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Diversification patterns in cosmopolitan earthworms: similar mode but different *tempo* ☆

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ABSTRACT

Comparative phylogeography of widespread species that span the same geographic areas can elucidate the influence of historical events on current patterns of biodiversity, identify patterns of co-vicariance, and therefore aid the understanding of general evolutionary processes. Soil-dwelling animals present characteristics that make them suitable for testing the effect of the palaeogeographical events on their distribution and diversification, such as their low vagility and population structure. In this study, we shed light on the spatial lineage diversification and cladogenesis of two widely-distributed cosmopolitan and invasive earthworms (*Aporrectodea rosea* and *A. trapezoides*) in their putative ancestral area of origin, the Western Palearctic, and a few populations in North America. Molecular analyses were conducted on mitochondrial and nuclear markers from 220 (*A. rosea*) and 198 (*A. trapezoides*) individuals collected in 56 and 57 localities, respectively. We compared the lineage diversification pattern, genetic variability and cladogenesis in both species. Our findings showed that both species underwent a similar diversification from the Western Mediterranean plates to (i) Northern Europe and (ii) the Iberian Peninsula, establishing their two main lineages. Their diversification was in concordance with the main palaeogeographical events in the Iberian Peninsula and Western Mediterranean, followed by a later colonization of North America from individuals derived exclusively from the Eurosiberian lineage. Their diversification occurred at different times, with the diversification of *A. rosea* being potentially more ancient. Cladogenesis in both species seems to have been modelled only by the Mediterranean plate shifts, ignoring historical climatic oscillations such as the Messinian salinity crisis. Their high genetic variability, strong population structure, lack of gene flow and stepping-stone-like cladogenesis suggest the existence of different cryptic lineages. Our results may indicate a recurrent event in invasive earthworms within their ancestral distribution areas in the Western Palearctic.

1. Introduction

Subterranean biological invasions have gone largely unnoticed until the last century. Particularly, exotic earthworms have traditionally escaped the attention of all but a few biologists interested in the distribution and ecology of soil invertebrates (Beddard, 1912; Eisen, 1900; Ehrenfeld and Scott, 2001), yet many earthworm species are widely distributed throughout the world. From a total of about 3700 described earthworm species, approximately 120 can be found worldwide: the so-called peregrine earthworms. This term was first coined by Michaelsen (1900), who acknowledged the wide distribution of several earthworm species and described their presence in geographically remote areas. Since the invention of agriculture, human-mediated transportation of earthworms has allowed exotic earthworms to reach and spread in geographical areas remote to those of their origin. To be transported successfully to other regions, an earthworm species or its cocoons must have a high tolerance to changing environmental conditions such as temperature or moisture. Therefore, the most successful earthworm travelers will be those with the maximum degree of tolerance for adverse soil conditions (James and Hendrix, 2004).

Exotic earthworms can have an important effect on the ecosystem processes from the lands they colonize. For example, they can out-compete and replace native earthworm species, and affect severely soil processes mediated by biological activities such as litter decomposition, nutrient mineralization or changes in soil structure. Competitive displacement is not well documented with

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experimental work, but it was apparent to many authors from the 'New World'. For instance, while Eisen (1900) and Stebbings (1962) observed that locations once home to endemic earthworms were becoming dominated by exotics in California and Missouri respectively, Smith (1928) observed the same in central Illinois regarding *Diplocardia communis* and *Lumbricus terrestris*. An extreme example is that of the rhinodrilid *Pontoscolex corethrurus*, which 'compacts (the soil) more than a bulldozer' (Chauvel et al., 1999).

Most peregrine earthworms can be grouped in three families: Lumbricidae, Megascolecidae and Rhinodrilidae. Approximately 30 species of Lumbricidae include 'by far the greatest number of peregrine forms', including species from the genera *Lumbricus*, *Allolobophora*, *Octolasion*, *Aporrectodea*, *Dendrobaena*, *Eisenia* or *Eiseniella* (Beddard, 1912). Peregrine lumbricids are indigenous from the Western Palearctic, although there is debate on the size of the region of origin for most earthworm taxa (Hendrix et al., 2008). From the approximately 385 species of Lumbricidae, the highest diversity occurs in the unglaciated regions of southern Europe, and only approximately 5% of those species have spread into northern areas either by natural dispersion or through human transport (Sims and Gerard, 1999). Despite the importance of peregrine earthworms to understand ecological processes and predict future changes in invaded lands, molecular studies have been mainly restricted to endemic soil species with well-known distributions (Chang and James, 2011; Pérez-Losada et al., 2011) and still little is known about the phylogeography and genetic variability of peregrine earthworms, with a handful of studies focusing on a few species such as *Allolobophora chlorotica* or *P. corethrurus* (e.g., Dupont et al., 2011, 2012; King et al., 2008). Phylogeographic inference in this kind of species is therefore key to delimit their ancestral distribution area, to infer the exact origin of the invasions in remote lands, and ultimately to understand general evolutionary processes in invasive species.

