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Phylogenomics resolves ambiguous relationships within Aciculata (Errantia, Annelida)

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\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

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 \textit{Histriobdella}, \textit{Struwela}, \textit{Lacydonia}, \textit{Pilargis} and the holopelagic worms \textit{Lopadorrhynchus}, \textit{Travisiopsis} and \textit{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: \textit{Lacydonia}, \textit{Typhloscolecidae}, \textit{Lopadorrhynchidae} and \textit{Phyllodocidae}. Nephtyiformia includes \\textit{Nephtyidae} and \\textit{Pilargidae}. We also broaden the delineation of Glyceriformia to include \textit{Sphaerodoridae}, \textit{Tomopteridae} and \textit{Glyceridea} (\textit{Glyceridae} + \textit{Goniidae}). Furthermore, our study demonstrates and explores how conflicting, yet highly supported topologies can result from confounding signals in gene trees.

\textbf{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 \textit{Platynereis dumerili} (Nereididae), the magnificent sand-striker worm, \textit{Eunice cf. aphroditois} (Eunicidae), the furry sea mouse, \textit{Aphroditia} (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.,...
(2015), the clade Eunicida + Phyllococida 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 Phyllococida 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. Phyllococida 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 Phyllococida, having unresolved phylogenetic placement (Martin et al., 2021; Rouse and Pleijel, 2001). Major taxa within Phyllococida 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 Phyllococida, 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...
phylogenetic analysis. These encompass Hesionidae (Fig. 1A), Chrysopetalidae (Fig. 1B), Sphaerodoridae (Fig. 1F), Aphroditidae (Fig. 1J), Pilarigidae, 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’ Histrionobdellidae (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 Histiobdella 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 transcriptome data. Vouchers for newly generated transcriptomes (highlighted in Table 1) 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 PRJNA744330.

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 (Kalyanamoorthy et al., 2017), partition merging (MF + MERGE) and

Table 1

<table>
<thead>
<tr>
<th>Higher Taxa</th>
<th>Species</th>
<th>Sampling locality/Source</th>
<th>Voucher</th>
<th>SRA number</th>
</tr>
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<tr>
<td>Aphroditidae</td>
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<td>A9830</td>
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<td>Sigalionidae</td>
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<td>Sweden</td>
<td>A10088</td>
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<tr>
<td>Polyroidae</td>
<td>Orested, 1843</td>
<td>Okinawa Trough (Zhang et al., 2017)</td>
<td>N/A</td>
<td>SRR4419843</td>
</tr>
<tr>
<td>Polyroidae</td>
<td>Harmothoe imbricata (Linnaeus, 1767)</td>
<td>Sweden (Andrade et al., 2015)</td>
<td>A1142</td>
<td>SRR2005364</td>
</tr>
<tr>
<td>Syllidae</td>
<td>Syllis gracilis Grube, 1840</td>
<td>Spain (Ribeiro et al., 2019)</td>
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<td>SRR8510622</td>
</tr>
<tr>
<td>Nephthyidae</td>
<td>Nephys sp.</td>
<td>Sweden</td>
<td>A12634</td>
<td>SRR15277958</td>
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<td>SRR15277955</td>
</tr>
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<td>A4322</td>
<td>SRR15277965</td>
</tr>
<tr>
<td>Chrysopetalidae</td>
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<td>A4325</td>
<td>SRR15277964</td>
</tr>
<tr>
<td>Nereididae</td>
<td>Platynereis dumerilii (Audouin &amp; Milne Edwards, 1833)</td>
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<td></td>
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<td>Salazar-Vallejo et al. 2019</td>
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<td>Lumbrineridae</td>
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<td>SRR2040483 SRR2040484</td>
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<tr>
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<td>Omphaliidae</td>
<td>Diopatra cuprea (Bosc, 1802)</td>
<td>US-SC (Struck et al., 2015)</td>
<td>N/A</td>
<td>SRR2040374 SRR2040376</td>
</tr>
<tr>
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<td>Sweden (Andrade et al., 2015)</td>
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<tr>
<td>Dorylaimidae</td>
<td>Opyrotoech a globopeltis Blake &amp; Hillig, 1990</td>
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<td>N/A</td>
<td>SRR2040502 SRR2040503</td>
</tr>
<tr>
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<td>Histiobdella sp.</td>
<td>Boston, US-MA</td>
<td>A13436</td>
<td>SRR15277957</td>
</tr>
</tbody>
</table>

