



## Original article

# Contrasting phylogeographic patterns of earthworms (Crassiclitellata, Lumbricidae) on near-shore mediterranean islands

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## ABSTRACT

Comparative phylogeography is a powerful methodological approach to understand the particular evolutionary phenomena that occur in islands. This method has been rarely applied to insular earthworm communities. These soil animals show a striking dichotomy in their phylogeographic patterns: deeply divergent, regionally structured lineages and widely distributed, genetically homogeneous lineages. An intensive earthworm sampling campaign in the Southern France archipelago of Hyères served as an opportunity to check for the existence of these patterns in the framework of a near-shore, continental archipelago. Molecular barcoding (COI sequencing) was performed for earthworm communities of 31 localities, and the seven with more informative distributions were chosen for phylogenetic inference and genetic diversity evaluation. A time-calibrated phylogeny was obtained to estimate a temporal framework for colonization and divergence within the islands, and haplotype networks were used to visualize relationships between populations in detail. Four species (*Allolobophora chlorotica*, *Aporrectodea nocturna*, *Scherotheca* cf. *dugesi* and *Scherotheca rhodana*) showed clades restricted to the archipelago and hints of Plio-Pleistocene *in situ* divergence. The other three species (*Aporrectodea rosea*, *Aporrectodea trapezoides* and *Eiseniella tetraedra*) lacked geographic structure, being nested in clades with individuals from distant countries; their arrival to the archipelago appeared consistent with human-mediated introduction. These contrasting phylogeographic patterns are discussed in the context of paleogeographic events affecting the Mediterranean in the Late Tertiary-Quaternary and biological traits of earthworms, with biparental sexual vs parthenogenetic reproduction as the main factor explaining them.

## 1. Introduction

Islands have been one of the main subjects of biogeography and evolutionary biology since the inception of these disciplines: the diverse degrees of isolation associated with them resulting in unique patterns of biodiversity [1]. The main phenomena observed on islands (colonization, paleoendemism and neoendemism [2,3] can not only be found at species level, but also between genetic lineages within species [4,5]. Thus, the evolutionary interest of islands lies at the interface between biogeography and phylogeography.

Comparative phylogeography, which relies on the joint study of the

distribution of lineages for several species that share distributional ranges, constitutes a powerful tool for testing phylogeographic hypotheses [6]. For instance, it enables exploration of the differences in the ability to cross physical barriers (such as the sea) related to ecological or life-history traits [7], and the rate at which genetic lineages are formed. In such studies, island populations are an invaluable source of information [8].

As one of the most important groups of soil fauna in terms of biomass and impact on the ecosystem [9], earthworms have been the focus of several phylogeographic studies. This interest is related to their particular biological characteristics. For example, although earthworms have

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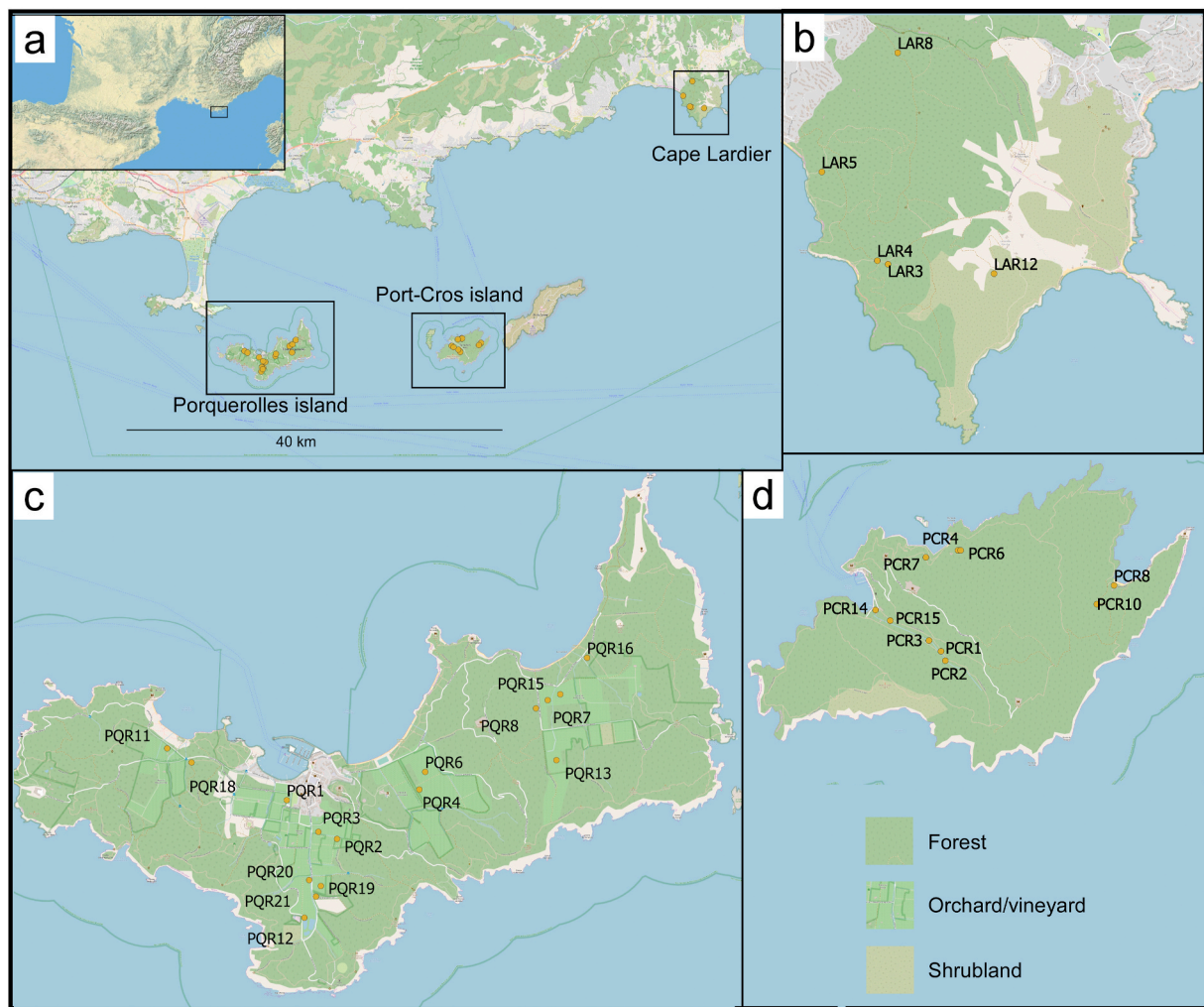
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low vagility and active dispersal ability (2.5–14 m per year [10]) several cosmopolitan species are distributed across the globe, often becoming invasive species due to human-mediated transport [11]. This paradox may be partially explained by the strong colonizing potential attributed by the parthenogenetic mode of reproduction [12] shared by many cosmopolitan species (*Aporrectodea trapezoides* Dugès (1828), *Aporrectodea rosea* Savigny (1826), *Dendrobaena octaedra* Savigny (1826), *Eiseniella tetraedra* Savigny (1826), *Pontoscolex corethrurus* Muller (1856) among others). Some studies have revealed two phylogeographic patterns that are consistent with the aforementioned traits (low dispersal ability but strong colonizing potential): strong population structure with deeply divergent regional lineages [13,14] and widely distributed, genetically homogeneous lineages [15–17].