To date, only one piece of work deals with the phylogeographic pattern of a peregrine species: the lumbricid *Aporrectodea trapezoides* (Duges, 1828) (Fernández et al., 2011). In that study, the authors found that the species was divided in two main clades, one distributed through the Eurosiberian part of Southern Europe and the other occupying only Mediterranean localities. The authors also found a high genetic diversity (with all haplotypes excepting one being exclusive to each locality), and a high degree of genetic differentiation between populations (Fernández et al., 2013). Building upon these results, the present study aims to further our knowledge on the evolutionary history of peregrine earthworms by (i) inferring the phylogeography and genetic variability of another peregrine species, *A. rosea* (Savigny, 1826), (ii) exploring the cladogenesis and lineage diversification of both *Aporrectodea* species in their ancestral distribution area: the Western Palearctic, and (iii) shedding light on the putative origin of the colonizing populations of *A. rosea* in North America.

2. Materials and methods

2.1. Sampling and molecular markers

A total of 126 adult earthworms of *A. rosea* were collected by hand from 44 localities in Spain, France, Italy and Algeria (Fig. 1, Suppl. Mat. S1). The animals were cleansed with distilled water, fixed in ca. absolute EtOH and stored at −20 °C. All the specimens have been deposited in the Oligochaete Cryo collection of Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid (DZAF, UCM).

A portion of integument (ca. 25 mg) was used for DNA extraction using DNeasy tissue kit (QIAGEN). Mitochondrial genes COI (cytochrome c oxidase subunit I), and 16S (including 16S rRNA and tRNA Leu, Ala, and Ser) and nuclear 28S (28S rRNA) and H3 (histone H3) were amplified. Primer sequences and PCR conditions are described in Supp. Mat. S2. Ninety-one sequences from *A. rosea* were retrieved from GenBank and BOLD databases and added to our analyses (only COI, Fig. 1, Suppl. Mat. S1). The DNA sequences were deposited in GenBank; the Accession Numbers are shown in Suppl. Mat. S1.

A total of 178 individuals belonging to *A. trapezoides* (Dugès, 1828) collected in 47 different localities and representing the two previously-described lineages by Fernández et al. (2011) were retrieved from GenBank (COI, COII, H3 and 28S rRNA). The mitochondrial gene 16S rRNA was newly sequenced for the *A. trapezoides* specimens included in this study. Furthermore, we collected and sequenced the above-mentioned genetic markers in 20 new individuals from new four localities in Algeria and Balearic islands (Fig. 1; Suppl. Mat. S1). Sequencing was performed as described above for *A. rosea*. Additional sequences from *Lumbricus terrestris* Linnaeus 1758, (Lumbricidae), *Hormogaster elisae* Álvarez, 1977 and *H. castillana* Qiu and Bouche, 1998 (Hormogastridae) for the same genetic markers were retrieved from GenBank for their use as outgroups in the phylogenetic analyses.

2.2. Overall genetic variability and gene flow estimation

For *A. rosea*, estimates of the variability of each gene, expressed as number of haplotypes (*Nh*), haplotype diversity (*H*), nucleotide diversity (π), number of segregating sites (*S*) and total number of mutations were calculated with DnaSP v5 (Librado and Rozas, 2009) Mean genetic differentiation between and within populations and localities was estimated using uncorrected *p*-distances. Pairwise F_{ST} for COI in *A. rosea* were calculated with Arlequin 3.5 (Excoffier and Lischer, 2010). We compared these values with those previously published for *A. trapezoides* in Fernández et al. (2011, 2013).

2.3. Phylogeographic analyses

Sequences of each gene were aligned using MUSCLE (Edgar, 2004) with default parameters. Phylogenetic analyses with the concatenated sequence of the four genes (2526 bp) included Bayesian inference (BI) and Maximum Likelihood (ML). The best-fit model of evolution for each gene was selected in Modeltest 3.7 (Posada and Crandall, 1998) under the Akaike Information Criterion (AIC). The general time reversible model of evolution, with proportion of invariable sites and a discrete gamma distribution (GTR + I + Γ) was selected for each data partition. For both analyses, datasets were partitioned by gene first, and then by codon position in the mitochondrial genes (two partitions: 1 + 2 codon positions, and 3rd codon position).

