



## Short Communication

# Worms that suck: Phylogenetic analysis of Hirudinea solidifies the position of Acanthobdellida and necessitates the dissolution of Rhynchobdellida



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## ABSTRACT

Annelids possessing a posterior sucker and a fixed number of somites – most famously leeches (Hirudinida), but also crayfish worms (Branchiobdellida) and salmonid parasites (Acanthobdellida) – form a clade; however, determining the relationships between these orders has proven challenging. Here, we compile the largest molecular phylogenetic dataset yet analysed for these groups, including new sequences for key taxa. We find robust model-based support for a clade formed by Hirudinida and Acanthobdellida, contrasting the largest prior studies. We determine that conflicting prior studies included contaminant sequences for *Acanthobdella peledina*. In addition to this broad-scale comparison, the size of our dataset grants us invaluable insight into the internal relationships of leeches and crayfish worms. Of particular importance, a largely marine clade of leeches (Piscicolidae and Ozobranchidae) is recovered as sister to all remaining Hirudinida. This necessitates the dissolution of the paraphyletic suborder Rhynchobdellida into two new suborders (Oceanobdelliformes and Glossiphoniiformes). Likewise, we decompose Arhynchobdellida into its respective suborders: Hirudiniformes, Erpobdelliformes, and the new, monotypic, Americobdelliformes.

## 1. Introduction

After over a century of controversy, it is now evident that leeches (Hirudinida), crayfish worms (Branchiobdellida), and leech-like salmonid parasites (Acanthobdellida) form a clade (see Table 1). Bloody swamp legends, personally-invasive infestations (hirudiniasis), and an extensive history of use in folk and authoritative medicine has cemented leeches in the public psyche (Sawyer, 1986). Additionally, many leeches are important predators, can be useful environmental indicators, and vector blood parasites to many vertebrates (Apakupakul et al., 1999; Sawyer, 1986). Comparatively unknown, branchiobdellidans form tight symbiotic relationships with their crayfish hosts (Skelton et al., 2013), making them captivating in their own turn. Some branchiobdellidans range from highly beneficial mutualists to deleterious parasites on their hosts (depending on their densities), and have accordingly emerged as ecological models (Skelton et al., 2013). Acanthobdellidans infest freshwater fishes (Bielecki et al., 2014a; Sawyer, 1986) that are important predators, and which draw

substantial fishing tourism to the northerly latitudes where these worms are found. Yet despite being voracious salmonid parasites, they are rarely collected – probably as they live in remote areas (Bielecki et al., 2014a) and have patchy distributions – and little is therefore known about their biology. Taken together, leeches, branchiobdellidans, and acanthobdellidans represent the entire scale of ectosymbiotic relationships (Siddall et al., 2001; Skelton et al., 2013). Discerning the relationships between these groups is essential for understanding their evolution in terms of major ecological and morphological transitions, and is fundamental for determining the origins of bloodfeeding and anticoagulants in a truly notable sanguivorous clade (see Siddall et al., 2015; Tessler et al., 2018a).

Odier (1823) drew an initial link between leeches and branchiobdellidans. Later, Livanow (1931) proposed that Acanthobdellida shared a common ancestor with Branchiobdellida and Hirudinida. Sawyer (1986) also proposed this relationship on the basis of a strong unifying morphological feature: the caudal sucker, which is critically important for precise movement and host attachment. The possession of

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**Table 1**

Previous phylogenetic hypotheses concerning the relationships between leeches (Hirudinida), crayfish worms (Branchiobdellida), and leech-like salmonid parasites (Acanthobdellida).

