



Phylogenomics resolves ambiguous relationships within Aciculata (Errantia, Annelida)

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ABSTRACT

Aciculata (Eunicida + Phyllodocida) is among the largest clades of annelids, comprising almost half of the known diversity of all marine annelids. Despite the group's large size and biological importance, most phylogenomic studies on Annelida to date have had a limited sampling of this clade. The phylogenetic placement of many clades within Phyllodocida in particular has remained poorly understood. To resolve the relationships within Aciculata we conducted a large-scale phylogenomic analysis based on 24 transcriptomes (13 new), chosen to represent many family-ranked taxa that have never been included in a broad phylogenomic study. Our sampling also includes several enigmatic taxa with challenging phylogenetic placement, such as *Histiobdella*, *Struwela*, *Lacydonia*, *Pilargis* and the holopelagic worms *Lopadorrhynchus*, *Travisopsis* and *Tomopteris*. Our robust phylogeny allows us to name and place some of these problematic clades and has significant implications on the systematics of the group. Within Eunicida we reinstate the names Eunicoidea and Oenonoidea. Within Phyllodocida we delineate Phyllodociformia, Glyceriformia, Nereidiformia, Nephtyiformia and Aphroditiformia. Phyllodociformia now includes: *Lacydonia*, Typhloscolecidae, Lopadorrhynchidae and Phyllodocidae. Nephtyiformia includes Nephtyidae and Pilargidae. We also broaden the delineation of Glyceriformia to include Sphaerodoridae, Tomopteridae and Glycerioidea (Glyceridae + Goniadidae). Furthermore, our study demonstrates and explores how conflicting, yet highly supported topologies can result from confounding signals in gene trees.

1. Introduction

With about 5900 named species Errantia contains almost half of the diversity of marine annelids (Pamungkas et al., 2019; Rouse et al., 2022). This includes some of the most iconic and well-known polychaete species such as the emerging model organism *Platynereis dumerilii* (Nereididae), the magnificent sand-striker worm, *Eunice* cf. *aphroditoides* (Eunicidae), the furry sea mouse, *Aphrodita* (Aphroditidae), and the venomous “blood worms” Glyceridae. However, despite the diversity and ecological importance of the group, most broad phylogenomic studies on annelids have had only limited sampling within Errantia (Andrade et al., 2015; Helm et al., 2018; Martín-Durán et al., 2021; Struck et al., 2015; Weigert et al., 2014). With the broad pattern of annelid relationships now becoming somewhat settled, more recently

phylotranscriptomic datasets have also been employed to resolve phylogenies within subclades of Annelida and it is evident that some relationships cannot be confidently resolved by small numbers of loci (Stiller et al., 2020; Tilic et al., 2020).

Errantia is an old grouping that was originally erected by Audouin and Milne-Edwards (1832) and the membership included Amphinomida, Eunicida and Phyllodocida. However, both Errantia and the complementary Sedentaria were largely abandoned in the later 20th century (Dales, 1962; Fauchald, 1977). The name was recently resurrected by Struck et al. (2011) and currently consists of three main clades: Eunicida, Phyllodocida and Protodriliformia. The name Aciculata was erected by Rouse and Fauchald (1997), and it was essentially synonymous with the older taxon Errantia. However, with the placement of Protodriliformia within Errantia (Andrade et al., 2015; Struck et al.,

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2015), the clade Eunicida + Phyllodocida needed a name and Andrade et al. (2015) reinstated the name Aciculata for this grouping. The focus of this study is on Aciculata as delineated in Andrade et al. (2015).

The respective monophyly of Eunicida and Phyllodocida is well-supported (Rouse and Fauchald, 1997; Struck et al., 2015). Each group was named by Dales (1962) and is characterized by distinct morphological features: Eunicida have a ventral muscularized proboscis with complex jaws and a peristomium forming a ring or rings. Phyllodocida have an axial muscular proboscis, ventrally positioned sensory palps, anterior enlarged cirri, and compound chaetae (when present) with a single ligament. The relationships within these clades however have been largely problematic with several family-ranked clades, especially within Phyllodocida, having unresolved phylogenetic

placement (Martin et al., 2021; Rouse and Pleijel, 2001). Major taxa within Phyllodocida have been previously erected, including Aphroditiformia (Fauchald, 1977), Glyceriformia (Fauchald, 1977), Nereidiformia (Fauchald 1977, Dahlgren et al. 2000), Nereidoidea (George and Hartmann-Schröder 1985; Glasby 1993), and Phyllodociformia (Fauchald, 1977), but apart from Aphroditiformia and Glyceriformia, these taxa presently have uncertain membership.

For this study we have analyzed twenty-four Aciculata transcriptomes, including thirteen that are new. The main aim of our study was to resolve the phylogeny of Phyllodocida, focusing on taxa that have been hard to place and often referred to as *incertae sedis*. We present a well-supported phylogeny of Aciculata, that includes representatives of seven family-ranked clades that have never been included in a broad



Fig. 1. Live photographs of specimens used in this study. **A** *Amphiduros pacificus* (Hesionidae) **B** *Chrysopetalum occidentale* (Chrysopetalidae), **C** *Pholoe baltica* (Sigalionidae), **D** *Tomopteris* sp. (Tomopteridae), **E** *Histriobdella* sp. (Histriobdellidae), **F** *Sphaerodorum gracilis* (Sphaerodoridae), **G** *Nephtys hombergii* (Nephtyidae), **H** *Travisiopsis* sp. (Typhloscolecidae), **I** *Struwela camposi* (Microphthalmidae), **J** *Laetmonice* cf. *iocasica* (Aphroditidae), **K** *Lacydonia* sp., **L** *Lopadorrhynchus* sp. (Lopadorrhynchidae).

phylogenomic analysis. These encompass Hesionidae (Fig. 1A), Chrysopetalidae (Fig. 1B), Sphaerodoridae (Fig. 1F), Aphroditidae (Fig. 1J), Pilargidae, *Lacydonia* (Fig. 1K), the enigmatic *Struwela* (Fig. 1I), the holopelagic Lopadorrhynchidae (Fig. 1L) and Typhloscolecidae (Fig. 1H) and from Eunicida the tiny “Charlie-Chaplin-worms” Histriobdellidae (Fig. 1E) that live as commensals with crustaceans. Our results have significant implications for the systematics of the group, allowing us to name and place some of these problematic clades that no longer need to be referred to as *incertae sedis*. Furthermore, we also show how conflicting, yet highly supported topologies can result from confounding signals in gene trees and discuss the challenges this creates in resolving phylogenetic uncertainties within Annelida.

