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## Original article



# Understanding the diversification and functional radiation of *Aporrectodea* (Crassiclitellata, Lumbricidae) through molecular phylogenetics of its endemic species

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## ABSTRACT

The genus *Aporrectodea* includes some of the most conspicuous earthworm species, but its taxonomic history is among the most complex within the family Lumbricidae. Molecular phylogenetic studies have produced some advances by assigning former *Aporrectodea* species to other monophyletic clades and by detecting species level lineages within the cosmopolitan *caliginosa-trapezoides* complex. However, little attention has been devoted to endemic taxa of *Aporrectodea* such as *Ap. rubra*, *Ap. arverna*, *Ap. gogna*, *Ap. balisa*, *Ap. velox*, *Ap. giardi voconca* and *Ap. longa ripicola*. These earthworms (and additional populations of *Ap. longa* and *Ap. nocturna*) were included in a molecular phylogenetic framework in order to reconstruct the ancestral range of the genus, as well as to help understand its diversification within its native range and to perform a systematic revision. Species delimitation, ancestral area reconstruction and Bayesian inference of the phylogenetic relationships were performed using a large gene sequence (COI) dataset and a narrower dataset composed of 5 mitochondrial and nuclear markers. Phylogenetic position and species delimitation indicated that *Ap. giardi voconca* and *Ap. longa ripicola* constitute species-level entities not closely related to *Ap. giardi* or *Ap. longa*, and they were thus redescribed as *Aporrectodea voconca* stat. nov. and *Aporrectodea ripicola* stat. nov. Ancestral area reconstruction enabled location of the origin of *Aporrectodea* in the Auvergne-Rhône-Alps, in Southeastern France. The study findings provide some insight into the evolution of functional traits in this ecologically successful genus. *Ap. rubra* and *Ap. arverna* (small, reddish, epigeic/epianecic) and *Ap. gogna* (very large, dark, anecic) were recovered as the earliest branching taxa, suggesting a complex evolution of functional traits within this genus.

## 1. Introduction

*Aporrectodea* Orley, 1885 is one of the earthworm genera best known by non-specialized biologists and naturalists. This may be partly because some of the species (e.g. *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea trapezoides* (Duges, 1828) and *Aporrectodea rosea* (Savigny, 1826), are amongst the most frequent and abundant in managed (such as crops, orchards and pastures) and natural habitats on a large range of soil types and climates around the world. Even though the origin of the genus is recognized to be in Europe, it includes cosmopolitan species (such as the aforementioned ones) which have been introduced in

several countries and become invasive in temperate and mountain soils. Either in their native or non-native range, they play an important role in soil functioning as demonstrated by numerous studies [1–3].

*Aporrectodea* has a particularly conflictive taxonomic history, inextricably linked over the last 140 years to that of *Allolobophora* Eisen, 1873, another remarkable genus in the family Lumbricidae. Initially, Örley [4] included *Enterion chloroticum* Savigny, 1826 and *Lumbricus trapezoides* Dugès, 1828 within *Aporrectodea*, without explicitly establishing a type species. The later choice [5] of *Enterion chloroticum* as the type species of *Allolobophora* (due to no original type species designation) led to the automatic establishment of *Lumbricus trapezoides* as the

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type species of *Aporrectodea*. Since then, several species have been transferred from one genus to the other, perhaps as a result of the imprecision of each of their original diagnoses. Additionally, Marcel Bouché [6] created the genus *Nicodrilus* Bouché, 1972 for *Enterion caliginosum* Savigny, 1826 (synonymous to *Ap. trapezoides* according to Bouché) and its most closely related species. This was justified by the fact that Orley's (not Dugès') description of *Ap. trapezoides* was incomplete and probably based on Hungarian material, thus arguably making it an unsuitable type species [7]. However, this argument was not accepted by many taxonomists (e.g. Gates [8], Csuzdi and Zicsi [9] and Reynolds [10]), who considered *Aporrectodea* a valid genus and *Nicodrilus* to be its junior synonym. Nonetheless, Marcel Bouché's intuition was sharp, and he grouped a very homogeneous set of species within *Nicodrilus*, while leaving other species within *Allolobophora sensu lato*. Later, he transferred some of these species to the new genus *Koinodrilus* Qiu and Bouché, 1998, the most conspicuous being *Ap. rosea*. However, the differences in the diagnoses of *Nicodrilus* and *Koinodrilus* (the position of the nephridial pores) are so small that *Koinodrilus* is also not widely accepted as a valid genus. To further complicate the issue, the genus *Eiseniona* Omodeo, 1956 was created to include several Central/Eastern European species such as *Eiseniona handlirschi* (Rosa, 1897) and *Eiseniona sineporis* (Omodeo, 1952); however, authors such as Blakemore [11] considered this genus a junior synonym of *Aporrectodea* and retained the species within the latter.

As with other systematic conundrums, the advent of molecular phylogenetics contributed to disentangling the *Aporrectodea/Allolobophora* knot. Domínguez et al. [12] recovered a well-supported clade including *Ap. caliginosa*, *Ap. trapezoides* and other species assigned by Marcel Bouché to *Nicodrilus*, while other species (*Ap. rosea*, *Aporrectodea georgii* (Michaelson, 1890), *Aporrectodea jassyiensis* (Michaelson, 1891)) were found to be unrelated. On the other hand, an *Allolobophora* clade including *Allolobophora chlorotica* (Savigny, 1826), *Allolobophora molleri* Rosa, 1889, *Allolobophora moebii* Michaelson, 1895 and *Aporrectodea dubiosa* Orley, 1881 was also strongly supported. Martínez-Navarro et al. [13] found that *Aporrectodea icterica* (Savigny, 1826) also belonged to a redefined *Allolobophora*, and that *Aporrectodea pseudoantipai* Qiu and Bouché, 1998 was closely related to *Eiseniona handlirschi*: the latter two appeared closely related to *Aporrectodea sensu stricto*, but it was unclear whether they should be considered a separate genus (which would correspond to *Eiseniona*).

Molecular phylogenetic studies have also focused on the *Ap. caliginosa* species complex itself [14,15]. Their results showed that, not only are *Ap. caliginosa* and *Ap. trapezoides* not the same species, but they both contain several species-level cryptic lineages. These lineages display different geographical ranges and dispersal histories [16,17]. The difficulty in genotyping old (or missing) type specimens or obtaining topotypes corresponding to a single lineage has so far hindered the assignment of the names *caliginosa* and *trapezoides* to lineages (and the taxonomic description of the others).