Citation	Data type	Optimality criterion	<i>Acanthobdella peledina</i> sequences	Notes
<i>Relationship recovered for focal taxa: Hirudinida sister to Acanthobdellida</i>				
Brinkhurst (1999)	Morphology	Parsimony		Evidence regarding the position of Branchiobdellida is equivocal: Brinkhurst states that it could also be the sister taxon to Lumbriculidae
Martin (2001)	18S	Parsimony	AF099948	The combined dataset (and morphological datasets) support this hypothesis. When analysed in isolation, the 18S gene tree resolves Acanthobdellida within Lumbriculidae
Marotta et al. (2008)	18S, morphology	Parsimony	AF099948	
Purschke et al. (1993)	Morphology	Parsimony		
<i>Relationship recovered for focal taxa: Hirudinida sister to Branchiobdellida</i>				
Erséus and Källersjö (2004)	18S	Parsimony	AY040680	Williams et al. (2013) note that a few of the branchiobdellidan sequences in this study are contaminants
Gelder and Siddall (2001)	18S, COI, morphology	Parsimony	AF099948 (18S) AF003264 (COI)	
Siddall et al. (2001)	18S, COI	Parsimony	???? (18S) ???? (COI)	
Apakupakul et al. (1999)	18S, COI	Parsimony	AF115978 (18S) AF003264 (COI)	
Rousset et al. (2008)	18S	Maximum Likelihood	AY040680	
Siddall and Bureson (1998)	COI	Parsimony	AF003264	Hiurdinida and Branchiobdella are the sister clade to Acanthobdellida and Lumbriculidae
<i>Relationship recovered for focal taxa: Alternative hypotheses</i>				
Kaygorodova and Sherbakov (2006)	18S	Parsimony	AF099948 AY040680	Acanthobdellida and Hirudinida form a sister group; this clade is part of a polytomy containing Branchiobdellida and Lumbriculidae A polytomy was recovered. Morphology was only scored from ovaries Hirudinida, Branchiobdellida, and Lumbriculidae form a polytomy; Acanthobdellida nests within Lumbriculidae Hirudinida and Acanthobdellida form a sister group, but Branchiobdellida is not sister to this clade: branchiobdellidans nest with polychaetes ( <i>Neanthes</i> and <i>Aphrodita</i> ). Martin (2001) notes that this placement is likely a result of long branch attraction, and therefore “erroneous” Acanthobdellida and Branchiobdellida form a clade
Bielecki et al. (2014b)	Morphology	Parsimony		
James and Davidson (2012)	18S	Bayesian	AY040680	
Rota et al. (2001)	18S	Parsimony	AF099948	
Trontelj et al. (1999)	18S	Parsimony	AF099948	

\* Sequences with a strikethrough line represent contaminant sequences (AF115978 [18S] BLASTs as chiton; AF003264 [COI] BLASTs as human), rendering the phylogenetic position of *Acanthobdella peledina* misleading for studies using said sequences.

a clitellum for cocoon formation, the fixed number of somites, and their ectosymbiotic lifestyles provide further evidence of shared ancestry (Siddall et al., 2001). When these, and other morphological characters, are used for phylogenetic reconstruction, the monophyly of these groups is recovered (see Table 1). Previous studies have cited morphological characters as evidence that acanthobdellidans might be the earliest-branching clade of Hirudinea (Sawyer, 1986; Siddall et al., 2001); however, most morphological phylogenetic studies have found that Acanthobdellida is sister to leeches (see Table 1).

Molecular phylogenetic analyses (some of which include morphological data) have similarly recovered the monophyly of this vermiform clade; though, conflict abounds regarding the internal relationships of the group. At present, our best understanding of the relationships within the clade stems from analyses combining 18S rDNA and COI sequence data, as these are the largest molecular datasets compiled thus far. These studies suggest that Branchiobdellida and Hirudinida form a clade to the exclusion of Acanthobdellida. Yet, not all studies incorporating molecular data have relied on COI and 18S sequences: many have focused on 18S alone, or a combination of 18S sequences and morphological data. Despite the fact that these studies rely on similar datasets, roughly half have recovered acanthobdellidans as the closest relatives of leeches. The varied morphological and molecular phylogenetic hypotheses are detailed in Table 1.

Within leeches, internal relationships are often unstable or poorly supported (e.g., Apakupakul et al., 1999; Borda and Siddall, 2004; Trontelj et al., 1999), and far less is known about the interrelationships of Branchiobdellida (Gelder and Siddall, 2001; Williams et al., 2013). Acanthobdellida encompasses only two described species (Bielecki et al., 2014a), and, to date, only *Acanthobdella peledina* has been

collected for molecular work (Table 1).

Here, we clarify the relationships between, and to some degree within, Acanthobdellida, Branchiobdellida, and Hirudinida. We combine prior datasets to include previously omitted taxa and to expand the molecular branchiobdellidan matrix generated by Williams et al. (2013), compile a large dataset for determining the relationships within leeches, and provide new sequences for key taxa: Acanthobdellida and Branchiobdella — a widespread taxon, and the type genus of Branchiobdellidae.