2. Material and methods

2.1. Taxon sampling and transcriptome sequencing

Twelve species of Phyllodocida and *Histriobdella* sp. were sampled for transcriptome sequencing (Fig. 1). In addition to these, eleven previously published transcriptomes were included in the analyses. Specimen details, voucher information and accession numbers for the sequence data are summarized in Table 1. COI Barcode sequences from the assembled transcriptomes were uploaded to GenBank (Table S1) and voucher specimens are deposited to the Benthic Invertebrate Collection of Scripps Institution of Oceanography (SIO-BIC), to facilitate future identification of taxa sequenced as part of this study.

Tissue samples were preserved in RNAlater quickly after the animals were collected and stored at -80°C until RNA extraction. For larger specimens, only the anterior region of the animals was sampled, for smaller specimens the whole individual was used for RNA extraction. For *Histriobdella* sp. multiple individuals, collected from a single host, were pooled during extraction. RNA extractions were performed from Trizol, using Direct-zol RNA Miniprep Kit with an in-column DNase treatment (Zymo Research). mRNA was isolated with Dynabeads mRNA Direct Micro Kit (Invitrogen).

RNA concentration was estimated using Qubit RNA broad range assay kit, and quality was assessed using RNA ScreenTape with an Agilent 4200 TapeStation on an Agilent Bioanalyzer 2100. Values were

used to customize library preparation protocols following manufacturer’s instructions. Library preparation was performed with the KAPA-Stranded RNA-Seq kit, targeting an insert size in the range of 200–300 bp. Quality, concentration and molecular weight distribution of libraries were assessed using a DNA ScreenTape, a Bioanalyzer 2100. Libraries were sequenced in multiplexed pair-end runs using 150 bp paired end Illumina HiSeq 4000, with 8 libraries per lane, resulting in an average sequencing depth of 48 million reads (range: 16 million – 108 million). To minimize read crossover, we used 10 bp sequence tags designed to be robust to indel and substitution errors (Faircloth and Glenn, 2012). All sequence data have been deposited in NCBI’s sequence read archive (SRA) (Table 1) with Bioproject accession number PRJNA743301.

2.2. Transcriptome Assembly and phylogenetic analyses

Sequence adapters and low-quality regions were removed from the raw reads of each species using Trimmomatic v.0.36 (Bolger et al., 2014) with default parameters. All transcriptomes were *de novo* assembled and orthologous gene sequences were identified using the automated pipeline Agalma v.1.0.1 (Dunn et al., 2013; Guang et al., 2017). In brief, Agalma assembles transcripts with Trinity (Grabherr et al., 2011), maps reads with Bowtie2 (Langmead and Salzberg, 2012) and identifies orthologs using the topology of gene phylogenies and DendroPy (Sukumaran and Holder, 2010). Assembly statistics and the number of genes identified by Agalma are summarized in Table S2.

To decide on the placement of the root, we conducted a preliminary analysis with an extended taxon sampling including 19 additional taxa spanning the diversity of annelids. We conducted a concatenated ML analysis with IQTREE with the same parameters as outlined below on a supermatrix with 70% occupancy (817 genes).

Maximum Likelihood (ML) phylogenetic analyses were conducted using a concatenated sequence matrix with 80% occupancy (242,492 AA sites; 854 genes). Unpartitioned sequence matrices were analysed with RAxML v.8.2.10 (Stamatakis, 2014), using the PROTGAMMAAUTO model setting, and 1000 non-parametric bootstrap inferences and 10 distinct randomized maximum parsimony trees were used as the starting point. Furthermore, amino acid substitution model selection (Kalyaanamoorthy et al., 2017), partition merging (MF + MERGE) and

Table 1

Voucher information and Sequence Read Archive (SRA) accession numbers for the transcriptome data. Vouchers for newly generated transcriptomes (highlighted in bold) were deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO BIC). BioProject accession number is PRJNA743301.

Higher Taxa	Species	Sampling locality/Source	Voucher	SRA number
Aphroditidae	<i>Laetmonice</i> cf. <i>iocasica</i> McIntosh, 1885	Costa Rica	A9830	SRR15277959
Sigalionidae	<i>Pholoe</i> <i>baltica</i> Ørsted, 1843	Sweden	A1008	SRR15277956
Polynoidae	<i>Lepidomorphopus</i> sp.	Okinawa Trough/(Zhang et al., 2017)	N/A	SRR4419843
Polynoidae	<i>Harmothoe imbricata</i> (Linnaeus, 1767)	Sweden/(Andrade et al., 2015)	A1142	SRR2005364
Syllidae	<i>Syllis gracilis</i> Grube, 1840*	Spain/(Ribeiro et al., 2019)	N/A	SRR8510622
Nephtyidae	<i>Nephtys</i> sp.	Sweden	A12634	SRR15277958
Pilargidae	<i>Pilargis verrucosa</i> Saint-Joseph, 1899	Sweden	N/A	SRR15277955
Hesionidae	<i>Amphiduros pacificus</i> Hartman, 1961	San Diego, US-CA	A4322	SRR15277965
Chrysopetalidae	<i>Chrysopetalum occidentale</i> Johnson, 1897	San Diego, US-CA	A4325	SRR15277964
Nereididae	<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)	N/A	N/A	SRR1742987
Microphthalmidae	<i>Struwela camposi</i> Salazar-Vallejo et al. 2019	Mexico	A13437	SRR15277962
Glyceridae	<i>Glycera dibranchiata</i> Ehlers, 1868	(Kocot et al., 2017)	N/A	SRR2057019
Sphaerodoridae	<i>Sphaerodorum gracilis</i> (Rathke, 1843)	Sweden	A1007	SRR15277954
Tomopteridae	<i>Tomopteris</i> sp.	San Diego, US-CA	A1182	SRR15277953
<i>Lacydonia</i>	<i>Lacydonia</i> sp.	Costa Rica	A10090	SRR15277960
Typhloscolecidae	<i>Travisioopsis</i> sp.	San Diego, US-CA	A9405	SRR15277963
Lopadorrhynchidae	<i>Lopadorrhynchus</i> sp.	San Diego, US-CA	A10669	SRR15277961
Phyllodocidae	<i>Phyllodoce medipapillata</i> Moore, 1909	San Diego, US-CA/(Andrade et al., 2015)	A5913	SRR2016923
Lumbrineridae	<i>Ninoe nigripes</i> Verrill, 1873	US-NC/(Struck et al., 2015)	N/A	SRR2040483 SRR2040484
Oeonidae	<i>Arabella</i> sp.	Panama/(Struck et al., 2015)	N/A	SRR2040140 SRR2040141
Onuphiidae	<i>Diopatra cuprea</i> (Bosc, 1802)	US-SC/(Struck et al., 2015)	N/A	SRR2040374 SRR2040376
Eunicidae	<i>Leodice torquata</i> (Quatrefages, 1866)	Spain/(Andrade et al., 2015)	A1168	SRR2005375
Dorvilleidae	<i>Ophryotrocha globopalpata</i> Blake & Hilbig, 1990	US-WA/(Struck et al., 2015)	N/A	SRR2040502 SRR2040503
Histriobdellidae	<i>Histriobdella</i> sp.	Boston, US-MA	A13436	SRR15277957