Study of those cosmopolitan representatives of *Aporrectodea* have highlighted the need to sample the native ranges of the species to pinpoint their geographic origin [17]. However, the endemic species of *Aporrectodea* (found within the putatively native range of the genus, France) have received little attention regarding molecular phylogenetics and phylogeography. The following species could reveal the centre of diversification of the genus and explain its evolutionary origin before human-mediated dispersal blurred the biogeographic patterns: *Aporrectodea rubra* (Vedovini, 1969), *Aporrectodea arverna* (Bouché, 1969), *Aporrectodea gogna* (Bouché, 1972), *Aporrectodea balisa* (Bouché, 1972) and *Aporrectodea velox* (Bouché, 1967). Interestingly, these species provide a wider outline of the functional and ecological diversity of the genus, including both relatively small, red pigmented forms (similar to *Lumbricus* Linnaeus, 1758) and large and strongly pigmented forms (reminiscent of *Scherotheca* Bouché, 1972). In addition, several endemic subspecies such as *Aporrectodea rubra acidicola* (Bouché 1972) (known from Provence), *Aporrectodea giardi voconca* (Bouché, 1972) (known

from Auvergne-Rhône-Alpes) and *Aporrectodea longa ripicola* (Bouché, 1972) (known from the Paris Basin up to the north of the Rhône Basin) have been described, although their taxonomic status remains unconfirmed.

The aim of this study was to include the aforementioned taxa for the first time in the multilocus phylogenetic framework of Lumbricidae, together with additional representatives of *Aporrectodea longa* (Ude, 1885) and *Aporrectodea nocturna* (Evans, 1946) comprising their native range. This will allow to: i) reconstruct the ancestral range of the genus *Aporrectodea*, ii) understand the diversification of the genus within its native range, and iii) perform a systematic revision of some of its taxa.

## 2. Material and methods

### 2.1. Specimens, sampling and morphological description

Specimens included in this study were collected between October and December 2021 in Auvergne-Rhône-Alpes, Bourgogne-Franche-Comté, Grand Est and Provence-Alpes-Côte d'Azur (France). The specific localities are listed in Table 1.

The earthworms were collected by digging and hand-sorting the soil, then rinsed with water and fixed in 70%. Once immobilized, the specimens were immediately transferred to 100% ethanol for further molecular analyses. Species classification and morphological diagnoses were conducted using the same set of external and internal morphological characters reported by Ref. [6].

The following main external morphological characters were considered: mean length, mean number of segments, mean weight (all three measured in adult, complete, ethanol fixed specimens), pigmentation, type of prostomium, position of papillae, position of first dorsal pore, position of spermathecal pores, position of clitellum and position of *tubercula pubertatis*. The following main internal anatomical characters were considered: 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, and number and position of spermathecae.

### 2.2. DNA sequencing and phylogenetic analysis

Two datasets differing in specimen and gene coverage were assembled.

Dataset 1 consisted of a phylogeographic sample of individuals from different populations of the target species (*Ap. rubra*, *Ap. rubra acidicola*, *Ap. arverna*, *Ap. gogna*, *Ap. balisa*, *Ap. velox*, *Aporrectodea giardi* (Ribaucourt, 1901), *Ap. giardi voconca*, *Ap. nocturna*, *Ap. longa*, *Ap. longa ripicola*) from different parts of their range (Table 1, Supplementary Table 1, Supplementary Fig. 1).

For each locality sampled, we selected up to five adult individuals per species for DNA barcoding. Small ventral integument tissue samples were assembled in 96-well plates and shipped to the Centre for Biodiversity Genomics at the University of Guelph (Ontario, Canada) for processing. After the total genomic DNA was extracted using a CTAB-based approach, the standard DNA barcode for animals [18] – a 658bp fragment of mitochondrial gene cytochrome *c* oxidase subunit 1 (COI) – was amplified using the primers C\_LepFolF-C\_LepFolR [19]. Sequencing reactions were carried out with the same primer pair, and the products were subjected to clean-up with PureSeq-MP (Aline Biosciences, Woburn, USA) before Sanger sequencing in a DNA sequencer (ABI 3730XL). Consensus sequences from automatically assembled contigs (subsequently reviewed and manually edited when needed) were uploaded to BOLD ([www.boldsystems.org](http://www.boldsystems.org)), along with trace files, specimen data and images. The DNA sequences, together with metadata are publicly available in the (to be provided) dataset.

Additional sequences for the species *Ap. giardi*, *Ap. nocturna*, and *Ap. longa* were downloaded from BOLD ([www.boldsystems.org](http://www.boldsystems.org), Supplementary Table 1), discarding misidentifications based on their clustering

**Table 1**  
Sampling localities of the species of *Aporrectodea* under study.

| Sample ID       | Species                            | Country | State/Province                       | Latitude | Longitude |
|-----------------|------------------------------------|---------|--------------------------------------|----------|-----------|
| DFM-0975 - 0979 | <i>Aporrectodea gogna</i>          | France  | Auvergne-Rhône-Alpes, Ain            | 46.216   | 5.340     |
| DFM-0980 - 0981 | <i>Aporrectodea giardi voconca</i> | France  | Auvergne-Rhône-Alpes, Ain            | 46.216   | 5.340     |
| DFM-0982 - 0984 | <i>Aporrectodea nocturna</i>       | France  | Auvergne-Rhône-Alpes, Ain            | 45.966   | 5.174     |
| DFM-0985 - 0986 | <i>Aporrectodea longa ripicola</i> | France  | Auvergne-Rhône-Alpes, Ain            | 45.966   | 5.174     |
| DFM-0987        | <i>Aporrectodea giardi voconca</i> | France  | Bourgogne-Franche-Comté, Côte-d'Or   | 47.062   | 4.760     |
| DFM-0988 - 0991 | <i>Aporrectodea longa ripicola</i> | France  | Bourgogne-Franche-Comté, Côte-d'Or   | 47.017   | 5.127     |
| DFM-0992 - 0996 | <i>Aporrectodea gogna</i>          | France  | Bourgogne-Franche-Comté, Doubs       | 47.170   | 6.168     |
| DFM-0997        | <i>Aporrectodea longa</i>          | France  | Bourgogne-Franche-Comté, Doubs       | 47.170   | 6.168     |
| DFM-0998 - 1002 | <i>Aporrectodea velox</i>          | France  | Bourgogne-Franche-Comté, Haute-Saône | 47.836   | 6.359     |
| DFM-1003 - 1007 | <i>Aporrectodea velox</i>          | France  | Grand Est, Vosges                    | 47.988   | 6.512     |
| DFM-1009 - 1010 | <i>Aporrectodea longa</i>          | France  | Grand Est, Haut-Rhin                 | 47.799   | 7.026     |
| DFM-1011        | <i>Aporrectodea longa ripicola</i> | France  | Grand Est, Haut-Rhin                 | 47.799   | 7.026     |
| DFM-1012 - 1015 | <i>Aporrectodea rubra</i>          | France  | Provence-Alpes-Côte d'Azur, Vaucluse | 43.963   | 5.337     |
| DFM-1021 - 1025 | <i>Aporrectodea balisa</i>         | France  | Provence-Alpes-Côte d'Azur, Vaucluse | 43.965   | 5.323     |
| DFM-1026 - 1030 | <i>Aporrectodea longa ripicola</i> | France  | Provence-Alpes-Côte d'Azur, Vaucluse | 43.903   | 4.968     |
| DFM-1046        | <i>Aporrectodea rubra</i>          | France  | Provence-Alpes-Côte d'Azur, Var      | 43.181   | 6.345     |
| DFM-1067 - 1071 | <i>Aporrectodea nocturna</i>       | France  | Provence-Alpes-Côte d'Azur, Var      | 43.587   | 6.538     |

with confidently identified specimens of the same species.