Bayesian inference was executed in MRBAYES v.3.1.2 (Huelsenbeck and Ronquist, 2005). Two runs, each with three hot and one cold chains, were conducted in MRBAYES for 20 million generations sampling every 2000th generation and using random starting trees. All sample points prior to the plateau phase (2500 trees) were discarded as 'burn in' after checking stationarity with Tracer v. 1.5 (Rambaut and Drummond, 2007). The remaining trees were combined in a 50% majority-rule consensus tree. Maximum likelihood (ML) analysis was run in RAxML v. 7.2.7 (Stamatakis, 2006) as implemented in T-REX (Boc et al., 2012). Nodal support was assessed with 1000 pseudoreplicates of non-parametric bootstrapping. Unlink nucleotide substitution model GTR + I + G was specified for each gene fragment, allowing the estimated to vary independently between each partition.

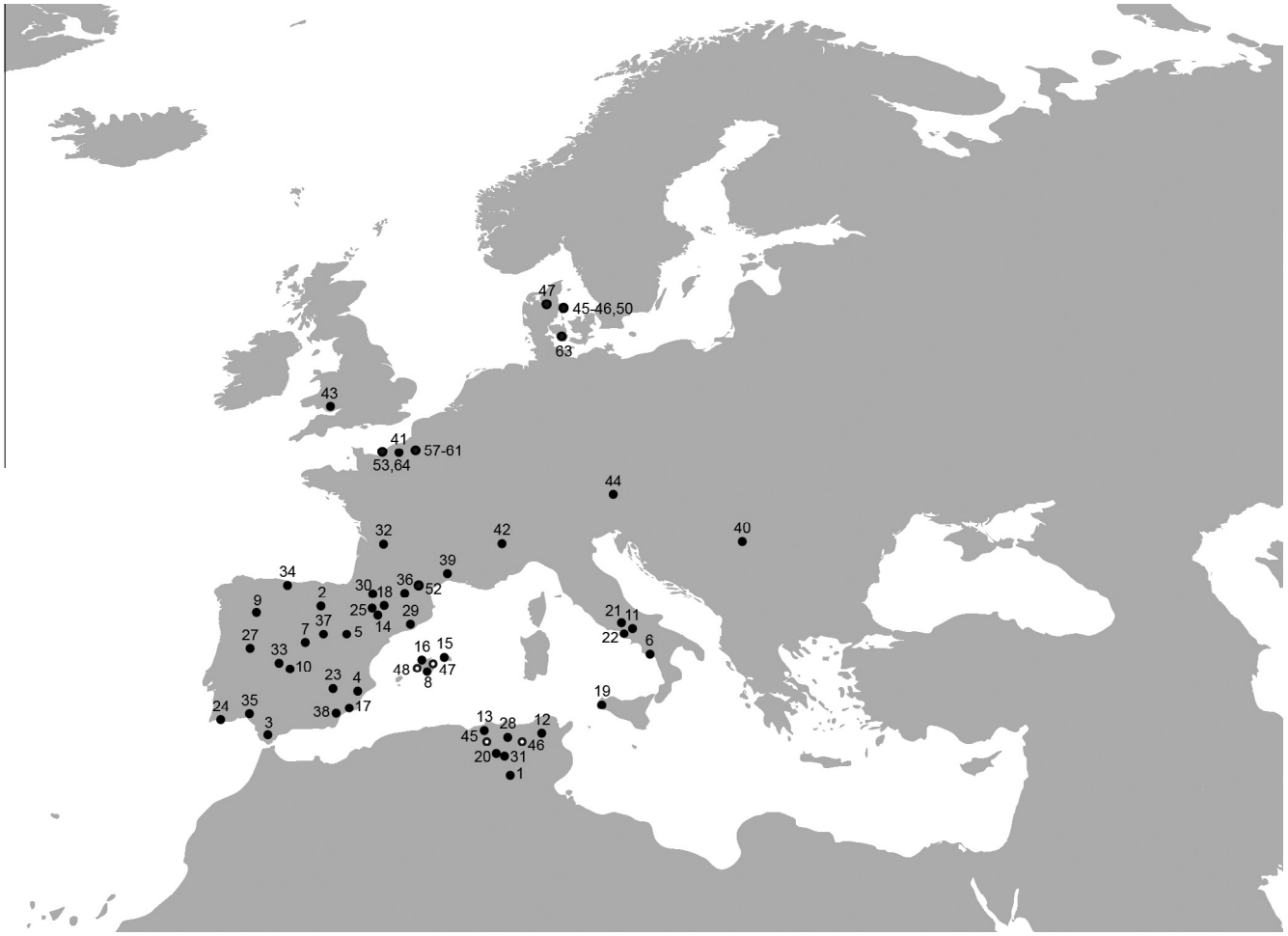

Fig. 1. Geographical location of the sampled populations. Black circles: localities of *A. rosea*. White circles: new localities of *A. trapezoides*, in addition to those provided in Fernández et al. (2011). The localities from North America are not shown. Numbers correspond to the localities in Supplementary Material S1.