## 2. Materials and methods

Although most of the data used here were downloaded from GenBank, eight additional specimens were collected and sequenced for this study: most notably *Acanthobdella peledina* and *Branchiobdella cf. kozarovi* (Supplementary Table 1). Worms were collected from hosts (i.e., grayling or crayfish) and stored in 90–100% ethanol or RNAlater (Ambion). Specimens were identified with specialized literature, and identifications were confirmed using COI (Supplementary Table 1).

A DNeasy Blood and Tissue Kit (Qiagen) was used to extract DNA. We amplified and sequenced COI, 16S, 18S, 28S, and ITS following Williams et al. (2013). All amplification reactions used Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare); 2.5 µl 1% dimethylsulfoxide was added for ribosomal loci. Primers used followed Williams et al. (2013), except for 18S primers “A”, “B”, and “Y” from Apakupakul et al. (1999) and primers from Tessler et al. (2018b) used for COI (only the typical barcode region was included in downstream analysis to avoid missing data). Thermocycler protocols followed prior studies (Apakupakul et al., 1999; Borda and Siddall, 2004; Williams et al.,

2013). Where COI was difficult to amplify, annealing temperatures were lowered to 42 °C. PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Inc.), ethanol precipitated, and cycle sequenced. Sequenced products were analyzed using an ABI 3730 (Applied Biosystems), and reconciled and edited using Geneious version 6.18. We then combined our sequences with prior studies' matrices (Ahn and Min, 2016; Apakupakul et al., 1999; Borda and Siddall, 2004; Füreder et al., 2009; Williams et al., 2013) (Supplementary Table 1). The COI sequence for *Hirudo medicinalis* (AF003272) was omitted from analyses due to peculiarities. Clitellates from Erséus et al. (2010) were selected as outgroup taxa. Several GenBank branchiobdellidan COI sequences necessitated primer trimming.

Phylogenetic methods represent a subset of the methods in de Carle et al. (2017) and are briefly detailed here. Combined matrices were aligned using MUSCLE (Edgar, 2004) with defaults. PartitionFinder2 (Lanfear et al., 2017) was used to determine models of evolution ("greedy" search, using the Akaike Information Criterion). The alignment was partitioned into four sets: COI 2nd position; ITS and COI 1st position; 18S, 28S, and COI 3rd position; and 16S. A GTR model and GAMMA distribution with a proportion of invariable sites for all partitions was suggested. Maximum likelihood (ML) analysis used RAxML ver. 8 (Stamatakis, 2014) on CIPRES (Miller et al., 2010). Tree searches used 1000 replicates with 25 initial GAMMA rate categories and final optimization using four GAMMA shape categories. Bootstraps were calculated using 1000 replicates (rapid algorithm). Bayesian Inference (BI) used MrBayes ver. 3.2.6 (Huelsenbeck and Ronquist, 2001) on CIPRES for 10 million generations (burn-in = 25%). Maximum parsimony (MP) analyses used TNT ver. 1.5 (Goloboff et al., 2008) with xmult (1000 replications each with five rounds of ratcheting) and 1000 bootstraps as support. An additional parsimony search constrained Hirudinida and Acanthobdellida as sister. Trees were rooted on Naididae, following Rousset et al. (2008).

### 3. Results and discussion

Model-based analyses recovered *Acanthobdella peledina* (Acanthobdellida) as sister to Hirudinida (93% ML bootstrap support [MBS]; 0.99 posterior probability [PP]; Fig. 1 and supplementary tree files). Although the results of the parsimony analysis recovered *A. peledina* as sister to Branchiobdellida, these results were not robust enough to refute the findings of the ML and BI analyses: support was low (Parsimony bootstrap support [PBS] = 5%), and when a sister group relationship between Acanthobdellida and Hirudinida was constrained, the length of the most parsimonious tree increased by only 4 steps (unconstrained = 19,693 steps; constrained = 19,697 steps). This finding contrasts several of the most recent, and largest, phylogenetic analyses investigating the relationships of these sucker-bearing worms (Apakupakul et al., 1999; Erséus and Källersjö, 2004; Gelder and Siddall, 2001; Rousset et al., 2008; Siddall et al., 2001; Siddall and Bureson, 1998). The studies that are concordant with our findings relied on 18S, morphological data, or a combination thereof (Brinkhurst, 1999; Marotta et al., 2008; Purschke et al., 1993). On closer examination, we found that the larger molecular studies incorporated contaminant sequences for *Acanthobdella peledina* (AY040701, AF115978, and AF003264), the only acanthobdellid currently available for molecular work. Our new sequences from a freshly collected specimen of *A. peledina* eliminate that problematic legacy. Furthermore, our findings leverage the most substantial dataset yet compiled for these taxa. We find strong support (MBS and PBS = 100%; PP = 1.00) for the monophyly of all three orders, and for these orders together comprising the sister group to Lumbriculidae (MBS = 100%; PP = 1.00; PBS = 97%), bolstering prior studies (e.g., Erséus and Källersjö, 2004; Siddall et al., 2001).

