French Mediterranean islands as a refuge of relic earthworm species: Cataladrilus porquerollensis sp. nov. and Scherotheca portcrosana sp. nov. (Crassiclitellata, Lumbricidae)

Daniel F Marchan, Thibaud Decaens, Darío J. Diaz Cosin, Mickael Hedde, Emmanuel Lapied, Jorge Domínguez

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French Mediterranean islands as a refuge of relic earthworm species: *Cataladrilus porquerollensis* sp. nov. and *Scherotheca portcrosana* sp. nov. (Crassiclitellata, Lumbricidae)

Daniel F. MARCHÁN [1,3,*], Thibaud DECAËNS [2,*], Darío J. DÍAZ COSÍN [3],
Mickaël HEDDE [4], Emmanuel LAPIED [5] & Jorge DOMÍNGUEZ [6]

[1,6] Grupo de Ecoloxía Animal (GEA), Universidade de Vigo, E-36310 Vigo, Spain.
[2] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France.
[3] Department of Biodiversity, Ecology and Evolution, Faculty of Biology,
Universidad Complutense de Madrid, Madrid, Spain.
[4] UMR Eco&Sols, INRAE–IRD–CIRAD–SupAgro Montpellier, 2 Place Viala,
34060 Montpellier Cedex 2, France.
[5] Taxonomia Biodiversity Fund, 7 rue Beccaria, 72012, Paris, France.

*Corresponding authors: daniel.fernandez.marchan@uvigo.es; thibaud.DECAENS@cefe.cnrs.fr
[3] Email: dadico@ucm.es
[4] Email: mickael.hedde@inra.fr
[5] Email: elapied@yahoo.com
[6] Email: jdguez@uvigo.es

[1] urn:lsid:zoobank.org:author:3B3731B6-B5FB-409A-A7A3-99FD0F96D688
[2] urn:lsid:zoobank.org:author:B61F61B2-3012-4526-8FF9-DC94D372AF77
[3] urn:lsid:zoobank.org:author:38538B17-F127-4438-9DE2-F9D6C597D044
[4] urn:lsid:zoobank.org:author:F4A219F7-7E75-4333-8293-3004B3CD62C5
[5] urn:lsid:zoobank.org:author:B1FB8658-DFC3-481C-A0BE-B8488A018611
[6] urn:lsid:zoobank.org:author:167575D5-D2CC-4B37-8B1D-0233E6B154E5

Abstract. The area comprising the Pyrenees, Northeast Spain, Southern France and Corsica-Sardinia supports a large part of the diversity of Lumbricidae earthworms, including most species of the endemic genera *Prosellodrilus*, *Cataladrilus* and *Scherotheca*. In this region, the probability of encountering new species for science is significant, especially in scarcely sampled localities. In this study, we describe two unidentified species recently collected in the Hyères Archipelago (France), which we assigned to the genera *Cataladrilus* and *Scherotheca* based on morphological characters and molecular phylogenetic analyses. Other species of *Scherotheca* from Montpellier (including the type species of the genus, *Sc. gigas gigas*) were included in the analysis to clarify their conflicting systematics. A reduced molecular marker set (*COI*, 16S, 28S and *ND1*) proved as successful as larger marker sets for identifying phylogenetic relationships within the Lumbricidae. Remarkable disjunctions between both *Cataladrilus porquerollensis* Marchán & Decäens sp. nov., *Scherotheca portcrosana* Marchán & Decäens sp. nov. and their most closely related relatives, suggesting a strong influence of paleogeographic events on the earthworm fauna of the area and a possible role of near-shore islands as refugia for relict taxa. Genetic distances and branch lengths supported the elevation of some subspecies of *Scherotheca* to

specific status as well as the retention of other subspecies, highlighting the importance of testing for such delimitation with molecular methods.

Introduction

In the Palearctic, Lumbricidae Rafinesque-Schmaltz, 1815 is the most diverse and widely distributed native earthworm family, including 44 genera and around 670 species (Blakemore 2008) (but around 45 species have been described since then). Due to the large number of endemic species and genera, this family is believed to have originated and diversified in southwestern Europe (Omodeo & Rota 2008). Several genera, including *Prosellodrilus* Bouché, 1972 and *Scherotheca* Bouché, 1972, occur in the Pyrenees, Northeast Spain (Catalonia, Aragón, Navarra), Southeastern France (Occitanie, Provence-Alpes-Côte d'Azur), Corsica-Sardinia and northeastern Italy (Qiu & Bouché 1998a, 1998c), hinting at a paleogeographic link between these terranes before the Oligocene–Miocene rifting and breakup (Bache *et al.* 2010).

The genus *Prosellodrilus* comprises around 32 species and subspecies (Blakemore 2012). This genus is subdivided into a conflictive subgenus system, proposed by Qiu & Bouché (1998a) and Baha & Berra (2001): *Prosellodrilus*, *Pyrenodrilus* Qiu & Bouché, 1998, *Kenleenus* Qiu & Bouché, 1998 and *Maghrebiella* Baha & Berra, 2001. Those subgenera were later elevated to genus-status by Blakemore (2012), with *Maghrebiella* amended to *Cadanera* Blakemore, 2012. A closely related genus with a more restricted range is *Cataladrilus* Qiu & Bouché, 1998. *Cataladrilus* comprises eight species distributed in the Eastern Pyrenees (Catalonia, Andorra and Pyrénées-Orientales) and two species of the subgenus *Latisinella* Qiu & Bouché, 1998 found in Soria (Spain) more than 200 km away from the main range of the genus (Qiu & Bouché 1998b). A molecular phylogenetic analysis of the family Lumbricidae, including representatives of both genera, supported this close relationship, not identifying them as monophyletic but rather highlighting them as intermixed within a single clade (Domínguez *et al.* 2015). In spite of this, a morphological distinction between them is rather clear, with *Cataladrilus* possessing anterior spermathecae (intersegments 9/10, 10/11) and *Prosellodrilus* possessing posterior spermathecae (between intersegments 12/13 and 14/15).

The genus *Scherotheca* includes 41 species and subspecies (Qiu & Bouché 1998c), half of which occur in Occitanie, Provence-Alpes-Côte d'Azur and Corsica. *Scherotheca gigas* (Dugès, 1828), the type species of the genus, includes several subspecies; most of them were established as species by Qiu & Bouché (1998d). Domínguez *et al*. (2015) included 5 representatives of *Scherotheca* in their phylogeny of Lumbricidae, two of which were unidentified species. In addition, the specimens identified as *Sc. gigas* were collected in Navarra (Spain), which is far removed from the type locality of the species (Montpellier) raising doubts about their identification. Thus, the systematics of this highly diverse genus would benefit from the corroboration provided by a comprehensive molecular phylogenetic analysis, which has not yet been performed.

Despite the intensive research on earthworm diversity in southern France, some endemic species in relatively remote, comparatively weakly explored areas may remain undescribed. One example of such an area is the Hyères Archipelago, which faces the coast of Provence. These continental islands remained

connected to Corsica and Sardinia until Late Chattian–Aquitanian – ca 24 Ma – when the first marine ingression preceded the rotation and drifting of the Corso-Sardinian microplate (Oudet *et al.* 2010). Due to their proximity to the coast, the islands were intermittently connected to the French mainland during the Pleistocene until 11 000–12 000 BC, when they became permanently isolated (Médail *et al.* 2013). The Port-Cros National Park was created in 1963 to protect the Hyères Archipelago, and two of its three main islands (Porquerolles and Port-Cros) constitute its core areas.