Putative species were delimited using ASAP [20] based on simple distances (p-distances), and the model with the lowest ASAP score was selected.

An ultrametric phylogenetic tree was obtained in BEAST 1.10.4 [21]. jModelTest v. 2.1.3 [22] was used to select the best-fit evolutionary model by applying the Akaike information criterion (AIC [23]) and the Bayesian information criterion (BIC [24]). GTR + I + G was selected as the best-fit evolutionary model. The analysis was conducted using a constant coalescent model and an uncorrelated lognormal relaxed clock. A relative calibration was specified for the root of the tree as a normal prior with mean = 1 and standard deviation = 0.05. A uniform distribution with an initial value of 0.002 and a range of 0.00005–0.02 was specified through the ucl. mean parameter, and a uniform distribution

with an initial value of 0.10 ranging from 0 to 10 was specified for the ucl. stdev parameter. Fifty million generations were specified for the Monte-Carlo Markov chain, sampling every 5,000th generation. The log file was visualized in Log Tracer v. 1.7 [25] to check for convergence and effective sampling sizes (ESS) greater than 100. The final tree was generated by TreeAnnotator v.1.10.4 [21]. with a burn-in of 2000 trees.

Ancestral area reconstruction was performed in RASP 4 [26] using the previous ultrametric tree as input, and the most suitable model was chosen using BioGeoBEARS (DIVALIKE + j) and allowing a maximum of three areas for each node. The areas considered were coded as follows: A (Bretagne), B (Normandie), C (Ile de France), D (Grand Est), E (Bourgogne-Franche-Comté), F (Nouvelle Aquitaine), G (Occitanie), H (Auvergne-Rhône-Alps), I (Provence-Alpes-Côte d'Azur), J (Spain, United Kingdom, Norway, Sweden, Denmark).

Dataset 2 included one representative of the different species-level taxa within *Aporrectodea* (including the information from the species delimitation analysis) with additional molecular markers.

Total genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), from ventral integument samples of approximate size 5 × 5 mm, from two representative specimens of each putative species. Regions of the nuclear 28S rRNA and mitochondrial 16S rRNA, NADH dehydrogenase (ND1) and 12S rRNA were amplified by polymerase chain reaction (PCR), with the primers and conditions described in Refs. [14,27] (Suppl. Table 2). PCR products were purified and sequenced by the C.A.C.T.I Genomics service (University of Vigo) and by Macrogen Spain. The DNA sequences are available in Genbank, under accession numbers (to be provided).

Previously existing sequences of *Aporrectodea sensu stricto*, as well as *Aporrectodea limicola* (Michaelsen, 1890), *Ei. handlirschi* and *Ap. pseudoantipai* and representatives of other crown Lumbricidae genera were downloaded from Genbank and included in the dataset as references.

Sequences were aligned using MAFFT v.7 [28] with default settings and concatenated (as no major incongruence existed between mitochondrial and nuclear markers) using BioEdit [29] resulting in a matrix of 3199 bp. The best fitting evolutionary model for each partition was selected using jModelTest v. 2.1.3. GTR + I + G was selected as the best-fit evolutionary model for COI, 28S, 12S and ND1 and HKY + I + G was selected for 16S.

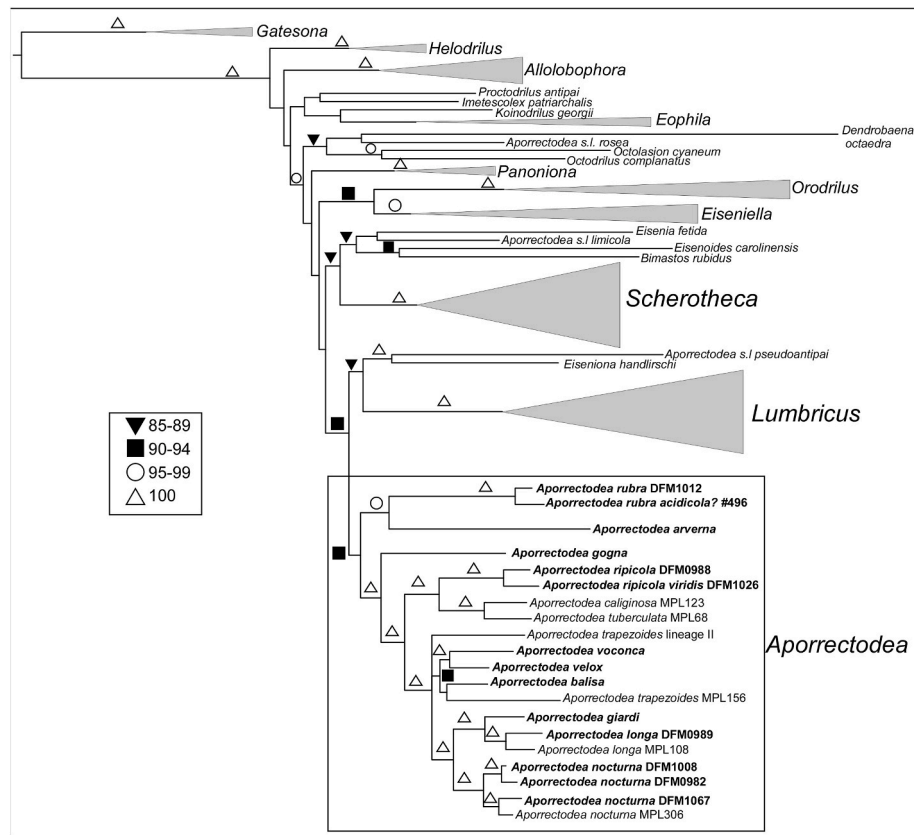
Bayesian inference of the phylogeny was estimated using MRBAYES v.3.1.2 [30] as implemented in CIPRES Science Gateway V. 3.3. Parameters were set to 50 million generations and sampled every 5,000<sup>th</sup> generation (10,000 trees). The best fitting evolutionary models were specified for each partition. 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 summarized on a 50% majority-rule consensus tree.

### 3. Results

#### 3.1. Species delimitation and phylogeny

The clustering hypothesis obtained by ASAP with the lowest ASAP score (5.5) consisted of 13 putative species (Supplementary Fig. 2). *Aporrectodea nocturna*, *Ap. arverna*, *Ap. balisa*, *Ap. gogna* and *Ap. velox* were each recovered as a single species. *Aporrectodea longa* was split in two putative species, and *Ap. longa ripicola* was recovered as two separate putative species (one of them corresponding to *Ap. longa ripicola viridis*). *Aporrectodea giardi* was recovered as a single species but *Ap. giardi voconca* was recovered as a separate putative species. Finally, *Ap. rubra* and *Ap. rubra acidicola* were shown as different putative species.