Island phylogeography of earthworm species is relatively understudied, with some works on the narrowly distributed *Postandrilus* species [18] and different species belonging to the *Metaphire-Amyntas* species group [19–22]. Islands provide an interesting opportunity to study the dispersal and colonization ability of different earthworm species by reducing the possibility of active dispersal. However, comparative phylogeographic studies have rarely been applied to earthworm communities on islands. Furthermore, ultrametric or time-calibrated phylogenies can be obtained from molecular markers, providing a relative or absolute time frame for the arrival and diversification of species and lineages to an island. This is particularly useful for continental islands, as certain species could already inhabit them before

isolation of the island from the mainland. However, history of connection and isolation of such islands is usually complex (the island of Rhodes as an example [23]). Besides continental remnants, there are two other possible origins for island earthworm fauna. Endemic earthworm species have been found in volcanic islands, showing the ability for small-scale overwater dispersal of some earthworm species [24,25]. As these earthworms appeared associated with rotting logs and fallen tree trunks, it is likely that this dispersal is facilitated by rafting logs and mainly found on small epigeic earthworms. In addition, human introduction is expected to have a large role in the presence of earthworm species on islands [11].

The Hyères archipelago was selected for a comparative phylogeographic study of its earthworm fauna due to several of its characteristics. It is situated in southeastern France, off the Provencal coast (Fig. 1). This region harbors high rates of endemism and genetic diversity [26,27] and has been suggested by some researchers as a potential area of diversification of Palearctic earthworms (Lumbricidae and Hormogastriidae [28,29]). As near-shore islands, they are expected to harbor neo-endemics, paleo-endemics and genetically differentiated populations [30]. The continental crust to which this archipelago belongs was located between Provence and the Corso-Sardinian microplate, and remained connected to Corsica and Sardinia until around 24 mya [31] and to the French mainland (with connection-isolation cycles during the Pleistocene) until 11,000–12,000 B. C [32]. Human settlement on the Hyères archipelago has been documented since the 1st century BC, by the



**Fig. 1.** Geographic location of the study area. a) Hyères archipelago and its location in Southeastern France (inset). Sampling points containing earthworms and their code on b) Cape Lardier c) Porquerolles island d) Port-Cros island.

Massaliote Greeks [32]. Since that time, the islands have been subjected to trade with the mainland and to agricultural use by the Romans, and several forts were built since the Middle Ages. The National Park of Port-Cros was founded in 1963, thus protecting the islands from further significant anthropogenic modifications.

The aim of this study was to test the phylogeographic patterns previously identified in earthworms (strong population structure in narrowly distributed divergent lineages vs widely distributed, genetically homogeneous lineages) within the framework of a near-shore, continental archipelago by i) assessing the genetic diversity and isolation from mainland populations of earthworm species composing the insular fauna; ii) obtaining an approximate temporal frame for the presence of the different species and genetic lineages in the islands and iii) comparing the phylogeographic patterns recovered for the studied species.