* *Syllis gracilis* represents a species complex and the specimen used for the transcriptome was identified as belonging to ‘lineage 8’ in Ribeiro et al. (2019).

subsequent ML analyses were conducted with IQTREE (Nguyen et al., 2015) with 1000 ultrafast bootstrap pseudoreplicates (Hoang et al., 2017).

In addition to ML analyses on the concatenated supermatrix, coalescent-based species tree was inferred using ASTRAL-III v.5.7.3 (Zhang et al., 2018b). Individual gene trees were estimated with IQTREE under the best-fit model for each alignment. Gene alignments were tested for model violation using the *-symtest* option in IQTREE (Naser-Khdour et al., 2019). Out of 9657 orthologous gene alignments identified by Agalma, 518 rejected SRH (stationary, reversible and homogeneous) assumptions and were excluded from the ASTRAL analysis. All the remaining 9139 gene trees were included as ASTRAL benefits from more data (Molloy and Warnow, 2018; Nute et al., 2018). Poorly supported branches with less than 10% bootstrap support were contracted in the input trees for ASTRAL as this can improve species tree inference (Zhang et al., 2018a). Input trees for ASTRAL were also filtered using TreeShrink (Mai and Mirarab, 2018), which implements an algorithm to identify and remove unexpectedly long branches caused by e.g. contamination, mistaken orthology, and misalignment. The ASTRAL tree inferred from this curated dataset revealed no effect on the topology and only some very minor differences in branch lengths and support values when compared to the uncurated dataset.

To assess if the phylogenetic signal differed between slow- and fast-evolving proteins, two submatrices were constructed from the 80% occupancy matrix with 854 genes. Individual gene trees were analyzed to rank each gene by evolutionary rate (total tree length/n terminals). The ranked genes were divided into the fast-evolving 50% and the slow-evolving 50%. The alignments were then concatenated and used to construct a maximum likelihood tree using IQTREE under the best-fit model chosen according to BIC (LG + F + R5). Support was assessed with 10,000 bootstrap pseudo-replicates.

2.3. Topology testing

To explore the strength of support for the five nodes that differed between the ASTRAL and ML trees, we used two approaches. To assess how much less likely alternative topologies were compared to the best ML tree from the concatenated dataset, we used topology tests as implemented in IQ-TREE using expected likelihood weights (Strimmer and Rambaut, 2002) and with the approximately unbiased (AU) test (Shimodaira, 2002). All tests performed 10,000 resamplings using the RELL method (Kishino et al., 1990). In each assessment, we changed only one branch to assess whether the alternative topology was statistically less supported than the best ML tree.

For the tests in a coalescent-based framework, we used ASTRAL as above but constrained the analysis to either the ML topology or the ASTRAL topology (Rabiee and Mirarab, 2020). This way, we could assess whether there is support for the alternative hypotheses proposed by the ML trees that were not recovered in the main ASTRAL analysis. We tested how support (measured as local posterior probability PP (Sayyari and Mirarab, 2016)) for the two topologies changed with additional data. A true relationship is expected to gain support with additional gene trees, while a spuriously recovered signal is more likely to remain supported with low support values. We randomly split the 9657 gene trees into subsets of 250, 500, 1000, to 9000 gene trees, and replicated each subset 20 times. From each subset, we built a species tree using ASTRAL v.5.6.9, while constraining to the topology of the ML or ASTRAL analysis. For the resulting species tree, we recorded PP support for each of the five nodes that differed between ASTRAL and ML trees.

3. Results

3.1. Data analyses and matrix Assembly

Assembly statistics and values to assess the quality of each transcriptome (number of assembled contigs, mean contig length, N50,

BUSCO values, and number of detected loci) are summarized in Table S2. The smallest number of reads in the total data set was the 16 million reads sequenced for *Lacydonia* sp. (assembled into 91,326 contigs), whereas the largest one was sequenced for *Histriobdella* sp. with 108 million reads (assembled into 45,904 contigs).

Total number of orthologous genes identified for all species was 9,617 (ranging from 972 in *Leodice torquata* to 6,223 in *Amphiduros pacificus*) (Table 1). For phylogeny reconstruction an 80% occupancy sequence matrix was created (242,492 AA sites; 854 loci). Over 60% of analyzed loci were present in all taxa except for *Leodice torquata* (302 loci) and *Glycera dibranchiata* (392 loci) (Fig. 2).

3.2. Phylogeny reconstruction based on supermatrices

The unpartitioned RAxML analysis, as well as the partitioned IQTREE analysis using integrated model selection and the separate analyses of slow-evolving and fast-evolving genes recovered the same topology (Fig. 2). All nodes had > 94% bootstrap support.

