Organisms Diversity & Evolution
https://doi.org/10.1007/s13127-024-00639-w

ORIGINAL ARTICLE

Ancient diversity within *Diporodrilus* (Crassiclitellata, Annelida) clarify the historical biogeography of Corso-Sardinian earthworms

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Received: 7 September 2023 / Accepted: 20 February 2024

Abstract

Corsica and Sardinia are amongst the largest islands of the Western Mediterranean. Their complex geological history included belonging to the European–Iberian continental margin (close to current-day Catalonia and Provence) and varying degrees of isolation for the last 30 million years, leading to peculiar, highly endemic faunas and floras. This is especially true for their earthworm faunas, which include endemic species of several Lumbricoidea genera and the endemic family (or subfamily) Diporodrilidae. Only three species have been described for the morphologically unique *Diporodrilus*, but there exists evidence for wide morphological variability within them and the existence of several species-level genetic lineages within Corsica. This work aimed to investigate the genetic diversity and phylogenetic relationships between the genetic lineages of *Diporodrilus* from Corsica and Sardinia (based on the sequences of 5 mito-nuclear markers), to perform an integrative systematics revision combining species delimitation techniques and morphological data, and to obtain a time-calibrated phylogeny of *Diporodrilus* and other Corso-Sardinian Lumbricoidea. Within 15 populations of the morphospecies *Diporodrilus omodeoi* and *Diporodrilus pilosus* across Corsica and Sardinia, 10 species-level genetic lineages were detected. Phylogenetic independence, high genetic divergence and morphological differences provided the support for the description of five new pseudocryptic species: *Diporodrilus rotundus* sp. nov., *Di. jorgei* sp. nov., *Di. minor* sp. nov., *Di. meridionalis* sp. nov. and *Di. telti* sp. nov. Time-calibrated phylogenetic inference estimated the age for genus *Diporodrilus* at 65.9 Mya; even if other Corso-Sardinian genera were significantly younger, all of them presented deep divergences predating the break-off of the microplate from the continent. The almost threefold increase in the known diversity of *Diporodrilus* stresses the need for comprehensive earthworm diversity inventories in both Sardinia and Corsica, and for studies on their ecological role and conservation status. The close correspondence between some geological and cladogenetic events suggest that the distribution of Corso-Sardinian earthworms could be used to inform standing geological controversies.

Keywords Western Mediterranean · Oligochaeta · Molecular phylogenetics · Time-calibrated phylogeny · Taxonomy

Introduction

Corsica and Sardinia are the fourth and second largest islands of the Western Mediterranean. They form a small microplate, sharing their geological history at least for the last 56 million years (Siravo et al., 2023), when they formed part of the European–Iberian continental margin adjacent to current-day Catalonia and Provence. The opening of the Liguro-Provençal basin (30–21 Ma) and rotation and drifting of the Corsican–Sardinian block (21–15 Ma) led to their migration into their current position (Siravo et al., 2023). Even though there is evidence for temporary connections to the continent during this process (and afterwards) (Sissingh, 2006), they have been predominantly isolated from the neighbouring land

This article is registered in Zoobank under: LSID urn:lsid:zoobank.org:pub:2016BE52-4F68-4053-8D21-C7F39A218C04.

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Published online: 15 March 2024

masses, leading to peculiar, highly endemic faunas and floras (Grill et al., 2007).

This uniqueness is also reflected in the earthworm faunas of these islands. Corsica and Sardinia are believed to hold vestiges of the early evolution of Lumbricoidea, as they host most of the known diversity of the hormogastrid genera *Hormogaster* Rosa, 1887 and *Norana* Marchán et al., 2018 (Novo et al., 2015), the early branching, endemic lumbricid genus *Pietromodeona* Qiu and Bouché, 1998, several early branching species of *Scherotheca* Bouché, 1972 (including the putatively related *Eumenescolex* Qiu and Bouché, 1998—Marchán et al., 2023) and the endemic family Diporodrilidae.

The single genus within the latter, *Diporodrilus*, Bouché, 1970 contains three currently recognised species (*Diporodrilus omodeoi* Bouché, 1970, *Diporodrilus bouchei* Omodeo, 1984 and *Diporodrilus pilosus* Bouché, 1970) which inhabit Corsica, Sardinia and both islands respectively. Their peculiar morphology and physiology include two rows of dorsal pores (as opposed to a single row of dorsal pores in Lumbricidae and no dorsal pores in Hormogastridae), a stout (proportionally short and wide) body and the ability to secrete large quantities of mucus when disturbed, which rapidly solidifies into a sticky mass after being in contact with the air (Fig. 1).

Bouché (1972) described a wide morphological variability in size and position of spermathecae within *Di. pilosus* and *Di. omodeoi*, describing a few varieties (which are considered invalid according to the International Code of Zoological Nomenclature). This already suggested a greater diversity within the genus than is reflected in the three currently valid taxa. Marchán et al. (2022a) confirmed this through a community-wide DNA barcoding survey in Corsica which allowed the detection of 5 distinct species-level genetic lineages within *Diporodrilus*. A similar phenomenon is to be expected in Sardinia, but large-scale sampling for genetic diversity has not yet been conducted in this part of the range of *Diporodrilus*.

This work aims to (i) investigate the genetic diversity and phylogenetic relationships between the genetic lineages of *Diporodrilus* from Corsica and Sardinia, (ii) to perform an integrative systematics revision (combining species delimitation based on molecular techniques and morphological data) describing previously overlooked taxa and (iii) to obtain a time-calibrated phylogeny of *Diporodrilus* and other Corso-Sardinian Lumbricoidea (Corso-Sardinian Hormogastridae and *Scherotheca*).

Material and methods

Specimens, sampling and morphological description

The 82 specimens described in this study were collected during a sampling survey carried out on the island of Sardinia between 2008 and 2012 and the island of Corsica in March-April 2021. The complete list of localities and morphospecies (understood as the species to which the individuals can be preliminarily assigned based on external morphology) is shown in Table 1 and Fig. 2.

Earthworms were collected by digging and hand-sorting the soil, then rinsed with water and fixed in 70% ethanol in order to obtain relaxed (as opposed to excessively retracted) specimens. Once immobile, specimens were transferred to 100% ethanol to enable further molecular analyses. Species classification and morphological diagnoses were conducted

Fig. 1 Individual of *Diporodrilus pilosus*, displaying their peculiar appearance with a stout body and mucus-filled coelom which gives them their white-yellow colour

Table 1 List of sampling localities of *Diporodrilus* and *Eumenescolex* included in this work

Locality	Code	Island	Latitude	Longitude	Morphospecies	*n*
Campu di Bonza	BONZ	Corsica	41.769651	9.124331	*Diporodrilus omodeoi*	10
Cagnano	CAGN	Corsica	42.876441	9.43541	*Diporodrilus omodeoi*	1
Olivese	OLIV	Corsica	41.845706	9.082436	*Diporodrilus omodeoi*	5
Zonza	ZONZ-A	Corsica	41.742614	9.194813	*Diporodrilus omodeoi*	15
Col San-Giovani (Cap Corse)	SGIO	Corsica	42.822759	9.432619	*Diporodrilus pilosus*	6
Sisco	SISC	Corsica	42.810296	9.461780	*Diporodrilus pilosus*	1
Strette de Saint-Florent	STFL	Corsica	42.67203	9.3446	*Diporodrilus pilosus*	2
Sainte-Lucie de Tallano	STLU	Corsica	41.697222	9.064167	*Diporodrilus pilosus*	3
Zonza	ZONZ-B	Corsica	41.742614	9.194813	*Diporodrilus pilosus*	11
Luras	LURA	Sardinia	40.963611	9.169722	*Diporodrilus pilosus*	1
Ola Nuraghe	OLA	Sardinia	40.308755	9.179346	*Diporodrilus pilosus*	2
Porto Rotondo	PROT	Sardinia	41.027324	9.529347	*Diporodrilus pilosus*	7
Telti	TELT	Sardinia	40.905508	9.38418	*Diporodrilus pilosus*	10
Tempio	TEMP	Sardinia	40.878209	9.046967	*Diporodrilus pilosus*	7
Olbia	OLB	Sardinia	40.948386	9.498756	*Eumenescolex gabriellae*	1

n number of studied specimens

using the same set of external and internal morphological characters reported by Bouché (1972).