## 2. Materials and methods

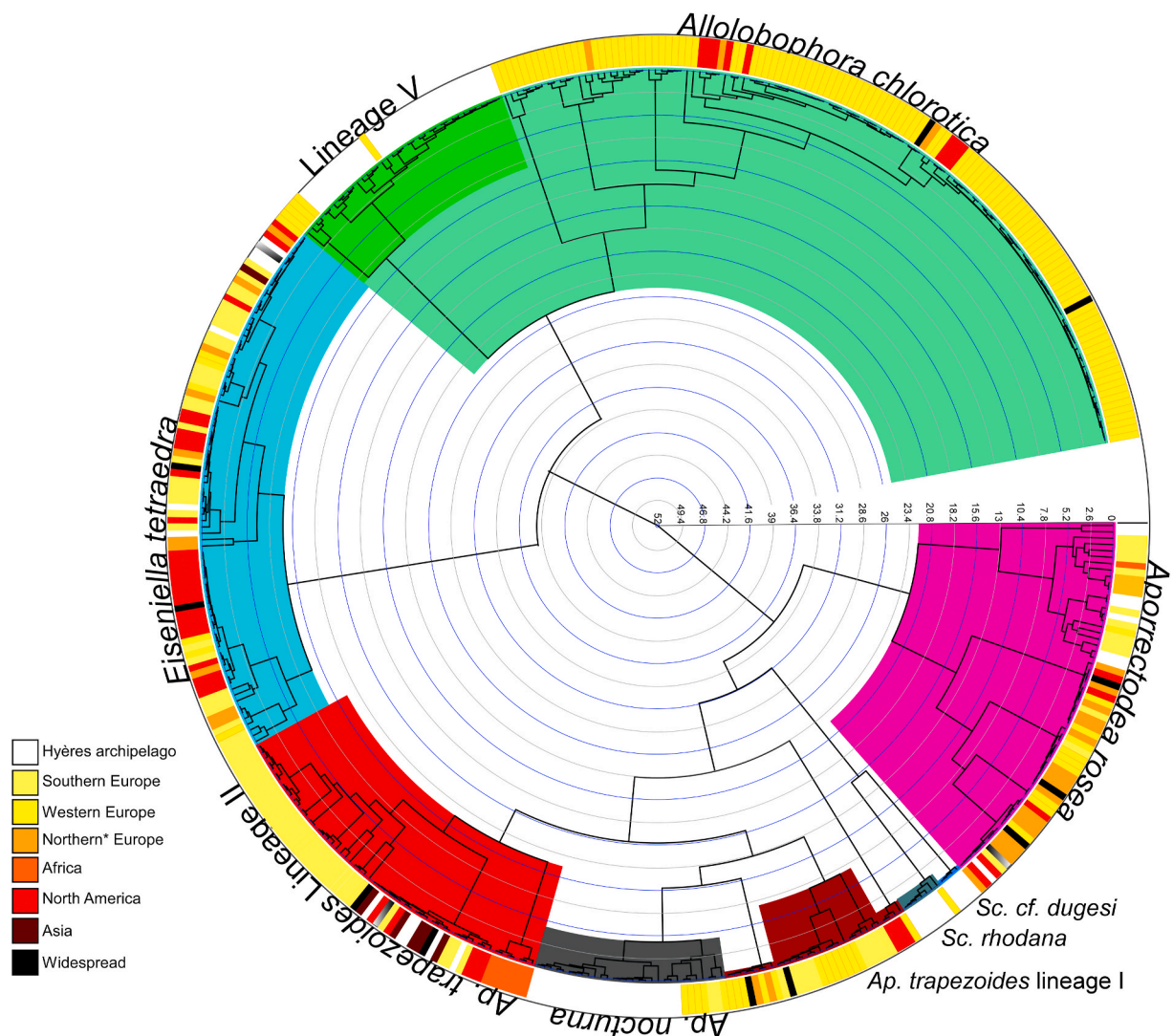
Sampling was performed on the islands of Porquerolles and Port-Cros, and at Cape Lardier (Fig. 1). Porquerolles island is 7.5 km long and 3.2 km wide, while Port-Cros island is 4.4 km long and 2.7 km wide.

Porquerolles and Port-Cros are separated by distances of 2.7 km and 8.4 km from the mainland, respectively; and 33 km and 23 km from Cape Lardier, respectively. The island of Port-Cros and the natural spaces of Porquerolles island constitute the core areas of the Port-Cros National Park, while the Cape Lardier is included in the buffer zone of the park.

A total of forty-nine localities were sampled in April 2018 by Decaens, Lapiéd, Maggia and Hedde [33], with 31 of them containing earthworms (Fig. 1 and Supplementary Table 1). Earthworms were collected by hand-sorting and fixed in 100% ethanol.

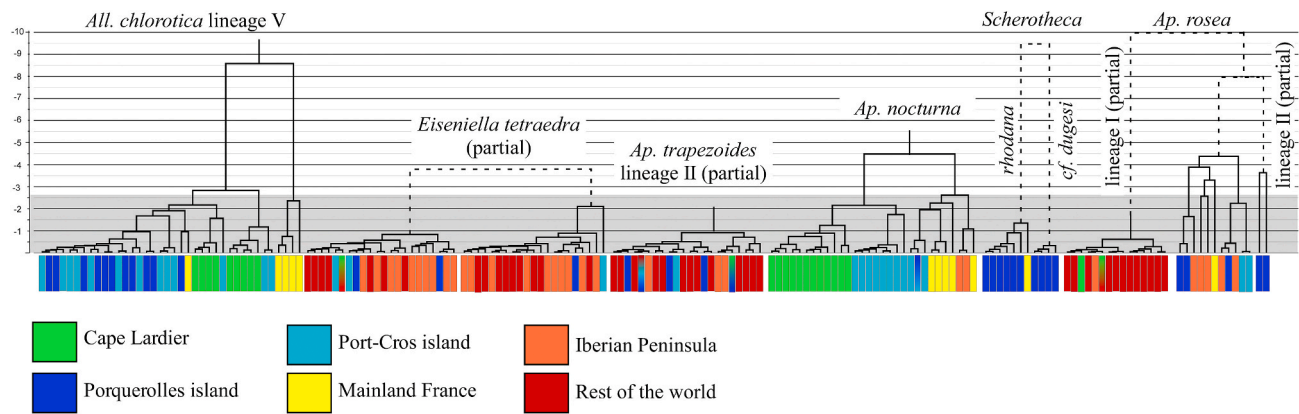
Total genomic DNA was extracted from ventral integument tissue samples, and the mitochondrial marker cytochrome c oxidase subunit 1 (COI) was amplified and sequenced at the Biodiversity Institute of Ontario using the primers from Ref. [34] and following the standard protocol established for the International Barcode of Life project (<http://ibol.org/>) (see details in Ref. [35]).

The seven best-represented lumbricid species were chosen for further analyses: *Allolobophora chlorotica* Savigny (1826) (62 specimens), *Ap. rosea* (19 specimens), *Aporrectodea nocturna* Evans (1946) (49 specimens), *Ap. trapezoides* (37 specimens), *E. tetraedra* (16 specimens), *Scherrothea cf. dugesi* Rosa (1895) (10 specimens) and *Scherrothea rhodana* Bouché (1972) (16 specimens). BOLD codes and/or Genbank



**Fig. 2.** Ultrametric tree based on COI sequences of specimens of *Allolobophora chlorotica*, *Aporrectodea rosea*, *Aporrectodea nocturna*, *Aporrectodea trapezoides*, *Eiseniella tetraedra*, *Scherrothea cf. dugesi* and *Scherrothea rhodana* sampled in the Hyères archipelago and reference sequences available in BOLD (Suppl. Table 2). *Ap. trapezoides* lineages I and II, and *All. chlorotica* lineage V according to Ref. [15,41]. Northern\* Europe: includes Serbia (as the only Eastern Europe sequence). Widespread: haplotypes shared between two or more geographic regions.





