochrome c oxidase subunit 1* (*COI*) and *cytochrome c oxidase subunit 2* (*COII*) were amplified for 6 species using the polymerase chain reaction (PCR); primers and length of the obtained amplicons are shown in Table 2. PCR condition were as follows: 94°C – 3', (94°C – 30'', X°C – 45'', 72°C – 1') **40** (<35 for *ND1*) cycles (see Table 2), 72°C – 5', 4°C – ∞. PCR products were purified and sequenced by the C.A.C.T.I Genomics service (University of Vigo).

DNA sequences obtained in this study are available in GenBank, and the accession numbers are shown in Table 3.

Sequences reported by Domínguez *et al.* (2015, 2018), Pérez-Losada *et al.* (2009, 2011, 2015), Paoletti *et al.* (2016), de Sosa *et al.* (2019), Bozorgui *et al.* (2019), Jiménez *et al.* (2021) and Marchán *et al.* (2020, in press) including representatives of most of the Lumbricidae genera and two members of the closest families (Hormogastridae and Criodrilidae) were downloaded from GenBank and used as a reference dataset. The list of species and reference to the publication on which they were provided are shown in Appendix 1.

Table 3.

Phylogenetic analyses and time-calibrated phylogenetic inference

Sequences were aligned with MAFFT v.7 (Katoh & Standley 2013) with default settings and concatenated with BioEdit (Hall 1999), resulting in a matrix of 4,183 bp (Suppl. Information Alignment S1). The best fitting evolutionary model for each partition was selected with jModelTest v. 2.1.3 (Darriba *et al.*, 2012) by applying the Akaike information criterion (AIC; Akaike, 1973) and the Bayesian information criterion (BIC; Schwarz, 1978). *tRNAs* were analyzed as a separate partition from *16S*. GTR + I + G was selected as best-fitting evolutionary model for *COI*, *28S* and *ND1*, GTR + G was selected for *12S*, and HKY+I+G was selected for *16S*, *tRNAs* and *COII*.

Bayesian Inference of the phylogeny was estimated with MrBayes v.3.2.6 (Ronquist *et al.*, 2012) as implemented in CIPRES Science Gateway V. 3.3 (Miller *et al.* 2010). The analysis was performed with default parameters, and each of the two independent runs was set to 50 million generations sampling every 5,000th generation (10,000 trees). Twenty-percent of the trees were discarded as burn-in, with remaining trees combined and summarized on a 50% majority-rule consensus tree. Maximum Likelihood phylogenetic inference was performed using RAxML-NG (Kozlov *et al.* 2019) in the CIPRES Science Gateway V. 3.3 platform, from 10 random starting trees and 1,000 rapid bootstrap replicates.

To generate a suitable starting tree for the time-calibrated phylogenetic inference, the Maximum Likelihood tree was converted into an ultrametric tree by non-parametric rate smoothing (NPRS) using the *chronopl* function in the R package *ape* v5.2. The maximum and minimum ages of the clades were the same as those used in the downstream BEAST analysis.

The final ultrametric tree was generated with BEAST v. 1.10 (Suchard *et al.*, 2018) using the NPRS tree as the starting tree. Each partition was trimmed with GBlocks (Castresana, 2000) under the less stringent parameters, with the best-fitting evolutionary model (shown above) as the evolutionary model for each.

The following calibration points were implemented as uniform priors:

-87–170 mya for the Criodrilidae + Lumbricidae + Hormogastridae clade

-82–147 mya for the Lumbricidae+Hormogastridae clade

These age intervals correspond to the 95% HPD (highest posterior density) intervals obtained by Marchán *et al.* (2017).

A Yule diversification model and an uncorrelated lognormal relaxed clock were specified. Three parallel runs were performed, each including 50 million generations, sampling every

5,000th generation. Tree and log files were combined in Logcombiner v.1.10 (Suchard *et al.*, 2018) by resampling at a lower frequency (15,000) and the 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 generations.

Ancestral area reconstruction

Ancestral area reconstruction was performed in RASP 4 (Yu *et al.* 2020), and the most suitable model was chosen using BioGeoBEARS (DEC+j) and allowing a maximum of 3 areas for each node. The areas considered were coded as follows: A-Iberian Peninsula, B-France, C-Tyrrhenian (Corsica, Sardinia and Italian Peninsula), D-Central Europe (including Alpine Italy), E- Carphato-Balkanic, F-Eastern Eurasia, G- North America. Species ranges were mainly obtained from DriloBase Taxo (<http://taxo.drilobase.org/>); for cosmopolitan/peregrine species, the original range was included when available in the literature, if not they were removed from the analysis. Species ranges are shown in Table 2 and Appendix 1.

Ecological Niche Modelling

Presence data (Table S1) were aggregated according to the clades recovered in the phylogenetic analyses (see Results) as follows: Northwestern Iberian, Mediterranean and Vindobonic.

The following large-scale variables, used in Marchán *et al.* (2016) for the large-bodied, endogeic Hormogastridae) were chosen as predictor variables:

-Bioclimatic variables (downloaded from Worldclim - <http://www.worldclim.org/> accessed 01/12/2020)

Mean Diurnal Range (BIO2, TRANGE)

Isothermality (BIO3, ISOTHER)

Mean Temperature of the Coldest Quarter (BIO11, TCOLD)

Precipitation of the Driest Month (BIO14, PRDRY)

-Topographical variable

Lithology (LITHO), represented by the PAR-MATDOM2 (Second level code for the dominant parent material of the STU) layer obtained from the European Soil Database Raster Library 1 km _ 1 km

(http://eussoils.jrc.ec.europa.eu/ESDB_Archive/ESDB_data_1k_raster_intro/ESDB_1k_raster_data_intro.html accessed 01/12/2020).

-Biotic variables

Vegetation and dominant land use (VEGET) were represented by the CORINE 2006 Land Cover layer (version 12/2009: <http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-clc2006-100-m-version-12-2009> accessed 01/12/2020).

Human disturbance (ANTHRO) was incorporated through the 'Human footprint' data set - representing the human influence on land surface (Sanderson et al. 2002).