Two undescribed earthworm species were collected during a sampling survey of the islands of Porquerolles and Port-Cros (Decaëns *et al.* 2020). Further detailed morphological diagnoses and molecular phylogenetic analyses confirmed the novelty of both taxa, but also revealed that they should be assigned to the genera *Scherotheca* and *Cataladrilus* respectively. The inclusion of closely related congeneric species in the molecular phylogenetic reconstruction provided further insight into the systematics of these genera. In this work we relate the results of both of these morphological and molecular analyses, and propose the formal description of the two new species under the names *Scherotheca portcrosana* Marchán & Decäens sp. nov. and *Cataladrilus porquerollensis* Marchán & Decäens sp. nov.

Material and methods

Specimens, sampling and morphological description

Specimens described in this work were collected in a sampling survey carried out in the Port-Cros National Park (France) in March 2018 (Decaëns *et al.* 2020). The rest of the specimens were collected in Montpellier (France) and its vicinities at different times between 2015 and 2019. The list of species and the localities where they were collected is shown in Table 1.

Earthworms were obtained by soil digging and hand-sorting, rinsed with water and fixed in 100% ethanol to enable further molecular analyses. Species classification and morphological diagnoses were carried using the set of external and internal morphological characters used by Qiu & Bouché (1998a, 1998b, 1998c, 1998d), and following the format established by Domínguez *et al.* (2018). Main external morphological characters were: average length, average number of segments, average weight, pigmentation, type of prostomium, setal arrangement, position of papillae, position of first dorsal pore, nephridial pore arrangement, position and development of male pores, position and development of female pores, position of spermathecal pores, position of clitellum, position of tubercula pubertatis. Main internal anatomical characters were: position of oesophageal hearts, 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, number and position of spermathecae.

Institutional acronyms

Earthworm holotypes and paratypes were deposited in the following institutions:
CEFE   =   Center of Functional and Evolutionary Ecology, Montpellier, France
UCMLT = Earthworm Collection of Universidad Complutense de Madrid, Spain

DNA isolation, sequencing and molecular analyses

Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) from ventral integument samples of approximately 5 × 5 mm. Molecular barcoding (cytochrome c oxidase subunit I – *COI*) was performed for all the specimens of *Ca. porquerollensis* Marchán & Decäens sp. nov. (30) and *Sc. portcrosana* Marchán & Decäens sp. nov. (9) at the Biodiversity Institute of Ontario using the primers from Folmer *et al.* (1994) and following the standard protocol established for the International Barcode of Life project (http://ibol.org/) (see details in Decaëns *et al.* 2016). Regions of the nuclear 28S rRNA and mitochondrial 16S rRNA, NADH dehydrogenase (*ND1*) and *COI* (3348 bp) were amplified for two specimens of *Ca. porquerollensis* Marchán & Decäens sp. nov. and *Sc. portcrosana* Marchán & Decäens

Table 1. List of species studied in this work and their sampling localities.

Species	Location	Coordinates	Habitat
Cataladrilus porquerollensis sp. nov.	Porquerolles island, France; Locality 1 (PQR1)	42.9988º N 6.20025º E	Meadow
	Porquerolles island, France; Locality 2 (PQR2)	42.9955º N 6.20609º E	Vineyard
	Porquerolles island, France; Locality 3 (PQR3)	42.9961º N 6.20393º E	Olive grove
Scherotheca portcrosana sp. nov.	Port-Cros island, France; Locality 1 (PCR3)	43.0042º N 6.39014º E	Evergreen oak forest
	Port-Cros island, France; Locality 2 (PCR6)	43.0119º N 6.39384º E	Mixed pine/ evergreen oak forest
	Port-Cros island, France; Locality 3 (PCR8)	43.0089º N 6.41176º E	Meadow
Scherotheca gigas gigas (Dugès, 1828)	Bois de Montmaur, Montpellier, France	43.6443º N 3.865º E	Pine forest
Scherotheca gigas mifuga Bouché, 1972	Montpellier ECOTRON, Prades le Lez, France	43.6817º N 3.8762º E	Mediterranean garrigue
Scherotheca gigas heraultensis Qiu & Bouché, 1998	Mont de la Gardiole, Vic-la-Gardiole, France	43.49484º N 3.77569º E	Mediterranean garrigue
Scherotheca rhodana Bouché, 1972	IUT de Montpellier, Montpellier, France	43.635411º N 3.853335º E	Urban mixed forest
Scherotheca monspessulensis idica Bouché, 1972	IUT de Montpellier, Montpellier, France	43.635411º N 3.853335º E	Urban mixed forest

sp. nov. and one specimen of *Sc. gigas gigas*, *Sc. gigas mifuga* Bouché, 1972, *Sc. gigas heraultensis* Qiu & Bouché, 1998, *Sc. rhodana* Bouché, 1972 and *Sc. monspessulensis idica* Bouché,1972 using the polymerase chain reaction (PCR), with primers and conditions described in Pérez-Losada *et al.* (2009, 2015). PCR products were purified and sequenced by the C.A.C.T.I Genomics service (University of Vigo).

DNA sequences obtained in this study (including the two new species and the above mentioned *Scherotheca* spp), as well as their associated meta-data and GenBank accession numbers are all available in the public dataset "DS-EWSPPCNP" on the BOLD bioinformatics platform (https://doi.org/10.5883/DS-EWSPPCNP).

Sequences reported by Domínguez *et al.* (2015, 2018), Pérez-Losada *et al.* (2009, 2011, 2015), Paoletti *et al.* (2016) and De Sosa *et al.* (2019), including representatives from most of the Lumbricidae genera and two members of the closest families (Hormogastridae Vejdovsky, 1884 and Criodrilidae Michaelsen, 1900) were downloaded from GenBank and used as a reference dataset. Included species are shown in Appendix 1.

Sequences were aligned with MAFFT ver. 7 (Katoh & Standley 2013) with default settings and concatenated with BioEdit (Hall 1999), resulting in a matrix of 3348 bp. The best fitting evolutionary

model for each partition was selected with jModelTest ver. 2.1.3 (Darriba *et al.* 2012) by applying the Akaike information criterion (AIC; Akaike 1973), and Bayesian information criterion (BIC; Schwarz 1978). GTR+I+G was selected as best-fitting evolutionary model for *COI*, 28S and *ND1*, and HKY+I+G was selected for 16S.

Maximum Likelihood analysis was performed with RaxML-HPC ver. 8 (Stamatakis 2014) as implemented in the CIPRES Science Gateway ver. 3.3 (Miller *et al.* 2010), using GTR+I+G for each data partition with 10 alternative runs and estimating the support for the resulting topologies by 1000 rapid bootstrap replicates. Bayesian Inference of the phylogeny was estimated with MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) as implemented in the CIPRES Science Gateway ver. 3.3. Parameters were set to 50 million generations sampling every 5000th generation (10 000 trees). Two independent runs with four chains each were performed and 20% of the trees were discarded as burn-in. The remaining trees were combined and summarized on a 50% majority-rule consensus tree. Clade support (Bootstrap and Posterior probability) values over 70% and 90% respectively were considered as high (see Marchán *et al.* 2018; De Sosa *et al.* 2019).

Uncorrected average pairwise distances between the newly described species and their closest relatives for the molecular markers *COI* and 16S were calculated in MEGA X (Kumar *et al.* 2018) in order to support their status as separate species.