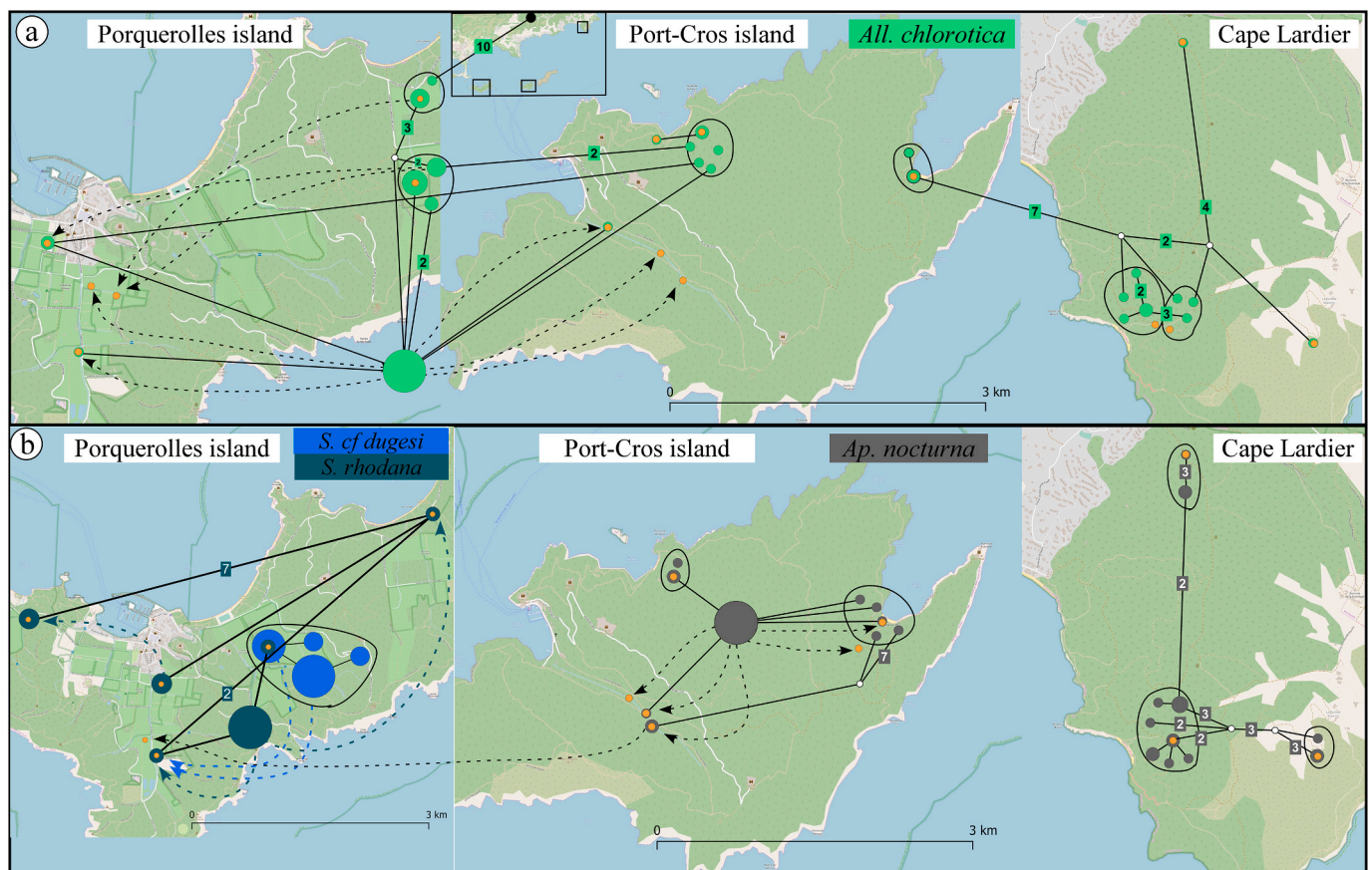
**Fig. 3.** Expanded view of the phylogenetic relationships of specimens of *Allolobophora chlorotica*, *Aporrectodea rosea*, *Aporrectodea nocturna*, *Aporrectodea trapezoides*, *Eiseniella tetraedra*, *Scherotheca cf. dugesi* and *Scherotheca rhodana* sampled in the Hyères archipelago (and closely related individuals from different locations around the world), obtained from the ultrametric tree based on COI sequences (Fig. 2). Lineage II and lineage 5 according to Ref. [15,42]. Terminals marked with an asterisk indicate sequences obtained for this study. Grey background squares indicate Pleistocene transgression-regression cycles (2.6 mya- 12,000 B.C.).

accession numbers for the sequences obtained for these species are shown in Suppl. Table 1. Sequences and associated metadata are also available on BOLD in the public dataset DS-PHYLPNPC (dx.doi.org/10.5883/DS-PHYLPNPC).

All COI sequences available in the Barcode of Life Data systems (BOLD) (including Genbank-datamined sequences) for the seven species

were downloaded and combined with the sequences generated for this work to build a reference dataset. BOLD codes and/or Genbank accession numbers for these sequences are shown in Suppl. Table 2 and are also available in the BOLD dataset DS-PHYLPNPC.