The following main external morphological characters were considered: mean length, mean number of segments, mean weight, type of prostomium, position of papillae, position of spermathecal pores, position of first bilateral coelomic pores, position of clitellum and position of *tubercula pubertatis*. The following main internal anatomical characters were considered: position of thickened septa, position and morphology of calciferous glands, position of crop, position of gizzard, type of typhlosole, shape of nephridial bladders, number and position of seminal vesicles, and number and position of spermathecae.

Specimens are stored at the following institutions:

Eco&Sols: Eco&Sols laboratory, INRAE, Montpellier
MNHN: National Natural History Museum of Paris
UCM-LT: Universidad Complutense de Madrid, Faculty of Biology, Earthworm collection
Universidade de Vigo

DNA sequencing

COI sequences covering all Corsican specimens of *Diporodrilus* are reported in Marchán et al. (2022a) and are available, together with GenBank accession numbers and associated metadata, in the "*Diporodrilus* from Corsica and Sardinia" dataset (to be provided) of the Barcode of Life Data systems (BOLD; Ratnasingham & Hebert 2007).

The molecular analysis was completed for additional markers and to include the Sardinian *Diporodrilus* plus a specimen of *Eumenescolex gabriellae* (Omodeo, 1984). Total genomic DNA was extracted from ventral integument samples, of approximate size 5 × 5 mm, with the SPEEDTOOLS TISSUE DNA Extraction kit (Biotools) from two representative specimens of each putative species. Regions of the nuclear 28S rRNA and mitochondrial 16S rRNA, NADH dehydrogenase (ND1) and COI were amplified by polymerase chain reaction (PCR), with the primers and conditions described in Pérez-Losada et al. (2009, 2015). PCR products were purified, and sequenced by Macrogen (Madrid). The DNA sequences are available in Genbank, under accession numbers (to be provided) and in BOLD dataset (to be provided.

Species delimitation and phylogeography

Average uncorrected pairwise distances within and between morphospecies were calculated using MEGA 11. Putative species were delimited using ASAP (Puillandre et al., 2021), a method which has been shown to be the most efficient to delimit earthworm OTUs based on COI sequences (Goulpeau et al., 2022). The chosen model was the one with the lowest possible ASAP score which did not split populations with genetic distances under 9% or with no morphological differences.

Haplotype networks were obtained for each of the putative species through PopArt (Leigh & Bryant, 2015).

Phylogenetic analyses and time-calibrated phylogenetic inference

Sequences from representatives of most of the Lumbricidae and Hormogastridae genera (with a focus on the Corso-Sardinian *Scherotheca*, *Hormogaster* and *Norana*) and an outgroup (Oligochaeta: Haplotaxida: Criodrilidae) were downloaded from Genbank and used as a reference data set (indicated in Suppl. Fig. 1).

Fig. 2 Sampling localities of *Diporodrilus* included in this work. Codes correspond to Table 1. Black diamond: *Dipodrilus omodeoi* morphospecies; White circle: *Diporodrilus pilosus* morphospecies. Note that in ZONZ both morphospecies were found

Sequences were aligned with MAFFT v.7 (Katoh & Standley 2013) with default settings. As no significant topological incongruence was detected between the different markers, they were concatenated with BioEdit (Hall, 1999), resulting in a matrix of 3266 bp. 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). GTR + I + G was selected as the best-fitting evolutionary model for COI, 28S and ND1 and HKY + I + G was selected for 16S.

Bayesian inference of the phylogeny was estimated using MRBAYES v.3.1.2 (Ronquist & Huelsenbeck, 2003) as implemented in CIPRES Science Gateway V. 3.3. Parameters were set to 50 million generations and sampled every 5000th generation (10,000 trees). Two independent runs were performed, each with four chains, and 20% of the trees were discarded as burn-in. The remaining trees were combined and summarised 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 1000 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 (COI, 16S and 28S) 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, which correspond to the 95% HPD (highest posterior density) intervals from Marchán et al. (2017), were implemented as uniform priors:

(i) 93-180 mya for the Lumbricoidea (Criodrilidae, Diporodrilidae, Lumbricidae and Hormogastridae)
(ii) 68-130 mya for the Hormogastridae
(iii) 69-128 mya for the Lumbricidae

An additional calibration point, reflecting the putative vicariance associated with the rifting-drifting of Sardinia from the Iberian margin (Siravo et al., 2023) was implemented as a lognormal distribution (mean = 20, stdev = 20, offset = 18) for the *Norana regina* (Catalonia)–*Norana emiliae* (Sardinia) clade. As very similar (differing only 1 to 5 My) but slightly older divergence age estimates were obtained with this calibration point, this analysis was preferred as it would be expected to better reflect the geological events of the Western Mediterranean.

A Yule diversification model and an uncorrelated lognormal relaxed clock were specified. A uniform distribution with initial value = 0.01, ranging from 0 to 100 was specified through the ucld. mean parameter, and a uniform distribution with initial value = 0.10, ranging from 0 to 10 was specified for the ucld. stdev parameter. Two parallel runs were performed, each including 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 a lower frequency (10,000) and the results were visualised 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.

Results

Species delimitation and phylogeography

Two species partitions (or species hypotheses) obtained the highest ASAP score (3.0), delimiting 12 and 10 species respectively. The second one was chosen (Fig. 3) as it avoided the splitting of two populations with a pairwise genetic distance of 6% (TEMP and LURA) and of a single population (with an internal genetic distance of 4%) in two putative species: in both cases, no morphological differences supported the rejected splits.

COI average uncorrected pairwise distances (Fig. 3) were remarkably high between populations assigned to different putative species (12.7–24.0%), and were of similar magnitude for populations within the same putative species and for individuals of the same population (0.2–6.3%).

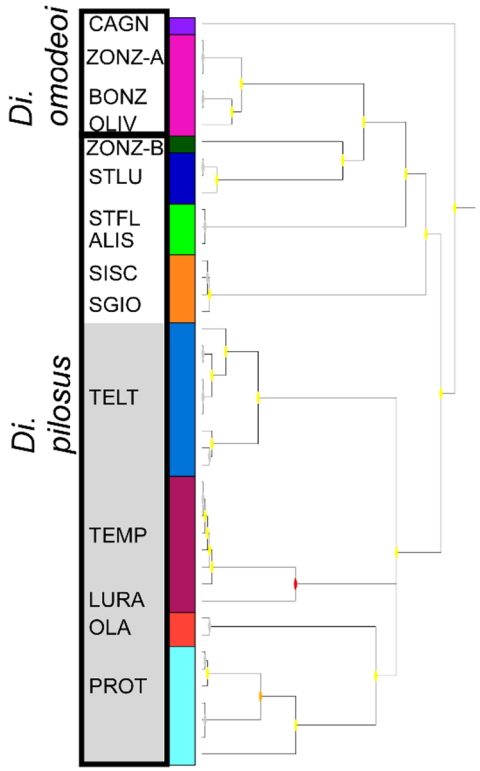
The phylogeographic analysis based on COI haplotype networks (Fig. 4) showed strong population structure, with populations 5 to 10 km apart not sharing haplotypes and being separated by 5 to 38 mutational steps. The populations belonging to the *Diporodrilus pilosus* morphospecies also displayed high genetic diversity, with several haplotypes (up to 6) separated by up to 22 mutational steps; the only exception was ZONZ, where 11 individuals shared the same haplotype. The latter was also found for the *Diporodrilus omodeoi* morphospecies, with a single haplotype within large samples (5–15 specimens).