Ecological niche models were obtained using the R package 'SSDM' (Schmitt et al. 2017). Ensemble species distribution models (ESDMs) were built by combining the algorithms ('MAXENT', 'GLM', 'CTA', 'MARS', 'SVM', 'GAM', 'GBM', 'ANN') producing kappa values greater than 0.5, with 5 repetitions for each algorithm.

Results

Phylogenetic analysis and time-calibrated phylogenetic inference

Bayesian phylogenetic inference (Figure 1) placed the studied taxa (*Kritodrilus calarensis*, *Kritodrilus micrurus*, *Ethnodrilus setusmontanus*, *Allolobophora hrabei*, *Kritodrilus mrazeki* and *Kritodrilus osellai*) within an early-branching clade sister to another one comprising *Castellodrilus*, *Zophoscolex*, *Ethnodrilus*, *Prosellodrilus* and *Cataladrilus*. The former clade split into two moderately supported clades: one of them comprised *Pietromodeona januaeargenti*, *Kr. osellai* and *Postandrilus* representatives, while the other comprised the Northwestern Iberian genera *Galiciandrilus* and *Compostelandrilus*, the Vindobonic species *A. hrabei* and *Kr. mrazeki*, and the other *Kritodrilus* species (*calarensis* and *micrurus*) together with *Et. setusmontanus*.

Thus, *Kritodrilus* in the most inclusive sense appears to be polyphyletic, with some representatives placed within loosely-related clades and a well-delimited and strongly supported clade including the generotype (*K. calarensis*) as well as a species originally assigned to a different genus (*Et. setusmontanus*).

Figure 1

The time calibrated tree (Figure 2) enabled comparison of the estimated ages of the different genus-level clades within the Lumbricidae. Although the crown age of *Kritodrilus sensu stricto* appeared relatively young (8Ma, 4.8-12.3 95% Highest Posterior Density interval) and similar to the crown age of *Postandrilus* (13.7Ma, 9.2-19.4), its divergence from its sister clade was

remarkably early (58.6Ma, 46.2-76.3). Crown ages of the Vindobonic clade (*A. hrabei* + *Kr. mrazeki*) and the two Northwestern Iberian genera *Galiciandrilus* and *Compostelandrilus* were comparatively ancient (41Ma, 27.5-54.4; 38.9Ma, 26.7-51.9 and 42.7Ma, 30.6-56.1 respectively), with the splits between these genera occurring relatively close in geological time (53.6Ma, 40.4-68.2 and 50.8Ma, 37.5-64.7).

The most recent common ancestor of all Lumbricidae (A, see Figure 2) was estimated to have occurred at 71.3Ma (55.5-87.5), which places it in the Maastrichtian. A clade restricted to the Iberian Peninsula, France and Sardinia (with the exception of the Vindobonic taxa) -B- and a widespread clade with genera distributed across Eurasia and North America -G- were estimated to have diverged at 67Ma (53.5-84), close to the K-T boundary. The most recent common ancestor of the Carpato-Balkan clade and the majority of the Lumbricidae genera (G) was estimated to have occurred at 64Ma (49.6-79.3). Within clade B, the most recent common ancestor of the Franco-Iberian clade D (*Castellodrilus*, *Zophoscolex*, *Ethnodrilus*, *Proselodrilus* and *Cataladrilus*) and clade C was estimated to have occurred at around 63.3Ma (49.2-78).

Kritodrilus sensu stricto and clade F (comprising the Vindobonic species, *Galiciandrilus* and *Compostelandrilus*) are estimated to have diverged at 58.6Ma (46.2-76.3Ma), while the most recent common ancestor of the disjunct clades within F was estimated to have occurred at 53.6Ma (40.4-68.2Ma) corresponding to the Late Paleocene-Early Eocene.

Figure 2

Ancestral area reconstruction

The Iberian Peninsula, France and Corsica-Sardinia were inferred as the most likely ancestral area of Hormogastridae and Lumbricidae (Fig. 2). The ancestral area of Lumbricidae (node A) was located at Corsica-Sardinia, with subsequent dispersal and vicariance: the Iberian Peninsula was identified as the ancestral area of the clade comprising all Lumbricidae except *Diporodrilus*. Another dispersal-vicariance event would have led to the ancestral area of clade G, in the Carpato-Balkan region. Within clade G, the ancestral area for most of the nodes corresponded to Central Europe, with further dispersal or dispersal-vicariance events explaining ancestral areas in the Iberian Peninsula (*Iberoscolex*, *Satchellius*), Corsica (*Scherotheca*) and North America (*Bimastos*+*Eisenoides*).

The ancestral area of most nodes within clade B corresponded to the Iberian Peninsula. In node D, a dispersal-vicariance event would have separated *Castellodrilus* (ancestral range: Iberian Peninsula) and *Zophoscolex*+*Ethnodrilus*+*Proselodrilus*+*Cataladrilus* (ancestral range:

France). Further dispersal-vicariance would have led to an Iberian ancestral area for *Cataladrilus*.

The ancestral area for clade E (comprising *Kritodrilus*, *Vindoboscolex* gen. nov., *Galiciandrillus* and *Compostelandrilus*) was probably located in the Iberian Peninsula, with consecutive dispersal-vicariance events leading to the French and Central Europe ancestral areas of *Kritodrilus* and *Vindoboscolex* gen. nov. (respectively).

Ecological Niche Modelling

The three ecological niche models obtained displayed high predictive power, with high AUC and kappa values (0.89-0.96 and 0.7-0.92 respectively), high sensitivity and specificity (0.90-1 and 0.88-0.92 respectively) and low omission rates (0.00-0.10) (Table S2).

The geographical representation of the predicted suitability values is shown in Fig. 3. The predicted highly suitable areas was significantly broader than the known range of the studied taxa, but the difference was particularly remarkable for the Mediterranean clade. There was a large amount of overlap between the highly suitable areas of the Mediterranean and Northwestern Iberian clades, while the highly suitable area predicted for the Vindobonic clade was clearly isolated from the other two areas.