* Syllis gracilis represents a species complex and the species 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 homogeneus) 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 (Shimodaira, 2002). All tests performed 10,000 resamplings using the ML tree from the concatenated dataset, we used topology tests as with 10,000 bootstrap pseudo-replicates. To assess model chosen according to BIC (LG (Shimodaira, 2002)).

We tested how support (measured as local posterior probability PP) 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 oenonid 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); Eunicoida to the Eunicidae plus Onuphidae clade and the name Oenonidea for the Lumbrineridae plus Oenonidae 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; Travisiosis sp. and Lopadorrhynchus sp.

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

Aphroditiformia, with Lastmonice cf. iocash McIntosh, 1885 (Aphroditidae) as sister to a clade including Pholoe balica Orsted, 1843 and two polymorphic species Lepadotomopterum sp. and Harmotohe inimbicata (Linnaeus, 1767). The syllid Syllis gracilis Grube, 1840 was supported as the sister to Aphroditiformia.

Nephytiformia is a new name we use to include Pilargidae and Nephytidae, based on Pilargis verrucosa Saint-Joseph, 1899 and Nephys 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 campshi Salarz-Vallejo et al. 2019 and this clade was the sister to Glyceriformia + Phyllodociformia. We do name the well-supported clade comprising of Chrysoptetalidae and Hesionidae as Hesionoidea, which has an ending signifying superfamly rank, and the authority is Grube (1850).

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.

Within Eunicida Ophryatomyra globopallata Blake & Hilbig, 1990 formed a clade with the eunicid 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 artificial 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 Ophryatomyra globopallata Blake & Hilbig, 1990 formed a clade with the eunicid 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 oenonid 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); Eunicoida to the Eunicidae + Onuphidae clade and the name Oenonidea for the Lumbrineridae + Oenonidae clade.
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 *Glyceria dibranchiata* and *Sphaerodorum gracilis* (Fig. 1F) grouped together (Fig. 2).

In contrast to the supermatrix analysis the ASTRAL result showed *Platyneris* 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 *Platyneris*, 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 Nereditidae 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 2.15e-33). The position of Nereditidae 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 pro-bosics with a complex jaw apparatus that is mineralized or sclerotized (Tzetlin 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, Oenonidae Kinberg, 1865, Lumbrineridae Schmarda, 1861, Dorvilleidae Chamberlin, 1919 and the two smaller enigmatic clades Histiobdellidae Vaillant, 1890 and *Hartmaniella* Imajima, 1977. Hartmaniellidae is monotypic for *Hartmaniella* with the former being superfluous, we therefore only use

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**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 of 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 + Oenonidae and then Dorvilleidae (possibly together with Histriobdellidae) 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) Eunicoidae, which we adopt here. The synapomorphies that unite Eunicoidae are: eulabidognath maxillae, five prostomial appendages, peristomial cirri, and subacular hooks in median and posterior parapodia (Budaeva and Zanol, 2020).