Results

Molecular phylogenetic analyses

The phylogenetic relationships recovered by the Bayesian Inference (Fig. 1, Appendix 2) and Maximum Likelihood analyses were generally consistent. A clade containing *Cataladrilus* and *Prosellodrilus* was well supported, but the relationship between them was not fully resolved. *Cataladrilus porquerollensis* Marchán & Decäens sp. nov. was recovered as a sister species to *Ca. monticola* Qiu & Bouché, 1998. *Scherotheca portcrosana* Marchán & Decäens sp. nov. was recovered within a well-supported,
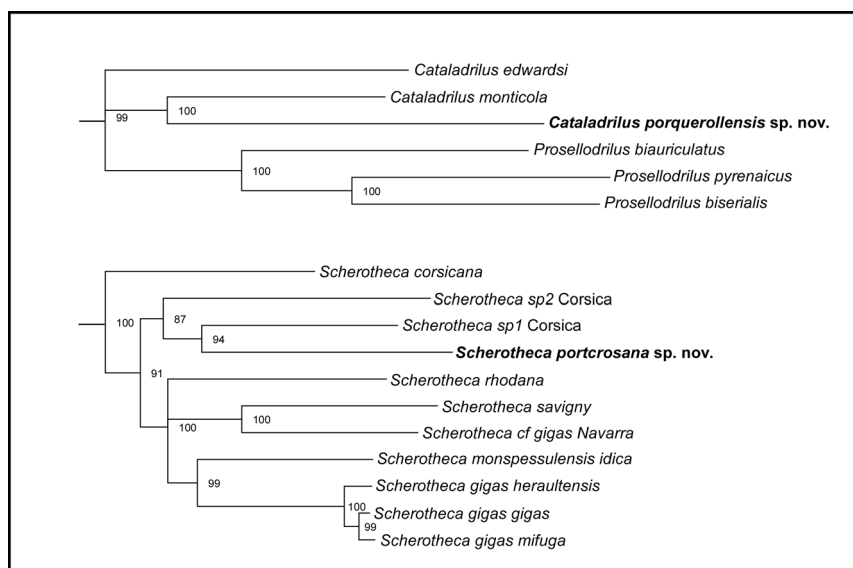
Fig. 1. Detail of the clades including the species *Cataladrilus porquerollensis* Marchán & Decäens sp. nov. and *Scherotheca portcrosana* Marchán & Decäens sp. nov. obtained by Bayesian phylogenetic analysis of the concatenated sequence of molecular markers *COI*–16S–*ND1*–28S. The complete phylogenetic tree is shown in Appendix 2. Posterior probability support values are shown besides corresponding nodes.

monophyletic *Scherotheca*, as a sister to two undescribed species from Corsica. In addition, *Sc. rhodana*, *Sc. monspessulensis idica*, *Sc. gigas gigas* and *Sc. savignyi* Guerne & Horst, 1893 appeared separated from each other by comparatively long branches, while *Sc. gigas heraultensis* and *Sc. gigas mifuga* formed a well-supported, comparatively shallow clade with *Sc. gigas gigas*. The specimen from Navarra identified as *Sc. gigas* by Domínguez *et al*. (2015) was recovered as a sister taxon to *Sc. savignyi.*

Uncorrected Average Pairwise Genetic (UAPG) distances for both the *COI* and 16S molecular markers are given in Table 2. The UAPG distances for *COI* between *Ca. porquerollensis* Marchán & Decäens sp. nov. and its closest relatives (representatives of *Cataladrilus* and *Prosellodrilus*) ranged from 13.4 to 18.3%. The UAPG distance for 16S was noticeably lower with congeneric *Ca. monticola* Qiu & Bouché, 1998 (12.3%) than with species belonging to *Prosellodrilus* (18.5%) and *Scherotheca* (18.6–22%).

The UAPG *COI* distances between *Sc. portcrosana* Marchán & Decäens sp. nov. and other representatives of the genus ranged from 11.8% (*Scherotheca* sp. 1 Corsica) to 14.6% (*Sc. rhodana*). The UAPG 16S distances with congeneric species ranged between 11.8% (*Scherotheca* sp. 1 Corsica) to 15.8% (*Scherotheca* sp. 2 Corsica), the values of which were much lower than the distances with other genera (*Prosellodrilus* and *Cataladrilus*, 18.3–21.3%).

The UAPG distances between *Sc. gigas gigas* and the subspecies *mifuga* and *heraultensis* for the molecular markers *COI* (1.4–3.7%) and 16S (2.9–3.2%) were much lower than with the other species of *Scherotheca* (*COI*: 11.1–12.9%, 16S: 9.3–13.5%).

Systematics

Phylum Annelida Lamarck, 1802
Class Oligochaeta Grube, 1850 / Clitellata Michaelsen, 1919
Order Megadrili Benham, 1890 / Haplotaxida Michaelsen, 1900
Family Lumbricidae Rafinesque-Schmaltz, 1815

Genus ***Cataladrilus*** Qiu & Bouché, 1998

Type species

Cataladrilus monticola Qiu & Bouché, 1998

Diagnosis

Lumbricinae Rafinesque-Schmaltz, 1815 of small to medium size. Longitudinal furrows in peristomium and pygidium. Closely paired or separate chaetae. Aligned nephridial pores. Spermathecal pores simple in 9/10, 10/11 (exception *Cataladrilus multhitecus* Qiu & Bouché, 1998: 7/8–10/11). Male pores in ½ 15 with developed porophores. Calciferous gland in 11–15, usually with diverticles in 11. Gizzard in 17–19 (exception *Cataladrilus annulatus* Qiu & Bouché, 1998: (18)19–21). Typhlosole simple, bifid or multifid. Nephridial bladders U-shaped, reclinate (exception *Cataladrilus multhitecus* Qiu & Bouché, 1998: V-shaped – "fourchué"). Two pairs of seminal vesicles in 11, 12 (exception *Cataladrilus mrsici* Qiu & Bouché, 1998: three pairs in 9, 11, 12).

Cataladrilus porquerollensis Marchán & Decäens sp. nov.
urn:lsid:zoobank.org:act:DE99E6A6-65EF-496A-AEAA-4F6FBAFA19CB
Fig. 2, Table 3

Table 2. Uncorrected average pairwise genetic (UAPG) distances between the species described in this work (*Cataladrilus porquerollensis* Marchán & Decäens sp. nov. and *Scherotheca portcrosana* Marchán & Decäens sp. nov.) and their closest relatives for the molecular markers *COI* (under diagonal) and 16S (over diagonal).

	Ca. porquerollensis sp. nov.	*Ca. edwarsi*	*Ca. monticola*	*Pr. pyrenaicus*	*Pr. biserialis*	*Sc.* sp 2 Corsica	*Sc.* sp 1 Corsica	*Sc. corsicana*	*Sc. portcrosana* sp. nov.	*Sc. gigas gigas*	*Sc. gigas mijuga*	*Sc. gigas heraultensis*	*Sc. monspessulensis idica*	*Sc. rhodana*	*Sc. savignyi*	*Sc.* cf. *gigas* Navarra
Ca. porquerollensis sp. nov.	0.6	–	12.3	18.5	–	21.0	21.9	19.7	20.8	22.0	22.6	21.4	18.6	18.8	19.4	19.4
Ca. edwarsi	13.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ca. monticola	14.1	13.6	–	13.7	–	15.5	15.9	16.2	18.3	19.7	19.5	18.4	15.7	14.1	17.2	15.3
Pr. pyrenaicus	15.9	15.6	14.7	–	–	20.4	20.5	20.1	21.3	24.7	24.3	24.3	19.4	19.6	22.5	20.0
Pr. biserialis	18.3	14.2	14.3	15.2	–	–	–	–	–	–	–	–	–	–	–	–
Sc. sp 2 Corsica	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sc. sp 1 Corsica	14.4	15.3	17.1	16.4	–	–	–	13.0	11.8	12.1	12.4	11.3	12.5	11.8	12.6	12.7
Sc. corsicana	15.3	13.6	15.2	17.4	–	–	13.0	–	13.1	11.7	11.9	12.0	11.9	13.0	12.7	11.9
Sc. portcrosana sp. nov.	15.5	12.3	13.7	15.2	–	–	11.8	13.1	0.2	12.9	13.0	12.3	12.4	14.6	13.8	12.8
Sc. gigas gigas	15.8	14.9	16.9	17.4	–	–	12.1	11.7	12.9	–	1.4	3.7	11.1	12.0	12.2	12.6
Sc. gigas mijuga	16.4	14.9	17.8	17.6	–	–	12.4	11.9	13.0	1.4	–	3.4	11.0	12.3	11.9	12.1
Sc. gigas heraultensis	17.6	14.8	17.1	16.8	–	–	11.3	12.0	12.3	3.7	3.4	–	11.6	12.4	13.1	12.1
Sc. monspessulensis idica	16.0	15.8	16.1	17.2	–	–	12.5	11.9	12.4	11.1	11.0	11.6	–	12.2	12.4	11.3
Sc. rhodana	15.0	16.5	15.9	17.2	–	–	11.8	13.0	14.6	12.0	12.3	12.4	12.2	–	13.4	12.2
Sc. savignyi	16.3	15.1	16.7	18.8	–	–	12.6	12.7	13.8	12.2	11.9	13.1	12.4	13.4	–	9.0
Sc. cf. *gigas* Navarra	15.7	16.0	16.0	15.4	–	–	12.7	11.9	12.8	12.6	12.1	12.1	11.3	12.2	9.0	–

