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Published in:
Molecular Phylogenetics and Evolution

DOI:
10.1016/j.ympev.2021.107339

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
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Citation for published version (APA):
Tilic, E., Stiller, J., Campos, E., Pleijel, F., & Rouse, G. W. (2022). Phylogenomics resolves ambiguous relationships within Aciculata (Errantia, Annelida). Molecular Phylogenetics and Evolution, 166, [107339]. https://doi.org/10.1016/j.ympev.2021.107339
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 Histioella, Struwea, Lacydonia, Pilargis and the holopelagic worms Lopadorrhynchus, Travisiopsis 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 Eunicoida and Oenonoida. 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 Sphaerodorididae, Tomopteridae and Glyceroidae (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 Eunice cf. aphroditois (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 subclasses 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 Amphimida, 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.,...
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

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Fig. 1. Live photographs of specimens used in this study. A Amphidurus pacificus (Hesionidae) B Chrysopetalum occidentale (Chrysopetalidae), C Pholoe baltica (Sigalionidae), D Tomopteris sp. (Tomopteridae), E Histriobdella sp. (Histriobdellidae), F Sphaerodorum gracilis (Sphaerodoridae), G Nephys hombergii (Nephryidae), H Travisiopsis sp. (Typhloscolecidae), I Struwela camposi (Microphthalmidae), J Laemonice cf. tocasica (Aphroditidae), K Lacydonia sp., L Lopadorrhynchus sp. (Lopadorrhynchidae).
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phylogenetic analysis. These encompass Hesioniidae (Fig. 1A), Chrysopetalidae (Fig. 1B), Sphaerodoridae (Fig. 1F), Aphroditidae (Fig. 1J), Pilargidae, Lacydonia (Fig. 1K), the enigmatic Struwela (Fig. 1I), the holopelagic Lopodorrhynchidae (Fig. 1L) and TYPHOLOIDECI (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 Histrionobdella 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.

Table 1

| Higher Taxa   | Species                      | Sampling locality/Source     | Voucher          | SRA number                        |
|---------------|------------------------------|------------------------------|------------------|-----------------------------------|
| Aphroditidae  | Laemonice cf. locasica McIntosh, 1885 | Costa Rica                  | A9830            | SRA15277959                      |
| Sigalionidae  | Pholoe baltica               | Sweden                       | A1008            | SRA15277956                      |
| Polyphagidae  | Lepistostomopus sp.          | Okinawa Trough/(Zhang et al., 2017) | N/A             | SRA419843                         |
| Polyphagidae  | Harmotaheim hicera (Linnaeus, 1767) | Sweden/(Andrade et al., 2015) | A1142            | SRA205364                         |
| Sylidae       | Sylia gracilis Grube, 1840*  | Spain/(Ribeiro et al., 2019) | N/A             | SRA8510622                        |
| Nephthyidae   | Nephys sp.                   | Sweden                       | A12634           | SRA15277958                      |
| Pilargidae    | Pilargis verrucosa Saint-Joseph, 1899 | Sweden                  | N/A             | SRA15277955                      |
| Hentionidae   | Amphidurus pacificus Hartman, 1961 | San Diego, US-CA | A4322            | SRA15277965                      |
| Chrysopetidida | Chrysopetula occidentale Johnson, 1897 | San Diego, US-CA | A4325            | SRA15277964                      |
| Nereididae    | Playnarios dambeli (Audouin & Milne Edwards, 1833) | N/A                          | N/A             | SRA1742987                        |
| Microphthalamida | Struwela campisi | Salazar-Vallejo et al 2019 | Mexico            | A13437                          | SRA15277962 |
| Glycerida     | Glycera dinbranchiata Ehlers, 1868 | (Kocot et al., 2017)         | N/A             | SRA2050719                       |
| Sphaerodoridae | Sphaerodorus gracilis (Rathke, 1843) | Sweden | A1007 | SRA15277954 |
| Tomopteridae  | Tomopteris sp.               | San Diego, US-CA            | A1182            | SRA15277953                      |
| Lacydonia     | Lacydonia sp.                | Costa Rica                   | A10090           | SRA15277960                      |
| Typhlophagidae | Travissia sp.                | San Diego, US-CA            | A9405            | SRA15277963                      |
| Lopodorrhynchida | Lopodorrhynchus sp.        | San Diego, US-CA            | A10669           | SRA15277961                      |
| Phylodocidae  | Phylodoco medipapillata Moore, 1909 | San Diego, US-CA | A5913            | SRA15277962                      |
| Lumbrineridae | Ninus nigripes Verrill, 1873 | US-NC/(Struck et al., 2015) | N/A             | SRA2040483 SRR2040484            |
| Oenonidae     | Ara bella sp.               | Panama/(Struck et al., 2015) | N/A             | SRA2040140 SRR2040414            |
| Omphalidae    | Dioptra cuprea (Bosc, 1802)  | US-SC/(Struck et al., 2015) | N/A             | SRA2040374 SRR2040376            |
| Eunicidae     | Lepidose toxus (Quatrefages, 1866) | Spain/(Andrade et al., 2015) | A1168            | SRA2005375                       |
| Dorvilleidae  | Ophryotrocha globopatula Blake & Hilgib, 1990 | US-VA/(Struck et al., 2015) | N/A             | SRA2040502 SRR2040503            |
| Histrionobdellida | Histrionobdella sp. | Boston, US-MA               | A13436           | SRA15277957                      |

* 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 homogenous) assumptions and were excluded from the ASTRAL analysis. All the remaining 9139 gene trees were included as ASTRAL benefits from the sister to Aphroditiformia. 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 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 ononiid 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 + Onuphididae and the name Oenonoidae for the Lumbrineridae + Ononidae clade.