The second well-supported clade within Eunicida contains Lumbrineridae and Oenonidae. 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 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 Oenonidae. This was also favored by earlier morphological hypotheses and was given the name Oenonoida 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 Oenonidae 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 Eunicoidae either on its own (ASTRAL) or together with Histriobdella sp. (RAxML/IQTREE). 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 Histriobdella, our phylogenomic analysis is the first attempt to place Histriobdellidae within Eunicida using molecular data. In all ML analyses with the concatenated supermatrix Histriobdellidae 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 (Histriobdella 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 Histriobdellidae 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), Histriobdella grouped together with other long-branched taxa outside Aciculata. This indicates that confounding long-branch effects may be causing the placement of Histriobdella 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 Histriobdella within Eunicida (Fig. S2, S4), indicating that there seems to be little signal in the Histriobdella data combined with many mutations that are not shared with any other Eunicida. Alas, given the relatively long branches of both Ophryotrocha globopalpata and Histriobdella, and the conflicting topologies, we cannot confidently resolve the phylogenetic position of Histriobdellidae, 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 Eunicoida, then again Fauchald and Rouse (1997) interpreted the jaws differently and considered them to be closer to Oenonoida. 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: Euleptethidae Chamberlin, 1919; Aphroditidae Malmgren, 1867; Sigalionidae Kinberg, 1856; Acocetidae 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; Micronphalidae Hartmann-Schröder, 1971; Glyceridae Grube, 1850; Goniadidae Kinberg, 1866; Sphaerodoridae Malmgren, 1867; Tomopteridae Johnston, 1865; Lacydonia Marion, 1874 (=monotypic Lacydo- niidae); Paralayconia Fauvel, 1913 (=monotypic Paralayconidae); Typhloscolecidae Uljanin, 1878; Lopadorrhynchidae Claparede, 1868; Phyllodocidae Orsted, 1843; Yndolacidae Stemp-Bowitz, 1887 and Ponto- dora Greff, 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 (Laetomonie e. icosaica), Sigalionidae (Pholeo ballica) 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 *Pilaris verrucosa* together form the sister taxon to the rest of this clade. The position of Pilarigidae has long been problematic. Several studies based on both morphology (Fitzhugh and Wolf, 1990; Glasy, 1993; Licher and Westheide, 1994; Pleijel and Dahlgren, 1998) and molecular data (Roussé et al., 2007) have often placed them within Nereidiformia (or Nereididea). Rouse and Fauchald (1997) placed Pilarigidae as sister to Sphaerodoridae. In Struck et al.’s (2007) phylogeny of annelids based on Sanger sequenced data, Pilarigidae were either in a trichotomy together with Syllidae and Nephtyidae, or as sister to the two others. The position of *Pilaris verrucosa* is highly supported in all analyses we performed, and we therefore use the new name Nephytiformia for the Pilarigidae + Nephtyidae clade.

As with Pilarigidae, 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). Glasy (1993) and Pleijel and Dahlgren (1998), both based on morphology, identified a sister-group relationship between Chrysopetalomorpha and Hesionidea. All our analyses also place Chrysopetalum occidentale in a well-supported clade with Amphidium pacificum, therefore we name the clade consisting of Hesionidea and Chrysopetalomorpha, Hesionoida (Grube 1850). The position of Nereididae differed depending on the analysis we performed: the supramatrix analyses grouped Platynereis together with *Struwela*, whereas ASTRAL recovered Nereidiformia (Nereididea + Hesionidea). The latter relationship was also obtained by Glasy (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, Uncopedarke*, *Westheideus*, *Hesionella* and *Fridericella*. 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 Hesionidea and so not likely to be a subgroup of this clade as seen also in Salazar-Vallejo et al. (2019).

Glyceridae (*Glyceria dibranchiata*, Sphaerodoridae (*Sphaerodorororum 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 Glycerioidea for Goniidelidea + 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; Typhlosolecidae (*Travisiosis* sp.) and Lopadorrhynchidae (*Lopadorrhynchus* sp.) always grouped together with the Phyllodocidae terminal (*Phyllocoelo 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 Typhlosolecidae and Lopadorrhynchidae resulting from analyses published using chimeric sequence data (Struck and Halanych, 2010), which was demonstrated and further discussed in Nygen and Pleijel (2011). The topology we recover here using a much larger and comprehensive phylogenomic dataset also corroborates the results of Nygen 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 Levinson, 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. Josefina Stillier: 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/10.17632/9vvdjztcb.1

Appendix A. Supplementary material

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