The tree in Fig. 2 was rooted with Eunicida forming a clade including *Histriobdella*. This decision was based on the tree topology recovered in the extended analyses with broader taxon sampling of annelids (Fig. S1-S4), which matched the tree shown in Fig. 2, apart from the placement of *Histriobdella*. In the expanded analyses *Histriobdella* grouped with a clade of Diurodrilidae, Dinophilidae and Myzostomida, or with Sabellidae which all have notably long terminal branches and may be a result of artifactual long branch attraction (see Andrade et al. 2015). The numerous morphological similarities that Histriobdellidae share with members of Eunicida make this a reasonable assumption.

Within Eunicida *Ophryotrocha globopalpata* Blake & Hilbig, 1990 formed a clade with the epibiotic *Histriobdella* and this clade was recovered as the sister-group to the remaining Eunicida. The lumbrinerid *Ninoe nigripes* Verrill, 1873 (incorrectly listed on the SRA archive as *Ninoe nigrens*) was the sister to the oeononid *Arabella*, and the onuphid *Diopatra cuprea* (Bosc, 1802) grouped together with the eunicid *Leodice torquata* (Quatrefages, 1866) (Fig. 2). Here we apply two previously erected names (Orensanz 1990): **Eunicoidea** to the Eunicidae + Onuphidae clade and the name **Oeononoidea** for the Lumbrineridae + Oeononidae clade.

Within Phyllodocida several clades were recovered that we apply names to, based on support and congruence with the ASTRAL result:

Phyllodociformia, consisting here of *Lacydonia* sp., *Phyllodoce medipapillata* Moore, 1909 and two holopelagic taxa; *Travisioopsis* sp. and *Lopadorrhynchus* sp.

Glyceriformia is delineated here to include *Sphaerodorum gracilis* (Rathke, 1843), *Glycera dibranchiata* Ehlers, 1868 and *Tomopteris* sp. Bootstrap support for Glyceriformia was 95% for RAxML and 94% for IQTree respectively.

Aphroditiformia, with *Laetmonice* cf. *iocasica* McIntosh, 1885 (Aphroditidae) as sister to a clade including *Pholoe baltica* Örsted, 1843 and two polynoid species *Lepidonotopodium* sp. and *Harmothoe imbricata* (Linnaeus, 1767). The syllid *Syllis gracilis* Grube, 1840 was supported as the sister to Aphroditiformia.

Nephtyiformia is a new name we use to include Pilargidae and Nephtyidae, based on *Pilargis verrucosa* Saint-Joseph, 1899 and *Nephtys* sp. grouping together with full support.

Hesionoidea. We could not consistently recover a clade that could be referred to as Nereidiformia. In the ML analysis *Platynereis dumerilii* (Audouin & Milne Edwards, 1833) grouped together with *Struwela camposi* Salazar-Vallejo et al. 2019 and this clade was the sister to Glyceriformia + Phyllodociformia. We do name the well-supported clade comprising of Chrysopetalidae and Hesionidae as Hesionoidea, which has an ending signifying superfamily rank, and the authority is Grube (1850).