The Bayesian (Fig. 1) phylogenetic tree recovered a monophyletic and well supported *Aporrectodea sensu stricto* comprising all of the studied species of *Aporrectodea* except *Ap. limicola* (closely related to *Eisenia*, *Eisenoides*, *Bimastos* and *Scherotheca*), *Ap. rosea* and *Ap. pseudoantipai*. The latter clustered with *Eisenionia handlirschi* in a strongly supported clade, more closely related to *Lumbricus* than to *Aporrectodea*.



**Fig. 1.** Phylogenetic relationships between *Aporrectodea* sensu stricto species and their closest relatives obtained by Bayesian inference based on the concatenated sequence of five molecular markers (COI, ND1, 16 S, 12S and 28S). Support (posterior probability) values are shown above the corresponding branches. The taxa added in this study are shown in bold.

*Aporrectodea rubra* and *Ap. arverna* were recovered as the earliest-branching clades within *Aporrectodea*, followed by *Ap. gogna*. The next clade comprised *Ap. caliginosa*, the closely related *Ap. tuberculata* and *Aporrectodea ripicola* stat. nov., the latter clearly separated from *Ap. longa*. One of the last two clades included *Aporrectodea voconca* stat. nov. (neatly separated from *Ap. giardi*), *Ap. velox*, *Ap. balisa* and one of the lineages of *Ap. trapezoides* (plus a second one according to Maximum Likelihood analysis); the other included *Ap. giardi* and *Ap. longa*, and an *Ap. nocturna* clade with strong internal divergence.

### 3.2. Ancestral area reconstruction

The topology of the ultrametric tree on which the Ancestral Area Reconstruction was based (Fig. 2) differed slightly from the Bayesian and ML tree in regard to the sister relationship of *Ap. rubra* and *Ap. gogna* (instead of *Ap. arverna*) and *Ap. velox* and *Ap. balisa* (instead of *Ap. voconca* stat. nov.). This was probably due to exclusion of the cosmopolitan species *Aporrectodea caliginosa*/*Aporrectodea tuberculata* and *Aporrectodea trapezoides*.

The oldest divergence was between *Ap. rubra* and *Ap. gogna*, and between both *Ap. voconca* stat. nov. and *Ap. nocturna* and their closest relatives. The divergence between *Ap. balisa*-*Ap. velox* and *Ap. giardi*-*Ap. longa* was comparatively recent.

*Aporrectodea rubra*, *Ap. ripicola* stat. nov., *Ap. nocturna* and *Ap. longa* displayed deeper internal branches than the other species of which several populations were sampled.

Ancestral area reconstruction (Fig. 2) estimated the most likely area of origin for the common ancestor of the studied *Aporrectodea* to be Auvergne-Rhône-Alps (Southeastern France). The ancestral area for most of the clades and species corresponded to Southeastern-Eastern France (Bourgogne-Franche-Comté, Auvergne-Rhône-Alps, Provence-

Alpes-Côte d'Azur), suggesting most of the diversification of the genus occurred in this region. The exception was the *Ap. giardi*-*Ap. longa* clade, whose ancestral area was estimated to be in north-central France (Ile de France). For *Ap. longa*, the ancestral area was estimated to be the surrounding countries (Spain, United Kingdom, Norway, Sweden, Denmark), but this is probably due to an overrepresentation of populations from outside of France.

## 4. Discussion

### 4.1. Systematic implications

The wealth of subspecies and varieties described by Bouché [6] for *Nicodrilus* (here *Aporrectodea*) already suggested a greater diversity of the genus than is usually recognized. Phylogenetic inference and species delimitation analyses supported that the subspecies *giardi voconca* and *longa ripicola* are not closely related to their parent taxa *Ap. giardi* and *Aporrectodea longa* thus should be elevated to species status.

#### 4.1.1. Proposed taxonomic changes

Phylum Annelida Lamarck, 1802.

Subphylum Clitellata Michaelsen, 1919.

Class Oligochaeta Grube, 1850.

Superorder Megadrili Benham, 1890.

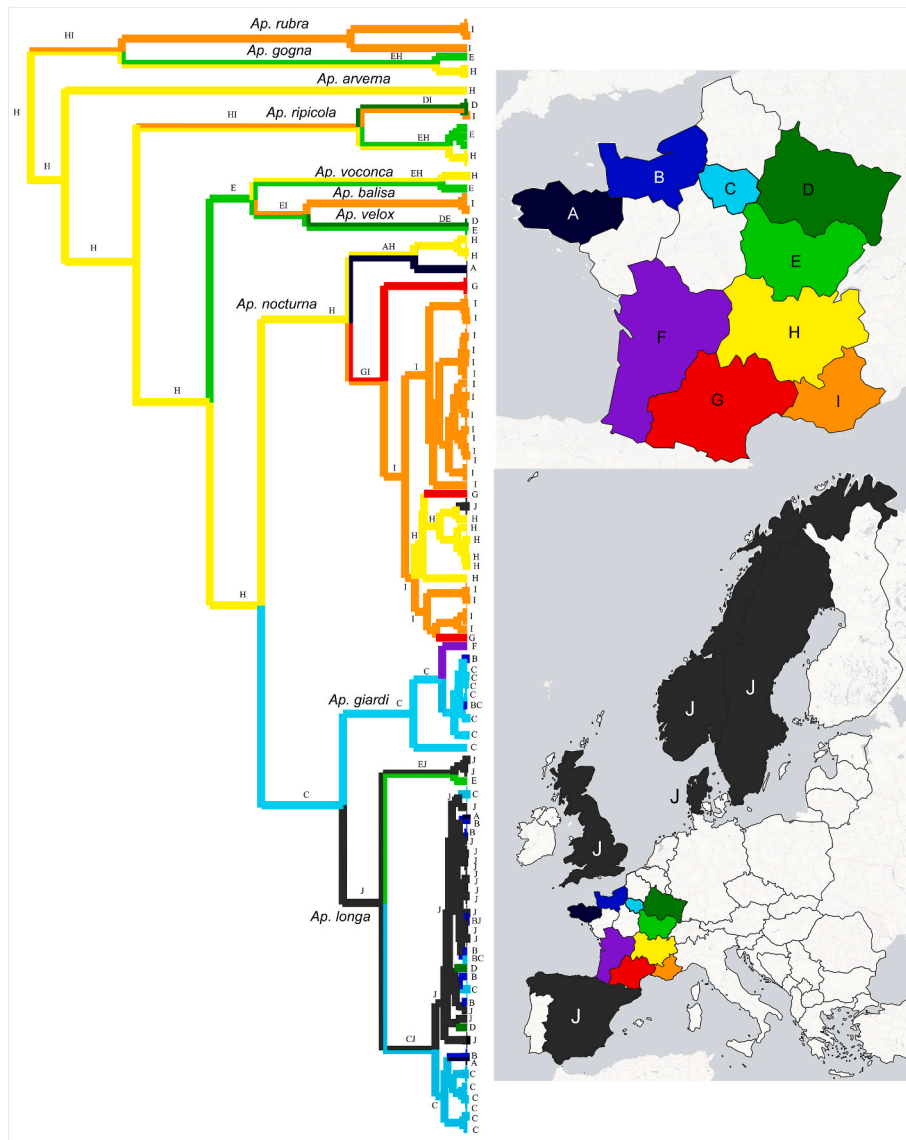
Order Crassiclitellata Jamieson, 1988.