Phylogenetic analyses and time-calibrated phylogenetic inference

The studied representatives of the *Diporodrilus* morphospecies were recovered within a strongly supported clade by maximum likelihood (Fig. 5, Suppl. Fig. 1) and Bayesian phylogenetic analyses, which showed identical topologies. This *Diporodrilus* clade appeared clearly separated from representatives of Hormogastridae and Lumbricidae. Representatives of the different putative species were separated by long branches. *Diporodrilus omodeoi* lineages were recovered as the sister clade to all other taxa, and the Corsican and Sardinian representatives of the *Diporodrilus pilosus* complex were recovered in reciprocally monophyletic clades.

Time-calibrated phylogenetic inference (Fig. 6) estimated the age for genus *Diporodrilus* at 65.9 Mya (49–86 95% HPD). The estimated ages for Lumbricidae and Hormogastridae genera was significantly younger (from 10.7 to 63 Mya, mean 41.1 Mya), the exception being *Compostelandrilus* Domínguez et al. (2018) (68.8 Mya, 53–88.4 95% HPD).

Most of the deepest divergence events within *Diporodrilus* were estimated to predate the break-off



	CAGN	ZONZ-A	BONZ	OLIV	ZONZ-B	STLU	STFL	ALIS	SISC	SGIO	TELT	TEMP	LURA	OLA	PROT
CAGN															
ZONZ-A	18.7														
BONZ	19.3	2.8													
OLIV	19.0	2.7	2.0												
ZONZ-B	19.7	17.1	18.5	18.6											
STLU	19.1	17.4	17.3	18.0	13.6	4.0									
STFL	21.0	16.4	16.6	16.9	16.7	17.0									
ALIS	21.2	16.3	16.5	16.8	16.9	17.1	0.2								
SISC	20.1	15.1	15.3	15.2	17.8	18.5	17.3	17.4							
SGIO	20.1	15.1	15.2	15.1	17.6	18.4	17.3	17.4	0.7	1.0					
TELT	21.9	18.5	18.8	18.8	18.3	18.2	17.6	17.8	17.7	17.3	2.0				
TEMP	24.0	18.3	17.8	18.6	16.7	19.2	19.2	19.5	18.2	17.8	13.5				
LURA	21.1	18.8	17.9	18.8	18.7	17.9	19.6	19.5	18.3	18.3	13.7	6.3			
OLA	21.2	18.8	18.1	18.6	17.8	17.6	17.8	18.0	19.4	19.2	15.2	13.6	14.9		
PROT	23.9	20.1	20.1	20.4	19.9	20.6	19.0	19.2	21.2	20.9	14.3	15.1	14.9	12.7	4.0

Fig. 3 Most fitting ASAP species delimitation hypothesis and COI uncorrected average pairwise distances for the studied populations of the morphospecies *Diporodrilus omodeoi* and *Diporodrilus pilosus*. White background indicates Corsican populations and grey background indicate Sardinian populations

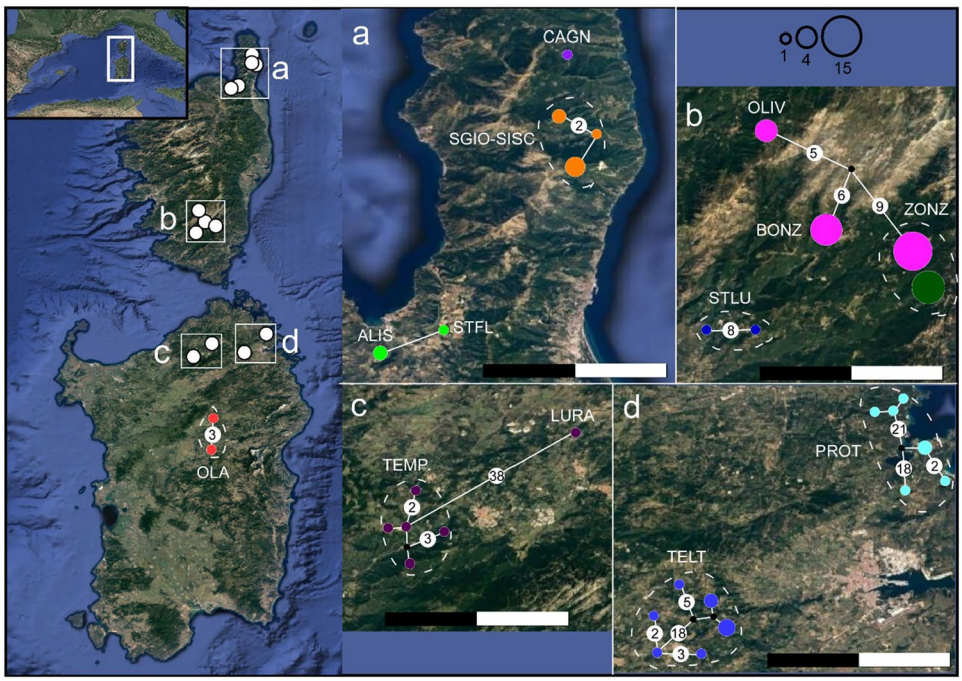


Fig. 4 Haplotype networks based on COI gene for the different putative species within the morphospecies *Diporodrilus omodeoi* and *Diporodrilus pilosus*. Colours correspond to Fig. 2. Circle size represent the number of individuals belonging to each haplotype (see top right for reference). Numbers represent the number of mutational steps between each sampled haplotype. Small black dots represent unsampled intermediate haplotypes. Dashed lines represent several haplotypes being found in the same locality. Scale bar = 10 km

