

Anchoring the systematics of Lumbricidae: phylogenomic reconstruction of above-genus level relationships through Anchored Hybrid Enrichment

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Introduction

Earthworm taxonomy has shown great instability since its inception. The implementation of molecular phylogenetics displayed potential for clarifying relationships between earthworm genera: in Lumbricidae, Sanger-sequenced “legacy markers” have allowed to recover genus-level clades and to identify polyphyletic genera. However, the relationships between some genera are usually recovered as polytomies, with some species behaving as rogue taxa. Anchored Hybrid Enrichment (AHE) is a recent source for phylogenomic analyses, providing hundreds of unique orthologous loci from across the genome with several other advantages (like informativeness across different taxonomic scales, cost effectiveness, no necessity of special preservation protocols).

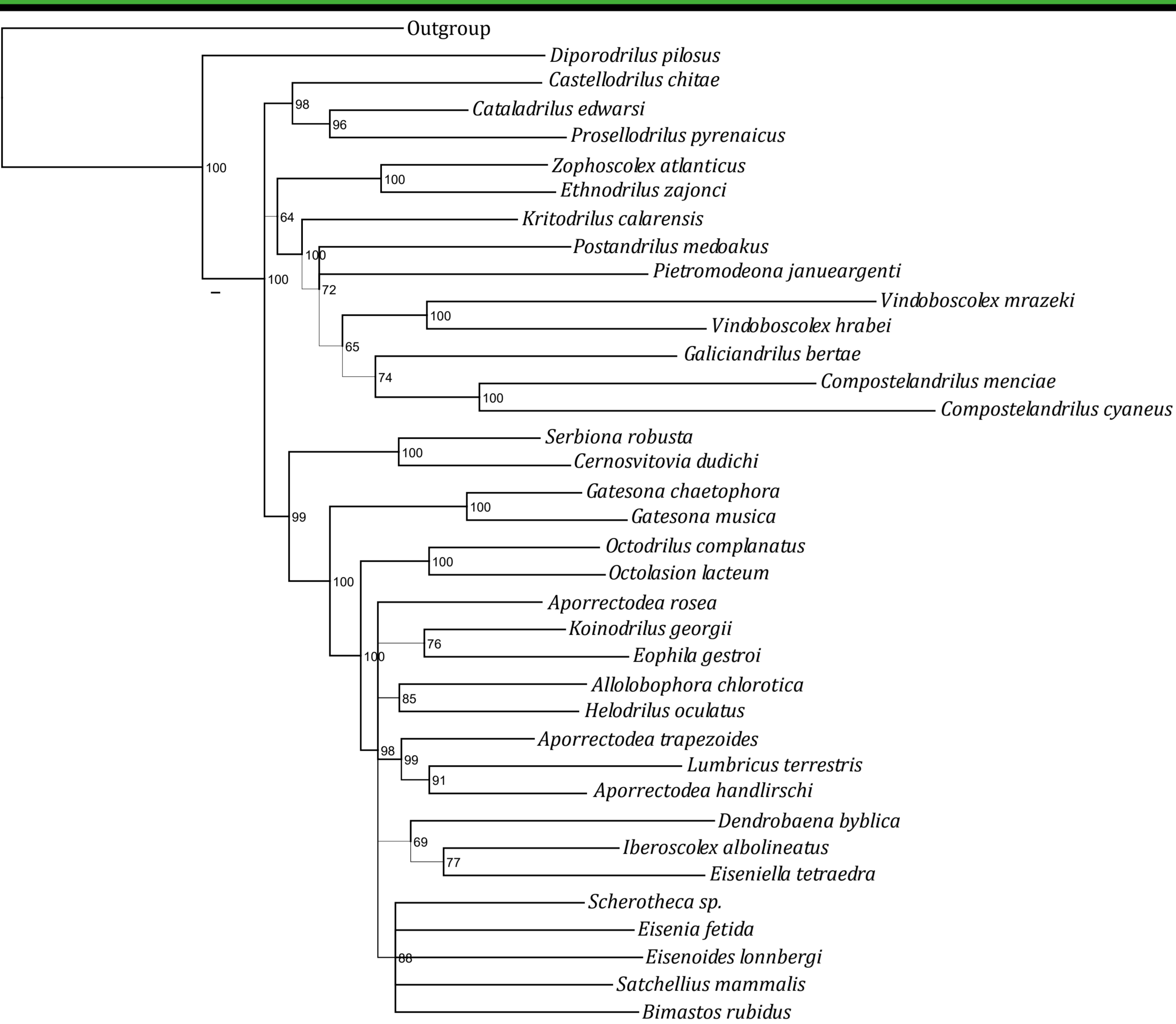
Materials and methods

Representatives of 31 Lumbricidae genera and outgroups were chosen in order to validate recent genus-level changes and to provide a solid “backbone” for the systematics of the family. Library preparation and AHE enrichment were performed at the Center for Anchored Phylogenomics. The enriched libraries were sequenced at the Translational Lab at the FSU College of Medicine on an Illumina NovaSeq6000 sequencer.

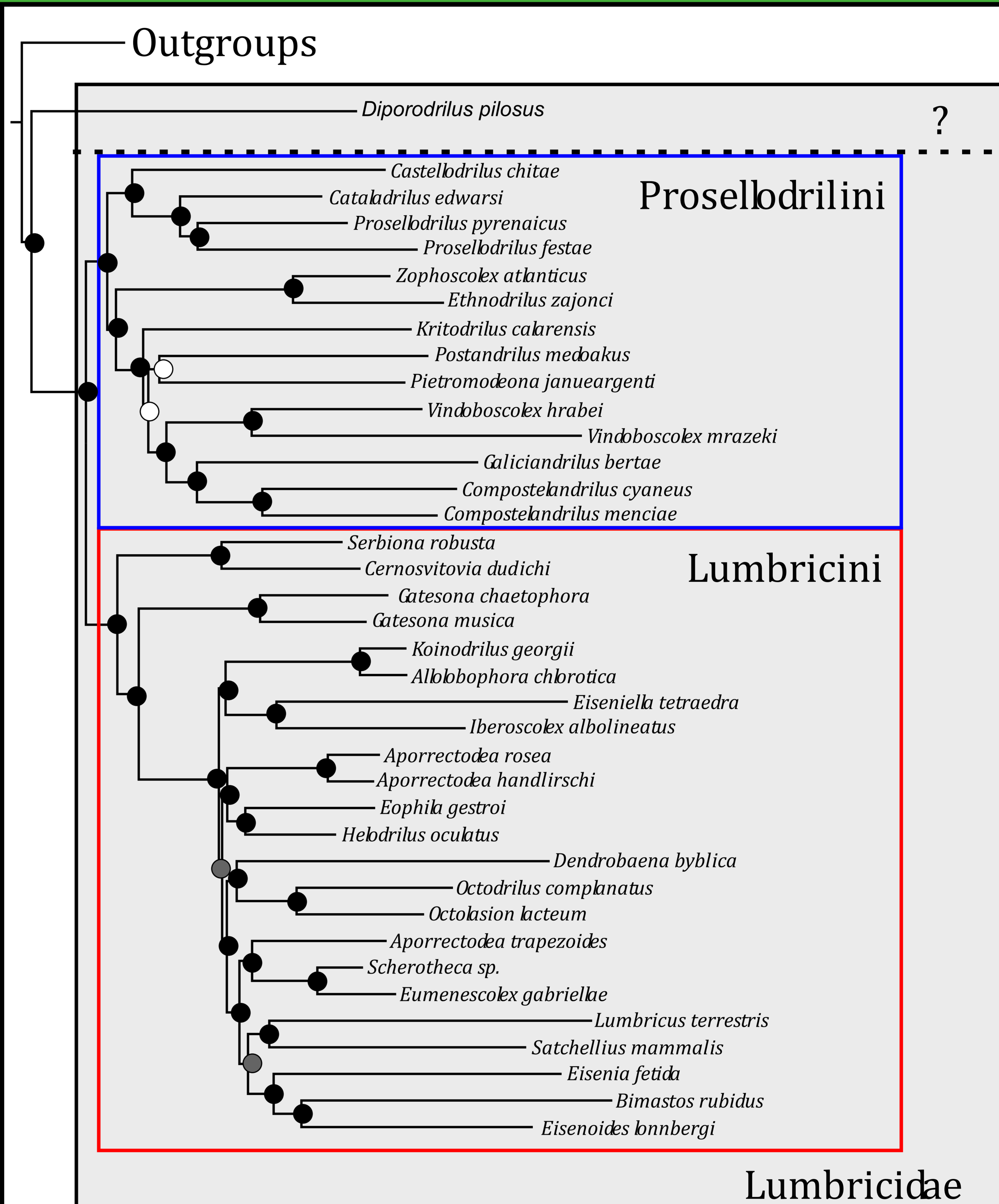
Phylogenetic trees were estimated using a Maximum Likelihood approach, as implemented in RAxML v2.2.3 with the GTR+G model of sequence evolution partitioned by locus for the **concatenated alignment**.