The relative contributions of the predictor variables to each model are shown in Table S2. Isothermality and Lithology were amongst the three most influential variables for the three clades, and Mean Diurnal Range was one of the three most influential variables for the Mediterranean and Northwestern Iberian clades (replaced by Human disturbance for the Vindobonic clade).

Figure 3

Discussion

Systematic implications

The inclusion of the type species of *Kritodrilus* (*K. calarensis*) within a well-delimited, strongly supported clade together with *K. micrurus* and *Ethnodrilus setusmontanus* suggest that the genus should be restricted to these species, excluding *K. mrazeki* and *K. osellai*. The morphological similarity and geographical distribution suggest that *K. tetryae* should probably be included in *Kritodrilus* proper, while Iberian species assigned to *Kritodrilus* (*K. ruffoi*, *K. pseudorrosea*) would be expected to form a clade with *K. osellai*. The latter should be confirmed by molecular phylogenetic analysis, as *K. pseudorrosea* was also assigned to

Iberoscolex (Qiu and Bouché 1998X). In any case, *K. osellai* and its closest relatives should be described as a new genus, as they do not belong to *Dendrobaena* (the genus they were originally assigned to): the generotype *Dendrobaena octaedra* Savigny 1826 belongs to a distant clade, as shown by the phylogenetic trees. However, reassignment is not advisable until the aforementioned species (and others like *Iberoscolex microepigeus* Qiu and Bouché, 1998, type species of its genus) are analysed.

The Vindobonic species (*Allolobophora hrabei*, *Kritodrilus mrazeki*) formed an independent, well-supported clade with an estimated time of divergence consistent with other genus-level clades. This, together with their morphological resemblance, justifies the establishment of a new genus to include both species.

Phylum Annelida Lamarck, 1802

Class Oligochaeta Grube, 1850/Clitellata Michaelsen, 1919

Order Megadrili Benham, 1890/Haplotaxida Michaelsen, 1900

Family Lumbricidae Rafinesque-Schmaltz, 1815

Genus *Kritodrilus* Bouché ,1972

Type species:

Octolasion calarensis Tetry, 1944

Species included:

Kritodrilus calarensis Tetry, 1944

Kritodrilus micrurus Zicsi and Csuzdi, 1999

Kritodrilus setusmontanus (Qiu and Bouché,1998) comb. nov.

Kritodrilus tetryae? Zicsi and Csuzdi, 1999

Remarks

K. tetryae was provisionally included in the genus due to the morphological similarity and geographical proximity with the confirmed representatives. *K. osellai* was excluded as it belonged to a different clade in molecular phylogenetic analyses. *K. ruffoi* and *K. pseudorrosea* were provisionally excluded as their morphological similarity and geographical proximity to *K. osellai* suggest they are more closely related to the latter than to *K. calarensis*. All three aforementioned species (*osellai*, *ruffoi*, *pseudorrosea*) differ from *Kritodrilus* by having more or less intensive red pigmentation, and are temporally placed back to their original genus

Dendrobaena. However, our results suggest that they represent a different, as yet undescribed genus.

Genus *Vindoboscolex* gen. nov. Marchán 2021

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Type species:

Eophila hrabei Cernosvitov, 1935

Species included:

Vindoboscolex hrabei comb. nov. (Cernosvitov, 1935)

Vindoboscolex mrazeki comb. nov. (Cernosvitov, 1935)

Diagnosis:

Medium to large-sized (80-300 mm) and thin (4-6 mm) Lumbricidae, with up to 615 segments with more or less intensive porphyrin based red pigmentation. Prostomium pro-epilobous. Setae closely paired or separate. First dorsal pore in segments 6/7-7/8. Male pores in segments ½ 15 with well-developed porophore. Spermathecal pores simple, in 9/10 and 10/11. Clitellum between segments 29 and 60. *Tubercula pubertatis* between segments 37 and 54. Calciferous glands in segments 11-14 with lateral diverticula in 11. Typhlosole large, multilobate. Two pairs of small, globular spermathecae opening in 9/10 and 10/11. Two or three pairs of seminal vesicles in segments (9), 11 and 12. Nephridial vesicles after clitellum bilobous. Cross section of longitudinal musculature pinnate.

Differential diagnosis:

Vindoboscolex shares with *Kritodrilus* sensu stricto the shape of the nephridial bladders (bilobous) and the presence of calciferous diverticula in segment 11, but it is clearly separated from *Kritodrilus* sensu stricto by the presence of porphyrin pigment (*Kritodrilus* species are unpigmented) and pinnate musculature (vs fasciculate musculature).

Vindoboscolex can also be differentiated from its closest relatives *Galiciandrilus* and *Compostelandrilus* by the presence of porphyrin pigment (*Galiciandrilus* and *Compostelandrilus* species are unpigmented).

Remarks.

In the classical system of the Lumbricidae (Pop 1941) the setal arrangement was one of the most important characters separating different genera. However, it has recently been shown that several genera include species both with widely and narrowly paired setae, as in *Dendrobaena* Eisen, 1873 (Zicsi and Csuzdi 1986) *Dendrodrioides* Kvavadze, 2000, *Bimastos* Moore, 1893 (Csuzdi et al. 2017) or *Castellodrilus* (Jiménez et al. 2021). The new genus *Vindoboscolex* follows the same pattern, highlighting the high degree of homoplasy of the the setal position in the family Lumbricidae.

Historical biogeography and evolution

The time-calibrated phylogeny of the Lumbricidae, together with the paleogeographic settings of the corresponding geological times, enables reconstruction of the early evolution of the family. These evolutionary scenarios should be regarded as working hypotheses, as the estimated times of divergence showed wide HPD intervals due to the use of external calibration points in the absence of ingroup fossil record. However, the congruence between some estimated times of divergence ages and vicariant events (separation between Corsican and French *Scherotheca* at around 27 Ma, diversification of Balearic *Postandrilus* after 13.6 Ma) supports the general fit of the time-calibrated phylogeny to the historical biogeography of Lumbricidae.