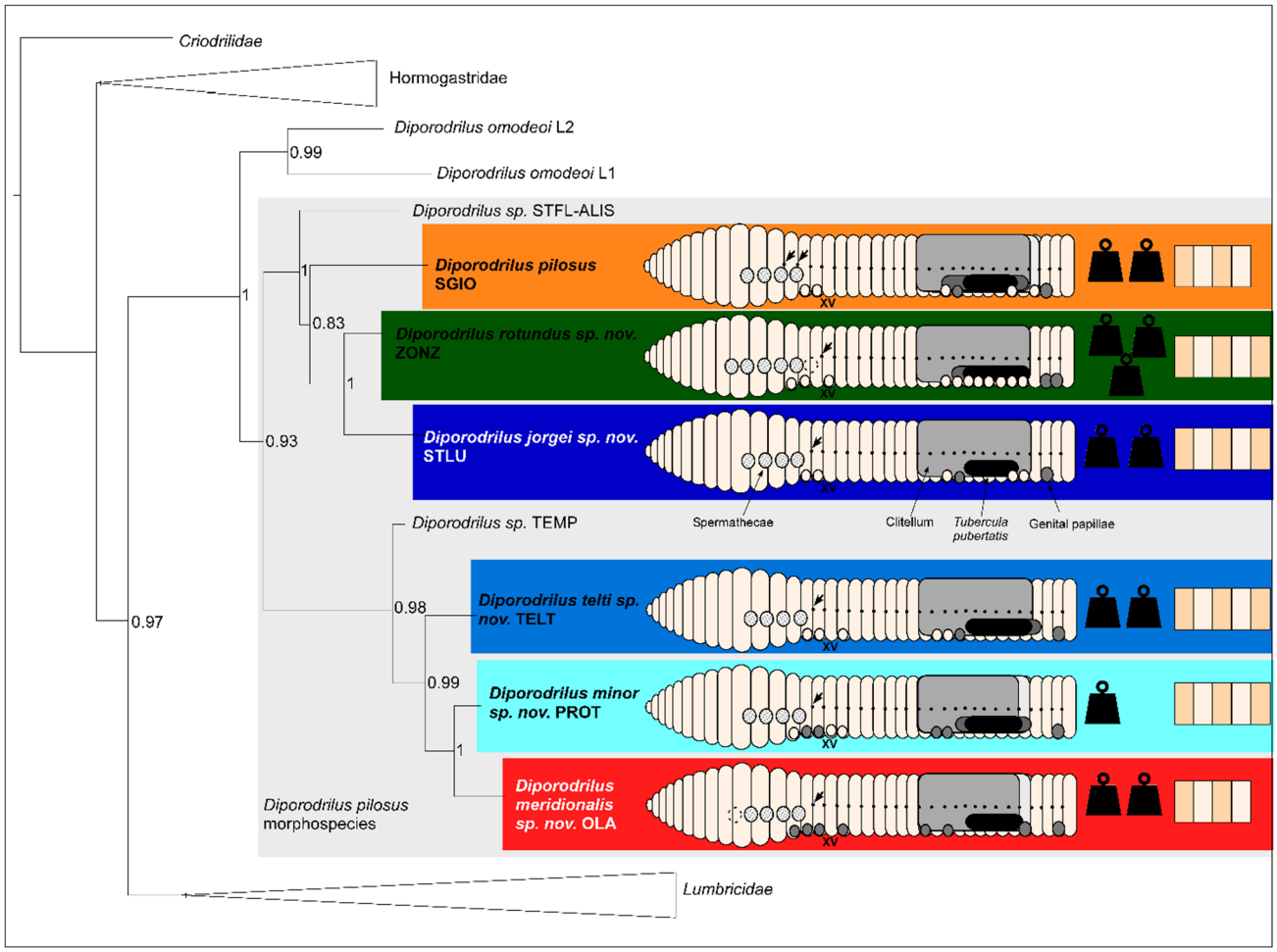


Fig. 5 Detail of the phylogenetic tree obtained by maximum likelihood phylogenetic analysis of the concatenated sequence of molecular markers COI–16S–ND1–28S (see full tree in Suppl. Fig. 1). Bootstrap values are shown besides the corresponding nodes. External and internal morphological differences between the species of *Diporodrilus* are displayed besides the corresponding branches. Dashed circles represent position of spermathecae only found in some individuals. Structures shown in light and dark shades (clitellum and *tubercula pubertatis*) represent the maximum and minimum extension of these characters. Dark grey genital papillae were observed in all individuals while cream ones were only present in some individuals. First pair of dorsal pores is indicated by a black arrow. Black weights indicate body weight: one weight—under 2 g; two weights—between 2 and 3 g; three weights—exceeding 3 g. Rectangles to the right of the image represent the proportionally smaller or larger average number of segments

and rotation of the Corso-Sardinian microplate (from 30 to 15 Mya), including the split between the Corsican and Sardinian clades. The same was observed for *Hormogaster* and *Norana*, for which only their species-level divergence events overlapped with the period of tectonic activity. For the Corsican *Scherotheca* clade and *Eu. gabriellae* (recovered by the analysis within *Scherotheca*), their estimated divergence (including the 95% HPD interval) from their closest continental relative predated the start of Corso-Sardinian rifting and drifting from the continent, but their internal diversification partially overlapped with this paleogeographic event.

The Messinian salinity crisis (MSC, 5.96–5.33 Mya) did not show clear overlap with the most significant cladogenetic events, except for the divergence of the Algerian population of *Hormogaster redii* Rosa, 1887 from its Sardinian relatives.

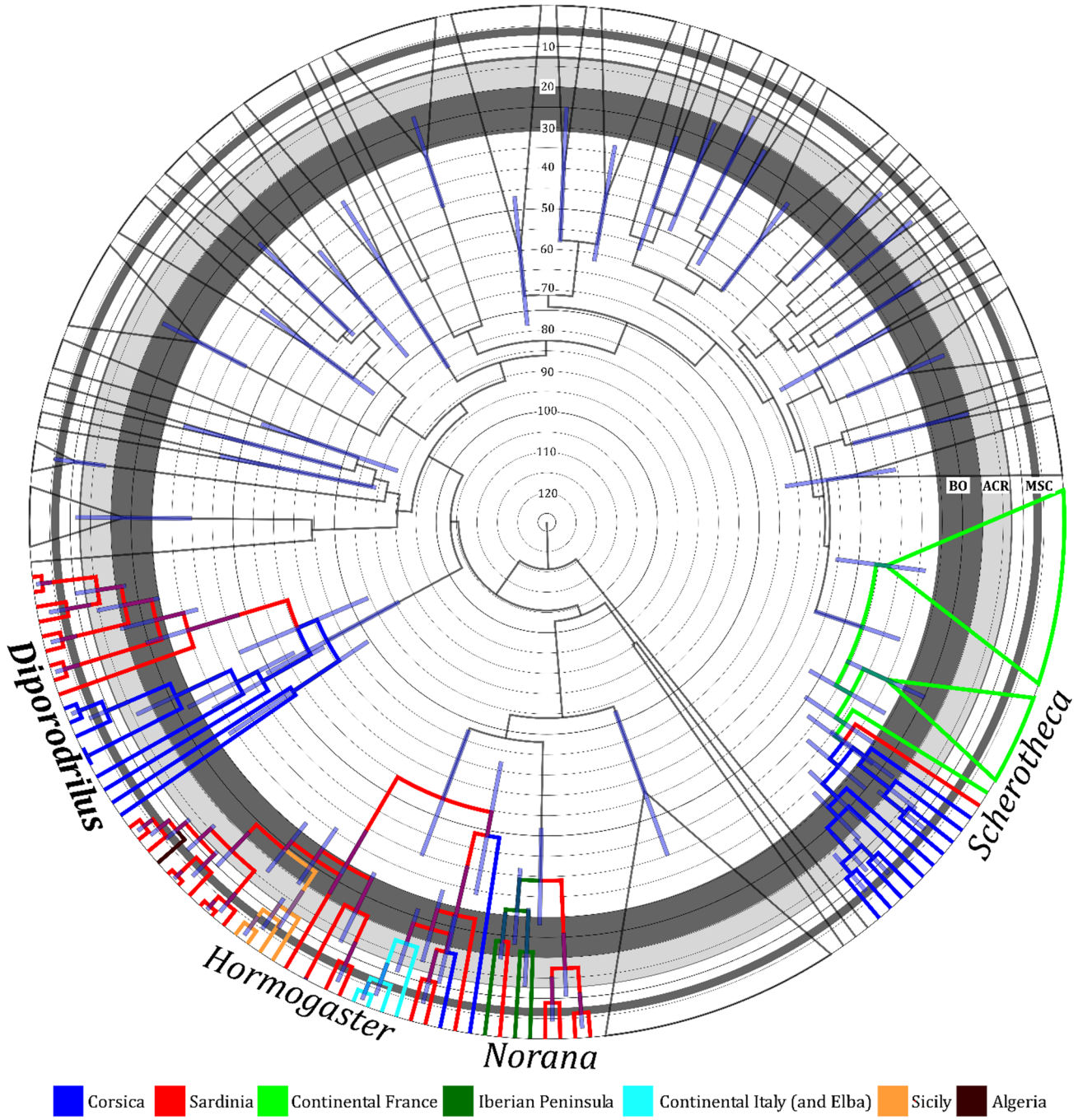


Fig. 6 Estimated times of divergence of Lumbricidae, Hormogastridae and Diporodrilidae genus-level clades. The Corso-Sardinian taxa (*Diporodrilus*, *Hormogaster*, *Norana* and *Scherotheca*) and their continental relatives are shown in different colours (see legend). Vertical blue bars indicate 95% highest posterior density intervals. Gray rings indicate the approximate geological ages corresponding to the most relevant paleogeographical events involving Corsica and Sardinia. BO: Break-off/rifting of the Corso-Sardinian microplate (30–12.5 Mya). ACR: Anti-clockwise rotation of the Corso-Sardinian microplate (20–12.5 Mya). MSC: Messinian Salinity Crisis (5.96–5.33 Mya)