Diagnosis

Specimens of *Cataladrilus porquerollensis* Marchán & Decäens sp. nov. can be distinguished from the other known species of *Cataladrilus* by the position of the clitellum in segments (19)20–32,33(34) and tubercula pubertatis in segments (28)29–31 (Table 3). They resemble *Cataladrilus* (*Latisinella*) *mrsici* in the position of tubercula pubertatis and small body size, but differ in the position of the clitellum (22–32 in *Ca. mrsici*), two pairs of seminal vesicles (three in *Ca. mrsici*) and paired chaetae (separate in *Ca. mrsici*).

Etymology

The species name is derived from Porquerolles, the island where this species was found.

Material examined

Holotype

FRANCE • adult; Provence-Alpes-Côte d'Azur, Var, Porquerolles Island; 42.9961º N, 6.20393º E [Locality 3 (PQR3) (Table 1)]; 14 Mar. 2018; T. Decaëns, E. Lapied, M. Hedde and M. Zwicke leg.; olive grove; BOLD Sample ID: EW-PNPC-0098; UCMLT.

Paratypes

FRANCE – **Provence-Alpes-Côte d'Azur** • 10 specs; same collection data as for holotype; BOLD SampleID: EW-PNPC-0036, EW-PNPC-0037, EW-PNPC-0038, EW-PNPC-0039, EW-PNPC-0040, EW-PNPC-0096, EW-PNPC-0097, EW-PNPC-0099, EW-PNPC-0100, EW-PNPC-0268; UCMLT • 10 specs; same collection data as for holotype; BOLD SampleID: EW-PNPC-0041, EW-PNPC-0042, EW-PNPC-0043, EW-PNPC-0044, EW-PNPC-0045, EW-PNPC-0101, EW-PNPC-0102, EW-PNPC-0103, EW-PNPC-0104, EW-PNPC-0105; CEFE • 1 spec.; Var, Porquerolles Island; 42.9988º N, 6.20025º E [Locality 1 (PQR1) (Table 1)]; 14 Mar. 2018; T. Decaëns, E. Lapied, M. Hedde and M. Zwicke leg.; meadow; BOLD SampleID: EW-PNPC-0173; CEFE • 6 specs; Var, Porquerolles Island; 42.9955º N, 6.20609º E [Locality 2 (PQR2) (Table 1)]; 14 Mar. 2018; T. Decaëns, E. Lapied, M. Hedde and M. Zwicke leg.; vineyard; BOLD SampleID: EW-PNPC-0127, EW-PNPC-0128, EW-PNPC-0129, EW-PNPC-0130, EW-PNPC-0131, EW-PNPC-0132; UCMLT • 2 specs; same collection data as for preceding; BOLD SampleID: EW-PNPC-0133, EW-PNPC-0134; CEFE.

Morphological description

External morphology

Body pigmentation absent in live specimens. White-beige homogeneous color in fixed specimens (Fig. 2).

Average length 48 mm (41–51 mm, n = 5 adults); diameter 2 mm (1.8–2.2 mm, n = 5 adults); body cylindrical in cross-section; average number of segments 196 segments (166–239, n = 5 adults; 239 segments in the holotype). Average weight (alcohol fixed specimens): 0.14 g (0.12–0.17 g, n = 5 adults). Prostomium epilobous. Longitudinal furrows in first 2–3 segments. First dorsal pore at the intersegmental furrow 10/11–11/12. Nephridial pores aligned in *b*. Spermathecal pores at the intersegmental furrows 9/10, 10/11 in *c*. Male pores in segment 15, surrounded by a well-developed porophore. Female pores inconspicuous. Clitellum saddle-shaped in segments (19)20–32,33(34). Tubercula pubertatis in segments (28)29–31. Chaetae very small and closely paired, with interchaetal ratio *aa*: 9, *ab*: 1, *bc*: 3, *cd*: 1, *dd*: 7 at segment 40. Chaetophores/genital papillae in segments 11, 13.

Internal anatomy

Septa 5/6–9/10 thickened and muscular. Hearts in segments 6–11, oesophageal. Calciferous glands in segments 11–13, without clear diverticles or enlargements. Crop in segments 15–16, gizzard in segments

Table 3. Morphological characters of *Cataladrilus porquerollensis* Marchán & Decäens sp. nov. and morphologically similar relatives within the genera *Cataladrilus* Qiu & Bouché, 1998 and *Prosellodrilus* Bouché, 1972. Length expressed in mm, weight expressed in mg.