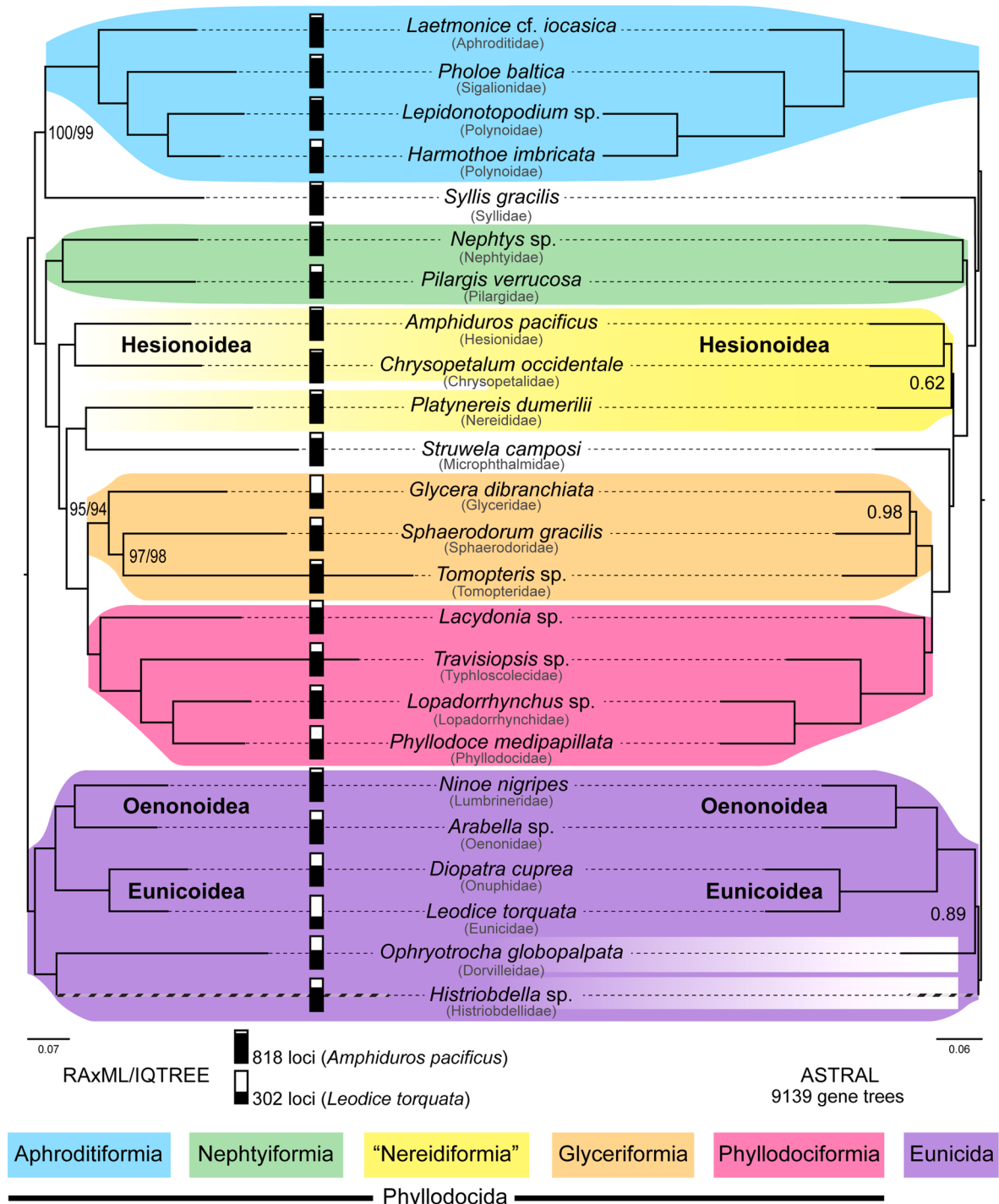


Fig. 2. Phylogeny of Aciculata based on transcriptomic data. The tree on the left shows the topology recovered from maximum likelihood inference on the supermatrix with 80% taxon occupancy (242,492 AA sites; 854 genes). The black bars are proportional to the number of genes present for each terminal. The same topology was also found when slow-evolving and fast-evolving genes were analyzed separately. The tree on the right shows the results of the ASTRAL analysis summarizing all 9139 gene trees. Bootstrap support values and ASTRAL local posterior probabilities are only shown on the tree if a node was not recovered with full support. Note that the delineation of Glyceriformia is expanded to include Tomopteridae and Sphaerodoridae, former Glyceriformia (Glyceridae + Goniadidae) therefore becomes Glyceroidea. We also bring back the names Eunicioidea (Eunicidae + Onuphidae) and Oenonoidea (Lumbrineridae + Oeononidae) both originally assigned by [Orensanz \(1990\)](#). Note the dashed line of *Histriobdella* indicating the conflicting topologies shown in Figs. S1-4, likely a result of long-branch artefacts.

3.3. Coalescent-based species tree analysis with ASTRAL

The ASTRAL topology remained stable when potentially extraneous sequences were filtered with TreeShrink. Most of the nodes had full support (Fig. 2). Though the topologies recovered from concatenation based supermatrix analyses and the coalescent-based species tree from

ASTRAL were broadly similar, there were few significant differences indicating a conflicting phylogenetic signal in the gene trees.

In the ASTRAL tree *Histriobdella* and *Ophryotrocha* did not group together. *Histriobdella* was the sister to the remaining Eunicida, which formed a clade that had an ASTRAL local posterior probability of 0.89. Within Glyceriformia the position of *Tomopteris* sp. (Fig. 1D) shifted

when *Glycera dibranchiata* and *Sphaerodorum gracilis* (Fig. 1F) grouped together (Fig. 2).

In contrast to the supermatrix analysis the ASTRAL result showed *Platynereis* forming a clade with the hesionid *Amphiduros pacificus* Hartman, 1961 and the chrysopetalid *Chrysopetalum occidentale* Johnson, 1897, which could be regarded as the taxon Nereidiformia (ASTRAL support 0.62), but further assessment of this is required given the conflict with the ML result (Fig. 2). Instead of grouping with *Platynereis*, the microphthalmid *Struwela camposi* was the sister group to a Glyceriformia + Phyllodociformia clade. Lastly, *Syllis gracilis* became the sister of all Phyllodocida except Aphroditiformia. The remaining topology was identical to that recovered by the maximum-likelihood analyses.

3.4. Topology testing

Two out of the five nodes that differed between the ML and the ASTRAL trees were found to result in significantly worse trees using the AU test (p-value less than 0.05). These involved the placement of Nereididae and Microphthalmidae (Fig. 3D, E). However, alternative topologies for three nodes, involving Syllidae, Sphaerodoridae and Histiobdellidae (Fig. 3A–C) were not significantly different and so cannot be ruled out based on the data. The ASTRAL subsetting analyses showed strong support in favor of the ASTRAL topology for four out of the five nodes (Fig. 3). It is notable that large amounts of data were needed to gain high PP support (>0.90) for most of these nodes. The position of Syllidae as the sister of Aphroditiformia was strongly supported when more than ~ 5000 gene trees were analyzed, while the ML hypothesis was only poorly supported (Fig. 3A). On the other hand, the AU test could not reject the alternative topology, with Syllidae as sister to the remaining Phyllodocida, over the ML topology (p-value 0.137). The ASTRAL position for Sphaerodoridae and for Histiobdellidae was strongly favored and support levels increased with additional data, while the support for the alternative topology decreased (Fig. 3B–C). However, the trajectory of the curves suggest that more than 9000 loci could be needed to obtain full support on those nodes. Both topological changes did not result in statistically different trees according to the AU test. The position of *Struwela* (Microphthalmidae) as the sister group to Nereididae as in the ML topology was strongly supported but a lower-

level support for the alternative topology as the sister to Glyceriformia + Phyllodociformia remained relatively constant (Fig. 3D). This contrasted with the results from the AU test, which indicated that the alternative position of *Struwela* led to strong statistical differences compared to the best ML tree (p-value 2.15×10^{-33}). The position of Nereididae was the only node where both alternative topologies had consistent and equal support and no obvious effect of increasing the number of analyzed gene trees existed (Fig. 3E). Interestingly, the AU test did detect statistically significant differences between the two topologies (p-value 0.0055).

4. Discussion

We presented a phylogenetic analyses of Aciculata based on transcriptomic data from 24 species that resulted in an overall well-supported and congruent topology. Using subsetting analyses, we show that some difficult nodes require large numbers of loci to obtain strong support, consistent with what was observed in other clades within annelids (Tilic et al. 2020, Stiller et al. 2020). Difficulty remains in some branches, which appear to be impacted by long branches and conflicts between analysis types. Nevertheless, the stability and support for a number of clades allows for a series of nomenclatural recommendations that are outlined below.