Family Lumbricidae Rafinesque-Schmaltz, 1815.

Tribe Lumbricini Qiu and Bouché 1998.

Genus *Aporrectodea* Orley, 1885.

#### 4.1.1.1. *Aporrectodea voconca* stat. nov. (Bouché, 1972)



**Fig. 2.** Ancestral area reconstruction for *Aporrectodea* sensu stricto populations from France and surrounding countries (excluding the cosmopolitan *Aporrectodea caliginosa*/*Aporrectodea tuberculata* and *Aporrectodea trapezoides*). Only the most likely reconstruction for each node is shown for key nodes. The areas considered were coded as follows: A (Bretagne), B (Normandie), C (Ile de France), D (Grand Est), E (Bourgogne-Franche-Comté), F (Nouvelle Aquitaine), G (Occitanie), H (Auvergne-Rhône-Alpes), I (Provence-Alpes-Côte d'Azur), J (Spain, United Kingdom, Norway, Sweden, Denmark).

*Nicodrilus terrestris voconcus* Bouché, 1972: 321.

*Aporrectodea giardi voconca* Blakemore, 2008: 21

#### 4.1.2. Redescription

**4.1.2.1. Diagnosis.** Specimens of *Aporrectodea voconca* can be distinguished from other known species of *Aporrectodea* by the position of the clitellum in segments XXVI-XXXVI, spectacle-shaped tubercula pubertatis in segments XXXII-XXXIV, strongly developed genital papillae in segments X, XII (sometimes in XI, XII) and a partially pennate typhlosole which changes to trifold around segment XXVII (Table 2).

**4.1.2.2. Etymology.** The name *voconca* refers to the Gallic tribe of the Vocontii, which inhabited the lower Rhône valley.

#### 4.1.3. Material examined

**Holotype.** France • Adult; Auvergne-Rhône-Alpes, Ain, Saint-Sorlin-en-Bugey 26-Feb-1968; Marcel Bouché leg. ; voucher 61-401-212;

deposited in MNHN.

**Additional material.** France • 3 adult specimens; Auvergne-Rhône-Alpes, Ain, Jasseron; latitude/longitude: 46.216/5.340; elevation: 373 m asl; 30-Oct-2021; T. Decaëns, D. Fernández Marchán leg. (2 specimens) BOLD Sample ID: DFM-0980, DFM-0981; deposited in ECO&SOLS. Bourgogne-Franche-Comté, Côte-d'Or, Bouze-les-Beaune; latitude/longitude, 47.062, 4.76; elevation, 490 m asl; 30-Oct-2021; T. Decaëns, D. Fernández Marchán leg. (1 specimen); BOLD Sample ID: DFM-0987; deposited in ECO&SOLS.

#### 4.1.4. Morphological description

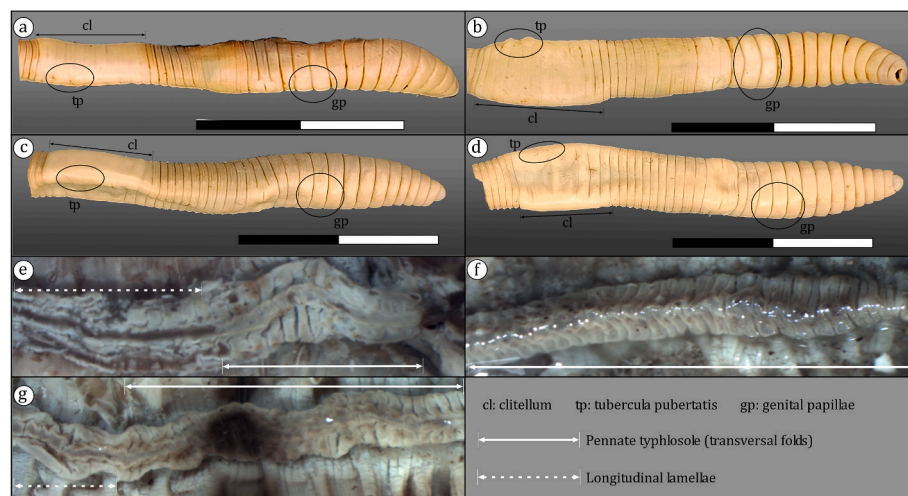
**4.1.4.1. External morphology.** Body pigmentation brown with antero-posterior and dorso-ventral gradients (Fig. 3).

Mean length (fixed specimens) 13 cm (12.1–13.7 cm, n = 2 adults); body cylindrical in cross-section, slightly flattened tail; mean number of segments 207 (n = 2 adults; 192 segments in the holotype). Mean weight (fixed specimens): 2.65 g (2.27–3.03 g, n = 2 adults). Prostomium epilobous, closed. Transverse furrows from segment VII or VII. First dorsal

**Table 2**

Morphological characters of *Aporrectodea voconca* stat. nov., *Aporrectodea ripicola* stat. nov. and the other species of *Aporrectodea* sensu stricto. Average length in centimeters, average weight in grams. 1st D.P: First dorsal pore. G.P: Genital papillae. T.P: Tubercula pubertatis. \* Obtained from the minimum and maximum values shown in Bouché [6].