An ultrametric phylogenetic tree was obtained in BEAST 1.10.4 [36]. jModelTest v. 2.1.3 [37] was used to select the best fit evolutionary



**Fig. 4.** Haplotype networks of (a) *Allolobophora chlorotica* (b) *Scherotheca cf. dugesi*, *Scherotheca rhodana* and *Aporrectodea nocturna* overlaid on the geographical locations of the species on the islands of Porquerolles and Port-Cros, and Cape Lardier. The inset shows the coast of Hyères. Connection limit between haplotypes corresponds to 95% probability (11 steps). White dots represent inferred missing haplotypes. Orange dots indicate sampling locations. Hatched arrows represent the presence of the same haplotype in different locations. Black outlines represent the presence of different haplotypes in the same location. Numbers represent mutational steps between each sampled haplotype. Size of the circles is proportional to the number of individuals sharing each haplotype. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



model: GTR + I + G was selected as best-fitting evolutionary model. The analysis was conducted under a Coalescent: constant size model and an uncorrelated lognormal relaxed clock. An external calibration obtained from Refs. [13] for the clade comprising the target species (42–74 million years) was specified for the root of the tree as a normal prior with mean = 58 and standard deviation = 10. A uniform distribution with initial value = 0.002, ranging from 0.00005 to 0.02 was specified through the ucl. mean parameter, and a uniform distribution with initial value = 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 [38] to check for convergence and effective sampling sizes (ESS) over 100. The final tree was generated by TreeAnnotator v.1.10.4 [36], with a burnin of 2000 trees.

Haplotype networks were obtained with TCS 1.21 [39] using a 95% probability connection limit, to identify disconnected networks. In addition, haplotype networks were inferred in PopART 1.7 [40] for their graphical representation.

Genetic diversity and population genetics parameters were obtained in DnaSP 6 [41] within each of the main species of the study: haplotypic diversity (H), nucleotide diversity ( $\pi$ ), Tajima's D, Fu's  $F_s$ , Fu & Li's  $D^*$ , Fu & Li's  $F^*$  and Ramos-Onsins  $R_2$ .

Sampling localities were classified according to their land use (managed - olive groves, meadows, vineyards, fig tree orchards, pond banks and lagoons - vs unmanaged - forests and water resurgences), and species were classified according to their phylogeographic pattern (regional vs cosmopolitan). Presence or absence of cosmopolitan species was inspected in order to identify a possible effect of habitat anthropization on phylogeographic patterns. A chi-square test was performed in IBM SPSS Statistics v. 24.

### 3. Results

The ultrametric tree based on the sequences of *All. chlorotica*, *Ap. rosea*, *Ap. nocturna*, *Ap. trapezoides*, *E. tetraedra*, *S. cf. dugesi* and *S. rhodana* (Fig. 2) displayed the differences between phylogenetic structure, phylogeography and relative age of clades for these species. *All. chlorotica* and *Ap. rosea* showed deeply divergent lineages with different distributions: a few of them were restricted to France or Southern-Western Europe (*All. chlorotica* lineage V sensu [42], *Ap. rosea* lineage II sensu [43]), while the others were widely distributed around the world. *Eiseniella tetraedra* showed younger lineages, all of them with mostly cosmopolitan distributions. *Ap. trapezoides* reflected the findings of [15], with a mostly-Mediterranean (except for a cosmopolitan

subclade) lineage II and a series of paraphyletic lineages (comprised within lineage I sensu [15]) with a Eurosiberian-widespread distribution and more closely related to *Ap. nocturna*. *Ap. nocturna* was recovered as a monophyletic lineage restricted to France, United Kingdom and the Iberian Peninsula. *S. cf. dugesi* and *S. rhodana* were recovered as shallow clades limited to the Hyères archipelago.

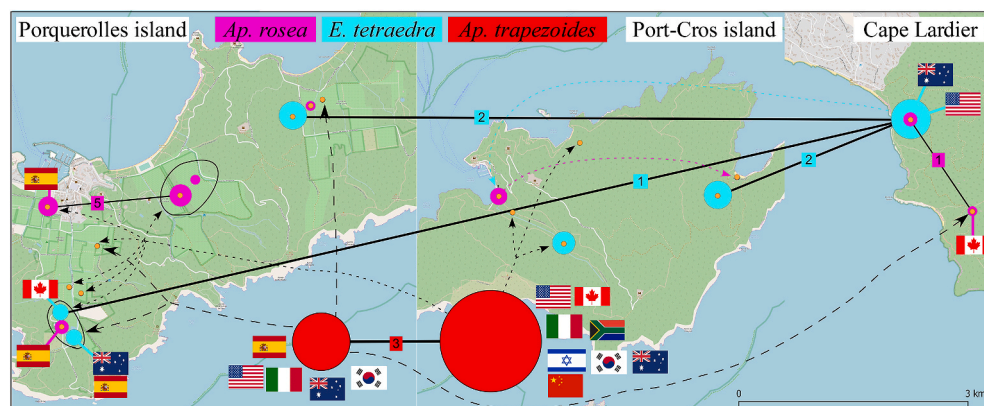
Detailed inspection of the clades comprising samples from the Hyères archipelago (Fig. 3) allowed to distinguish three phylogeographic patterns within this species: deeply divergent regional clades with relatively ancient divergence, younger and geographically restricted clades, and widely distributed lineages with young root ages.

*Ap. nocturna* and *All. chlorotica* (lineage 5) showed relatively similar phylogeographic patterns: individuals from Hyères were recovered as monophyletic groups with French mainland individuals as their closest relatives or as relatively basal branches. Estimated root age for *All. chlorotica* and *Ap. nocturna* regional clades were 2.8 mya (1.24–5.10, 95% highest posterior density) and 2.14 mya (0.8–4)–2.6 mya (1.12–4.65) respectively. Both species showed high genetic diversity within the Hyères archipelago. Most cladogenetic events within these clades overlapped with the Pleistocene period (2.59 mya – 10,000 B.C.). The main difference between both species was the isolation between the individuals from the different islands and the mainland: while in *Ap. nocturna* individuals from Cape Lardier formed a single clade neatly separated from individuals from Port-Cros island (which shared a single haplotype with Porquerolles), in *All. chlorotica* a close relationship between individuals from Cape Lardier and Port-Cros was recovered, and individuals from Porquerolles and Port-Cros islands appeared intermixed within the same clade.