4.1. Relationships within Eunicida and implications on the systematics of the group

Eunicida (Dales, 1962) is a well-defined clade of annelids whose members show a notable synapomorphy in the ventral muscular proboscis with a complex jaw apparatus that is mineralized or sclerotized (Tzvetlin and Purschke, 2005). These jaws can often be found in the fossil record (scolecodonts) and date the group back to the late Cambrian (Paxton, 2009). Extant Eunicida are grouped into seven major clades: Eunicidae Berthold, 1827, Onuphidae Kinberg, 1865, Oeononidae Kinberg, 1865, Lumbrineridae Schmarda, 1861, Dorvilleidae Chamberlin, 1919 and the two smaller enigmatic clades Histiobdellidae Vaillant, 1890 and *Hartmaniella* Imajima, 1977. *Hartmaniella* is monotypic for *Hartmaniella* with the former being superfluous, we therefore only use

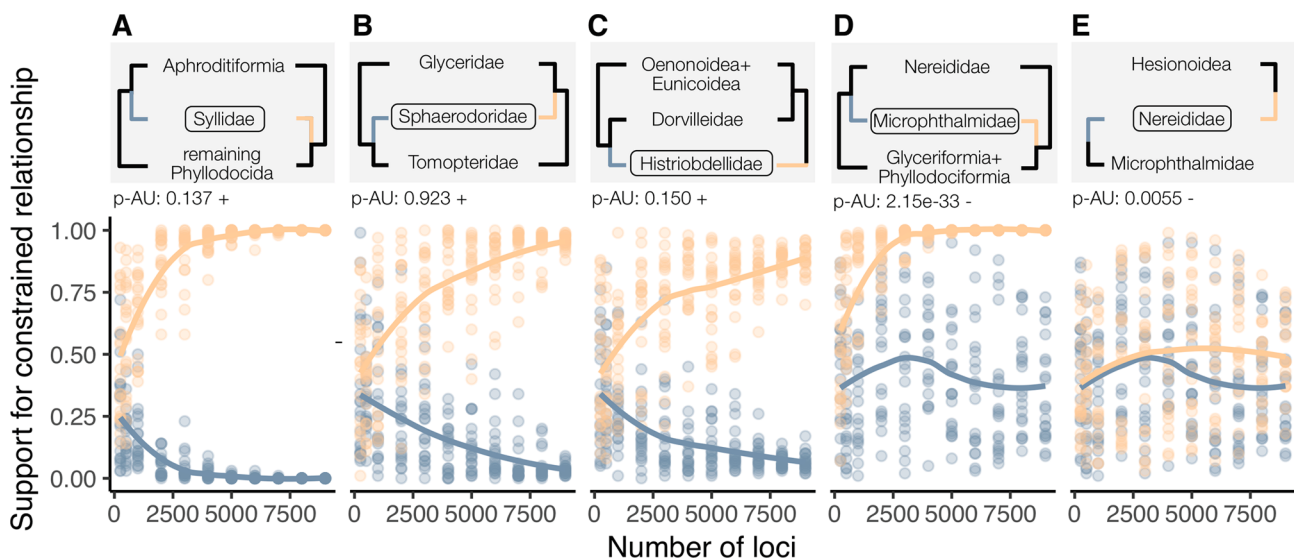


Fig. 3. Topology tests. Alternative hypotheses for the placement of five taxa were tested using constrained tree searches comparing the ML topology from RAXML (left) to the ASTRAL topology (right). The p-values from the approximately unbiased (AU) test are shown below the tree illustrations. The graphs show the local posterior probability (PP) of ASTRAL when constraining the topology to the ASTRAL (orange) or the RAXML (blue) topology. Constrained species trees were built from an increasing number of gene trees in 20 replicates and the PP support for each replicate is shown as dots, while the line is a local polynomial regression fit. Placement of **A** Syllidae, **B** Sphaerodoridae, **C** Histiobdellidae, **D** Microphthalmidae, **E** Nereididae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the latter in this paper.

Previous phylogenetic analyses based on Sanger sequencing and combined morphological data have been largely congruent in recovering the monophyly of most these clades (Budaeva et al., 2016; Struck et al., 2015, 2006; Tilic et al., 2016; Zanol et al., 2014). Studies that included *Pettiboneia* (Struck et al., 2006, 2002) failed to recover a monophyletic *Dorvilleidae*, but this was only based on limited sequence data (16S rRNA, 18S rRNA genes). Given the long branches and low support in these analyses further investigation was warranted.

Inferred relationships within Eunicida have become more stable with the introduction of phylogenomic datasets. Our results presented herein are congruent with Struck et al. (2015) in recovering a clade consisting of Eunicidae + Onuphidae, another clade with Lumbrineridae + Oeononidae and then *Dorvilleidae* (possibly together with *Histiobdellidae*) as sister to these two clades. The sister group relationship of Eunicidae and Onuphidae is well supported both on morphology (Budaeva et al., 2016; Fauchald, 1992; Orensanz, 1990; Paxton, 2009; Zanol et al., 2014) and on sequence data (Struck et al., 2015, 2006; Tilic et al., 2016). In contrast, Rouse and Fauchald (1997) had recovered Eunicidae as sister to Lumbrineridae + *Dorvilleidae* and Onuphidae as sister to this clade. This topology, however, is no longer supported and the monophyly of Eunicidae and Onuphidae has become undisputed. Orensanz (1990) had named this clade (ranked superfamily) Eunicoidea, which we adopt here. The synapomorphies that unite Eunicoidea are: eulabidognath maxillae, five prostomial appendages, peristomial cirri, and subacicular hooks in median and posterior parapodia (Budaeva and Zanol, 2020).

The second well-supported clade within Eunicida contains Lumbrineridae and Oeononidae. In previous phylogenetic analyses based on few Sanger-sequenced markers (Struck et al., 2006; Tilic et al., 2016) Lumbrineridae was recovered as the sister to all other Eunicida, however phylogenomic studies based on transcriptome data, both in Struck et al. (2015) and in this paper, now support the sister group relationship of Lumbrineridae and Oeononidae. This was also favored by earlier morphological hypotheses and was given the name Oenonoidea by Orensanz (1990) with a superfamily ranking and we also adopt it here.