|   | Av. length | # segments | Av. weight | 1st D.P          | G.P            | Clitellum                     | T.P.  | Typhlosole                  |
|---|------------|------------|------------|------------------|----------------|-------------------------------|---|-----------------------------|
| <i>Aporrectodea voconca</i> stat. nov.  | 13         | 192–222    | 2.65       | 11/12            | 10,12 or 11,12 | 26–36                         | 32–34 spectacle-shaped  | Pennate (up to 28), trifold |
| <i>Aporrectodea giardi</i>              | 20*        | 108–203    | 2.4        | (10/11)<br>11/12 | (9)10,11,12    | (1/n 26)27–35<br>(2/3 36)     | 32–34 spectacle-shaped  | Pennate (up to 32), trifold |
| <i>Aporrectodea ripicola</i> stat. nov. | 10.5       | 155–173    | 1.26       | 12/13,<br>13/14  | (9)10,11       | 1/2 28 (28)–1/2<br>35 (35)    | 32–1/2 34 oval shaped   | Pennate (up to 40+), simple |
| <i>Aporrectodea longa</i>               | 15*        | 160–200    | 1.65       | 12/13            | 9–11           | (1/2 27)28–35<br>(1/2 36)     | 32–34 gutter-shaped   | Pennate (up to 32), trifold |
| <i>Aporrectodea nocturna</i>            | 13.5*      | 180–250    | 1.68       | (8/9)/<br>10     | (9)10–12       | (1/y 27)28–34<br>(1/2 35)     | 31–33 spectacle-shaped  | Trifold-pentafid            |
| <i>Aporrectodea velox</i>               | 23.6       | 483        | 7          | (9/10)<br>10/11  | (9)10–12       | (1/n 24)25–1/2<br>36 (36)     | 1/n 30–34 gutter-shaped   | Pennate (up to 32), bifid T |
| <i>Aporrectodea gogna</i>               | 17.3       | 215–240    | 6.45       | 11/12            | 11,13,14       | 24–37                         | 1/2 31–35 gutter-shaped   | Pennate                     |
| <i>Aporrectodea balisa</i>              | 21.3       | 280–315    | 5.4        | 11/12            | 9–11           | (1/n 26)27–35<br>(1/n 36)     | (1/n 29)1/2 30–1/2 34 (34) band-shaped, ventral protuberances in 31, 33 | Pennate                     |
| <i>Aporrectodea rubra</i>               | 5.5        | 95–115     | 0.5        | (4/5,5/<br>6)6/7 | (9,10,11,12)   | (25)1/3 25–1/2<br>32 (1/a 33) | (1/y 26)1/3 27–30 (1/n 31) gutter-shaped                                | Pennate                     |
| <i>Aporrectodea arverna</i>             | 5.9        | 115–126    | 0.8        | (9/10)<br>10/11  | 10,11 (12)     | (25)1/n 26–34<br>(1/y 35)     | (1/2 31)2/3 31–33 band-shaped, ventral protuberances in 1/4 32, 1/4 33  | Pennate                     |
| <i>Aporrectodea caliginosa</i>          | 5.5        | 130–165    | 0.4        | (9/10)<br>10/11  | 9–11           | (26)27–34 (1/a<br>35*)        | 31–33 spectacle-shaped  | Pentafid                    |
| <i>Aporrectodea trapezoides</i>         | 5.9        | 130–170    | 0.6        | (8/9)/<br>10     | 9–11           | 27–34                         | 31–33 arc-shaped band   | Pennate (up to 32), trifold |



**Fig. 3.** External morphology of *Aporrectodea voconca* stat. nov. (a,b) and *Aporrectodea ripicola* stat. nov. (c,d) in lateral (a,c) and ventral (b,d) views; typhlosole of e) *Aporrectodea voconca* stat. nov. f) *Aporrectodea ripicola* stat. nov. and g) *Aporrectodea longa*. Scale bar: 2 cm.

pore at intersegmental furrow 11/12. Nephridial pores inconspicuous aligned in C close to b. Spermathecal pores at intersegmental furrows 9/10 and 10/11 in c. Male pores in segment XV, surrounded by a well-developed porophore. Female pores on the posterior part of segment XIV, inconspicuous. Clitellum saddle-shaped in segments XXVI–XXXVI. Spectacle-shaped tubercula pubertatis in segments XXXII–XXXIV. Chaetae closely paired. Strongly developed chaetophores/genital papillae in segments X, XII (sometimes in XI,XII).

**4.1.4.2. Internal anatomy.** Septa 6/7–8/9 strongly thickened, 9/10–11/12 slightly thickened. Lateral hearts in segments VI–XI. Calciferous glands in segments X–XIV, with paired diverticula in X. Crop in segments XV–XVI, gizzard in segments XVII–XVIII. Typhlosole pennate (composed of transverse folds resembling ribs) from the start to segments XXVII–XXVIII, trifold (composed of three longitudinal lamellae) from that point to the end (Fig. 3). 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 IX, X, XI and XII, with the latter two pairs being larger. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Two pairs of globular spermathecae in segments IX and X. Nephridial bladders inverted J shaped, proclinate in anterior and posterior segments.

#### 4.1.4.3. *Aporrectodea ripicola* stat. nov. (Bouché, 1972)

*Nicodrilus longus ripicola* Bouché, 1972: 325.

*Aporrectodea longa ripicola* Blakemore, 2008: 22

#### 4.1.5. Redescription

**4.1.5.1. Diagnosis.** Specimens of *Aporrectodea ripicola* can be

distinguished from other known species of *Aporrectodea* by the position of the clitellum in segments 1/2 XXVIII (XXVIII)-1/2 XXXV (XXXV), oval-shaped tubercula pubertatis in segments XXXII-1/2 XXXIV, partially pennate typhlosole which changes to simple beyond segment XL and frequent green pigmentation (Table 2).

**4.1.5.2. Etymology.** The name *ripicola* refers to the preference of this earthworm for moist soils.

**4.1.5.3. Material examined. Holotype.** France • Adult; Centre-Val de Loire, Indre et Loire, Loches 07-Nov-1968; Marcel Bouché leg. Voucher 64-1021-0231; deposited in MNHN.

**Additional material.** France • 12 adult specimens; Provence-Alpes-Côte d'Azur, Vaucluse, Caumont-sur-Durance; latitude/longitude: 43.903/4.968; elevation: 49 m asl; 25-Nov-2021; T. Decaëns, D. Fernández Marchán, Y. Capowiez leg. (5 specimens); BOLD Sample ID: DFM-1026, DFM-1027, DFM-1028, DFM-1029, DFM-1030; deposited in ECO&SOLS. Auvergne-Rhône-Alpes, Ain, Crans; latitude/longitude, 45.966/5.174; elevation, 299 m asl; 29-Oct-2021; T. Decaëns, D. Fernández Marchán leg. (2 specimens); BOLD Sample ID: DFM-0985, DFM-0986; deposited in ECO&SOLS. Bourgogne-Franche-Comté, Côte d'Or, Pouilly-sur-Saone; latitude/longitude, 47.017/5.127; elevation, 177 m asl; 30-Oct-2021; T. Decaëns, D. Fernández Marchán leg. (4 specimens); BOLD Sample ID: DFM-0988, DFM-0989, DFM-0990, DFM-0991; deposited in ECO&SOLS. Grand Est, Haut-Rhin, Bourbach-le-Haut; latitude/longitude, 47.799/7.026; elevation, 572 m asl; 03-Nov-2021; T. Decaëns, D. Fernández Marchán leg. (1 specimen); BOLD Sample ID: DFM-1011; deposited in ECO&SOLS.

#### 4.1.6. Morphological description

**4.1.6.1. External morphology.** Body pigmentation faint brown to brown with antero-posterior and dorso-ventral gradients, sometimes with a green hue which is more intense in the tail region (Fig. 3).