The relationships between leeches, crayfish worms, and acanthobdellidans help to illuminate the evolution of key morphological and ecological characters of these groups. Given the putative plesiomorphic

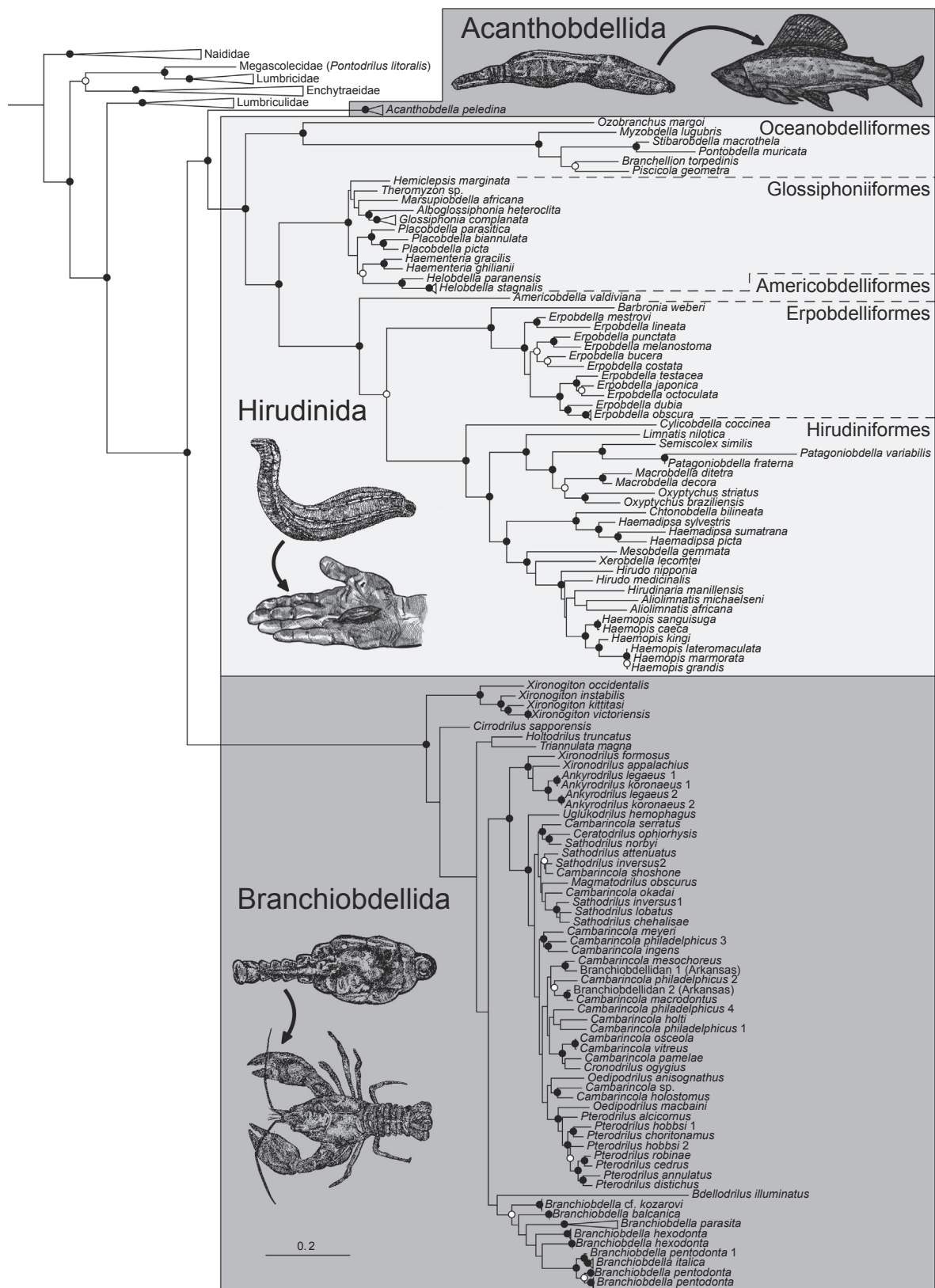
state of leeches as bloodfeeders (Apakupakul et al., 1999; Siddall et al., 2015) and the ectoparasitic, partially bloodfeeding, lifestyle of Acanthobdellida, it is unsurprising that these taxa should be united. This suggests a single origin of vertebrate parasitism within Clitellata, which corroborates comparative studies (Apakupakul et al., 1999; Borda and Siddall, 2004; Trontelj et al., 1999), as well as evidence that a number of anticoagulants are shared across distantly related leech lineages (Siddall et al., 2015; Tessler et al., 2018a). The potentially relictual characters found in acanthobdellidans - such as chaetae and somite septa (Purschke et al., 1993) - therefore appear to have been lost multiple times or evolved independently. Significantly, these groups share a plesiomorphic freshwater condition (Lumbriculidae, Branchiobdellida, Acanthobdellida, and many leeches inhabit freshwater environments), as predicted by a prior comparative study (Rousset et al., 2008).