2.4. Continuous phylogeography in space and time

A continuous phylogeographic analysis with relaxed random walks (Lemey et al., 2010) was conducted for both species independently using BEAST 2.0.1 (Drummond et al., 2012). Analyses were performed with COI (*A. rosea*) and COII (*A. trapezoides*) and with the concatenated dataset independently. As the colonization to North America was shown to be a recent phenomenon unrelated to landmasses movement (see Section 3), the North American specimens of *A. rosea* were excluded from this analysis. An uncorrelated lognormal clock model was set for each partition and a coalescence process as assumed for the tree prior. After prior optimization in a series of iterative test runs, Markov chains were run for 50 million generations, saving trees every 5000th generation. The results were visualized in Tracer v.1.5 (Rambaut and Drummond, 2007) and the burn-in was set to 2000 in TreeAnnotator version 2.0 (Drummond et al., 2012). The continuous phylogeographic pattern of dispersion in space was visualized with SPREAD (Bielejec et al., 2011). Unfortunately, the timing of the diversification processes cannot be inferred with accuracy due to a lack of fossils in oligochaetes and hindered by the exhibition of different mutation rates by different lineages (Novo et al., 2011), therefore the molecular dating based on a fixed rate of mutation is not particularly suitable in this animal group but at least gives us an idea about the potential age of diversification events. We applied the rates of mutation of 2.4 substitutions per million of years for mitochondrial genes calculated by Chang et al. (2008) and Pérez-Losada et al. (2011).

2.5. Lineage diversification and cladogenesis

Lineage diversification was explored and compared both in the concatenated dataset (using the ultrametric tree generated with BEAST 2.0 for the continuous phylogeographic analysis) and in the COI (*A. rosea*) or COII (*A. trapezoides*) dataset, as it includes a higher number of individuals. We applied the same mutation rate as in the previous analyses (i.e., 2.4 substitutions per million of years). Temporal shifts in diversification rates were explored with the R package LASER (Rabosky, 2006). Multiple diversification models were fitted to the ultrametric trees, including two constant rate (birth–death and pure birth) and seven variable rate models: Yule 2 rates, Yule 3 rates, DDL, DDX (density-dependent logistic and exponential model, respectively), exponentially declining speciation, exponentially declining extinction and variable speciation and extinction (see Section 3). The fit of the alternative models (including two constant rates and seven variable rate models) was compared by means of the Akaike Information Criterion (Posada and Buckley, 2004). The best-fit model was selected to calculate the diversification parameters, including 95% profile-likelihood confidence intervals. A lineage-through-time (LTT) plot, relative cladogenesis tests and the γ-statistic for measuring diversification bias towards the root ($\gamma < 0$) or the tips ($\gamma > 0$) (Pybus and Harvey, 2000) were also conducted with the same R package. The existence of lineage-specific multiple shifts in birth and death rates was explored with the R package MEDUSA (Alfaro et al., 2009).

2.6. Ancestral state reconstruction

Ancestral state reconstructions were explored through both a ML and a BI approach for both species using RASP (Ali et al., 2012). As in the previous analysis, the North American specimens were excluded from the ancestral reconstruction. Ranges were coded in 3 terminals corresponding to the two main clades obtained in the phylogeographic analyses plus the samples belonging to the Western Mediterranean microplates (Valencian Community, Balearic Islands, Algeria and localities placed in the Betic-Rifan system), as the continuous phylogeographic analysis indicated this area as the original area of diversification (see Section 3). In the Bayesian analysis, two runs of 10 million generations were run with a sampling every 10,000 generations. The maximum likelihood approach was performed through the dispersal-extinc tion-cladogenesis model with dispersal constraints set to 1.0, which implies that landmasses were connected and is therefore the most conservative approach.

3. Results

3.1. Lack of gene flow and overall genetic variability: Aporrectodea rosea is even more variable than A. trapezoides

Our results revealed a high genetic variability within *A. rosea*. The mitochondrial markers showed a higher variability than the nuclear ones. We found 88 haplotypes in COI and 35 in 16S rRNA. Both the haplotype and nucleotide diversity were high for these two markers (Table 1).