Characters	*Cataladrilus porquerollensis* sp. nov.	*Cataladrilus (Latisinella) mrsici*	*Cataladrilus albus*	*Prosellodrilus dactylothecus*
Length	45–51	35	120–145	92
N. segments	196	168	229–297	207
Weight	140	102	1526	409
Pigmentation	Absent	Absent	Absent	Absent
Prostomium	Epilobous	Epilobous	Epilobous	Epilobous
Setae	Closely paired	Separate	Closely paired	Closely paired
Setal arrangement	9:1:3:1:7	5:5:5:4:8	15:1:5:1:15	20:1:10:1:30
Papillae	11, 13	Absent	28–34	13, 14, 15
First dorsal pore	10/11–11/12	6/7	6/7	5/6
Nephridial pores	Aligned, *b*	Aligned, *b*	Aligned, *b*	Aligned, *b*
Male pores	½ 15, *b*	½ 15, *b*	½ 15, *b*	⅔ 15
Female pores	½ 14, inconspicuous	½ 14, *b*	½ 14, *b*	½ 14, *b*
Spermathecal pores	9/10, 10/11 simple in *c*	9/10, 10/11 simple in *c*	9/10, 10/11 simple in *d*	13/14, 14/15 in *c*
Clitelum	(19)20–32, 33(34)	22–32	22–34	19–32
Tubercula pubertatis	(28)29–31	29–31	(½ 29)30–34	22–25
Oesophageal hearts	6–11	6–11	6–11	6–11
Calciferous glands	11–13	11–14, dilated in 12, 13	11–14, diverticles in 11	11–14, diverticles in 11
Crop	15–16	15–16	15–16	15–16
Gizzard	17–18	17–18	17–18	17–18
Typhlosole	Bifid	Simple	Bifid	Bifid
Nephridial bladders	U-shaped, reclinate	U-shaped, reclinate	U-shaped, reclinate	U-shaped, reclinate, proximal ampulla
Seminal vesicles	11, 12	9, 11, 12	11, 12	11, 12
Spermathecae	Simple, globular in 10, 11	Simple, pyriform in 10, 11	Simple, small globular in 10, 11	Simple, pedunculate in 14, 15
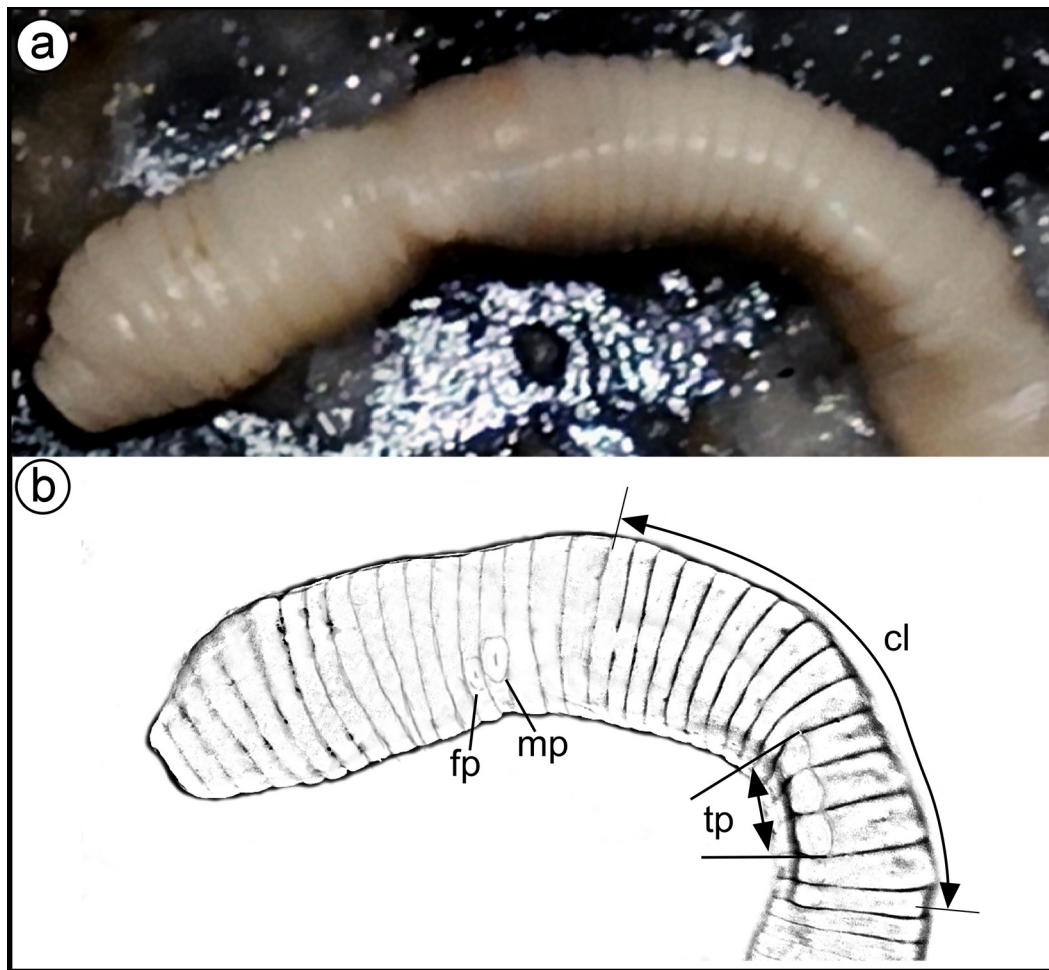
Fig. 2. External morphology of *Cataladrilus porquerollensis* Marchán & Decäens sp. nov. **a**. Fixed specimen. **b**. Schematic drawing. Abbreviations: cl = clitellum; fp = female pore; mp = male pore; tp = tubercula pubertatis.

17–18. Typhlosole bifid with a small intermediate ridge of varying development. Male sexual system holandric, testes and funnels (not enclosed in testes sacs, but with sperm present) located ventrally in segments 10 and 11. Two pairs of small seminal vesicles in segments 11 and 12, with free seminal masses filling most of these segments. Ovaries and female funnels in segment 13, ovarian receptacles (ovisacs) in segment 14. Two pairs of globular, intracelomic spermathecae in segments 9 and 10 (intersegments 9/10, 10/11). Nephridial bladders U-shaped, reclinate in segments 14, 20.

Distribution and ecology

Cataladrilus porquerollensis Marchán & Decäens sp. nov. is known from the island of Porquerolles in the Hyères Archipelago, France. This species has been found in meadows, vineyards and olive groves, thus appears to inhabit moderately to highly anthropized habitats.

Genus *Scherotheca* Bouché, 1972

Type species

Scherotheca gigas Dùges, 1828.

Diagnosis

Lumbricinae of large to very large size, post-clitellar trapezoidal section. Pigmentation brown to dark brown. Prostomium epilobous, closed. Longitudinal furrows in the peristomium. Nephridial pores "en solfège" (irregularly distributed). Spermathecal pores at least in two intersegments, between 9/10 and 13/14, or 13/14 and 15/16, sometimes multiple. Anterior septa strongly thickened. Male pores in ½ 15, usually with porophores. Gizzard in 17–20 (21, 22). Typhlosole pinnate. Two or four pairs of seminal vesicles in (9, 10) 11, 12.

Scherotheca portcrosana Marchán & Decäens sp. nov.
urn:lsid:zoobank.org:act:663F18D2-A2EA-4B4E-8ED3-55A3FE5B7D57
Fig. 3, Table 4

Diagnosis

Specimens of *Scherotheca portcrosana* Marchán & Decäens sp. nov. can be distinguished from other known species of *Scherotheca* by the position of the clitellum in segments 26–35 (½ 36) and tubercula pubertatis in segments 30–33, position of spermathecae in 12, 13 in addition to a smaller body size and faint pigmentation (Table 4).

Etymology

The species name is derived from Port-Cros, the island inhabited by this species.

Material examined

Holotype
FRANCE • adult; Provence-Alpes-Côte d'Azur, Var, Port-Cros Island; 43.0089º N, 6.41176º E [Locality 3 (PCR8) (Table 1)]; 13 Mar. 2018; T. Decaens, E. Lapied, M. Hedde and M. Zwicke leg.; meadow; BOLD Sample ID: EW-PNPC-0174; UCMLT.

Paratypes
FRANCE – **Provence-Alpes-Côte d'Azur** • 1 spec.; Var, Port-Cros Island; 43.0042º N, 6.39014º E [Locality 1 (PCR3) (Table 1)]; 13 Mar. 2018; T. Decaens, E. Lapied, M. Hedde and M. Zwicke leg.; evergreen oak forest; BOLD SampleID: EW-PNPC-0111; UCMLT • 5 specs; same collection data as for preceding; BOLD SampleID: EW-PNPC-0117, EW-PNPC-0119, EW-PNPC-0120, EW-PNPC-0121, EW-PNPC-0122; CEFE • 1 spec.; Var, Port-Cros Island; 43.0119º N, 6.39384º E [Locality 2 (PCR6) (Table 1)]; 13 Mar. 2018; T. Decaens, E. Lapied, M. Hedde and M. Zwicke leg.; mixed pine/evergreen oak forest; BOLD SampleID: EW-PNPC-0237; UCMLT • 1 spec.; same collection data as for preceding; BOLD SampleID: EW-PNPC-0242; CEFE.