*S. cf. dugesi* and *S. rhodana* formed two small clades exclusive to Porquerolles island. *S. rhodana* from Porquerolles island diverged from conspecifics from Montpellier at around 1.33 mya (0.38–2.85) and showed a root age of 0.90 mya (0.28–1.85), while *S. cf. dugesi* showed an estimated root age of 0.3 (0.06–0.80) mya.

*Ap. rosea*, *E. tetraedra* and *Ap. trapezoides* Mediterranean lineage (LII sensu Fernández et al. [15]) shared a different phylogeographic pattern, as individuals from the Hyères archipelago were nested within clades containing individuals from geographically distant countries.

In *Ap. rosea* and *E. tetraedra* several deeply divergent lineages were found to inhabit the archipelago, with some of them showing a Franco-Iberian distribution, some present in distant countries and the archipelago (even sharing haplotypes) and a few found exclusively in Hyères. Within *Ap. rosea*, lineage I sensu [43], the estimated root age for the clade including specimens from Cape Lardier and geographically distant countries was 130,000 years (52,000–280,000); for lineage II the

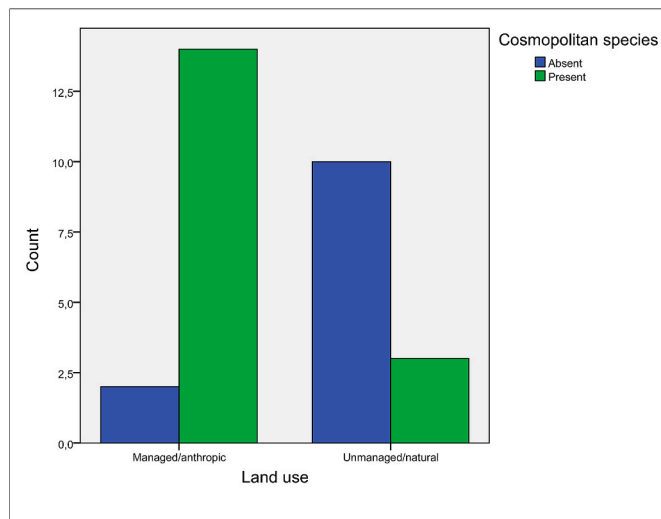


**Fig. 5.** Haplotype networks of *Aporrectodea rosea*, *Eiseniella tetraedra* and *Aporrectodea trapezoides* overlaid on their geographical locations on the islands of Porquerolles and Port-Cros, and Cape Lardier. Connection limit between haplotypes corresponds to 95% probability (11 steps). White dots represent inferred missing haplotypes. Orange dots indicate sampling locations. Hatched arrows represent the presence of the same haplotype in different locations. Black outlines represent the presence of different haplotypes in the same location. Flags represent the presence of the same (or closely related) haplotype in different countries. Numbers represent mutational steps between each sampled haplotype. Size of the circles is proportional to the number of individuals sharing each haplotype. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**

Genetic diversity and population genetics parameters obtained for each of the main species of the study. P values were non-significant ( $>0.10$ ) in all cases, H: Haplotype diversity,  $\pi$ : nucleotide diversity, D: Tajima's D, Fs: Fu's Fs, D\*: Fu & Li's D\*, F\*: Fu & Li's F\*, R2: Ramos-Onsins R2.

	H	$\pi$	D	Fs	D*	F*	R2
<i>All. chlorotica</i>	0.879	0.0157	-1.38178	-3.605	-1.06042	-1.41717	0.068
<i>Ap. nocturna</i>	0.831	0.0208	-0.61859	-0.367	-0.84967	-0.91281	0.0975
<i>S. rhodana</i>	0.683	0.0057	-0.21977	0.771	1.0815	0.83059	0.1383
<i>S. cf. dugesi</i>	0.711	0.0014	-0.4313	-1.02	-0.8049	-0.79808	0.1602
<i>E. tetraedra</i>	0.817	0.0229	-0.67559	6.94	0.49013	0.18388	0.1299
<i>Ap. rosea</i>	0.877	0.0844	-0.05075	15.168	0.8807	0.70149	0.1589
<i>Ap. trapezoides</i>	0.356	0.0012	0.92181	2.565	0.78344	0.95257	0.1778



**Fig. 6.** Presence or absence of “cosmopolitan” earthworm species (*E. tetraedra*, *Ap. rosea* and *Ap. trapezoides*) in managed/anthropic vs unmanaged/natural land uses.

estimated root ages for the clades including samples from the archipelago were 0.40 mya (0.06–1.06), 2.23 mya (0.76–4.6) and 3.62 (0.85–8.25). For *E. tetraedra* estimated root ages of clades comprising local and global samples were 400,000 years (155,000–670,000), 490,000 years (164,000–1 mya) and 460,000 years (172,000–970,000), with an endemic lineage diverging from the rest at 2 mya (0.82–4.01).

In *A. trapezoides*, only two closely related lineages were found to inhabit the archipelago, both of them also found worldwide. Estimated divergence age between these lineages was 0.90 mya (0.35–1.18). Estimated age of divergence of clades within these lineages were 700,000 (247,000 years –1.4 mya) and 500,000 (158,000 years–1 mya).

Haplotype networks displayed phylogeographic patterns congruent with the ones shown by the phylogenetic tree, but at a finer scale (Figs. 4 and 5).

Both *All. chlorotica* and *Ap. nocturna* showed high local genetic diversity, with several disconnected haplotype networks (11 or more mutational steps). While in *All. chlorotica* four connections were found between the islands or the islands and the mainland (even with a single haplotype being shared between Port-Cros and Porquerolles), in *Ap. nocturna* closely related haplotypes appear confined to the island of Port-Cros (except a single haplotype also found in Porquerolles) or to Cap Lardier. In both cases a star-like structure (with a frequent, widely distributed haplotype connected to several rare haplotypes) was observed in the islands, while on the mainland several rare haplotypes appear interconnected in complex networks. Several populations with different haplotypes (even from disconnected networks) were found in Cape Lardier and on the islands.