In general, the genetic variability observed for *A. rosea* was higher in all markers than that exhibited by *A. trapezoides*. Unlike *A. trapezoides*, all haplotypes in *A. rosea* were exclusive to each locality, and no haplotypes were found in widely separated localities, as occurred with one of the haplotypes of *A. trapezoides* (see Fernández et al., 2011).

F_{ST} values in all cases were very high for both mitochondrial genes (>0.7), indicating a lack of gene flow between populations (data not shown).

3.2. Similar phylogeographic patterns in Aporrectodea rosea and A. trapezoides

In *A. rosea*, both Bayesian inference and maximum likelihood tree topologies recovered two well-supported main lineages that mirrored the biogeographical distribution of the populations: Eurosiberian and Mediterranean. The first lineage included populations from the central and Northern part of the Iberian Peninsula, together with the French and Italian populations. The second lineage was formed by several localities in the centre of the Iberian Peninsula and all the populations from Southern Spain, showing a Mediterranean-like distribution. The results of these analyses were very similar to those recovered in the continuous phylogeographic tree (Fig. 2). Only one population from Southern Spain clustered in lineage I (RMOAB).

Continuous Bayesian phylogeographic inference supported a similar scenario of spatial dispersal in both species: an ancestral population located in the Western Mediterranean gave rise to a first clade that radiated into the Eurosiberian Europe and Italy, and a second clade that expanded towards the centre and South of the Iberian Peninsula (Fig. 2). In *A. rosea*, diversification would have started in an area located close to Corso-Sardinian plate, while *A. trapezoides* started to radiate from the eastern part of the Iberian Peninsula, including the present-day Valencian province and the Balearic islands, with a consequent intraspecific dispersal into multiple adjacent areas. Bayesian reconstruction of ancestral areas favored the ancestral area being in the terrane occupied by the Mediterranean land including the Western Mediterranean microplates (Balearic islands, Valencian community, Algeria and the Betic-Rifan system) as the most probable area for the ancestors of both species (Fig. 2).

3.3. Origin of the introduced A. rosea populations in North America

All the North American specimens from Iowa, Ohio and Canada shared haplotypes with Eurosiberian populations mainly from France but also from Denmark, including localities from Seine Maritime, Svendborg, Aarhus, the French Alps and Yvetot (Fig. 2). None of them showed any haplotypes exclusive of locality.

3.4. Tracking the tempo of diversification

Cladogenesis of an ancestral population followed a rapid 'stepping-stone' process (Suppl. Mat. S2). Lineage accumulation in both species occurred at a constant rate through time. A birth–death (BD) model provided the best fit for the data sets of both species. All other tested models, including diversity-dependent diversification and variable speciation or extinction through time (SPVAR, EXVAR and BOTHVAR), received lower support. The values recovered for r and ε when we allowed rates of speciation and extinction to vary among lineages using the MEDUSA algorithm were very similar to those of the BD model (Table 2). The value of extinction obtained in the BD model was high in both cases.

The relative cladogenesis test was non-significant in both species. MEDUSA did not detect a significant increase in the net diversification rate in any node. The gamma statistic was non-significant in both analyses ($\gamma = 12.187$, $p = 1$; $\gamma = 2.975$, $p = 0.99$ for *A. rosea* and *A. trapezoides*, respectively).

The molecular dating indicated that the diversification of *A. rosea* precedes that of *A. trapezoides* (Fig. 2); although the error bars for the diversification of both lineages in both species overlap (20–200 My in *A. rosea*; 5–26 My in *A. trapezoides*) the 95%

Table 1
Overall genetic variability for the sequenced markers in *A. rosea* (COI, 16S, H3 and 28S rRNA) and *A. trapezoides* (16S rRNA). The values for previously sequenced markers in *A. trapezoides* (COII, H3 and 28S rRNA) can be checked in Fernández et al. (2011).

	A. rosea				*A. trapezoides*
	COI	16S rRNA	H3	28S rRNA	16S rRNA
Length of sequence alignment	651	728	305	859	759
Total number of mutations	257	198	114	75	174
No. Polymorphic sites	182	143	75	64	132
G + C content	0.441	0.442	0.639	0.634	0.453
Haplotype diversity	0.943 ± 0.025	0.912 ± 0.024	0.834 ± 0.021	0.481 ± 0.058	0.991 ± 0.023
Nucleotide diversity	0.098 ± 0.004	0.085 ± 0.003	0.037 ± 0.003	0.007 ± 0.001	0.080 ± 0.003
Mean number of pairwise differences	58.324 ± 21.787	34.957 ± 18.987	9.465 ± 3.987	2.003 ± 0.567	27.997 ± 16.934