Systematic results

Results of the molecular phylogenetics and species delimitation analyses and morphological characters support 7 of the 10 species-level lineages of *Diporodrilus* as independent, pseudocryptic species (species which present subtle morphological differences upon reexamination following molecular delimitation). The remaining 3 currently lack

Table 2 Main morphological characters which separate the known species of *Diporodrilus*

Species	Av. length	Av. # segments	Av. weight	*Papillae*	1st C.P.	Sperm. pores	Clitelum	T.P.
Diporodrilus omodeoi	(2.5–3.5)	(110–135)	(0.14–0.18)	12, 14, 24, 25, 28, 29, 32	11/12	8/9–10/11	1/3 21–1/3 32	1/2 27–1/2 31
Diporodrilus bouchei	(3.3–4.4)	(120–143)	?	12, 13, 20 (22)	12/13	8/9–10/11 (11/12)	20–29, 30	25–29
Diporodrilus pilosus	6.9 (6.6–8.1)	136 (131–142)	2.4 (1.1–2.8)	(13–15) 16 (31, 33) **34**	11/12, 12/13*	9/10–12/13	(22) 23–32 (33)	(24) 26 (27)–31 (32)
Diporodrilus rotundus sp. nov.	5.2 (4.5–5.8)	173 (170–177)	3.1 (2.5–3.5)	(12–15, 25, 32) **34, 35**	14/15	8/9–12/13 (13/14)	23–32 (1/n 33)	(26) 27–32
Diporodrilus jorgei sp. nov.	7.2	170	2.31	(13, 14, 25) 26 (31, 33) **34**	13/14	9/10–12/13	23–32	27–31
Diporodrilus minor sp. nov.	4.7 (3.9–5)	162 (156–172)	1.7 (1.31–2.04)	(12) 13, 14 (15,16) 24, 25, **35**	13/14	9/10–(11/12) 12/13	23–31 (32)	(26) 27–31(32)
Diporodrilus meridionalis sp. nov.	6.9 (6.6–8.1)	136 (131–142)	2.4 (1.1–2.8)	12–14, 16, 23, 24, **32, 35**	13/14	(8/9) 9/10–12/13	23–31, 32	27–31
Diporodrilus telti sp. nov.	5.9 (5.3–6.9)	163 (150–171)	2.7 (2.5–2.9)	(13, 14, 25) 26 (31, 33) **34**	13/14	9/10 (10/11)–(11/12) 12/13	(22) 23–32 (33)	(25) 26 (27)–31 (32)

Av. length average length, *Av. # segments* average number of segments, *Av. weight* average weight, *1st C.P.* first pair of celomic pores, *Sperm. Pores* spermathecal pores, *T.P.* tubercula pubertatis

*According to Bouché, 1972

enough morphological information to perform robust taxonomic acts. Thus, taxonomic description is provided for the former.

Phylum Annelida Lamarck, 1802
Class Clitellata Michaelsen, 1919
Sub-class Oligochaeta Grube, 1850
Order Crassiclitellata Jamieson, 1988
Family Diporodrilidae Bouché, 1970
Genus *Diporodrilus* Bouché, 1970
Type species: *Diporodrilus pilosus* (Bouché, 1970)
Species included:

- *Diporodrilus bouchei* Omodeo, 1984
- *Diporodrilus omodeoi* Bouché, 1970
- *Diporodrilus pilosus* Bouché, 1970
- *Diporodrilus rotundus* Marchán and Decaëns sp. nov.
- *Diporodrilus jorgei* Marchán and Decaëns sp. nov.
- *Diporodrilus minor* Marchán and Novo sp. nov.
- *Diporodrilus meridionalis* Marchán and Novo sp. nov.
- *Diporodrilus telti* Marchán and Novo sp. nov.

Remarks: *Diporodrilus* contain further species-level genetic lineages which could not be described due to the lack of adult specimens (lineages from Strette de Saint-Florent/Rypisilve de l'Aliso and Tempio) or insufficient morphological differences (two lineages within *Di. omodeoi*). Further sampling could allow to formally describe them, adding 3 more species to the genus. Even though invalid, *Diporodrilus omodeoi* var. *posthecus*—a variety described in Bouché (1972) suggest the existence of a putatively undescribed species which does not fit with the lineages studied in this work.

Diporodrilus pilosus Bouché, 1970

Studied material — Topotypes. France • 3 adult specimens, 4 juveniles; Corsica, Haute Corse, Sisco, Sisco; latitude/longitude: 42.810296/9.461780; elevation: 139 m asl; 31-Mar-2021; T. Decaëns, D. Fernández Marchán leg. (1 specimen); BOLD Sample ID: DFM-0101; deposited at MNHN; Corsica, Haute Corse, Sisco, Col San-Giovani; latitude/longitude: 42.822759/9.432619; elevation: 344 m asl; 31-Mar-2021; T. Decaëns, D. Fernández Marchán leg. (6 specimens); BOLD Sample ID: DFM-0375, DFM-0376, DFM-0377, DFM-0378, DFM-0379, DFM-0380; deposited at ECOSOLS.

Diagnosis. — *Diporodrilus pilosus* can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the position of the clitellum in segments (XXII) XXIII–XXXII (XXXIII), from *Diporodrilus rotundus* sp. nov, *Diporodrilus minor* sp. nov.,

Diporodrilus meridionalis sp. nov. and *Diporodrilus telti* sp. nov by the position of the last genital papillae in XXXIV and from *Diporodrilus jorgei* sp. nov. by the smaller average number of segments (136 vs. 170) and position of first paired celomic pores (11/12, 12/13 vs. 13/14) (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus pilosus* as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Average length (fixed specimens) 6.9 cm (6.6–8.1, $n = 3$); body cylindrical truncated abruptly at the rear end; average number of segments 136 (131–142, $n = 7$). Average weight (fixed specimens), 2.4 g (1.1–2.8, $n = 3$). Prostomium epilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 11/12 (12/13 according to Bouché, 1970). Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows 9/10–12/13 in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments (XXII) XXIII–XXXII (XXXIII). *Tubercula pubertatis* indistinct in (XXV) XXVI (XXVII)–XXXI (XXXII). *Setae* very small and closely paired. Genital papillae/chaetophores in (XIII, XIV, XXV) XXVI (XXXI, XXXIII), strongly developed in XXXIV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X–XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Four pairs of globular intraparietal/supraparietal spermathecae in intersegments 9/10–12/13, simple. Nephridial bladders digitoid.

Distribution and ecology — *Diporodrilus pilosus* was found in two localities near Sisco, in the region of Cap Corse (northernmost part of the island of Corsica), under Mediterranean chaparral vegetation.

Remarks - This species corresponds to *Diporodrilus pilosus* L3 in the checklist of Corsican earthworms of Marchán et al. (2022a, b).

Diporodrilus rotundus Marchán and Decaëns sp. nov.

LSID urn:lsid:zoobank.org:act:A60FDB29-C271-4871-B079-5784F34BC439.

Type material — Holotype. France • 1 adult specimen; Corsica, Corse du Sud, Zonza; latitude/longitude: 41.743/9.195; elevation: 784 m asl; 04-Apr-2021; T. Decaëns, D. Fernández Marchán leg. (1 specimen); BOLD Sample ID: DFM-226; deposited at MNHN.

Paratypes. France • 4 adult specimens, 6 juveniles; Corsica, Corse du Sud, Zonza; latitude/longitude: 41.743/9.195; elevation: 784 m asl; 04-Apr-2021; T. Decaëns, D. Fernández Marchán leg. (10 specimens); BOLD Sample ID: DFM-216, DFM-217, DFM-218, DFM-219, DFM-220, DFM-221, DFM-222, DFM-223, DFM-224, DFM-225; deposited at Eco&Sols.