Mean length (fixed specimens) 10.5 cm (9.2–11.9 cm,  $n = 3$  adults); head and clitellum cylindrical in cross-section, tail strongly flattened dorso-ventrally; mean number of segments 167 ( $n = 3$  adults; 173 segments in the holotype). Mean weight (fixed specimens): 1.26 g (0.99–1.60 g,  $n = 3$  adults). Prostomium epilobous, closed. Transverse furrows starting at segment V or VI. First dorsal pore at intersegmental furrow 12/13 or 13/14. Nephridial pores inconspicuous aligned in C close to b. Spermathecal pores at intersegmental furrows 9/10 and 10/11 in c. Male pores in segment XV, surrounded by a well-developed porophore. Female pores on the posterior part of segment XIV, inconspicuous. Clitellum saddle-shaped in segments 1/2 XXVIII (XXVIII)-1/2 XXXV (XXXV), oval-shaped tubercula pubertatis in segments XXXII-1/2 XXXIV. Chaetae closely paired. Chaetophores/genital papillae or glandular areas in segments (IX) X, XI.

**4.1.6.2. Internal anatomy.** Septa 6/7–11/12 slightly thickened. Lateral hearts in segments VI-XI. Calciferous glands in segments X-XIV, with paired diverticula in X. Crop in segments XV-XVI, gizzard in segments XVII-XVIII. Typhlosole pennate (composed of transversal folds resembling ribs) from the start to segments XL or beyond, simple from that point (Fig. 3). 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 IX, X, XI and XII, with the latter two pairs being larger. Ovaries and female funnels in segment XIII, ovarian receptacles (ovisacs) in segment XIV. Two pairs of globular spermathecae in segments IX and X, intra-septal. Small nephridial bladders inverted J shaped, proclinate in anterior and posterior segments.

**4.1.6.3. General remarks.** Representative specimens of *Ap. rubra acidicola* and *A. ripicola* stat nov. var. *viridis* were separated from their parent

species by species delimitation analysis. However, in the absence of clear morphological differences (*Ap. rubra acidicola* mainly differed in its soil preferences and *Ap. ripicola viridis* in its stronger green pigmentation), it is more reasonable not to elevate them to species. In addition, *viridis* was originally a variety described after 1960, and according to the International Code of Zoological Nomenclature the name is considered unavailable.

A remarkably high intraspecific genetic diversity was observed within *Ap. nocturna*, which had already been hinted by the local scale comparative phylogeography of Marchán et al. [31]. However, species delimitation did not separate the intraspecific lineages into putative species, which suggests that they do not constitute cryptic species.

The opposite case was found for *Ap. longa*, with two deeply divergent lineages which were delimited as different putative species. Those lineages were already described by Martinsson et al. [32], who found the existence of a barcoding gap but no evidence of cryptic speciation from nuclear markers (ITS2 and Histone 3).

The different diversification patterns of the two aforementioned species may simply be due to a more incomplete sampling of the range of *Ap. longa*, resulting in a genetic gap between the observed lineages. However, other evolutionary causes (such as different dispersal-extinction histories or different isolation patterns) may have been involved and appear worthy of further exploration.

*Aporrectodea longa* and *Ap. nocturna*, which have previously been suggested to form a single species according to molecular analyses [14], appeared as well-separated taxa in our analyses. This may be due to the increased geographical sampling and more complete coverage of their intraspecific diversity. However, a few individuals identified as *Ap. longa* appeared nested within *Ap. nocturna*. Interestingly, Bouché [6] described *Ap. longa* var. *ambiguus* with a very similar morphology to *Ap. nocturna*. Our results suggest that those earthworms were actually morphs of *Ap. nocturna* which resembled *Ap. longa*.

The typhlosole had previously been described as pennate (“penné”) for most of the species of *Aporrectodea* sensu stricto in Bouché [6], except for *Ap. giardi* and *Ap. caliginosa* (bifid). For *Aporrectodea longa*, it was described as “pennate, sometimes organized in 2 or three lamellae”, while for *Ap. nocturna* it was described to be “pennate or formed by 2, 3 or 5 longitudinal lamellae”. Detailed observation of the typhlosole of representatives of those species has revealed a more complex scenario: *Ap. giardi*, *Ap. longa*, *Ap. voconca* stat. nov., *Ap. ripicola* stat. nov., *Ap. velox* and *Ap. trapezoides* (Eurosiberian lineage) possess pennate typhlosoles (composed of transverse folds resembling ribs) which change into three to one longitudinal lamellae at somewhat specific positions. For *Ap. voconca* stat. nov. and *Ap. ripicola* stat. nov., in which this transition is displaced forwards or backwards relative to the other species, this character can be considered of taxonomic utility. Within a genus with a limited amount of taxonomically informative characters, this discovery might help to resolve the troublesome systematics of the different lineages of *Ap. caliginosa* and *Ap. trapezoides*. Furthermore, the division of the typhlosole in two types of organization suggests that there may be some regionalization and functional specialization of the intestine in the genus *Aporrectodea*, a possibility which has not been previously explored and that could be related to the ecological flexibility of these species.

*Aporrectodea* is one of the most conspicuous genera within human managed habitats in its native range (Europe) and also in temperate latitudes all around the globe. Its complicated, unclear taxonomy has hindered soil ecology and applied research for decades, with results which are not really cross-comparable due to the different treatment of the taxa. The taxonomic advances in this work should pave the way for more rigorous inclusion of those species into the knowledge of soil functioning, with only the status of a few species remaining to be solved (see 4.3.).



#### 4.2. Biogeographic and evolutionary implications

Ancestral area reconstruction based on the endemic *Aporrectodea* species restricted to France enabled location of the origin of this successful and globally distributed genus in the Auvergne-Rhône-Alpes, in Southeastern France. The peri-Alpine region appears to have been an important area in the diversification and evolution of Lumbricini (*Lumbricus* [33], *Allolobophora* [13]), which is supported by this result. The closely related genus *Scherotheca* has been suggested to have originated between Provence and Corsica [34], although more complete sampling of the Provençal species will be necessary to confirm this possibility. These non-overlapping geographical origins could explain the surprisingly parallel evolution of both genera and their distinct biogeographical patterns.

It is worth mentioning that Ancestral Area Reconstruction methods heavily depend on sampling extent, and that the possibility of the actual ancestral area not having been sampled (and thus not being taken into account in the analysis) is never 0. This is unlikely for the whole genus *Aporrectodea*, as the earliest branching taxa (which contain the most information about the ancestral area) possess very restricted ranges; however, this could be the case for other more widely distributed species (such as *Ap. giardi* or *Ap. longa*).

Biogeographic reconstruction also showed that most of the diversification of the genus *Aporrectodea* occurred in the area of origin, before the genus expanded across the rest of France and neighbouring countries. However, a second centre of diversification appears to be located in the North of France (Ile de France), where the common ancestor of *Ap. giardi* and *Ap. longa* was inferred to have lived. Interestingly, this region is further north than the limit usually reconstructed for permafrost during the Last Glacial Maximum (LGM, ca. 33,000–15,000 years ago [35]): it is usually believed that earthworm species and populations occurring further north than this limit became extinct, and those areas were repopulated at a later date [6]. The very high genetic diversity detected in this northern area (including two distinct lineages of *Ap. longa*) suggests two hypotheses: either the species diversified in-situ and survived in glacial refuges, or northern France was recolonized independently from the south by separate lineages. This surprising result may be explained by the limited sampling of *Ap. giardi* (and to a lesser extent *Ap. longa*) ranges. It is also possible that the inclusion of several populations of these species from a region with very high human activity (Ile de France) could have distorted the results due to the likely repeated transfer of specimens from distant regions. A wider, denser coverage of the ranges of *Ap. giardi* and *Ap. longa* could offer further insight into these hypotheses.