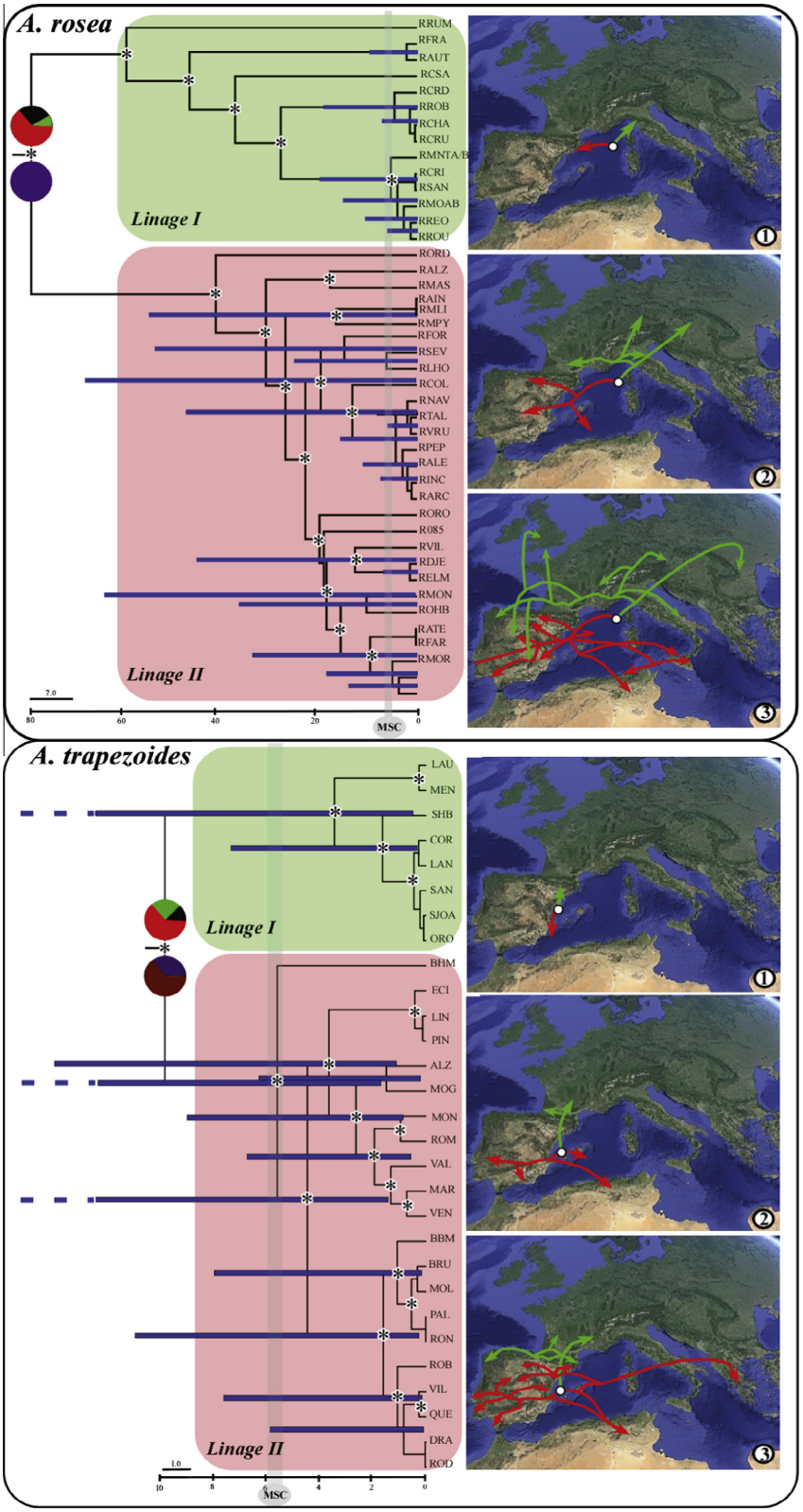

Fig. 2. Left: Bayesian evolutionary tree of *A. rosea* (up) and *A. trapezoides* (down) inferred from continuous phylogeographic analysis as performed in BEAST analysis of the concatenated datasets. Stars indicate posterior probabilities and bootstrap values higher than 90/0.8 (BI/ML). The two main clades recovered are shown in green (lineage I) and red (lineage II). In *A. rosea*, the clade represented by a black circle include the following localities: RFRA, RAUF, RIOW, ROHI, RHOJ, RGUE, RONT, RSBR, RFOU. The clade represented by a black square include the following localities: RSVEN, RSEN, RAAR, RLAN, RYVE, RMAR, RREO, RROU. Grey bar indicate the duration of the Messinian salinity crisis (MSC). Ancestral reconstruction of the basal node of both species is shown as a pie chart with sectors representing relative probability of the ranges of the hypothetical ancestor. Upper circle: RASP analysis (red: Mediterranean range; green: Eurosiberian range; black: Western microplates range). Lower circle: Lagrange analysis (purple represents Mediterranean + Eurosiberian ranges; dark red represents Mediterranean + Western microplates ranges). Right: Reconstruction of dispersal hypothesis in space for both species after the continuous phylogeographic model with random walks. Arrows indicate directionality of the expansion in both lineages (green: lineage I; red: lineage II). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Fit of models to the log-lineage through time curve for both species (*A. rosea*/*A. trapezoides*). DDL and DDX refer to density-dependent logistic and exponential models, respectively. In bold, optimal model and values for each parameter. $r = \lambda - \mu$ = net diversification rate. $\varepsilon = \mu/\lambda$ = extinction fraction. k = speciation rate of variation. z = extinction rate of variation. st = shift time (in Mya). λ = initial speciation rate. μ = final extinction rate.

Model	No. variables	ln*L*	AIC	dAIC	Value variables	MEDUSA
Pure birth	1	−30.748/21.533	63.496/−41.066	5.266/7.508	*r* = 0.072/0.488	
Birth–death	**2**	**−25.482/26.287**	**58.964/−48.574**	**0/0**	**ε = 0.816/0.970**	**ε = 0.816/0.970**
					***r* = 0.026/0.035**	***r* = 0.026/0.035**
DDL	2	−30.749/21.533	65.497/−39.065	5.267/9.509	*r* = 0.073/0.488	
					k = 68095/490679	
DDX	2	−28.529/25.226	61.058/−46.452	3.047/2.122	*r* = 0.022/0.100	
					z = −0.738/−0.682	
Yule 2 rates	2	−26.743/25.194	59.487/−44.387	1.261/4.187	st = 4.241/1.641	
					*r*1 = 0.053/0.246	
					*r*2 = 0.134/0.745	
Yule 3 rates	3	−25.939/26.045	61.879/−42.091	0.457/6.483	st1 = 10.061/1.641	
					st2 = 4.241/1.612	
					*r*1 = 0.062/0.246	