Etymology. — The species name refers to the heavier, broader and proportionally stouter body of this earthworm compared to other species of the genus.

Diagnosis. — *Diporodrilus rotundus* sp. nov. can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the position of the clitellum in segments XXIII–XXXII (1/n XXXIII), and from all other species of *Diporodrilus* by the position of the last genital papillae in XXXIV, XXXV and position of spermathecae in 8/9–12/13(13/14) (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus rotundus* sp. nov. as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Average length (fixed specimens) 5.2 cm (4.5–5.8, $n = 3$); body cylindrical truncated abruptly at the rear end; average number of segments 173 (170–177, $n = 3$). Average weight (fixed specimens), 3.1 g (2.5–3.5, $n = 3$). Prostomium epilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 14/15. Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows 8/9–12/13 (13/14) in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments XXIII–XXXII (1/n XXXIII). *Tubercula pubertatis* indistinct in (XXVI) XXVII–XXXII. *Setae* very small and closely paired. Genital papillae/chaetophores in (XII–XV, XXV–XXXII), strongly developed in XXXIV, XXXV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X–XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Five or six pairs of globular intraparietal/supraparietal spermathecae in intersegments 8/9–12/13(13/14), simple. Nephridial bladders digitoid.

Distribution and ecology — *Diporodrilus rotundus* was found in a single location near Zonza, in southern Corsica, in a pine forest.

Remarks - This species corresponds to *Diporodrilus pilosus* L2 in the checklist of Corsican earthworms of Marchán et al. (2022a, b).

Diporodrilus jorgei Marchán and Decaëns sp. nov.

LSID urn:lsid:zoobank.org:act:8E83D68E-40F4-4FE6-97C9-4CA33D85C30E.

Type material — Holotype. 1 adult specimen; Corsica, Corse du Sud, Sainte-Lucie de Tallano; latitude/longitude: 41.697222/9.064167; elevation: 444 m asl; 21-Abr-2008; M. Novo, R. Fernández leg. (1 specimens); BOLD Sample ID: DIP3; deposited at MNHN.

Paratypes. France • 1 adult specimen; Corsica, Corse du Sud, Sainte-Lucie de Tallano; latitude/longitude: 41.697222/9.064167; elevation: 444 m asl; 21-Abr-2008; M. Novo, R. Fernández leg. (1 specimens); BOLD Sample ID: DIP4; deposited at UCM-LT; Corsica, Haute Corse, Sisco, Col San-Giovani; latitude/longitude: 41.697222/9.064167; elevation: 444 m asl; 01-Mar-2014; J. Domínguez leg. (1 specimen); BOLD Sample ID: MPL357; deposited at Universidade de Vigo.

Etymology. — This species is named in honour of Prof. Jorge Domínguez, renowned earthworm researcher who included this species in a molecular phylogenetics analysis for the first time.

Diagnosis. — *Diporodrilus jorgei* can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the position of the clitellum in segments XXIII–XXXII, from *Diporodrilus rotundus* sp. nov, *Diporodrilus minor* sp. nov., *Diporodrilus meridionalis* sp. nov. and *Diporodrilus telti* sp. nov by the position of the last genital papillae in XXXIV, and from *Diporodrilus pilosus* by the larger average number of segments (170 vs. 136) (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus jorgei* sp. nov. as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Holotype length (fixed specimen) 7.2 cm; body cylindrical truncated abruptly at the rear end; number of segments in holotype 170. Holotype weight (fixed specimen), 2.31 g. Prostomium epilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 13/14. Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows 9/10–12/13 in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments XXIII–XXXII. *Tubercula pubertatis* indistinct in XXVII–XXXI. *Setae* very small and closely paired. Genital papillae/chaetophores in (XIII, XIV, XXV) XXVI (XXXI, XXXIII), strongly developed in XXXIV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X-XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Four pairs of globular intraparietal/supraparietal spermathecae in intersegments 9/10–12/13, simple. Nephridial bladders digitoid.

Distribution — *Diporodrilus jorgei* sp. nov. was found in Sainte-Lucie de Tallano, in southern Corsica.

Diporodrilus minor Marchán and Novo sp. nov.

LSID urn:lsid:zoobank.org:act:6834D1A5-DAB9-432C-8356-1502D6C3C517.

Type material — Holotype. Italy • 1 adult specimen; Sardinia, Sassari, Porto Rotondo; latitude/longitude: 41.027324/9.529347; elevation: 12 m asl; 31-Jan-2010; M. Novo, R. Fernández leg. (1 specimens); BOLD Sample ID: DIP10; deposited at MNHN.

Paratypes. Italy • 4 adult specimens, 2 juveniles; Sardinia, Sassari, Porto Rotondo; latitude/longitude: 41.027324/9.529347; elevation: 12 m asl; 31-Jan-2010; M. Novo, R. Fernández leg. (6 specimens); BOLD Sample ID: DIP9, DIP24, DIP25, DIP26, DIP27, DIP28; deposited at UCM-LT.

Etymology. — The species name refers to the lower weight of this earthworm compared to other similar species of the genus.

Diagnosis. — *Diporodrilus minor* can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the

position of the clitellum in segments XXIII-XXXI(XXXII), from *Diporodrilus rotundus* sp. nov, *Diporodrilus minor* sp. nov., *Diporodrilus meridionalis* sp. nov. and *Diporodrilus jorgei* sp. nov by the presence of a genital papillae in XXXV but not in XXXII or XXXIV, and from *Diporodrilus telti* sp. nov by the lower average weight (1.7 g vs. 2.7 g) (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus minor* sp. nov. as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Average length (fixed specimens) 4.7 cm (3.9–5, $n=4$); body cylindrical truncated abruptly at the rear end; average number of segments 162 (156–172, $n=4$). Average weight (fixed specimens), 1.7 g (1.31–2.04, $n=3$). Prostomium proepilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 13/14. Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows 9/10–(11/12)12/13 in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments XXIII–XXXI (XXXII). *Tubercula pubertatis* indistinct in (XXVI) XXVII–XXXI (XXXII). *Setae* very small and closely paired. Genital papillae/chaetophores in (XII) XIII, XIV, (XV, XVI), XXIV, XXV, strongly developed in XXXV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X–XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Four (rarely three) pairs of globular intraparietal/supraparietal spermathecae in intersegments 9/10–(11/12)12/13, simple. Nephridial bladders digitoid.

Distribution — *Diporodrilus minor* sp. nov. was found in Porto Rotondo (northern Sardinia), at 170 m from the coast and very close to sea level.

Diporodrilus meridionalis Marchán and Novo sp. nov.

LSID urn:lsid:zoobank.org:act:1CF9E63D-87D9-48F4-915B-AAFF3F78C69A.

Type material — Holotype. Italy • 1 adult specimens; Sardinia, Centrale Sarda, Ola Nuraghe; latitude/longitude: 40.308755/9.179346; elevation: 360 m asl; 31-Jan-2010; M. Novo, R. Fernández leg. (1 specimen); BOLD Sample ID: DIP5; deposited at MNHN.

Paratypes. Italy • 1 adult specimens; Sardinia, Centrale Sarda, Ola Nuraghe; latitude/longitude: 40.308755/9.179346; elevation: 360 m asl; 31-Mar-2010; M. Novo, R. Fernández leg. (1 specimen); BOLD Sample ID: DIP6; deposited at UCM-LT.

Etymology. — The species name refers to its type locality being the southernmost recorded for *Diporodrilus*.