Within Phylodocida several clades were recovered that we apply names to, based on support and congruence with the ASTRAL result: Phyllodociformia, consisting here of Lacydonia sp., Phyllodoco medipapillata Moore, 1909 and two holopelagic taxa; Travisiospis 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 Laetmonice cf. iocasica McIntosh, 1885 (Aphroditidae) as sister to a clade including Pholoe balica Orsted, 1843 and two polymorph species Lepidotoptopodium sp. and Harmothoe imbricata (Linnaeus, 1767). The syllid Syllis gracilis Grube, 1840 was supported as the sister to Aphroditiformia.

Nephthyiformia is a new name we use to include Pilarigidae and Nephthyidae, based on Pilaris 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 Struwella camposi Salazar-Vallejo et al. 2019 and this clade was the sister to Glyceriformia + Phyllodociformia. We do name the well-supported clade comprising of Chrysopterilidae and Hesionidae as Hesionoidea, which has an ending signifying superfamily rank, and the authority is Grube (1850).
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 treeHistriobdella 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.

Note the dashed line of Histriobdella indicating the conflicting topologies shown in Figs. S1-4, likely a result of long-branch artefacts.
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 Amphiduchos pacificus Hartman, 1961 and the chrysopetalid Chrysoptetalum 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 Glyceriforma + Phyllocociforma 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 Histriobdellidae (Fig. 3A–C) were not significantly different and so cannot be ruled out based on the data. The ASTRAL subssetting analyses showed strong support in favor of the ASTRAL topology for four out 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 Histriobdellidae 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 Glyceraiforma + Phyllocociforma 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 e-08). The position of Ner- eididae 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 subssetting 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-boscis 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 Histriobdellidae 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 Histriobdellidae, 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; Tzetlin, 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) Eunicoida, which we adopt here. The synomorphies that unite Eunicoida 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 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 Eunicidae + Oenonidea 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 more 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 Oenonoidae. 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 muscleous proboscis, the loss of dorso-lateral folds, the ventrally positioned sensory papules, 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: Euleptetheidae Chamberlin, 1919; Aphroditidae Malmgren, 1867; Sigalionidae Kinberg, 1856; Acetidae Kinberg, 1856; Iphionidae Kinberg, 1856 and 1856; Pilargidae Saint-Joseph, 1899; Chrysopetalidae Ehlers, 1864; Hesionidae Grube, 1850; Nereididae Blainville, 1818, 1818; Microphalmaidae Hartmann-Schröder, 1971; Glyceridae Grube, 1850; Goniadiidae Kinberg, 1866; Sphaerodoridae Malmgren, 1867; Tomopteridae Johnston, 1865; Lacydonia Marion, 1874 (=monotypic Lacydoniidae); Paralacydonia Faunal, 1913 (=monotypic Paralacynidae); Typhloscolecidae Uljanin, 1878; Lopadorrhynchidae Claparede, 1866; Phyllodocidae Orsted, 1843; Yndolaciidae Stéphow-Bowitz, 1879 and Pontodoridae 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 (Laetemoneicelosacica), Sigalionidae (Pholeo baltica) and Polynoidea (Lepadonotopodium 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. Nephys 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; Glasy, 1993; Licher and Westheide, 1994; Pleijel and Dahlgren, 1998) and molecular data (Rouset et al., 2007) have often placed them within Nereidiformia (or Nereididae). 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 Nephthyidae, 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 + Nephthyidae 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). Glasy (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 Amphidium pacificus, therefore we name the clade consisting of Hesionidae and Chrysopetalidae, Hesionoida (Grube 1850). The position of Nereididae differed depending on the analysis we performed: the supertree analyses grouped Platynereis together with Struwela, whereas ASTRAL recovered Nereidiformia (Nereididae + Hesionidae). 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, Uncopodarke, Westheideus, 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 (Sphaerodorura 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 Glyceroidae for Goniadiidae + 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 (Travisioptis sp.) and Lopadorrhynchidae (Lopadorrhynchus sp.) always grouped together with the Phyllodocidae terminal (Phyllocooea medi-papillata). 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 Halanych, 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 Yndolacidae.

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. Josefín 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.

Acknowledgements

The authors are grateful to Avery Hatch and Marina McCowan for their help with the molecular lab work and to Charlotte Seid for handling and cataloging specimens at the Benthic Invertebrate Collection of Scripps Institution of Oceanography (SIO-BIC). Sampling by E. Campos to collect S. camposi were granted by projects UAB-CONACYT SS2903-Q, UABC-CONANP/SEMARNAP (contract INE/E067RF7UCANP7AD-05700) and UANL/UABC/UdelMar-SEP-PRODEP, exotic species of Mexico. Arturo Ramirez is also thanked for the sampling of Struwela camposi. Many thanks also to Gonzalo Giriibet and the members of his lab for their hospitality during the collection Histriobdella.

Data availability statement

Data for this article is submitted to Mendeley Data: https://doi.org/10.17632/9vvdjztcht.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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