Within Hirudinida, we resolve a clade consisting of Ozobranchiidae and Piscicolidae as the earliest-branching lineage (MBS = 100%; PP = 1.00; PBS = 83%). The topology reported by most of the largest prior studies recovers Glossiphoniidae as the sister taxon to all remaining leeches (Apakupakul et al., 1999; Borda and Siddall, 2004; Siddall and Bureson, 1998; but see Martin, 2001; Rousset et al., 2008; Trontelj et al., 1999). In either case, it is clear that the traditionally-accepted order Rhynchobdellida (proboscis-bearing leeches) is paraphyletic, and that Arhynchobdellida (leeches without proboscises) is nested within this grade. As hypothesized and detailed by Trontelj et al. (1999), this suggests that the proboscis was lost in a lineage of leeches (i.e., arhynchobdellids) whose buccal apparatuses developed into stylets (i.e., Salifidae), tripartite jaws (i.e., Hirudiniformes), or jawless forms (e.g., Erpobdellidae). Furthermore, for some hirudiniform lineages, the 3-jawed apparatus was secondarily completely (i.e., Semiscolosciidae and some Haemopidae) or partially lost (i.e., two-jawed *Chthonobdella* spp. and single-jawed *Tyrannobdella rex*). It is worth noting that the tripartite muscular structure of the "rhynchobdellid" proboscis is morphologically similar to that of the feeding apparatus found in all other leeches (Brinkhurst, 1999; Sawyer, 1986). Moreover, acanthobdellidans exhibit a rudimentary proboscis, adding further credence to the notion that the ancestral leech was proboscis-bearing (Sawyer, 1986).

After decades of studies finding preliminary evidence that higher taxa within Hirudinida are paraphyletic (Apakupakul et al., 1999; Borda and Siddall, 2004; Siddall and Bureson, 1998), our expanded phylogenetic dataset finally allows for a much needed reclassification of leeches above the family level. Given the paraphyly of Rhynchobdellida, we have chosen to abandon this taxon, along with Arhynchobdellida. Instead, we expand upon existing subordinal classifications (e.g., Hirudiniformes), establishing three new suborders to better reflect our current phylogenetic understanding. For full taxonomic changes, see the Taxonomy section.

We also recover several noteworthy relationships within Branchiobdellida. The last major phylogenetic study of this group focused exclusively on North American taxa (Williams et al., 2013), not including previously published sequence data for other branchiobdellidans (Füreder et al., 2009; Gelder and Siddall, 2001). Here, we incorporate the broadest geographic and taxonomic sampling possible with currently-available data. Significantly, we find that the European genus *Branchiobdella* (the type genus) nests among North American taxa. Although this genus is monophyletic (MBS = 76%; PP = 0.99; PBS = 43%), the same is not true of many North American genera (e.g., *Sathodrilus*), or even species (e.g., *Cambarincola philadelphicus*), in this and prior studies (Williams et al., 2013). In agreement with Williams et al. (2013), we find that the subfamilies Branchiobdelinae, Bdellodrilinae, and Xironodrilinae are paraphyletic; and that, while much of Cambarincolinae is monophyletic, one species - *Triannulata magna* - nests outside the clade. Future work should aim to resolve these many taxonomic issues.