The most recent common ancestor of all studied Lumbricidae would have inhabited Corsica-Sardinia, which was located within a continuous landmass together with France and Northeastern Spain and isolated from surrounding landmasses at the time (Maastrichtian, Upper Cretaceous, Fig. 2). Interestingly, Novo et al. (2015) estimated that the ancestral area of Hormogastridae was located in Southern France and the Northeastern Iberian Peninsula: this suggests the two sister families could have originated by vicariance within that ancient terrane.

During the late Cretaceous and early Paleocene, paleogeographic changes (uplift of the European Cenozoic Rift System, ECRIS -Ziegler and Dèzes, 2007) led to the connection of the Franco-Ibero-Corso-Sardinian landmass to the adjacent Rhenish and Bohemian massifs and the East European Platform. This would have enabled the eastern expansion of Lumbricidae lineages around this time. This is consistent with the ancestral areas inferred for clade G (Fig. 2) and its internal clades. The biogeographical scenario reconstructed by our analysis does not exactly correspond with that expected from such a West-East colonization route, as the earlier-branching ancestral area (Carpatho-Balkan) is located further than the Central European area. However, this could be explained by a more crownward phylogenetic position of the Carpatho-Balkan clade (comprising *Cernosvitovia*, *Serbiona*, *Karpatodinariona* and related taxa) or the extinction/failure to sample of Central European taxa.

The same paleogeographic event is also compatible with the inferred historical biogeography of clade E: from an ancestral area located in the Iberian Peninsula, it would have expanded to Southeastern France (*Kritodrilus*) and to Central Europe (*Vindoboscolex*), with the Northwestern Iberian *Galiciandrilus* and *Compostelandrilus* as remnants of the original range. However, the 1700 km disjunction between the current ranges of *Vindoboscolex* and its Iberian relatives is difficult to understand. One possible explanation can be found in the large global climatic changes during the Tertiary. There is a significant overlap between the estimated times of divergence between these clades (including *Kritodrilus*) and the climatic event known as the Paleocene-Eocene Thermal Maximum (at around 55.5 Ma -Bowen et al. 2015). Superimposed on a general trend of long-term global warming, this episode resulted in increased temperatures of up to 8° C. The resulting climatic conditions were very different from those found from the Eocene to the present day. It is possible that, under these conditions, suitable habitat for the ancestors of the disjunct clades was distributed continuously across their ranges, unlike at present (Fig. 3). Under a progressively cooler, drier climate, the ecological niche of these taxa would have diverged and become more restricted, explaining their current isolation. Intermediate species would probably have become extinct, contributing to the biogeographical gap.

It is worth noting that most cladogenetic events within other lumbricid genera (for example *Castellodrilus*, *Octolasion-Octodrilus*, *Allolobophora* s.s. *Aporrectodea* s.s., *Scherotheca* or *Eophila*) are younger than the estimated ages of divergence between the species of *Vindoboscolex*, *Galiciandrilus* and *Compostelandrilus*. This could signal a replacement of the latter by the former genera in earthworm fauna. In order to support this hypothesis, further information about the interaction between these species (including putative competition) and about the environmental variables influencing their distribution is required.

Finally, the recovery of *Pietromodeona* and *Kritodrilus* sensu lato *osellai* as sister taxa of *Postandrilus* could solve the biogeographic riddle of the latter being isolated from their relatives in the Balearic Islands (Pérez-Losada et al. 2011). Said work identified *Galiciandrilus* and *Postandrilus* as sister taxa, thus demonstrating a strong disjunction between them. However, the authors hypothesized the presence of intermediate forms in the Eastern Iberian Peninsula. The addition of further representatives of this clade (*Compostelandrilus*, -described in 2018-, *Vindoboscolex* -described here-, *Kritodrilus* sensu stricto and *Kritodrilus* sensu lato *osellai*) apparently filled the gaps in the evolutionary and biogeographic scenario of these earthworms. The main range of *Pietromodeona janueargenti* is located in Sardinia, and *Kritodrilus* sensu lato *osellai* inhabits Southeastern Spain (Teruel, Valencia): these terranes and the Balearic Islands were contiguous and connected land masses until around 30Ma. Time-calibrated

phylogeny estimated an earlier split between these species and *Postandrilus*, possibly due to unrepresented, potentially closer relatives (e.g. *Kritodrilus sensu lato ruffoi*). Phylogeographic sampling of *Pietromodeona janueargenti*, *Kritodrilus sensu lato osellai* and *Kritodrilus sensu lato ruffoi* would help to pinpoint the phylogenetic and geographic origin of *Postandrilus* species and their colonization of the Balearic Islands.

Conclusions

Molecular phylogenetic analysis of the type species (and additional ones) of *Kritodrilus* allowed to delimit it as a Southeastern France endemic genus. This genus is phylogenetically close to other early-branching Franco-Iberian endemic genera.

Species not included in this work (*K. tetryae*, *K. ruffoi* and *K. pseudorrosea*), and also the generotype of *Iberoscolex* (*Ib. microepigeus*) should also be analysed to complete the taxonomic revision of the genus.

The close phylogenetic relationship of *Kritodrilus*, the Northwestern Iberian *Galiciandrilus* and *Compostelandrilus*, and the newly described genus *Vindoboscolex* (comprising *V. hrabei* and *V. mrazeki*, from Czech Republic, Slovakia, Austria and Hungary) is biogeographically puzzling due to the large disjunction between their ranges. The estimated time of divergence of these clades enables proposal of a hypothetical connection between the expansion and retraction of their range and global climatic changes from the Paleocene-Eocene to this day. The phylogenetic uniqueness and restricted range of these relict genera highlights the need for research on their conservation status and putative necessary conservation measures.

Ancestral area reconstructions of representatives of most Lumbricidae genera provided a promising overview of the historical biogeography of the family. On one hand, some genera within the ancestral range of the family (Corsica, Sardinia and the Iberian Peninsula) should be sampled more thoroughly (i.e. *Proselodrilus*, *Cataladrilus*, *Zophoscolex*, *Iberoscolex*, *Eumenescolex*) to better represent their diversity. On the other hand, unsampled species from Central Europe, the ancestral area for a large portion of known Lumbricidae genera should be included in further studies to confirm the biogeographical importance of this area. In addition, some Eastern Europe-Middle East-Central Asia species and genera (such as *Perelia*, *Healyella*, *Dendrobaena* and *Eisenia*) remain underrepresented in molecular phylogenetic analyses, and examination of these would shed some light on the expansion and evolution of Lumbricidae further from their center of origin.

