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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.