					*r*2 = 0.032/3.848	
					*r*3 = 0.134/0.715	
Exponentially declining speciation	3	−27.515/26.274	61.030/−46.549	2.033/2.483	λ = 0.151/1.195	
					k = 0.001/0.001	
					μ = 0.120/1.153	
Exponentially declining extinction	3	−27.485/26.287	60.971/−46.573	2.003/2.001	λ = 0.140/1.183	
					z = 52.696/9351.81	
					μ = 0.114/1.147	
Variable speciation and extinction	5	−27.515/26.275	63.030/−44.550	2.033/4.024	λ = 0.151/1.195	
					k = 0.001/0.001	
					z = 52.800/332.819	
					μ = 0.120/1.153	

confidence intervals in the clades within the lineages are generally older in *A. rosea*. Also, several diversification events included the Messinian salinity crisis in both species, suggesting that this crisis may not have had an effect in the overall diversification of both species. However, the extensive width of the 95% confidence intervals and the short duration of the Messinian salinity crisis make difficult any solid inference.

4. Discussion

4.1. High cryptic diversity in cosmopolitan earthworms: the rule more than the exception?

Although to date the studies dealing with phylogeography or population genetics of cosmopolitan earthworms is scarce and most of them are focused on small geographic areas, they all seem to have in common high values of genetic diversity. For example, Torres-Leguizamon et al. (2014) found 15 different COI haplotypes in *A. icterica* from 134 individuals collected in an area of approximately 100 m^2 in northern France. Likewise, Dupont et al. (2011) found 54 haplotypes in COI for *Allolobophora chlorotica* in northern Europe and UK. Another example includes 24 COI haplotypes found in *Dendrobaena octaedra* collected throughout Finland (Knott and Haimi, 2010). The only study with a comparable spatial scale to the present work is that of Fernández et al. (2011) for *A. trapezoides*, where 37 haplotypes for COII were recovered in populations distributed in 11 countries. In this study, we showed that the genetic diversity for *A. rosea* is higher than that exhibited by *A. trapezoides* in a similar spatial scale. These common patterns of a high genetic diversity seem to indicate that the sampled localities are a part of the ancestral area of distribution. Moreover, by comparing the genetic variability in general and the sequenced haplotypes in particular in the ancestral distribution areas and the remote colonized lands, we could potentially identify with accuracy the exact location where biological invasions come from in each case. Therefore these results set a background of knowledge that is key to understand the success of invasive peregrine species and to model and predict earthworm invasions.