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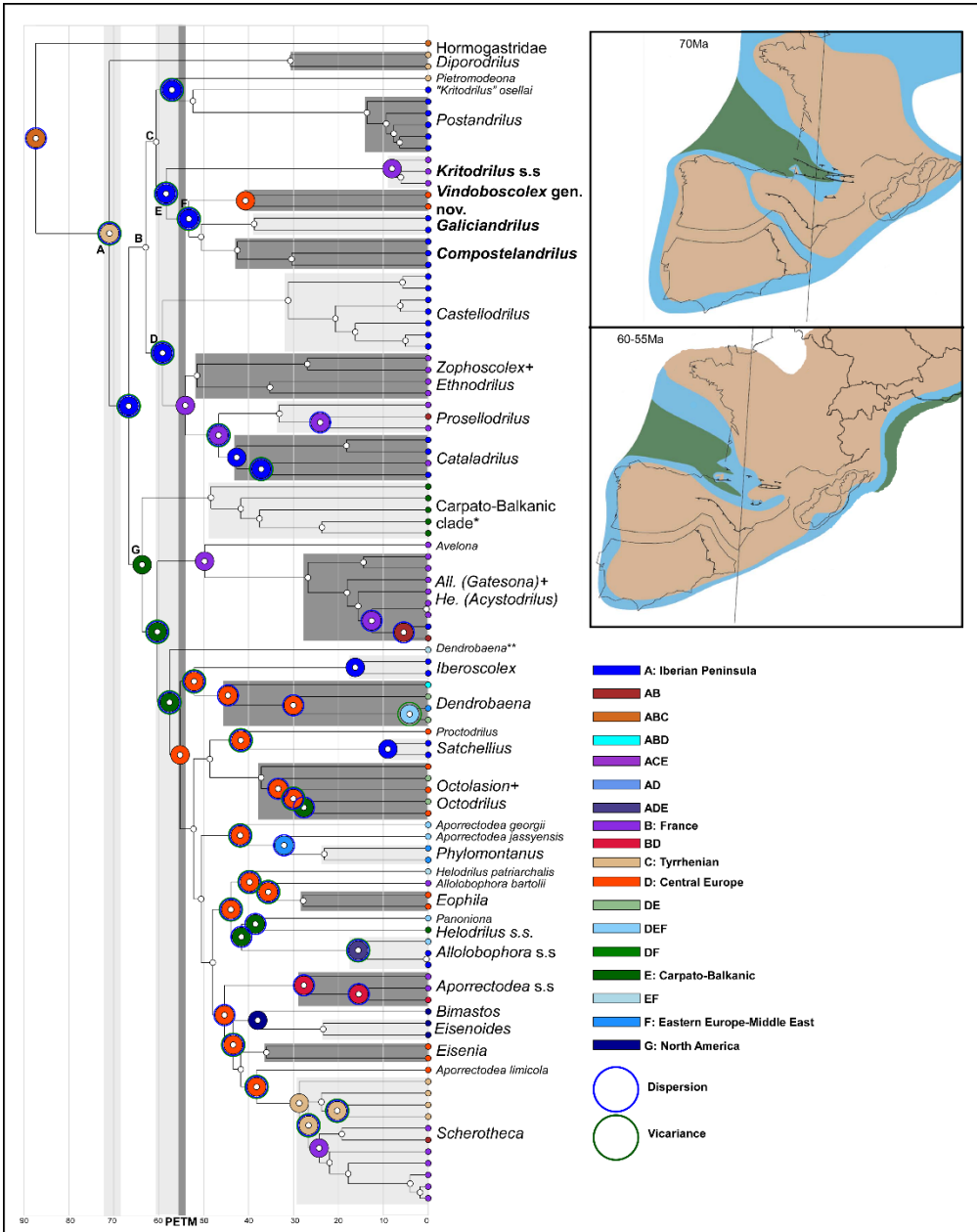
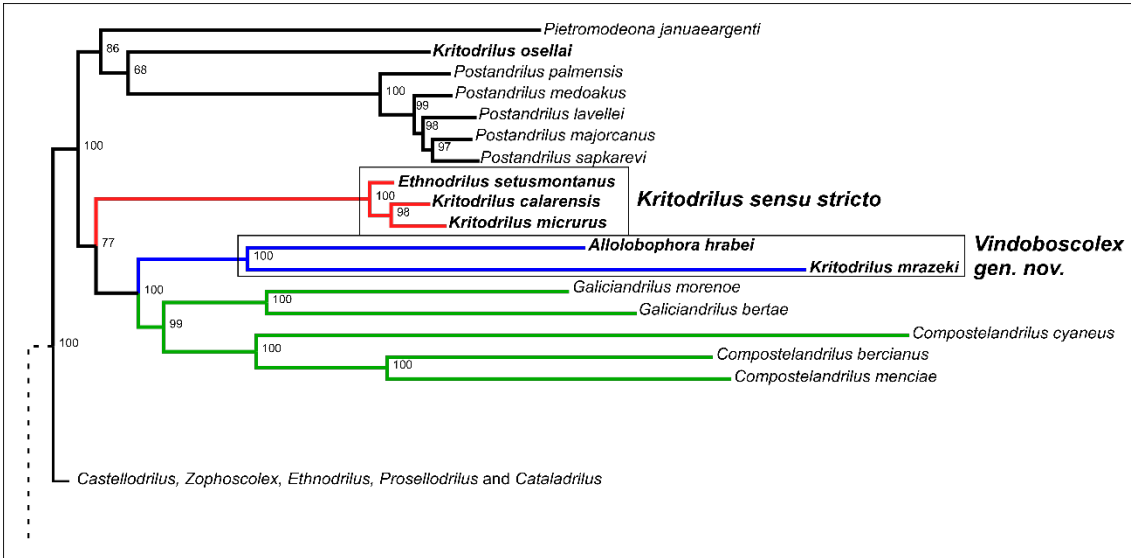
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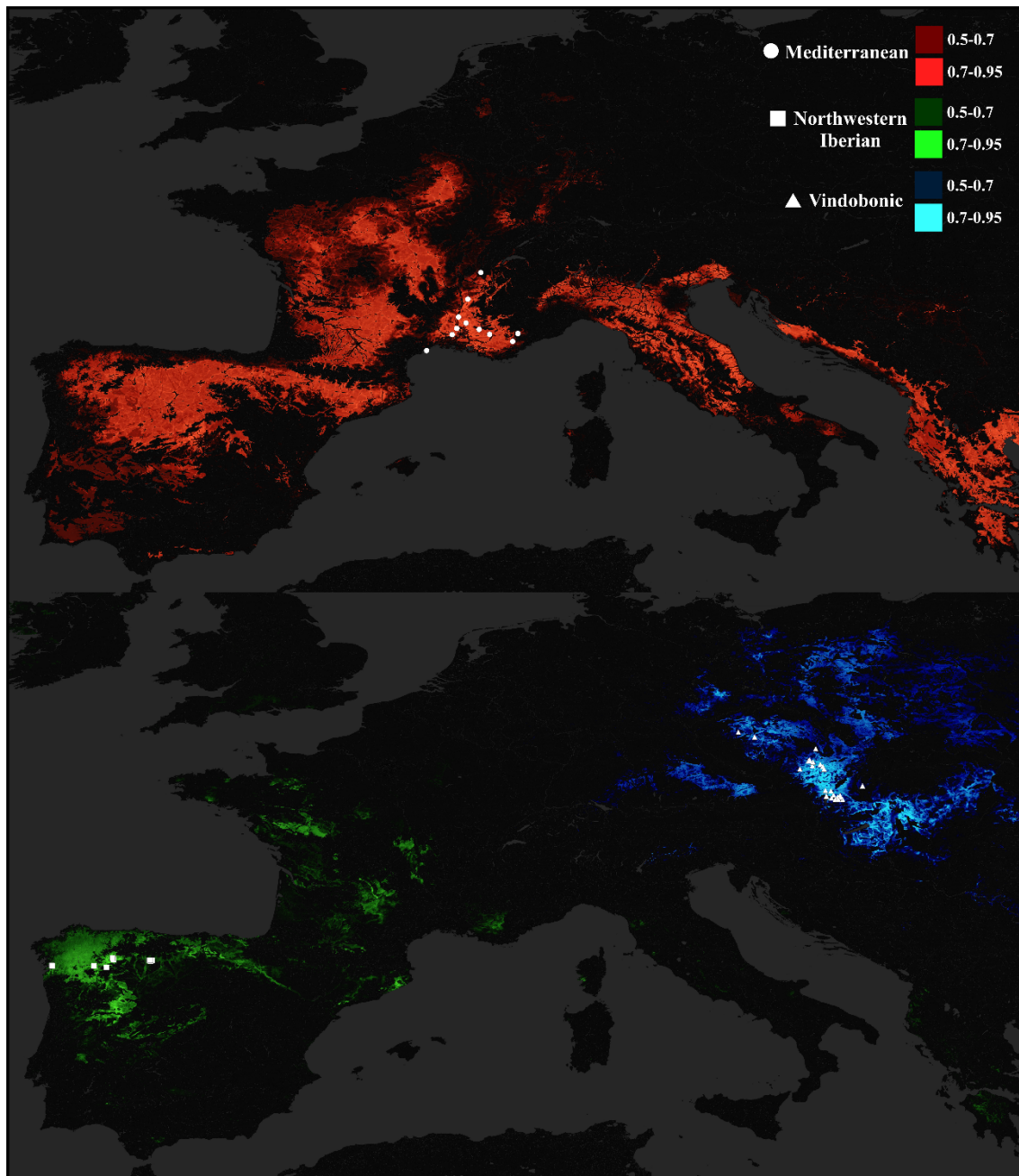
Figure legends

