Molecular Phylogenetics and Evolution 94 (2016) 473–478

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Phylogenomic analyses of a Mediterranean earthworm family (Annelida: Hormogastridae) ☆

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ARTICLE INFO

Article history:
Received 18 May 2015
Revised 19 October 2015
Accepted 24 October 2015
Available online 31 October 2015

Keywords:
Next generation sequencing
Transcriptomics
Oligochaeta
Gene conflict
Phylogeny

ABSTRACT

Earthworm taxonomy and evolutionary biology remain a challenge because of their scarce distinct morphological characters of taxonomic value, the morphological convergence by adaptation to the uniformity of the soil where they inhabit, and their high plasticity when challenged with stressful or new environmental conditions. Here we present a phylogenomic study of the family Hormogastridae, representing also the first piece of work of this type within earthworms. We included seven transcriptomes of the group representing the main lineages as previously-described, analysed in a final matrix that includes twelve earthworms and eleven outgroups. While there is a high degree of gene conflict in the generated trees that obscure some of the internal relationships, the origin of the family is well resolved: the hormogastrid *Hemigastrodrilus* appears as the most ancestral group, followed by the ailoscolecid *Ailoscolex*, therefore rejecting the validity of the family Ailoscolecidae. Our results place the origin of hormogastrids in Southern France, as previously hypothesised.

1. Introduction

Hormogastridae includes middle to large-sized earthworms, currently comprising four genera, 31 species and subspecies or varieties, all endemic to the Western Mediterranean (Omodeo and Rota, 2008) and adapted to the driest soils, thanks to their lack of dorsal pores and aestivation capacity (Diaz Cosin et al., 2006). They have been recorded in Spain, France, Italy, Maghreb, and islands such as Sicily, Corsica and Sardinia (Fig. 1) and their distribution and evolutionary relationships have been probably affected by the palaeogeographical and palaeoecological events in the Mediterranean basin (Novo et al., 2015a). Hormogastrid diversity peaks at the NE Iberian Peninsula and SE France. This area has been hypothesised as the possible ancestral area for the family in a recent biogeographical study based on ancestral area reconstructions (Novo et al., 2015a). The known diversity is currently divided into four genera: *Hormogaster* Rosa, 1887 (19 species), *Hemigastrodrilus* Bouché, 1970 (1 species), *Vignysa* Bouché, 1970 (2 species) and *Xana* Díaz Cosín et al., 1989 (1 species). Phylogenetic trees inferred by Novo et al. (2011) recovered seven well-supported clades (Fig. 1): (i) *Xana*; (ii) *Hemigastrodrilus*; (iii) *Vignysa*; (iv) *Hormogaster elisae* in the central area of the Iberian Peninsula; (v) *Hormogaster* species from the NE area of the Iberian Peninsula; (vi) *Hormogaster* species within the *H. pretiosa* group and related from Catalonia and S Sardinia; and (vii) *Hormogaster* from the Tyrrhenian area (*H. redii* and *H. samnitica*). These results highlighted the paraphyly of the genus *Hormogaster* but the relationships among the main well-supported seven clades remained equivocal. Additionally, James and Davidson (2012) proposed the placing of Ailoscolecidae, containing only the species *Ailoscolex lacteospumosus,* in the synonymy of Hormogastridae. The same authors, recovered *Hemigastrodrilus* as sister clade of Lumbricidae with molecular analyses, whereas Bouche (1970) indicated the presence of Morren glands within this genus, as it is shown in lumbricids, but not in hormogastrids (Bouche, 1972). *Hemigastrodrilus* has been placed within Hormogastridae in different positions using molecular markers (e.g. Novo et al., 2015a). As a consequence, clarifying the phylogenetic position of both *Ailoscolex* and *Hemigastrodrilus* is necessary.

In the last few years, next generation sequencing (NGS) techniques have undergone a substantial development and its

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☆ This paper was edited by the Associate Editor Marcos Perez-Losada.

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http://dx.doi.org/10.1016/j.ympev.2015.10.026
1055-7903/