Our findings exemplify the value of reassessing conclusions drawn



**Fig. 1.** Phylogenetic relationships recovered within and between leeches (Hirudinida), crayfish worms (Branchiobdellida), and leech-like salmonid parasites (Acanthobdellida) using maximum likelihood. Triangular, collapsed branches represent multiple representatives of the same species. Support values (summaries of 1000 bootstrap pseudoreplicates) are presented as circles on nodes: filled in circles are BS 90–100, while empty circles are BS 70–89. For each order, a generalized image of a worm in that order and its host is displayed.



by early phylogenetic studies, as well as the utility of previously-published data for resolving relatively deep nodes, and the importance of assessing the quality of those data using expanded sequence databases. Our work synthesizes the findings of prior studies, resolving equivocal relationships within Hirudinea and lending systematic stability to the group after decades of dispute. We recover strong support for the monophyly of the clade, and for its placement as sister to Lumbriculidae; we reaffirm Acanthobdellida as the closest relative of Hirudinida, overturning results from several large molecular phylogenetic analyses; we illustrate the need for revised classification within Branchiobdellida; and we enact broad-scale taxonomic changes to reconcile leech systematics with evolutionary history.

#### 4. Taxonomy

**Class:** Clitellata Michaelsen, 1919

**Subclass:** Hirudinea Lamarck 1818

**Order:** Acanthobdellida Livanow, 1905

**Order:** Branchiobdellida Holt, 1965

**Order:** Hirudinida Siddall et al., 2001

**Suborder:** Americobdelliformes, *nov.* Siddall, de Carle, and

Tessler

**Family:** Americobdellidae Caballero, 1956

**Suborder:** Erpobdelliformes Sawyer, 1986

**Suborder:** Hirudiniformes Caballero, 1952

**Suborder:** Glossiphoniiformes, *nov.* Tessler and de Carle

**Family:** Glossiphoniidae Vaillant, 1890

**Suborder:** Oceanobdelliformes, *nov.* Tessler and de Carle

**Family:** Ozobranchidae Pinto, 1921

**Family:** Piscicolidae Johnston, 1865

**Remarks on taxonomy:** The taxonomic scheme we present reflects an updated understanding of the relationships between major leech lineages following our phylogenetic analyses. We erect two new suborders (Oceanobdelliformes and Glossiphoniiformes) to replace Rhynchobdellida Blanchard, 1984, which we and other researchers resolve as paraphyletic (Apakupakul et al., 1999; Borda and Siddall, 2004; Siddall and Bureson, 1998; Trontelj et al., 1999). Of the former rhynchobdellids, Oceanobdelliformes comprises the primarily marine and brackishwater families Ozobranchidae and Piscicolidae, while Glossiphoniiformes are exclusively freshwater. Glossiphoniiformes are also unique among leeches in exhibiting parental care: their soft cocoons are protected by the parent - either by attachment to the venter or brooding - and, in some cases, the juveniles are protected after hatching, until they obtain their first meal (Sawyer, 1986). Accordingly, Oceanobdelliformes are diagnosable as leeches with a proboscis that lack parental care. Americobdelliformes is a simple extension of the Americobdellidae that was necessitated by *Americobdella valdiviana* being recovered as sister to the Erpobdelliformes and Hirudiniformes. For our taxonomic list of ranks, we choose only to list the families subordinate to our newly established suborders and not previously established suborders (e.g., Hirudiniformes), as it is beyond the scope of this current work. For similar reasons, we refrain from commenting on classifications within Branchiobdellida; however, it is clear that further taxonomic investigation is needed within this order.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ympev.2018.05.001>.