Figure 1. Detail of the Bayesian inference of the phylogenetic relationships between the *Kritodrilus* species under study, *Allolobophora hrabei* and their closest relatives (the whole phylogenetic tree displaying the relationships with representatives of Lumbricidae is shown in Suppl. Material 4). The clades highlighted in colour correspond to the groups used for the Ecological Niche Modelling analysis: Mediterranean (red), Vindobonic (blue) and Northwestern Iberian (green).

Figure 2. Ancestral area reconstruction and estimated times of divergence of Lumbricidae genus-level clades. The target taxa (*Kritodrilus*, Vindobonic species and Northwestern Iberian species) are shown in bold. Important nodes referred to in the main text are indicated by the letters A-G. Only the most likely reconstruction for each node is shown for key nodes. Inferred dispersal or vicariance events are shown by blue and green rings respectively. Vertical grey stripes indicate the approximate geological ages corresponding to the paleogeographical settings of the Western Mediterranean shown to the right (modified from Ziegler and Dèzes 2007; Vacherat et al. 2017). PETM: Paleocene-Eocene Thermal Maximum. *Carpatobalkanian clade: includes representatives of *Cernosvitovia*, *Serbiona* and *Karpatodinariona*. ** Representatives of *Dendrobaena* were split into two different clades.

Figure 3. Ecological Niche Models for the “Mediterranean” clade (comprising *Kritodrilus* sensu stricto species) and its sister clades, “Northwestern Iberian” (*Galiciandrilus* and *Compostelandrilus* species) and “Vindobonic” (*hrabei* and *mrazeki*). Only high suitability values (over 0.5) are shown. Dots, squares and triangles depict the presence localities used as input for the models.





List of supporting information

Supporting information alignment S1. Alignment of the molecular markers *COI* (1-606), *COII* (607-1287), *I6S* (1288-1934), *tRNAs* (1935-2150), *28S* (2151-2986), *12S* (2987-3293), *ND1* (3294-4183).