*S. cf. dugesi* and *S. rhodana* showed similar haplotype networks with restricted distributions, however *S. rhodana* was more widely distributed

across Porquerolles island, with haplotypes separated by a higher number of mutational steps.

*E. tetraedra* and *Ap. rosea* displayed several disconnected haplotype networks (3 and 5 respectively) with a very low number of haplotypes within each (1–4). The most complex haplotype network belonged to *E. tetraedra*, which displayed a star-like structure and connected haplotypes on both islands and the mainland (plus a single haplotype present in both Cape Lardier and Port-Cros island). Most sub-networks contained haplotypes present in other countries, in the Iberian Peninsula only for *Ap. rosea* and worldwide for *E. tetraedra*.

For *Ap. trapezoides*, two closely related haplotypes (separated by three mutational steps) present in several countries (United States, Canada, Australia, Italy, China, South Korea, Israel and South Africa) were widespread across the islands and mainland. Both haplotypes showed non-overlapping distributions, except for a single location.

Genetic diversity and population genetics parameters estimated for the main species are shown in Table 1. Haplotype diversity (H) was generally high, the lowest value corresponding to *Ap. trapezoides* (0.356) and the highest value to *All. chlorotica* (0.879). Nucleotide diversity ( $\pi$ ) was moderate to high (0.0157–0.0844) with the exceptions of *Ap. trapezoides*, *S. cf. dugesi* and *S. rhodana* (0.0012, 0.0014 and 0.0057 respectively).

The H- $\pi$  relationships for *All. chlorotica*, *Ap. nocturna*, *E. tetraedra* and *Ap. rosea*, (both values being high) corresponded to stable populations or secondary contact between differentiated lineages. In *S. cf. dugesi* and *S. rhodana*, H- $\pi$  relationships (high-low) matched the expectation for high demographic expansion from a small effective population, while *Ap. trapezoides* showed a H- $\pi$  relationship (low-low) typical of recent bottlenecks or founder effects.

None of the estimated demographic parameters (Tajima's D, Fu's Fs, Fu & Li's D\*, Fu & Li's F\* and Ramos-Onsins R2) were statistically significant.

A chi-square test showed statistically significant differences in the presence of “cosmopolitan” species (*E. tetraedra*, *Ap. rosea* and *Ap. trapezoides*) in managed vs natural habitats ( $p = 0.001$ ): these species were significantly more frequent in olive groves, meadows, vineyards, fig tree orchards, pond banks and lagoons than in different types of forests and water resurgences (Fig. 6).

#### 4. Discussion

Time-calibrated phylogenies are intrinsically troublesome for earthworms (and other soft-bodied invertebrates) due to the almost non-existent fossil record to use as calibration. Geological events have been used [18,44], with other approaches relying on outgroup fossils [13] or external node ages [45]. Here, external node ages obtained from a robust calibration based on annelid and clitellate fossils [13] avoided the use of *a priori* substitution rates, which are known to differ between different species of the same family [44]. Estimated substitution rates did indeed vary significantly (Suppl. Table 3), but they did not show a clear pattern of parthenogenetic species having faster or slower rates, agreeing with [46]. While this approach still requires a cautious interpretation of the estimated node ages, it provides a useful framework which would not be available otherwise. Thus, regardless of the different downsides of

molecular calibration in earthworms, it is advisable to make a compromise between reliability and stagnation of knowledge.

Limitations of single-locus mitochondrial phylogeographic analyses have been previously indicated: as an uni-parental, non-recombining marker, conclusions derived from it does not necessarily match those inferred from the nuclear genome. Deep mitochondrial lineages have been found in populations with admixture shown by nuclear markers, and their mixing within populations is possible by large population sizes maintaining such high mitochondrial divergence (i.e. incomplete lineage sorting) [47,48]. As the current work does not involve assumptions of species delimitation, hybridization or introgression, but focuses on geographical origin of haplotypes, any bias introduced by only considering maternal lineages should have a relatively low impact. This is supported by the continued use of COI in the last years as source of phylogeographic information for several studies in different animal groups [49–52], remaining a powerful tool for genetic diversity assessment of wide samplings of communities.

Comparative phylogeographic analysis of the earthworms inhabiting the Hyères archipelago revealed contrasting patterns related to age of divergence, geographic scale of genetic diversity and biological traits of the different species.

The time-calibrated phylogeny showed two distinct temporal frames for the establishment of the genetic lineages of earthworms on the archipelago. The first corresponds to ancient colonization and divergence *in situ* and is represented by four of the studied species. For *All. chlorotica*, estimated ages and multiple connections between islands or between islands and the mainland seem consistent with colonization by dispersal during the marine regression-transgression cycles associated with the Pleistocene [32]. For *Ap. nocturna*, isolation from the mainland populations after the Pliocene sea-level rise and subsequent dispersal from Port-Cros lineages during the Pleistocene appear more likely. *S. rhodana* appears to have diverged from mainland relatives during the Pleistocene. Several studies have shown the strong effect of Plio-Pleistocene [53–56] on shaping the genetic structure of different animal groups in the Mediterranean area. However, few have illustrated such an effect on island biota (but see Ref. [57]).

The second temporal frame matches recent human-mediated introduction and dispersal into the islands, inferred for *E. tetraedra*, *Ap. rosea* and *Ap. trapezoides*. These three species are well-known as cosmopolitan species inhabiting landmasses far from their Palearctic native ranges [16,58], and are generally assumed to be easily dispersed by humans. The increased presence of these species in managed habitats found in the archipelago supports the role of agriculture on their arrival and settlement.

A comparative phylogeographic study of the herpetofauna of Cyprus [59] showed a similar pattern of ancient colonization by land bridges and recent human-induced introductions, despite significant differences with the archipelago studied in this work (longer distance to the mainland, older land bridges, bigger island surface).

The limited sampling of mainland populations within this work could confound the observed colonization patterns if insular populations resulted from secondary colonization from unsampled coastal populations. While the possibility cannot be discarded, several barcoding studies were previously performed in the region of Var and Southeastern France, and the resulting sequences were included in the analyses. Nonetheless, additional sampling in the coast would be helpful to confirm these results.