The placement of *Dorvilleidae* within Eunicida using molecular phylogenies has been more problematic and challenging owing to long branch artefacts (Struck et al., 2006). In Struck et al. (2006) *Dorvilleidae* did not form a clade. Tilic et al. (2016) recovered Oeononidae and *Dorvilleidae* as sister taxa, though this had very low support. Struck et al. (2015) included transcriptomic data for several *Dorvilleidae* terminals, which were recovered as monophyletic and as the sister to remaining Eunicida. Our analyses only included a single *Dorvilleidae* terminal (*Ophryotrocha globopalpata*), which was the sister taxon to Eunicoidea + Oenonoidea either on its own (ASTRAL) or together with *Histiobdella* sp. (RAxML/IQ-TREE). Even though the ASTRAL position was favored based on our topology testing, this placement requires further investigation, with better taxon sampling within *Dorvilleidae* which might help resolve the incongruencies between the two analyses.

Though we were not able to unambiguously resolve the placement of *Histiobdella*, our phylogenomic analysis is the first attempt to place *Histiobdellidae* within Eunicida using molecular data. In all ML analyses with the concatenated supermatrix *Histiobdellidae* was recovered as the sister taxon to *Dorvilleidae*. However, there was some confounding signal in the gene trees, as the ASTRAL analysis supported a different topology (*Histiobdella* as sister to the remaining Eunicida), which was strongly favored in subsetting analyses over the ML topology (Fig. 3C) also with full support. An affinity of *Histiobdellidae* with *Dorvilleidae* has also been suggested based on some morphological similarities of their jaws (Tzetlin et al., 2020; Tzetlin, 1980). In the extended analyses of annelids (Fig. S1–S4), *Histiobdella* grouped together with other long-branched taxa outside Aciculata. This indicates that confounding long-branch effects may be causing the placement of *Histiobdella* among taxa with long terminal branches. This matches what was observed for other annelid taxa with symbiotic or parasitic

lifestyles (Andrade et al. 2015). Removing long-branched taxa was also not sufficient to place *Histiobdella* within Eunicida (Fig. S2, S4), indicating that there seems to be little signal in the *Histiobdella* data combined with many mutations that are not shared with any other Eunicida. Alas, given the relatively long branches of both *Ophryotrocha globopalpata* and *Histiobdella*, and the conflicting topologies, we cannot confidently resolve the phylogenetic position of *Histiobdellidae*, and this still warrants further investigation.

The placement of *Hartmaniella* within Eunicida still remains a mystery. Orensanz (1990) suggested that *Hartmaniella* has its sister group within Eunicoidea, then again Fauchald and Rouse (1997) interpreted the jaws differently and considered them to be closer to Oenonoidea. With only three named species and very limited records, and a complete lack of molecular data nothing is resolved about their placement within Eunicida.

4.2. Relationships within Phyllodocida and implications on the systematics of the group

Members of Phyllodocida (Dales, 1962) are characterized by an axial muscular proboscis, the loss of dorso-lateral folds, the ventrally positioned sensory palps, anterior enlarged cirri, and the presence of compound chaetae with a single ligament (Rouse and Fauchald, 1997). Relationships within the group, however, have been hard to resolve both based on morphology (Rouse and Pleijel, 2001) but also due to the limited transcriptomic data available to date. The group is arguably one of the most diverse annelid clades that includes around 20 “family-ranked” taxa. These are: Eulepethidae Chamberlin, 1919; Aphroditidae Malmgren, 1867; Sigalionidae Kinberg, 1856; Acoetidae Kinberg, 1856; Iphionidae Kinberg, 1856 and Polynoidae Kinberg, 1856, that together form Aphroditiformia; Syllidae Grube, 1850; Nephtyidae Grube, 1850; Pilargidae Saint-Joseph, 1899; Chrysopetalidae Ehlers, 1864; Hesionidae Grube, 1850; Nereididae Blainville 1818, 1818; Microphthalmlidae Hartmann-Schröder, 1971; Glyceridae Grube, 1850; Goniadidae Kinberg, 1866; Sphaerodoridae Malmgren, 1867; Tomopteridae Johnston, 1865; *Lacydonia* Marion, 1874 (=monotypic *Lacydoniidae*); *Paralacydonia* Fauvel, 1913 (=monotypic *Paralacydoniidae*); Typhloscolecidae Uljanin, 1878; Lopadorrhynchidae Claparede, 1868; Phyllodocidae Örsted, 1843; Yndolaciidae Støp-Bowitz, 1987 and *Pontodora* Greeff, 1879 (=monotypic *Pontodoridae*).

Our phylogenomic analyses of transcriptome data has the largest taxon sampling within Phyllodocida to date, representing many of the above-mentioned major lineages and resolves, with high support, many of the relationships within this diverse clade. Our results have significant implications on the systematics of the group. We reinstate and change the delineations of some existing names and name new clades when no preexisting ones were available. All new and amended taxon names are above family rank, therefore the rules and regulations of the ICZN do not apply.

One of the largest clades within Phyllodocida is Aphroditiformia, the scale-worms, the relationships of several family-ranked taxa within this clade is addressed in recent studies by Gonzalez et al., (2018); Norlinder et al., (2012); Zhang et al., (2018b). Our dataset includes four Aphroditiformia terminals representing Aphroditidae (*Laetmonice* cf. *iocasica*), Sigalionidae (*Pholoe baltica*) and Polynoidae (*Lepidonotopodium* sp. and *Harmothoe imbricata*). Though limited in sampling, our topology within Aphroditiformia is congruent with previous studies (Gonzalez et al., 2018; Zhang et al., 2018b) in recovering Aphroditidae as sister to the latter two taxa. Our concatenated supermatrix analysis placed Aphroditiformia as sister to Syllidae, a placement that has also been found in phylogenomic analyses of Weigert et al. (2014) and Struck et al. (2015). In contrast to this, Syllidae was recovered as the sister to all Phyllodocida excluding Aphroditiformia in our ASTRAL analysis, and this alternative topology could not be rejected with the AU test, which indicates that future work is required to fully resolve the placement of Syllidae within Phyllodocida.