Supporting information Table S1. Presence data for the Mediterranean, Northwestern Iberian and Vindobonic clades used for Ecological Niche Modelling.

Supporting information Table S2. Ecological niche model evaluation, algorithm evaluation and relative variable importance for the ENMs obtained for the Mediterranean, Northwestern Iberian and Vindobonic clades.

Supporting information Figure S1. Bayesian inference of the phylogenetic relationships of the studied Lumbricidae representatives based on the concatenated sequence of molecular markers *COI*, *COII*, *16S*, *tRNAs*, *28S*, *12S*, *ND1*.

Data Availability Statement

The data that support the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, accession numbers MZ300976- MZ300981, MZ299008- MZ299023, MZ299138- MZ299139 and MZ299146- MZ299151.

Tables

Table 1. Specimen list with species, number of studied specimens (N) and method (M: morphology, DNA: molecular phylogenetics) and Genbank accession numbers. UCM-LT: Universidad Complutense de Madrid Lombrices de Tierra (earthworm collection of the Complutense University of Madrid). HMNH: Hungarian Museum of Natural History. *28S*: 28S rRNA; *16S*: 16S rRNA; *tRNAs*: tRNAs Ala, Leu and Ser; *12S*: 12S rRNA; *ND1*: *NADH dehydrogenase subunit 1* gene; *COI*: *cytochrome c oxidase subunit 1* gene; *COII*: *cytochrome c oxidase subunit 2* gene.

Vouchers	Analysis (N)	<i>COI</i>	<i>COII</i>	<i>16S</i>	<i>tRNAs</i>	<i>12S</i>	<i>ND1</i>	<i>28S</i>
<i>Kritodrilus calarensis</i> UCM-LT 70051	M (6) DNA (1)	MZ300979	-	MZ299011	MZ299143	-	MZ299149	MZ299021
<i>Kritodrilus micrurus</i> UCM-LT 70052	M (9) DNA (1)	MZ300980	-	MZ299012	MZ299144	-	MZ299150	MZ299022
<i>Ethnodrilus setusmontanus</i> UCM-LT 70001	M (1) DNA (1)	MZ300978	-	MZ299010	MZ299142	-	MZ299148	MZ299020
<i>Allolobophora hrabei</i> HMNH-12621, 16316/1, 16478	M (3) DNA (1)	MZ300976	MZ299138	MZ299008	MZ299140	MZ299014	MZ299146	MZ299018
<i>Kritodrilus mrazeki</i> UCM-LT 70053	M (2) DNA (1)	MZ300977	-	MZ299009	MZ299141	MZ299015	MZ299147	MZ299019
<i>Kritodrilus osellai</i> UCM-LT 70054	M (1) DNA (1)	MZ300981	MZ299139	MZ299013	MZ299145	MZ299016	MZ299151	MZ299023

Table 2. Localities of the newly sampled and species ranges used for the Ancestral Area Reconstruction analysis. A: Iberian Peninsula; B: France; D: Central Europe (including Veneto).

Vouchers	Geographic origin	Range	Latitude	Longitude
<i>Kritodrilus calarensis</i> UCM-LT 70051	Malataverne, Drôme, Rhône-Alps, France	B	44.496	4.73943333
<i>Kritodrilus micrurus</i> UCM-LT 70052	Buisson, Drôme, Rhône-Alps, France	B	44.29884	4.98591
<i>Ethnodrilus setusmontanus</i> UCM-LT 70001	Sète, Occitaine, Hérault, France	B	43.396677	3.693158
<i>Allolobophora hrabei</i> HMNH-12621, 16316/1, 16478	Várbalog, Hungary	D	47.838056	17.055556
<i>Kritodrilus mrazeki</i> UCM-LT 70053	Podyjí National Park, Czech Republic	D	48.877472	15.837667
<i>Kritodrilus osellai</i> UCM-LT 70054	Villalba de los Morales, Teruel, Aragón, Spain	A	40.851678	-1.392155

Table 3. Amplified gene fragments (DNA), primer sequences, annealing temperature (AT) and amplicon length in base pairs (AL). 28S: 28S rRNA; 16S: 16S rRNA; *tRNAs*: tRNAs Ala, Leu and Ser; 12S: 12S rRNA; *ND1*: *NADH dehydrogenase subunit 1* gene; *COI*: *cytochrome c oxidase subunit 1* gene; *COII*: *cytochrome c oxidase subunit 2* gene.

DNA	Forward primer	Reverse primer	AT	AL
<i>12S</i>	12SF4: CAGCTTGTGTACTGCCGTCGTAAG	12SR2: GCAATGTTTTTGTAAACAGTCG	55°C	974
<i>16S-tRNAs</i>	16SF2: CGACTGTTTAACAAAACATTGC	16SR2: GTTTAAACCTGTGGCACTATTC	50°C	869
<i>28S</i>	RD33F: GAAGAGAGAGTTCAAGAGTACG	RD5b: CCACAGCGCCAGTTCTGCTTAC	50°C - 49°C	952
<i>COII</i>	COIIF1: GGCACCTATTTGTTAATTAGG	COIIR2: GTGAGGCATAGAAATACACC	47°C - 45°C	771
<i>COI</i>	LCO1490: GGTCAACAAATCATAAAGATATTGG	HCO12198: TAAACTTCAGGGTGACCAAAAATCA	45°C	710
<i>NDI</i>	ND1F2: GAATAGTGCCACAGGTTTAAAC	ND1R1b: TTAACGTCATCAGAGTTATC	45°C	947

Appendices

Appendix 1. List of species included in the reference dataset, species ranges used for the Ancestral Area Reconstruction analysis and reference to the original publication which provided the sequences. A: Iberian Peninsula; B: France; C: Tyrrhenian (Corsica, Sardinia, Peninsular Italy); D: Central Europe (including Veneto); E: Carpato-Balkans; F: Eastern Europe-Middle East; G: North America. -: Cosmopolitan/peregrine species that were excluded from the Ancestral Area Reconstruction analysis.