Diagnosis. — *Diporodrilus meridionalis* sp. nov. can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the position of the clitellum in segments XXIII–XXXI, XXXII and from the rest of the species of the genus by the presence of genital papillae in XXXII and XXXV (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus meridionalis* sp. nov. as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Average length (fixed specimens) 6.9 cm (6.6–8.1, $n=3$); body cylindrical truncated abruptly at the rear end; average number of segments 136 (131–142, $n=7$). Average weight (fixed specimens), 2.4 g (1.1–2.8, $n=3$). Prostomium epilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 13/14. Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows (8/9) 9/10–12/13 in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments XXIII–XXXI, XXXII. *Tubercula pubertatis* indistinct in XXVII–XXXI. *Setae* very small and closely paired. Genital papillae/chaetophores in XII–XIV, XVI, XXIII, XXV, strongly developed in XXXII, XXXV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X–XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Four or five pairs of globular intraparietal/supraparietal spermathecae in intersegments (8/9) 9/10–12/13, simple. Nephridial bladders digitoid.

Distribution — *Diporodrilus meridionalis* sp. nov. was found in a single location near Ola Nuraghe, in central Sardinia.

Diporodrilus telti Marchán and Novo sp. nov.

LSID urn:lsid:zoobank.org:act:E5901457-1DE0-4334-84C4-987D1557C2F0.

Type material — Holotype. Italy • 1 adult specimen; Sardinia, Sassari, Telti; latitude/longitude: 40.905508/9.38418; elevation: 216 m asl; 30-Jan-2010; M. Novo, R. Fernández leg. (1 specimen); BOLD Sample ID: DIP1; deposited at MNHN.

Paratypes. Italy • 3 adult specimens, 6 juveniles; Sardinia, Sassari, Telti; latitude/longitude: 40.905508/9.38418; elevation: 216 m asl; 30-Jan-2010; M. Novo, R. Fernández leg. (9 specimens); BOLD Sample ID: DIP2, DIP16, DIP17, DIP18, DIP19, DIP20, DIP21, DIP22; deposited at UCM-LT.

Etymology. — The species name refers to the type locality of the species, near the village and mount of the same name. Name in apposition.

Diagnosis. — *Diporodrilus telti* sp. nov. can be distinguished from *Diporodrilus omodeoi* and *Diporodrilus bouchei* by the position of the clitellum in segments (XXII) XXIII–XXXII (XXXIII), from *Diporodrilus rotundus* sp. nov, and *Diporodrilus jorgei* sp. nov. by the position of the last genital papillae in XXXV, from *Diporodrilus meridionals* sp. nov. by the absence of a strongly developed genital papillae in XXXII and from *Diporodrilus minor* sp. nov. by the greater average length and body weight (5.9 vs. 4.7 cm and 2.7 vs. 1.7 g) (Table 2).

COI uncorrected average pairwise distances and topology of multilocus molecular phylogenetic trees supports the status of *Diporodrilus telti* sp. nov. as independent from other morphologically related species.

Description

External morphology Body pigmentation absent, white-pink colour. Thick (sometimes iridescent) cuticle. Average length (fixed specimens) 5.9 cm (5.3–6.9, $n=4$); body cylindrical truncated abruptly at the rear end; average number of segments 163 (150–171, $n=4$). Average weight (fixed specimens), 2.7 g (2.5–2.9, $n=4$). Prostomium proepilobous, closed. Longitudinal furrows in segments 1 and 2. Dorsal pores absent; bilateral celomic pores in C, first pair in 13/14. Nephridial pores aligned, in 1/4 B. Spermathecal pores at intersegmental furrows 9/10(10/11)–(11/12)12/13 in C. Male pores in segment 15, surrounded by a well-developed porophore. Female pores in segment 14. Clitellum saddle-shaped in segments (XXII) XXIII–XXXII (XXXIII). *Tubercula pubertatis* indistinct in (XXV) XXVI (XXVII)–XXXI (XXXII). *Setae* very small and closely paired. Genital papillae/chaetophores in (XIII, XIV, XXV) XXVI (XXXI, XXXIII), strongly developed in XXXIV.

Internal anatomy Septa 5/6–9/10 thickened and muscular. Lateral hearts in segments VII–XI. Esophagus with villi projecting into the lumen. Calciferous glands in segments 1/2 X–XV, with no diverticula but dilated in segment X. Crop in segments XVI–XVII, gizzard in segments XIX–XX. Typhlosole pinnate. Two pairs of well-developed seminal vesicles in segments XI and XII. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Four (rarely three) pairs of globular intraparietal/supraparietal spermathecae in intersegments 9/10(10/11)–(11/12)12/13, simple. Nephridial bladders digitoid.

Distribution — *Diporodrilus telti* sp. nov. was found in a single location, in a sclerophyllous forest near Monte Telti, Sardinia.

Discussion

Systematic implications

The status of Diporodrilidae either as an independent family within Lumbricoidea or as a subfamily of Lumbricidae has been a matter of discussion since the description of its single genus, *Diporodrilus* (Bouché, 1970). Marchán et al. (2022b) obtained support for both hypotheses through the use of anchored hybrid enrichment (a phylogenomic technique); a single representative of *Diporodrilus* was included, though. The inclusion of a wider representation of the extant diversity of *Diporodrilus* has shown a different outlook. The length of the branches separating *Diporodrilus* and Lumbricidae are significantly longer than the branches separating the earliest branching lumbricid taxa, and *Diporodrilus* is older than all but one Lumbricoidea genera. This, together with the strong morphological differences in the base body plan of *Diporodrilus* and Lumbricidae (two rows of lateral celomic pores vs. one row of dorsal pores, nephridial vesicles which open to the exterior both through nephropores and shared collector channels vs through nephropores alone), offer enough support to confidently state that Diporodrilidae constitutes an independent family.

The low diversity of this family compared to Lumbricidae and Hormogastridae suggests that this family can be considered a relic. Yet, that diversity had been grossly underestimated due to *Diporodrilus* containing cryptic lineages and pseudocryptic species.

The existence of cryptic/pseudocryptic diversity within such an ancient genus is surprising, considering the long evolutionary time available for the development of morphological diversification. This could be explained by a very

restrictive ecological niche which would constrain phenotypic evolution (Smith et al., 2011) or by low interspecific competition (exacerbated by the insular isolation) not being an important driver of ecological and morphological diversification within this genus.

As in Marchán et al. (2020, 2023), integrative taxonomy including genetic information and inconspicuous or quantitative characters allowed to distinguish and describe five new species within the *Diporodrilus pilosus* morphospecies. It is not the first time that keen traditional earthworm taxonomists (Bouché, 1972) observed wide morphological variability within a species which has been later revealed to contain species-level lineages (see also the description of two "morphotypes" of *Lumbricus terrestris* in Bouché and Beugnot (1972) confirmed as different species by molecular methods in James et al. (2010)). The presence of an endemic, (apparently) endogeic earthworm species across Corsica and Sardinia also rose suspicions about it representing a single taxon. For *Diporodrilus*, poorly valued characters such as position of genital papillae or body size has proven again to hold important taxonomic information. As the biology of this earthworm group is still poorly known (including the biochemistry and function of their striking secretion), it is possible that other physiological or ecological differences exist between the newly described pseudocryptic species.

The discovery and description of *Di. rotundus* sp. nov., *Di. jorgei* sp. nov., *Di. minor* sp. nov., *Di. meridionalis* sp. nov. and *Di. telti* sp. nov. have several implications involving alpha taxonomy, soil ecology and soil diversity conservation.

The islands of Corsica and Sardinia remain understudied for earthworm diversity, as reflected by the almost threefold increase in diversity of *Diporodrilus*. While Marchán et al. (2022a) provided a comprehensive diversity survey of northern and eastern Corsica, a large part remains to be sampled and studied with the same approach, as is the larger island of Sardinia. It is to be expected that further species of *Diporodrilus* (but also of other endemic or native genera such as *Scherotheca*, *Hormogaster*, *Norana* and *Pietromodeona*) remain to be found and described.

Diporodrilus pilosus and the five new species possess a significant body size range, and they have been found in very different habitats: moderate altitude maritime pine forests, riparian forests and near-sea level Mediterranean chaparrals. This hints at some degree of ecological specialisation amongst them, yet their digging and feeding behaviour (which could be summed up by their functional group) have not been described even for the previously known species. Hence, further work remains to be done in order to understand the role of these earthworms in the unique ecosystems of Corsica and Sardinia.