The two main phylogeographic patterns (related to geographic structure of genetic diversity) identified in earthworms in previous studies were also found in the studied species: regional genetic structure vs worldwide shared haplotypes. The former was clearly observed in *All. chlorotica* lineage V and *Ap. nocturna*, which are distributed mostly in France but showed clades restricted to the Hyères archipelago and even within each island or the Cape Lardier. Although *E. tetraedra*, *Ap. rosea* and *Ap. trapezoides* were similar in the presence of shared haplotypes or lineages in distant locations around the world, some important

differences were observed. Clades of *Ap. rosea* “Mediterranean-like” Lineage II were shared between France, the Iberian Peninsula and the Hyères archipelago, but they were not present anywhere else in the world. For *E. tetraedra* and *Ap. trapezoides* (and *Ap. rosea* lineage I), lineages inhabiting the Hyères archipelago were found almost globally. Another difference was the genetic homogeneity within each species on the islands: several deeply divergent lineages were found in *Ap. rosea* and *E. tetraedra*, while *Ap. trapezoides* consisted on two closely related haplotypes from the same lineage. These differences may be explained by historical causes (geographical source and number of introduction events) but also by the different degrees of genetic diversity and genetic structure of the species in their native ranges: for example, it could be expected that *Ap. rosea* or *E. tetraedra* were introduced from previously diverse populations while *Ap. trapezoides* was likely transported from relatively homogeneous populations. This highlights the complexity of understanding the phylogeography of cosmopolitan species without a comprehensive knowledge of their phylogeography within their native range [43].

The mode of reproduction is an important life history trait that may explain those contrasting patterns. The three parthenogenetic species (*E. tetraedra*, *Ap. rosea* and *Ap. trapezoides*) showed evidence of recent human-mediated dispersal with moderate or strong genetic homogeneity across distant countries. Thanks to the ability of these species to reproduce in the absence of mates, parthenogenesis is expected to facilitate colonization of new habitats [12] even from single individuals or cocoons, which are the most likely means of anthropic transport. There are several more examples of the success of parthenogenetic earthworms as human-dispersed colonizers. For instance, *Ap. trapezoides* is the dominant species in the Palouse region, Washington-Idaho [60]. *Ap. rosea*, very frequent in the USA, shares haplotypes with populations from France and Denmark which supports their origin from these countries [43]. Moreover, *Ap. rosea*, *Ap. trapezoides* and *E. tetraedra* are among the most frequent and widely distributed exotic lumbricid earthworms in Canada [61]. A rich barcoding study of introduced earthworms in the USA and Canada [62] showed that *Ap. rosea* and *D. octaedra* (another parthenogenetic earthworm) are very frequent and represented by several lineages.

The other four species share biparental sexual reproduction, and their phylogeographic patterns were consistent with comparatively ancient occupation of the islands and strong regional genetic structure. This mode of reproduction does not necessarily preclude invasiveness or human-mediated introductions, as earthworms belonging to the genus *Lumbricus* are highly successful colonizers of North America [62,63] and *Aporrectodea caliginosa* Savigny (1826) is one of the most widespread species around the world [64]. However, biparental reproduction appears to make earthworms less prone to non-natural dispersal and settlement. For example, *All. chlorotica* is present in North America, but it is very rare and only represented by one of its many genetic lineages [62]. Another example is the higher success of *Amyntas corticis* Kinberg (1867) (parthenogenetic) as a colonizer of São Miguel island in Azores archipelago over *Amyntas gracilis* Kinberg (1867) (biparental) [21]. This suggests additional functional traits could have an influence on the suitability for human-mediated dispersal and colonization ability. It is also important to consider that some earthworm species show mixed modes of reproduction, like parthenogenesis with occasional amphimixis (as hinted by Refs. [65] for *A. trapezoides*): this could blur the correlation between reproductive mode and phylogeographic patterns depending on the frequency and importance of this phenomenon across the studied species.

Other functional traits that could influence phylogeographic patterns such as body size, behavior or habitat preferences are worth exploring. The largest earthworms in the Hyères archipelago, *S. rhodana* and *S. cf. dugesi*, are deep burrowing anecic species which makes the human transport of whole individuals or cocoons unlikely. However, for the other species these traits do not appear to have a clear effect. For example, *All. chlorotica* and *Ap. rosea* are small endogeic earthworms



which usually inhabit pastures, yet their phylogeographic patterns within Hyères are radically different. The same can be observed when comparing *Ap. nocturna* and *Ap. trapezoides*, which are very closely related mid-sized endo-anecic earthworms. As none of the studied species were epigeic earthworms intimately associated with rotten logs and fallen tree trunks (as is the case for most species of *Dendrobaena* or *Bimastos*), it seems unlikely overwater dispersal would be important for any of them (but experimental evidence would be needed to rule it out).

Papadopolou et al. [66] reported similar contrasting patterns between winged and flightless psammophilic and geophilic beetles in the Aegean archipelago with strong geographically structured and deeply divergent lineages vs widely distributed and shallow clades. In these insects, the interplay between habitat and dispersal ability was found to explain these patterns: the most ephemeral habitat appeared to promote dispersal and high population turnover, while the most stable habitat promoted persistence over time and vicariance (except in winged species, which lacked geographic structure).

## 5. Conclusions

Paleogeographic events, reproductive mode and human influence appear to be the main factors shaping the phylogeography of earthworms on Mediterranean near-shore islands. These patterns appear to be consistent with those observed for earthworms in other geographical contexts.

The present findings highlight the importance of comparative phylogeographic studies on earthworms, which have been shown to be closely related to paleogeographic events [67] but may also hold an unexplored potential for illustrating historical human migrations, trade routes and land uses [11].

## Data availability statement

Sequences and associated metadata are available on BOLD in the public dataset DS-PHYLPNPC ([dx.doi.org/10.5883/DS-PHYLPNPC](https://dx.doi.org/10.5883/DS-PHYLPNPC)).

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2020.103242>.

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