Phyllodocida excluding Syllidae and Aphroditiformia form a well-supported clade, which we choose not to name here pending resolution of the placement of Syllidae. *Nephtys* sp. and *Pilargis verrucosa* together form the sister taxon to the rest of this clade. The position of Pilargidae has long been problematic. Several studies based on both morphology (Fitzhugh and Wolf, 1990; Glasby, 1993; Licher and Westheide, 1994; Pleijel and Dahlgren, 1998) and molecular data (Rousset et al., 2007) have often placed them within Nereidiformia (or Nereidoidea). Rouse and Fauchald (1997) placed Pilargidae as sister to Sphaerodoridae. In Struck et al.'s (2007) phylogeny of annelids based on Sanger sequenced data, Pilargidae were either in a trichotomy together with Syllidae and Nephtyidae, or as sister to the two others. The position of *Pilargis verrucosa* is highly supported in all analyses we performed, and we therefore use the new name Nephtyiformia for the Pilargidae + Nephtyidae clade.

As with Pilargidae, Chrysopetalidae have never been included in a broad scale molecular analysis prior to this study. Molecular phylogenies assessing the relationships within Chrysopetalidae were not able to resolve the sister taxon of the group (Aguado et al., 2013; Jimi et al., 2019; Ravara et al., 2007). Glasby (1993) and Pleijel and Dahlgren (1998), both based on morphology, identified a sister-group relationship between Chrysopetalidae and Hesionidae. All our analyses also place *Chrysopetalum occidentale* in a well-supported clade with *Amphiduros pacificus*, therefore we name the clade consisting of Hesionidae and Chrysopetalidae, Hesionoidea (Grube 1850). The position of Nereididae differed depending on the analysis we performed; the supermatrix analyses grouped *Platynereis* together with *Struwela*, whereas ASTRAL recovered Nereidiformia (Nereididae + Hesionoidea). The latter relationship was also obtained by Glasby (1993) and Pleijel and Dahlgren (1998).

Struwela camposi is one of only two known *Struwela* species and was described only recently (Salazar-Vallejo et al., 2019). These animals are morphologically somewhat aberrant, with large hooks on the second segment, and both live in association with sand dollars. Salazar-Vallejo et al. (2019) have redelineated Microphthalmidae (and raised it to family rank) to include *Struwela* as well as *Microphthalmus*, *Hesionides*, *Uncopodarke*, *Westheideius*, *Hesionella* and *Fridericiella*. Furthermore, molecular sequence data also support the close affinity of *Struwela* and *Microphthalmus* (Rouse, unpubl.). Though we cannot resolve the placement of *Struwela* with certainty in our analyses, the transcriptome data we publish herein for this aberrant genus, makes it clear that it and Microphthalmidae are not closely related to Hesionidae and so not likely to be a subgroup of this clade as seen also in Salazar-Vallejo et al. (2019).

Glyceridae (*Glycera dibranchiata*), Sphaerodoridae (*Sphaerodorum gracilis*) and Tomopteridae (*Tomopteris* sp.) always formed a well-supported clade in our analyses. To avoid introducing a new name, we here expand the delineation of Glyceriformia to include both Sphaerodoridae and Tomopteridae and propose the superfamily-ranked taxon name Glyceroidea for Goniadidae + Glyceridae.

The last clade we recovered within Phyllodocida was Phyllodociformia, which was well-supported in all our analyses. We included *Lacydonia*, as well as the two holopelagic taxa; Typhloscolecidae (*Traviopsis* sp.) and Lopadorrhynchidae (*Lopadorrhynchus* sp.) always grouped together with the Phyllodocidae terminal (*Phyllococe medipapillata*). The placement of *Lacydonia* within Phyllodociformia is in accordance with previous morphology-based hypotheses, where they have been inferred as the sister group to Phyllodocidae (Pleijel and Dahlgren, 1998; Rouse and Fauchald, 1997). There has been some confusion regarding the placement of the holopelagic clades Typhloscolecidae and Lopadorrhynchidae resulting from analyses published using chimeric sequence data (Struck and Halanynch, 2010), which was demonstrated and further discussed in Nygren and Pleijel (2011). The topology we recover here using a much larger and comprehensive phylogenomic dataset also corroborates the results of Nygren and Pleijel (2011).

Though we were able to recover highly supported relationships for

most of the clades within Phyllodocida, few taxa where no transcriptome data was available remain as Phyllodocida *incertae sedis*. These are *Paralacydonia*, and two holopelagic taxa, *Pontodora* and *Yndolaciidae*.

4.3. Aciculata *incertae sedis* that still need assessment

Based on morphology several potential members of Aciculata, or at least Errantia, can be identified, which need to be assessed in future studies. This includes *Spinther* Johnston, 1845, Myzostomida Graff, 1877, *Aberranta* Wolf, 1987 and Nerillidae Levinsen, 1883 have always been hard to place within Annelida. No transcriptome data is available for *Aberranta* and previous phylotranscriptomic studies have been unsuccessful in confidently placing Myzostomida, Nerillidae and *Spinther*; that all have extremely long branches (Andrade et al., 2015; Struck et al., 2015; Weigert et al., 2014). This also appears to be the case for Histriobdellidae (Fig. S2). While our data shows that large numbers of loci can confidently resolve most relationships within Aciculata, the long branch of *Histriobdella* remains a problem. To break the long branch of *Histriobdella* and other Aciculata *incertae sedis*, future analyses with increased and broader taxon sampling could be useful, in addition to the analysis of non-coding data that could be less prone to confounding effects compared to protein coding data (Chen et al., 2017; Reddy et al., 2017).

CRedit authorship contribution statement

Ekin Tilic: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft. **Josefin Stiller:** Methodology, Formal analysis, Visualization, Writing – review & editing. **Ernesto Campos:** Resources. **Fredrik Pleijel:** Conceptualization, Resources, Writing – review & editing. **Greg W. Rouse:** Conceptualization, Resources, Investigation, Writing – review & editing, Funding acquisition, Supervision.

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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Data availability statement

Data for this article is submitted to Mendeley Data: <https://doi.org/10.17632/9vvdjztctb.1>

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2021.107339>.

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