The species of *Diporodrilus* appear to be relevant targets for conservation efforts, as they are highly endemic, geographically restricted and possess high evolutionary uniqueness. Rare, at-risk species perform well as indicator of biodiversity (Lawler et al., 2003), hence preserving *Diporodrilus* may be effective at targeting a wide range of taxa belonging to soil communities. The fragmentary knowledge on their distribution and ecological preferences precludes any conservation status assessment, which should be a target for Italian and French conservation agencies.

Historical biogeography of Corso-Sardinian earthworms

Time-calibrated phylogeny have shown *Diporodrilus* as the most ancient endemic genus of Corso-Sardinian earthworms. Sensationalist as it may sound, it is highly likely that the common ancestor of all *Diporodrilus* burrowed below the feet of the last dinosaurs. This is a reminder of how different the biotic, climatic and paleogeographic setting in which *Diporodrilus* evolved would have been: *Diporodrilus* could be a rare, precious legacy of that geological time.

Several earthworm clades (*Diporodrilus*, *Hormogaster* and *Norana*) evolved independently and diversified from their closest relatives while Corsica and Sardinia were part of the same landmass as the Iberian Peninsula and Southern France (before early Oligocene—30 Mya—Siravo et al., 2023). Interestingly, *Diporodrilus* currently possess no close relatives in the mainland, making difficult to pinpoint the ancestral area of the Lumbricoidea lineage leading to them. In contrast, *Hormogaster* (which possess no mainland species in Spain or France) is closely related to *Norana* (Marchán et al., 2018), which is distributed in Southern Sardinia and northeastern Iberian Peninsula (Catalonia). This suggest that the common ancestor of both genera lived in the area between Catalonia and Sardinia in their pre-Oligocene emplacement. A distinct lineage within *Scherotheca* had already started evolving in the area comprising southern Provence and Corsica (as shown by the divergence between *Scherotheca portcrosana* Marchán et al., 2020 and Corso-Sardinian *Scherotheca*) before the onset of break-off and rotation of the microplate, but its internal diversification could have been coeval with that geological process. The same can be said for the *Ho. redii* and *Hormogaster samnitica* Cognetti de Martis, 1914 complexes (known to harbour species-level lineages—Novo et al., 2015): their root and subsequent cladogenetic events strongly overlap with Corso-Sardinian rifting and drifting. There are few studies relating the intra- and interspecific diversification of earthworm groups to the phenomenon of insularity (but see Aspe & James, 2018): insular *Scherotheca*, *Ho. redii* and *Ho. samnitica* appear as ideal models for this line of research.

The pattern observed for Corso-Sardinian earthworms appear to differ from what is known of the islands' endemic fauna: according to Grill et al. (2007), lizards, salamanders, butterflies and beetles showed estimated divergences with

their closest relatives younger than the separation of the Corso-Sardinian microplate from the continent. Gattolliat et al. (2015) obtained similar estimations for several mayflies belonging to different genera. A possible explanation for this difference could be that those vertebrates and arthropods may mainly speciate in presence of physical barriers (i.e. reduced overseas dispersal) while earthworms usually present population structure and species-level divergence at small scale and in the absence of dispersal barriers (Marchán et al., 2017; Novo et al., 2010).

A close correlation between geological and cladogenetic events has been found for Corso-Sardinian earthworms, as previously found for other earthworms (Fernández et al., 2013). Novo et al. (2015) already hypothesised that the split between *Norana pretiosa* (Michaelsen, 1899) and *Norana najaformis* (Qiu & Bouché, 1998) could be a vicariant event related to the separation of their ranges. Some authors argued against the use of such an event to calibrate ultrametric phylogenies, as divergence could have predated the geological event and be unrelated to it. Yet, our results showed that the split between the Catalan/Sardinian sister species strongly overlap with the break-off of both terranes even if no geological calibration is implemented. Other significant events which match with corresponding splits are the end of Corsica rotation and its collision with the Apennines 18 Mya (Rosenbaum et al., 2002) and the dispersal of *Ho. samnitica* into Elba and mainland Italy, or the Messinian Salinity Crisis and the split between Sardinian and Algerian *Ho. redii*, with both being amongst the few windows of opportunity for those lineages to colonize the mainland.

In the light of such close ties between biogeography and paleogeography, distribution of Corso-Sardinian earthworms has potential to inform standing geological controversies. An outstanding example is the recent hypothesis of an independent Southern Sardinia which rotated and collided with a Northern Sardinia-Corsica block between 30 and 21 Mya (Siravo et al., 2023) at the Nuoro fault. This fault has a striking correspondence with the biogeography of Corso-Sardinian earthworms: *Diporodrilus* and *Eu. gabriellae* (and by extension, other insular *Scherotheca*) are not present to the south of this landmark, and *Norana* is absent to the north of the fault. The only endemic genus present at both sides of the fault is *Hormogaster*: even then, *Ho. samnitica* does not appear to the south of Nuoro fault, and there is a deep split between the southern and central–northern populations of *Ho. redii* (which suggest they could have crossed the fault after the collision).

Pietromodeona janueargenti (Cognetti de Martis, 1903), *Ho. redii* and *Ho. samnitica* (further populations from Tuscan islands, mainland Italy, Tunisia and Malta) and the mysterious *Eumenescolex simplex* (Zicsi, 1981) (only known from Matese, continental Italy) should be either more densely sampled or included in this framework to obtain more knowledge about the timing of their dispersal during the complex paleogeographic events of the Western Mediterranean in the Tertiary, including the MSC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13127-024-00639-w.

Acknowledgements A large part of the material examined during this study was collected during the naturalist expedition, "Our Planet Reviewed – Corsica 2019-2021". This survey was organised by the Muséum national d'Histoire naturelle (MNHN, Paris) in collaboration with and funded by the Collectivité de Corse and the Office Français de la Biodiversité (OFB). We are grateful to logistical partners who assisted with fieldwork in 2020: the Office de l'Environnement de la Corse (OCIC and CBNC), the Direction Régionale de l'Environnement de l'Aménagement et du Logement (DREAL) and the Conservatoire du Littoral (CdL). We are also grateful to Rodolphe Rougerie for coordinating DNA barcoding, and Evgeny Zakharov, director of Canadian Centre for DNA barcoding at CBG (University of Guelph, Canada), Nadya Nikolova, Suresh Naik and the laboratory staff for their support in DNA barcoding.

Author contributions DFM, TD and MN contributed to the study conception and design. Material preparation and data collection were performed by DFM, AMN, SG, TD and MN. Analysis were performed by DFM. The first draft of the manuscript was written by DFM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. DFM was supported by a Make Our Planet Great Again Postdoctoral grant from Campus France (mopga-postdoc-3—6111272103) and a María Zambrano Postdoctoral grant from the Spanish Government. MN was supported by Ramon y Cajal Fellowship (RYC2018-024654-I) from MCIN/AEI/https://doi.org/10.13039/501100011033. This research was funded by Grant PID2021-122243NB-I00, from MCIN/AEI/https://doi.org/10.13039/501100011033/FEDER.

Data availability We studied 82 specimens of *Diporodrilus* from Corsica (France) and Sardinia (Italy). Voucher specimens were deposited in the earthworm collections of Eco&Sols (INRAE, Montpellier, France), University of Vigo (Spain) and Department of Biodiversity, Ecology and Evolution, Complutense University of Madrid (UCM-LT, Madrid, Spain), as well as the National Natural History Museum (MNHN, Paris, France). In addition, all nucleotide sequences generated in this study were deposited in GenBank (accessions XX).

Declarations

Consent for publication All authors approved the final version of the manuscript for publication.

Competing interests The authors declare no competing interests.

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