Gene-specific trees were estimated from locus-specific alignments (with a single GTR+G model assumed). Those trees were used as input in ASTRAL v5.7 for **species tree estimation**.

A “legacy marker” reference dataset (28S, 16S, ND1, 12S and COI) was used in a Bayesian phylogenetic analysis to study the effect of the inclusion of the best AHE phylogenomic tree as constraint.



Phylogenetic tree based on the concatenated alignment of 5 “legacy” markers



Phylogenomic tree based on the concatenated alignment of 594 AHE loci

Results

Based on the **large number of non-duplicated sequenced loci (594)**, the **strong coverage** across all of the studied taxa and loci (90% of the loci were recovered for >97% of the samples) and the **robust phylogenetic results** obtained, **AHE** appears to be a **highly suitable** sequencing approach for **phylogenomic inference in lumbricid earthworms and closely related taxa**

Phylogenetic inference methods based on the AHE dataset showed **enhanced resolution of the deep nodes of the family Lumbricidae**.

Support for systematic revisions of Gatesona and Cernosvitovia (Marchán et al. 2021, Popovic et al. 2022)

Supported relationships from previous “legacy” marker phylogenetic analyses: *Zophoscolex*+*Ethnodrilus*, *Eiseniella*+*Iberoscolex*, *Eophila*+*Helodrilus*, *Dendrobaena*+*Octodrilus*+*Octolasion*, *Eisenia*+*Bimastos*+ *Eisenoides* (Jiménez et al. 2021, de Sosa et al. 2019, Csuzdi et al. 2017 Marchán et al. 2021a).

Close relationships between sister taxa (same genus?): *Koinodrilus georgii* and *Allolobophora chlorotica*; *Aporrectodea rosea* (*Koinodrilus*?) and *Aporrectodea handlirschi* (*Eiseniella*?), *Scherotheca* and *Eumenescolex*

Close relationships between sister taxa (similar ranges and ecology): *Scherotheca* and *Aporrectodea*; *Lumbricus* and *Satchellius*

Diporodrilidae-Lumbricidae vs Diporodrilinae within Lumbricidae: no unambiguous support for one or the other (difference between concatenated vs species tree estimation)

Tribes Prosellodrilini and Lumbricini re-defined. Supported by a shared morphological character state: Prosellodrilini have either calciferous glands dilatations or diverticula in 11 or absence of them, while Lumbricini have calciferous glands dilatations or diverticula in segment 10 (rarely absent).

The addition of the phylogenomic backbone changed the topology of the “legacy” Bayesian tree. Within Lumbricini several polytomies disappeared and some relationships not directly enforced by the constraint were now recovered or showed stronger support.

Conclusions

Anchored Hybrid Enrichment is a highly suitable phylogenomic approach to resolve the above-genus level relationships within Lumbricidae and closely related taxa.

The strongly supported relationships recovered in the AHE trees allowed to propose changes to the above-genus level systematics of the family Lumbricidae (tribes Prosellodrilini and Lumbricini).

The combination of a taxon-rich “legacy markers” dataset with an AHE phylogenomic backbone (with representatives from additional genera) appears as a short-term attainable goal for resolving Lumbricid systematics.