Morphological description

External morphology
Body pigmentation very faint brown-grey. White-beige with dorsal brownish mid-segment brown bands in fixed specimens (Fig. 3).

Average length 80 mm (75–85 mm, n = 2 adults); average diameter 7 mm (6.9–7.1 mm, n = 3 adults); body cylindrical in cross-section; average number of segments 163 (160–166, n = 2 adults; 166 segments in the holotype). Average weight (fixed specimens): 2.13 g (1.89–2.36 g, n = 2 adults). Prostomium epilobous, closed. Longitudinal furrows in segments 1 and 2. First dorsal pore at intersegmental furrow 5/6. Nephridial pores "en solfège" (irregularly distributed). Spermathecal pores at intersegmental furrows 12/13 and 13/14 in *c*. Male pores in segment 15, surrounded by a

well-developed porophore. Female pores on segment 14. Clitellum saddle-shaped in segments 26–35 (½ 36). Tubercula pubertatis in segments 30–33. Chaetae small and closely paired, with interchaetal ratio *aa*: 8, *ab*: 1, *bc*: 3.5, *cd*: 1, *dd*: 18 at segment 40. Chaetophores/genital papillae in segments 11, 12, 14, 27, 29 and 34–38.
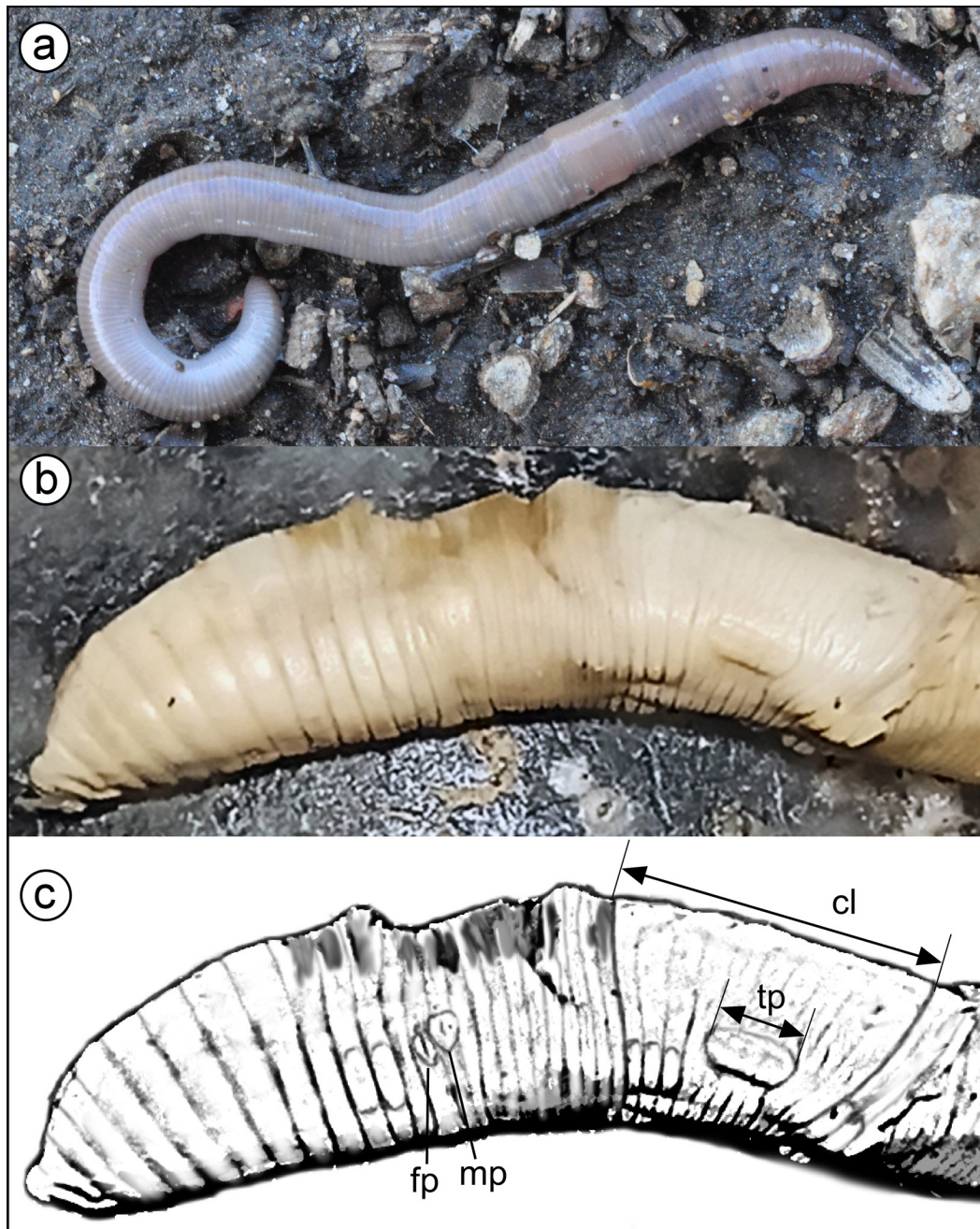
Fig. 3. External morphology of *Scherotheca portcrosana* Marchán & Decäens sp. nov. **a**. Live specimen (© T. Decaëns). **b**. Fixed specimen. **c**. Schematic drawing. Abbreviations: cl = clitellum; fp = female pore; mp = male pore; tp = tubercula pubertatis.

Table 4. Morphological characters of *Scherotheca portcrosana* Marchán & Decäens sp. nov. and morphologically similar relatives of genera *Scherotheca* Bouché, 1972 and *Eumenescolex* Qiu & Bouché, 1998. Length expressed in mm, weight expressed in mg.

Characters	*Scherotheca portcrosana* sp. nov.	*Scherotheca corsicana corsicana*	*Scherotheca portonana*	*Eumenescolex simplex*
Length	75–85	90–140	78–165	105–112
N. segments	160–166	177–215	193–208	99–154
Weight	1890–2360	2000–3500	1588–2821	?
Pigmentation	Brown (faint)	Dark brown	Brown	?
Prostomium	Epilobous	Epilobous	Epilobous	Pro-epilobous
Setae	Closely paired	Closely paired	Closely paired	?
Setal arrangement	8:1:3.5:1:18	8:1.5:4.5:1:19	11.6:1.6:8:1:33	?
Papillae	11–14, 27, 29, 34–38	9–11, 27–37	11–14, 34, 35	?
First dorsal pore	5/6	9/10	10/11	11/12
Nephridial pores	En solfège	En solfège	En solfège	?
Male pores	½ 15, *b*	½ 15, *b*	½ 15, *b*	?
Female pores	½ 14, *b*	½ 14, *b*	½ 14, *b*	?
Spermathecal pores	12/13, 13/14 simple in C	12/13, 13/14 simple in C	12/13, 13/14 simple in C	13/14 simple in C
Clitelum	26–35(½ 36)	(½ 26)1/27–½ 36(36)	(25)26–35	26–½ 36
Tubercula pubertatis	30–33	(½ 30) 31–½ 34(34)	(28)29–32(33)	29–½ 33
Oesophageal hearts	6/11	6/11	6/11	6–11
Calciferous glands	½ 10–14, diverticles in 10	½ 10–14, diverticles in 10	½ 10–14, diverticles in 10	?
Crop	15–16	15–16	15–16	?
Gizzard	17–19	17–19	17–19	17–19
Typhlosole	Pinnate	Pinnate	Pinnate	?
Nephridial bladders	U-shaped, reclinate	J-shaped, reclinate	J-shaped, reclinate	?
Seminal vesicles	9, 10, 11, 12	9, 10, 11, 12	9, 10, 11, 12	9, 10, 11, 12
Spermathecae	Simple, oval in 12, 13	Simple, oval in 13, 14	Simple, oval in 13, 14	?