4.2. North America was conquered by Eurosiberian specimens of A. rosea

All North American specimens of *A. rosea* shared haplotypes exclusively with earthworms from the Eurosiberian lineage, pointing towards a human-mediated introduction of earthworm potentially from France and Denmark in North America and Canada. Porco et al. (2013) found the same result, but they did not include any specimens from the Mediterranean lineage in their study. This pattern contrasts with the one observed for *A. trapezoides*: Fernández et al. (2011) found that the clone 1, belonging to the Mediterranean lineage of this species, was the one colonizing Australia. However, the authors did not include any specimens from the American continent. Further studies including new localities will help to shed light on this question.

4.3. Evidence of diversification from the Western Mediterranean microplates: the origin of European peregrine earthworms?

Both *A. trapezoides* and *A. rosea* are typical synanthropic species. Widely introduced extratropically by men, their present range comprises Europe, North and South America, Africa, Asia, Australia and New Zealand. As for the rest of peregrine lumbricids, their original area of distribution is the Western Palearctic, although the size of the region of origin for most earthworm taxa is a matter of debate (Hendrix et al., 2008). The results of this study seem to indicate that both species radiated from the Western Mediterranean, including the Iberian Peninsula, Northern Africa and Italy.

The existence of two highly-divergent and basally-separated lineages had been already observed in *A. trapezoides* (Fernández et al., 2011), but the cause underlying this fact was not explored. In this study we demonstrate that the same pattern was observed for *A. rosea*. According to the continuous phylogeographic analyses, diversification started in the Western Mediterranean microplates for both earthworm species. Diversification of the ancestor in *A. rosea* would have started between the Balearic Islands and Corsica. The common ancestor of both lineages in *A. trapezoides* would be located close to the Balearic-Rif microplate, radiating

from there into Algeria and Southern Spain. The existence of the same diversification process in two of the most widespread earthworm species is interesting and may indicate a common phenomenon in European earthworms present in Eurosiberian and Mediterranean areas. A first hypothesis explaining this fact could be that the Western Mediterranean microplates acted as a refugium during the glaciations.

A second hypothesis explaining the observed diversification patterns in both earthworm species would contemplate the Western Mediterranean microplates as the original range of distribution of these species that started to radiate and to follow the destiny of the geological events of the colonized and native areas. Other terrestrial animals have shown similar diversification patterns led by the complex changes induced by the Alpine Orogeny (e.g., Bidegaray-Batista and Arnedo, 2011). This event provoked the opening of the Western Mediterranean basin at the beginning of the Oligocene (ca 30 Ma), when the microplates within the Hercynian belt (Sardo-Corsican system, Calabro-Peloritan masiff, Balearic Islands, Betico-Rifan system and Kabylies) drifted to their present position; during the first stage of the back arc extension, the Balearic Islands and Great Kabylie drifted clockwise relative to Iberia, while the northern microplate assemblage (Sardinia, Corsica and Calabro-Peloritan massif) started drifting counterclockwise respecting the Eurasian plate (Andeweg, 2002; Rosenbaum et al., 2002a; Rosenbaum and Lister, 2004).

Curiously, the Western Mediterranean is also the distribution area of many endemic earthworms: the genus *Prosellodrilus* is endemic to Southern France, northeastern Iberia, Sardinia and Northern Africa, the genus *Postandrilus* distributed through the Balearic islands (excepting one species whose taxonomic status remains unclear, *P. galiciandrilus bertae*) and the whole family Hormogastridae is endemic to the Western Mediterranean (Northeastern Iberia, Eastern Italy, Sicily, Algeria, Corsica and Sardinia and South of France) (Bouché, 1972). Based on the present distribution of the autochthonous earthworm fauna, Omodeo and Rota (2008) hypothesized about the areas of origin of several earthworm genera. Following their ideas, the Pyrenees would be the homeland of the genera *Orodrilus*, *Ethnodrilus* and *Prosellodrilus*, the Sardo-Corsican system was the area of origin of *Diporodrilus* and part of *Prosellodrilus* and *Eumenoscolex*, the Betico-Rifan that of the *Allolobophora molleri* complex, and the family Hormogastridae and the genus *Scherotheca* would have diversified from the complete set of the emerged Western lands, excluding the Betico-Rifan microplates. After the results of this study, not only would be the Western Mediterranean the native area of many endemic species, but also could be the original land from which some peregrine, cosmopolitan earthworms (presumably with a higher adaptability and colonization ability) started to radiate. Further studies will shed light on the veracity of this hypothesis.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.07.017.

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