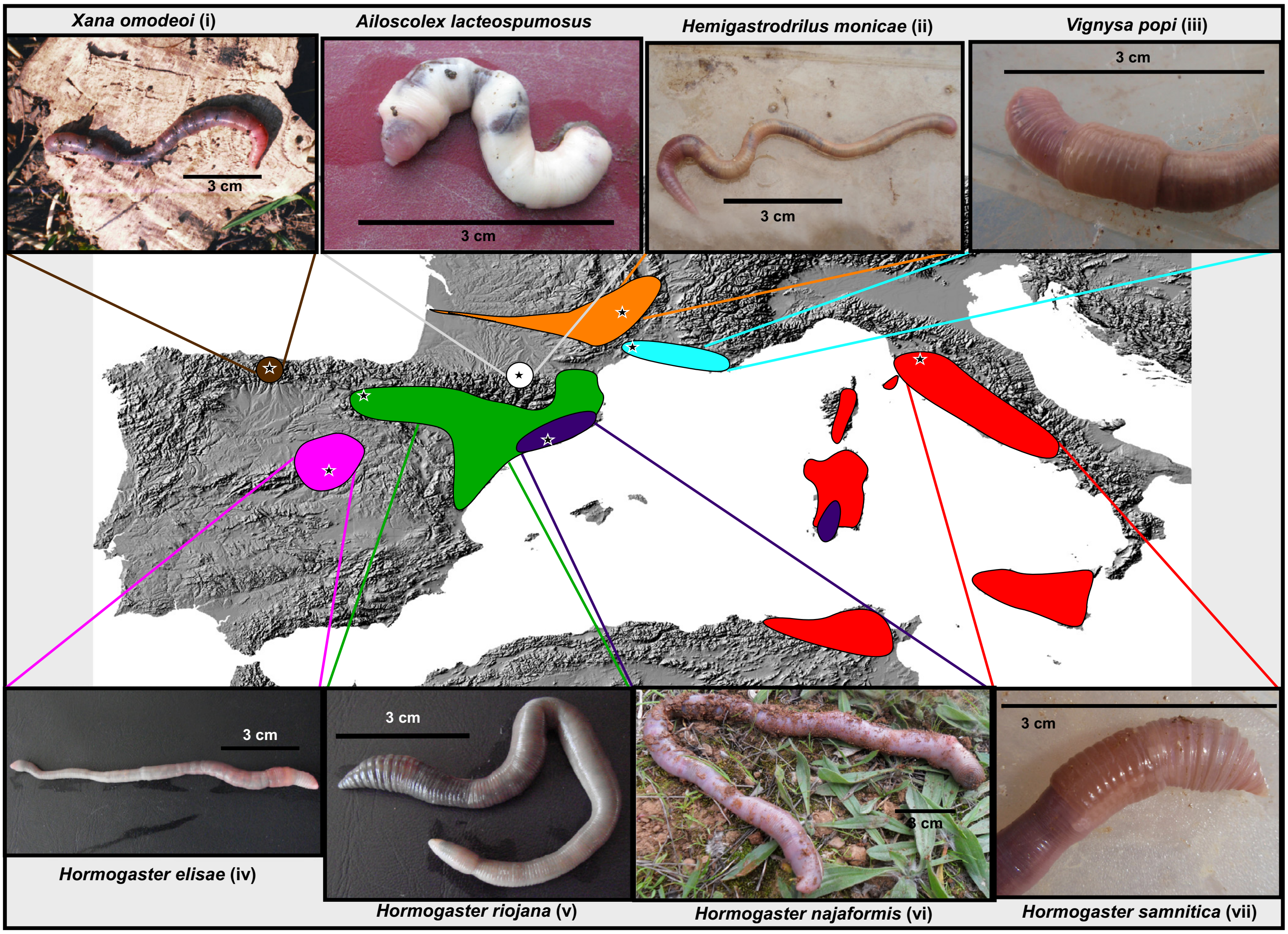

Fig. 1. Map showing the distribution of hormogastrids and the selected species for the phylogenomic analyses. Colour corresponds to that in trees by Novo et al. (2011) and to Fig. 2. A star indicates the sampling locality of the specimens. The number of clade following Novo et al. (2011), as described in the main text, is shown after the scientific name of the selected species. The species *Ailoscolex lacteospumosus* was not previously included in those phylogenetic analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

application in phylogenetics of non-model organisms has become key to understand complex evolutionary scenarios that could not have been resolved before based on morphology or in a handful of genes (e.g., Fernandez et al., 2014, spiders; Laumer et al., 2015, planarians). Therefore, this emerging technique seems promising to tackle unresolved evolutionary relationships such as the case of hormogastrid phylogeny. Our intention was to shed light on the phylogenetic relationships of the family Hormogastridae (plus the putatively related Ailoscolecidae) through phylogenomics by sampling a representative of each of the main clades recovered by Novo et al. (2011) and *Ailoscolex*. This study represents the first phylogenomic investigation within earthworms.

2. Materials and methods

2.1. Taxon sampling

Transcriptome reads from *Hormogaster samnitica* and *H. elisae* were already available and retrieved from Riesgo et al. (2012) and Novo et al. (2013) respectively. We sampled six new transcriptomes (five hormogastrids plus *Ailoscolex*) covering the remaining clades from Novo et al. (2011) (see Fig. 1). We selected earthworms from different families as close outgroups: *Pontoscolex corethrurus* (Rhinodrilidae); *Eisenia fetida* and *Lumbricus rubellus* (Lumbricidae) and *Amynthas gracilis* (Megascolecidae). Their transcriptomes are part of different studies, some of which are still under preparation and their unassembled reads were used (Supplementary File 1). Finally, we added eleven more taxa as distant outgroups, including sipunculans, polychaetes, molluscs and nemerteans whose assembled transcriptomes were available (Lemer et al., 2015; Weigert et al., 2014).

Sampling localities and coordinates for the earthworm specimens are shown in Supplementary File 1. Leftover tissues are deposited in the Oligochaete Cryo collection of the Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid (DZAF, UCM), Spain.

2.2. Library construction and transcript sequencing

Earthworms were preserved in RNA*later*® in the field. DNA was extracted and individuals were genotyped using cytochrome C oxidase subunit I (COI) primers LCO1490 and HCO2198 (Folmer et al., 1994). Sequences were compared to reference specimens previously dissected and taxonomically identified (see protocol and reference sequences in Novo et al. (2011)). For *Ailoscolex lacteospumosus*, seven molecular regions were amplified in order to further explore its relationship with the previously-sequenced hormogastrids and perform a topology test (see below): mitochondrial COI, 16S rRNA and tRNA Leu, Ala, and Ser (16S t-RNAs), one nuclear ribosomal gene (a fragment of 28S rRNA) and one nuclear