Internal anatomy

Septa 5/6–10/11 thickened and muscular. Hearts in segments 6–11, oesophageal. Calciferous glands in segments 10–14, with diverticula in segment 10. Crop in segments 15–16, gizzard in segments 17–19. Typhlosole pinnate. Male sexual system holandric, testes and funnels (not enclosed in testes sacs, but with sperm present) located ventrally in segments 10 and 11. Four pairs of reniform seminal vesicles in segments 9, 10, 11 and 12, with the latter two pairs being larger. Ovaries and female funnels in segment 13, ovarian receptacles (ovisacs) in segment 14. Two pairs of small globular spermathecae in segments 12 and 13 (intersegments 12/13, 13/14). Nephridial bladders U-shaped, reclinate in segment 30.

Distribution and ecology

Scherotheca portcrosana Marchán & Decäens sp. nov. is known from the island of Port-Cros in the Hyères Archipelago, France. This species has been found in meadows, pine and evergreen oak forests, thus appears to have a preference for natural habitats.

Discussion

Systematic implications

Molecular phylogenetic analyses and genetic distances clearly support the morphology-based inclusion of *Ca. porquerollensis* Marchán & Decäens sp. nov. within the genus *Cataladrilus*. It is worth noting that the relationship between *Cataladrilus* and *Prosellodrilus* is currently not fully resolved based on molecular information, however the morphological characters clearly separate both genera (Table 3) according to the number and position of spermathecae: two pairs in intersegments 9/10, 10/11 for *Cataladrilus* (except for *Ca. multithecus* Qiu & Bouché, 1998) instead of two to three pairs (sometimes multiple) in intersegments (12/13), 13/14, 14/15 for *Prosellodrilus* (Qiu & Boché 1998a, 1998b).

Several species from *Cataladrilus* and *Prosellodrilus* are yet to be included in a detailed molecular phylogenetic analysis, with emphasis on including representatives from the different subgenera (possibly genera for *Prosellodrilus* sensu lato according to Blakemore (2012). This will be necessary to resolve the reciprocal monophyly of both genera and the status of the subgenera/genera included within them.

Cataladrilus porquerollensis Marchán & Decäens sp. nov. resembles *Cataladrilus* (*Latisinella*) *mrsici*, the most in the position of their tubercula pubertatis (segments 29–31) and their clitellum (segments (19)20–32, 33(34) and 22–32 respectively), but they both differ in the total extension of the clitellum, body size, disposition of their chaetae (closely paired vs separate) and number of seminal vesicles (two pairs in segments 11, 12 vs 3 pairs in segments 9, 11, 12). *Cataladrilus porquerollensis* Marchán & Decäens sp. nov shows a remarkable external morphological similarity with *Allolobophora festae* Rosa, 1892 (sometimes attributed to genus *Prosellodrilus*), however, they can be easily distinguished by the position of their spermathecae (9/10, 10/11 vs 12/13, 13/14).

The inclusion of *Sc. portcrosana* Marchán & Decäens sp. nov. within *Scherotheca* is well supported by the phylogenetic trees. While French mainland species of *Scherotheca* appear superficially very different from *Sc. portcrosana* Marchán & Decäens sp. nov. (large body size, intense brown-grey pigmentation, very long clitellum), species of *Scherotheca* from Corsica share with *Sc. portcrosana* Marchán & Decäens sp. nov. their relatively small body size, pale brown pigmentation and comparatively short clitellum. Indeed, molecular analyses revealed a closer affinity of *Sc. portcrosana* Marchán & Decäens sp. nov. with the Corsican *Scherotheca* over the mainland species.

Scherotheca portcrosana Marchán & Decäens sp. nov. appears very close to *Sc. corsicana corsicana* Pop, 1947 and *Sc. portonana* Qiu & Bouché, 1998 according to their similar position of clitellum and tubercula pubertatis (Table 4). However, it differs from both species by the exact position of clitellum

and tubercula pubertatis, as well as being slightly smaller and less pigmented. *Scherotheca portcrosana* Marchán & Decäens sp. nov. also shows a remarkable resemblance to *Eumenescolex simplex* Zicsi, 1981 (initially described as *Sc. corsicana simplex* then transferred due to its single pair of spermathecae), but they clearly differ in the number and position of their spermathecae (in intersegments 12/13, 13/14 vs 13/14). This similarity highlights the importance of including representatives of *Eumenescolex* Qiu & Bouché, 1998 in a molecular phylogenetic framework to test the close relationship among both genera that was previously suggested by Qiu & Bouché (1998d).

Qiu & Bouché (1998d) elevated 9 subspecies of *Scherotheca* to species while maintaining or creating others. Genetic distances within the clade comprising *Sc. gigas gigas* from Montpellier and *Sc. gigas heraultensis* and *Sc. gigas mifuga* are way below the usual threshold established for divergence between conspecific taxa (less than 10% for *COI*, Chang *et al.* 2009), supporting the subspecific status of these taxa; while *Sc. rhodana* (described as *Sc. gigas rhodana*) appears to be as divergent from *Sc. gigas gigas* as from *Sc. monspessulensis idica* or other species of the genus (with genetic distances above the aforementioned threshold). Thus, the morphological criteria followed by Qiu & Bouché (1998d) appear supported by molecular data. However, larger sample sizes would be necessary to confirm this statement. These findings suggest that molecular markers are suitable for delimiting species and subspecies within *Scherotheca*. If this approach performs consistently across different animal taxa, it may be advisable to implement it widely in order to avoid the dismissal of unrecognized species-level taxa in ecological studies and biodiversity assessment (for different examples see Rutgers *et al.* 2016).

Scherotheca cf. *gigas* (from Domínguez *et al.* 2015) collected from Navarra appear to be unrelated to *Sc. gigas gigas* from Montpellier, but closely related to *Sc. savignyi*. These earthworms may belong to one of the former subspecies of *Sc. gigas* such as *Scherotheca aquitania* Bouché, 1972, which is relatively common in Northeastern Spain (Qiu & Bouché 1998d). Preliminary re-inspection of these specimens showed external morphological characters compatible with *Sc. aquitania*. Thus, the inclusion of French specimens of *Sc. aquitania* (ideally from the type locality) would enable confirmation of this hypothesis. This is just one of the many points highlighting the need for a comprehensive molecular phylogeny of the genus *Scherotheca*. Robust systematics of the many species of this genus in northeastern Spain-southern France-Corsica-Italy would contribute to the following: a) a better understanding of the impact on ecosystems of these large, deep burrowing anecic earthworms, which have been found to be important in both agricultural and unmanaged habitats (Gavinelli *et al.* 2018), b) assessment of the conservation status of the genus, with several taxa being narrowly restricted and potentially vulnerable to human impacts (Rida & Bouché 1995) and c) insight into the historical biogeography of native earthworm families in this geologically complex area.

Phylogeographic implications for *Cataladrilus* and *Scherotheca*

The discovery of a species of *Cataladrilus* in the Hyères Archipelago is puzzling from a phylogeographic point of view. The main range of *Cataladrilus* is restricted to Catalonia (Spain) and Andorra, with two species in neighboring locations in France separated from the Hyères Archipelago by 340 km. This disjunct distribution could be explained by a failure to find closely related species in the geographic gap, which would suggest that southeastern France could still harbor other undescribed species. This, however, seems quite unlikely if considering the intense sampling already done by Marcel Bouché at the end of the past century (Bouché 1972). Alternatively, *Ca. porquerollensis* Marchán & Decäens sp. nov. may be a relict from a formerly wider distribution. This would be consistent with paleogeographic events in the area: the Eastern Pyrenees and Provence were connected through an exposed Gulf of Lion up to Late Oligocene–Early Miocene (around 24 Ma) when a wide marine ingression separated them up to the present (except for the duration of the Messinian Salinity Crisis) (Sissingh 2006). The possibility of *Cataladrilus* and *Prosellodrilus* being synonyms (as the lack of reciprocal monophyly may suggest)

would not change the observed disjunction, as the closest species of *Prosellodrilus* (*Pr. tikalus* Qiu & Bouché, 1998) is found 225 km to the west of Porquerolles.