Name	State	Reference
<i>Allolobophora (Gatesona) chaetophora</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora (Gatesona) sp1</i>	B	Marchán et al. In press
<i>Allolobophora (Gatesona) sp2</i>	B	Marchán et al. In press
<i>Allolobophora (Gatesona) sp3</i>	B	Marchán et al. In press
<i>Allolobophora (Gatesona) sp4</i>	AB	Marchán et al. In press
<i>Allolobophora (Gatesona) sp5</i>	A	Marchán et al. In press
<i>Allolobophora bartoli</i>	B	Marchán et al. In press
<i>Allolobophora chlorotica</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora dacica</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora dubiosa</i>	DEF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora mehadiensis</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora moebii</i>	A	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Allolobophora mollei</i>	A	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Allolobophora robusta</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015

<i>Aporrectodea caliginosa</i>	-	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Aporrectodea georgii</i>	DEF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Aporrectodea jassyiensis</i>	DEF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Aporrectodea limicola</i>	D	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Aporrectodea longa</i>	ABD	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Aporrectodea nocturna</i>	B	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Aporrectodea rosea</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Aporrectodea trapezoides</i>	-	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Aporrectodea tuberculata</i>	B	Pérez-Losada et al. 2009, 2015; Domínguez et al. 2015
<i>Avelona ligra</i>	B	Marchán et al. In press
<i>Bimastos rubidus</i>	G	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Carpetania elisae</i>	ABC	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Castellodrilus alavanensis</i>	A	Jiménez et al. 2021
<i>Castellodrilus chitae</i>	A	Jiménez et al. 2021
<i>Castellodrilus eurythrichos</i>	A	Jiménez et al. 2021
<i>Castellodrilus hongae</i>	A	Jiménez et al. 2021
<i>Castellodrilus ibericus</i>	A	Jiménez et al. 2021
<i>Castellodrilus opisthoporus</i>	A	Jiménez et al. 2021
<i>Castellodrilus pulvinus</i>	A	Jiménez et al. 2021
<i>Cataladrilus edwardsi</i>	A	Jiménez et al. 2021
<i>Cataladrilus monticola</i>	A	Domínguez et al. 2015; Pérez-Losada et al. 2015

<i>Cataladrilus porquerollensis</i>	B	Marchán et al. 2020
<i>Cataladrilus zhongi</i>	A	Jiménez et al. 2021
<i>Cernosvitovia dudichi</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Cernosvitovia rebeli</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Compostelandrilus bercianus</i>	A	Domínguez et al. 2018
<i>Compostelandrilus cyaneus</i>	A	Domínguez et al. 2018
<i>Compostelandrilus menciae</i>	A	Domínguez et al. 2018
<i>Criodrilus lacuum</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena attemsi</i>	DE	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena illyrica</i>	DE	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena jastrebensis</i>	F	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena octaedra</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena pentheri</i>	EF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena pygmaea</i>	ABD	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Dendrobaena veneta</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Diporodrilus pilosus</i>	C	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Diporodrilus sp.</i>	C	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eisenia andrei</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eisenia balatonica</i>	D	Domínguez et al. 2015; Pérez-Losada et al. 2015

<i>Eisenia fetida</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eisenia lucens</i>	D	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eiseniella tetraedra</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eisenoides carolinensis</i>	G	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eisenoides lonnbergi</i>	G	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Eophila gestroi</i>	D	De Sosa et al. 2019
<i>Eophila tellini</i>	D	De Sosa et al. 2019
<i>Ethnodrilus zajonci</i>	B	Jiménez et al. 2021
<i>Galiciandrilus bertae</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Galiciandrilus morenoe</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Helodrilus (Acystodrilus) cortezi</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Helodrilus (Acystodrilus) sp.</i>	B	Marchán et al. In press
<i>Helodrilus cernosvitovianus</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Helodrilus patriarchalis</i>	EF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Lumbricus castaneus</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Lumbricus rubellus</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Iberoscolex albolineatus</i>	A	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Iberoscolex oliveirae</i>	A	Domínguez et al. 2015; Pérez-Losada et al. 2015

<i>Octodriloides boninoi</i>	D	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octodrilus complanatus</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octodrilus exacystis</i>	E	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octodrilus gradinescui</i>	DE	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octodrilus pseudotranspadanus</i>	DE	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octodrilus transpadanus</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octolasion cyaneum</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octolasion lacteum</i>	-	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Octolasion montanum</i>	D	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Panoniona leoni</i>	DEF	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Philomontanus baloutchi</i>	F	Bozorgui et al. 2019
<i>Philomontanus sarii</i>	F	Bozorgui et al. 2019
<i>Pietromodeona januaeargenti</i>	C	De Sosa et al. 2019
<i>Postandrilus lavellei</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Postandrilus majorcanus</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Postandrilus medoakus</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Postandrilus palmensis</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015
<i>Postandrilus sapkarevi</i>	A	Pérez-Losada et al. 2011, 2015; Domínguez et al. 2015

<i>Proctodrilus antipai</i>	D	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Proselodrilus biauriculatus</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Proselodrilus biserialis</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Proselodrilus pyrenaicus</i>	AB	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Satchellius gatesi</i>	A	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Satchellius madeirensis</i>	A	-
<i>Scherotheca corsicana</i>	C	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Scherotheca gigas</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Scherotheca gigas gigas</i>	B	Marchán et al. 2020
<i>Scherotheca gigas heraultensis</i>	B	Marchán et al. 2020
<i>Scherotheca gigas mifuga</i>	B	Marchán et al. 2020
<i>Scherotheca monspessulensis idica</i>	B	Marchán et al. 2020
<i>Scherotheca portcrosana</i>	B	Marchán et al. 2020
<i>Scherotheca rhodana</i>	B	Marchán et al. 2020
<i>Scherotheca savigny</i>	B	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Scherotheca sp. 1</i>	C	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Scherotheca sp. 2</i>	C	Domínguez et al. 2015; Pérez-Losada et al. 2015
<i>Zophoscolex atlanticus</i>	B	Jiménez et al. 2021
<i>Zophoscolex graffi</i>	B	Jiménez et al. 2021
<i>Zophoscolex micellus</i>	B	Jiménez et al. 2021