In relation to this same conundrum, *Ap. gogna* and *Ap. velox* are narrow endemics whose range is restricted to an area located in the region putatively affected by permafrost during the LGM. Martínez Navarro et al. [13] suggested that the Val de Saône-Vosges area (which they inhabit) could have acted as a glacial refuge for endemic Lumbricidae, as distinct vegetation types (such as ombrotrophic bogs) were present in the area during the LGM [36]. The relatively deep branches between the two sampled populations of *Ap. gogna* are more consistent with pre-glacial diversification and survival *in situ*, while the extremely low genetic divergence between the two populations of *Ap. velox* suggests post-glacial colonization or speciation associated with glacial isolation. Very little is known about the distribution of these two species: *Ap. gogna* was previously known from a single locality, but in this work a second population was found, located 120 km further to the north. Likewise, a second population of *Ap. velox* was found during our sampling. In this case, Bouché [6] already indicated that the range of the later may be much larger, having observed unusually large casts in localities separated by 250 km. However, the deep-burrowing and fast-retreating behaviour of these species have greatly restricted sampling.

The identification of the putative ancestral area of *Aporrectodea* and several of its species provides information about their native range and

their ability to adapt to different environmental conditions and biotic interactions. Studies based on this new knowledge could improve our understanding of the effect of globalization and climate change on the role of those important ecosystem engineers in soils.

Reconstruction of the whole phylogenetic tree of *Aporrectodea* offers insight into the evolution of functional traits in this ecologically successful genus. The earliest branching taxa, *Ap. rubra* and *Ap. arverna*, are small, reddish, epigeic/epianecic species [37]. This may lead to the conclusion that the ancestral life form of the genus corresponded to those character states, surprisingly similar to several *Lumbricus* species, *Eisenia*, *Bimastos* and *Satchellius*, which belong to the same clade [38]. The scenario is complicated by the other early branching species, *Ap. gogna*, which has an unusually large body (the second largest within the present definition of the genus), very dark pigmentation and a fully anecic lifestyle. The other species that shares such specialized adaptation, *Ap. velox*, appears in the crown group and is not closely related. Hence, even though there appears to be a general trend towards larger body size and a more anecic lifestyle within the genus (as shown by the *nocturna-giardi-longa* clade), extreme phenotypes were reached convergently in two unrelated lineages. The only endogeic, (mostly) unpigmented species (*Ap. caliginosa* and *Ap. tuberculata*) appear to be derived taxa closely related to the epianecic *Ap. ripicola*, so that this lifestyle and trait combination can be considered to be derived within *Aporrectodea*. Marchán et al. [33] found that most of the early-branching species of *Scherotheca* are unpigmented endogeics or faintly pigmented endoanecics, suggesting the ancestral state of this genus may be different from that of *Aporrectodea*. However, more comprehensive sampling of the diversity of *Scherotheca* must be included in phylogenetic analyses to enable construction of a robust hypothesis. The acquisition of trait syndromes explaining life forms (ecological categories, sensu [6,37]) within closely related lumbricid genera is an intriguing topic, and the progressively more complete molecular dataset on these taxa is bringing us closer to understanding the situation. The relationship between earthworm functional traits, ecological categories and their effect on soil functioning is a growing focus of interest for soil biologists and could lead to a greatly improved leverage of their ecosystem services.

#### 4.3. Remaining questions

As this work focused on endemic species of *Aporrectodea*, *Ap. caliginosa* and *Ap. trapezoides* were purposefully left aside. A large amount of species delimitation, neotyping and description remains to be performed with those peregrine, taxonomically troublesome species. The location of a putative diversification centre for the genus in Southeastern France suggests that intensive sampling and genotyping of *Ap. caliginosa* and *Ap. trapezoides* in this region could help to disentangle their systematics.

Several Central European species assigned to *Aporrectodea* or *Eisenionia*, depending on the authors (*sineporis*, *smaragdina*, *pannoniella*, *bohiniiana*, *kozjekensis*, *predalpina*, etc.), remain to be included in a molecular phylogenetics frame. With inclusion of the above-mentioned Central European species, we could attempt to resolve this question as their clustering with *Ap. trapezoides* or with *Eisenionia handlirschi* would place them in their appropriate genus.

Two species which have been recurrently assigned to *Aporrectodea* but which do not appear to be closely related (thus remaining within *Aporrectodea* sensu lato) are *Ap. rosea* and *Ap. limicola*. *Aporrectodea rosea* has appeared as a rogue taxon (i.e. with uncertain position in a phylogenetic tree) in several molecular phylogenetic analyses [12,14] but the addition of rare species (*Panionia satchelli* (Bouché, 1972)) suggests that it may be related to *Panionia* [13] (but not according to the results of our work). In any case, the *Ap. rosea* species complex remains to be properly delimited and described. It appears to constitute its own genus and should receive a new name in the near future. *Aporrectodea limicola* has consistently been recovered as a sister taxon to *Scherotheca*, while not being nested within it. The inclusion of this

species in a phylogenomic (such as Anchored Hybrid Enrichment [38]) dataset could confirm this relationship and justify the need for a new name for *Ap. limicola*.

## 5. Conclusions

Multilocus phylogenetic analyses supported the status of two subspecies of *Ap. longa* and *Ap. giardi* as *Ap. ripicola* stat. nov. and *Ap. voconca* stat. nov., while *Ap. longa*, *Ap. nocturna* and *Ap. giardi* were confirmed as separate species. The typhlosole of *Aporrectodea* species appears to be more complex than initially thought and adopts different character states between species, which could allow further advances to be made in their taxonomy. The ancestral area for the genus *Aporrectodea* was inferred to be the southeastern perialpine region of France, with most of the diversification of the genus occurring in the same area. The earliest branching taxa (*Ap. arverna*, *Ap. rubra* and *Ap. gogna*) suggest a complex evolution of functional traits within *Aporrectodea*, with small epianecic and large anecic forms in its early evolution.

This work has resulted in a homogeneous *Aporrectodea*, with just a few Central European species (likely belonging to *Eisenionia*), *Ap. rosea* and *Ap. limicola* remaining systematically unresolved in *Aporrectodea* sensu lato, and the species complexes *Ap. caliginosa* and *Ap. trapezoides* to be delimited and formally redescribed.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Daniel Fernandez Marchan reports financial support was provided by Campus France. Marta Novo reports financial support was provided by Spain Ministry of Science and Innovation. Jorge Domínguez reports financial support was provided by Government of Galicia Department of Culture Education and Universities.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejsobi.2023.103559>.

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