The closer relationship between *Sc. portcrosana* Marchán & Decäens sp. nov. and the Corsican *Scherotheca* than with French mainland *Scherotheca* is also unexpected. However, several species of plants, arthropods and vertebrates are common to the Hyères Archipelago and Corsica (Médail *et al*. 2013). *Scherotheca portcrosana* Marchán & Decäens sp. nov. may have diverged from its relatives before or during the rifting and drifting of the Corso-Sardinian microplate around 24 Ma (Oudet *et al*. 2010). Corsica remained connected to the French mainland through the rotation to its current position (Sissingh 2006), which would have allowed later migration to the Hyères Archipelago of a common ancestor of *Sc. portcrosana* Marchán & Decäens sp. nov. and Corsican *Scherotheca*. However, under this second scenario, one would expect to find other relatives in eastern Provence (where the land bridge was supposed to join both areas).

The area of Provence has been strongly disturbed by human activities, especially in the coastal area, while the small islands like Porquerolles and Port-Cros remained relatively well preserved. It is possible that these islands acted as refugia while continental relatives got extinct or relegated to relict habitats. This phenomenon has already been suggested to explain the distribution of the gecko *Euleptes europaea* Gené, 1839, which is frequent in Corsica and Sardinia and rare in the coastal islands of Provence and Liguria, probably after a range retraction in the mainland (Delauguerre *et al*. 2011). Further survey focusing on relic natural ecosystems in the littoral area (Cap Lardier) or further into the mainland (i.e., Sainte Baume, Plaine des Maures, Massif de l'Esterel) could be performed to test this hypothesis.

Whatever explicative hypothesis is retained, these disjunct distributions, as previously observed in other earthworm genera (Pérez Losada *et al*. 2011; Domínguez *et al*. 2018; Marchán *et al*. 2018), highlight the strong connection between paleogeographic events and earthworm evolution and divergence. A more robust approach to time-calibrated phylogenies in earthworms (hindered by the lack of body fossils) and the integration of different paleogeographic reconstructions could illuminate both the origin of Palearctic earthworms (Hormogastridae, Lumbricidae) and the geological history of the Western Mediterranean terranes from the Late Cretaceous to the Neogene.

Acknowledgments

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Appendix 1 (continued on next page). Species included in the reference dataset.

Species	Publication
Allolobophora chaetophora Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora chlorotica Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora dacica Pop, 1938	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora dubiosa Orley, 1881	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora mehadiensis Rosa, 1895	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora moebii Michaelsen, 1895	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Allolobophora molleri Rosa, 1889	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Allolobophora robusta Rosa, 1895	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Aporrectodea caliginosa Savigny, 1826	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Aporrectodea georgii Michaelsen, 1890	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Aporrectodea jassyensis Michaelsen, 1891	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Aporrectodea limícola Michaelsen, 1890	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Aporrectodea longa Ude, 1885	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Aporrectodea nocturna Evans, 1946	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Aporrectodea rosea Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Aporrectodea trapezoides Duges, 1828	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Aporrectodea tuberculata Eisen, 1874	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Carpetania elisae Álvarez, 1977	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Cataladrilus edwarsi Qiu & Bouché, 1998	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Cataladrilus montícola Qiu & Bouché, 1998	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Cernosvitovia dudichi Zicsi & Sapkarev, 1982	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Cernosvitovia rebeli Rosa, 1897	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Compostelandrilus bercianus Domínguez, Aira, Porto, Díaz Cosín & Pérez-Losada, 2017	Domínguez *et al.* 2018
Compostelandrilus menciae Domínguez, Aira, Porto, Díaz Cosín & Pérez-Losada, 2017	Domínguez *et al.* 2018
Criodrilus lacuum Hoffmeister, 1845	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena attemsi Michaelsen, 1902	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena cf. *biblica* Rosa, 1893	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena illyrica Cognetti de Martiis, 1906	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena jastrebensis Mrsic & Sapkarev, 1987	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena octaedra Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena pentheri Rosa, 1905	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena pygmaea Friend, 1923	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Dendrobaena veneta Rosa, 1886	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Diporodrilus pilosus Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenia andrei Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenia balatonica Pop, 1943	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenia fétida Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenia lucens Vaga, 1857	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eiseniella tetraedra Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015

Appendix 1 (continued). Species included in the reference dataset.

Species	Publication
Eiseniona albolineata Díaz Cosín, Trigo & Mato, 1989	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eiseniona oliveirae Rosa, 1894	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenoides carolinensis Michaelsen, 1910	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eisenoides lonnbergi Michaelsen, 1894	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Eophila crodabepis Paoletti *et al.*, 2016	Paoletti *et al.* 2016, De Sosa *et al.* 2019
Eophila gestroi Cognetti de Martiis, 1905	Paoletti *et al.* 2016, De Sosa *et al.* 2019
Eophila tellinii Rosa, 1888	Paoletti *et al.* 2016, De Sosa *et al.* 2019
Galiciandrilus bertae Díaz Cosín, Mato & Mascato, 1985	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Galiciandrilus morenoe Díaz Cosín, Calvin & Mato, 1985	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Helodrilus cernosvitovianus Zicsi, 1967	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Helodrilus cortezi Qiu & Bouché, 1998	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Helodrilus patriarcalis Rosa, 1893	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Lumbricus castaneus Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Lumbricus rubellus Hoffmeister, 1843	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodriloides boninoi Omodeo, 1962	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodrilus complanatus Duges, 1928	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodrilus exacystis Rosa, 1896	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodrilus gradinescui Pop, 1938	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodrilus pseudocomplanatus Omodeo, 1962	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octodrilus transpadanus Rosa, 1884	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octolasion cyaneum Savigny, 1826	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octolasion lacteum Orley, 1885	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Octolasion montanum Wessely, 1905	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Panoniona Leoni Michaelsen, 1891	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Postandrilus lavellei Qiu & Bouché, 1998	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Postandrilus majorcanus Qiu & Bouché, 1998	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Postandrilus medoakus Qiu & Bouché, 1998	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Postandrilus palmensis Qiu & Bouché, 1998	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Postandrilus sapkarevi Qiu & Bouché, 1998	Pérez-Losada *et al.* 2011, 2015; Domínguez *et al.* 2015
Proctodrilus antipai Michaelsen, 1891	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Prosellodrilus biauriculatus Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Prosellodrilus biserialis Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Prosellodrilus pyrenaicus Cognetti, 1904	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Scherotheca cf. *gigas* Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Scherotheca corsicana Pop, 1947	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Scherotheca savignyi Bouché, 1972	Domínguez *et al.* 2015; Pérez-Losada *et al.* 2015
Scherotheca sp 1	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Scherotheca sp 2	Pérez-Losada *et al.* 2009, 2015; Domínguez *et al.* 2015
Zophoscolex cyaneus Briones & Díaz Cosín, 1993	Domínguez *et al.* 2018

Appendix 2. Phylogenetic tree obtained from the concatenated sequence of *COI*–16S–*ND1*–28S molecular markers.