#### References

- Ahn, D.-H., Min, G.-S., 2016. First report of the branchiobdellidan *Holtodrilus truncatus* (Annelida: Clitellata) found on the freshwater atyid shrimp *Neocaridina* sp. from Korea. *J. Species Res.* 5, 459–462.
- Apakupakul, K., Siddall, M.E., Bureson, E.M., 1999. Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Mol. Phylogenet. Evol.* 12, 350–359.
- Bielecki, A., Cichocka, J.M., Jelen, I., Świątek, P., Plachno, B.J., Pikula, D., 2014a. New data about the functional morphology of the chaetiferous leech-like annelids *Acanthobdella peledina* (Grube, 1851) and *Paracanthobdella livanowi* (Epshtein, 1966) (Clitellata, Acanthobdellida). *J. Morphol.* 275, 528–539.
- Bielecki, A., Świątek, P., Cichocka, J.M., Siddall, M.E., Urbisz, A.Z., Plachno, B.J., 2014b. Diversity of features of the female reproductive system and other morphological characters in leeches (Clitellata, Hirudinida) in phylogenetic conception. *Cladistics* 30, 540–554.
- Borda, E., Siddall, M.E., 2004. Arhynchobdellida (Annelida: Oligochaeta: Hirudinida): phylogenetic relationships and evolution. *Mol. Phylogenet. Evol.* 30, 213–225.
- Brinkhurst, R.O., 1999. Lumbriculids, branchiobdellidans and leeches: an overview of recent progress in phylogenetic research on clitellates. *Hydrobiologia* 406, 281–290.
- de Carle, D., Ocegüera-Figueroa, A., Tessler, M., Siddall, M.E., Kvist, S., 2017. Phylogenetic analysis of *Placobdella* (Hirudinea: Rhynchobdellida: Glossiphoniidae) with consideration of COI variation. *Mol. Phylogenet. Evol.* 114. <http://dx.doi.org/10.1016/j.ympev.2017.06.017>.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Erséus, C., Källersjö, M., 2004. 18S rDNA phylogeny of Clitellata (Annelida). *Zool. Scr.* 33, 187–196.
- Erséus, C., Rota, E., Matamoros, L., De Wit, P., 2010. Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Mol. Phylogenet. Evol.* 57, 849–858.
- Füredy, L., Summerer, M., Brandstätter, A., 2009. Phylogeny and species composition of five European species of *Branchiobdella* (Annelida: Clitellata: Branchiobdellida) reflect the biogeographic history of three endangered crayfish species. *J. Zool.* 279, 164–172.
- Gelder, S.R., Siddall, M.E., 2001. Phylogenetic assessment of the Branchiobdellidae (Annelida, Clitellata) using 18S rDNA, mitochondrial cytochrome c oxidase subunit I and morphological characters. *Zool. Scr.* 30, 215–222.
- Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- James, S.W., Davidson, S.K., 2012. Molecular phylogeny of earthworms (Annelida: Crassicitellata) based on 28S, 18S and 16S gene sequences. *Invertebr. Syst.* 26, 213–229.
- Kaygorodova, A., Sherbakov, D. Yu., 2006. Molecular phylogenetic study of the systematic position of Baikalian oligochaetes in Clitellata. *Russ. J. Genet.* 42, 1390–1397.
- Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* 34, 772–773.
- Livanow, N., 1931. Die Organisation der Hirudineen und die Beziehungen dieser Gruppe zu den Oligochaeten. *Ergeb. Fortschritte Zool* 7, 378–484.
- Marotta, R., Ferraguti, M., Erséus, C., Gustavsson, L.M., 2008. Combined-data phylogenetics and character evolution of Clitellata (Annelida) using 18S rDNA and morphology. *Zool. J. Linn. Soc.* 154, 1–26.
- Martin, P., 2001. On the origin of the Hirudinea and the demise of the Oligochaeta. *Proc. R. Soc. B: Biol. Sci.* 268, 1089–1098.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, 2010 Gateway Computing Environments Workshop (GCE). 10.1109/gce.2010.5676129.
- Odier, A., 1823. Mémoire sur le Branchiobdelle, nouveau genre d'annélides de la famille des hirudinéés. *Mémoires de la Société d'Histoire Naturelle de Paris* 1, 69–78.
- Purschke, G., Westheide, W., Rohde, D., Brinkhurst, R.O., 1993. Morphological re-investigation and phylogenetic relationship of *Acanthobdella peledina* (Annelida, Clitellata). *Zoomorphology* 113, 91–101.
- Rota, E., Martin, P., Erséus, C., 2001. Soil-dwelling polychaetes: enigmatic as ever? Some hints on their phylogenetic relationships as suggested by a maximum parsimony analysis of 18S rRNA gene sequences. *Contrib. Zool.* 70, 127–138.
- Rousset, V., Plaisance, L., Erséus, C., Siddall, M.E., Rouse, G.W., 2008. Evolution of habitat preference in Clitellata (Annelida). *Biol. J. Linn. Soc. Lond.* 95, 447–464.
- Sawyer, R.T., 1986. Leech Biology and Behaviour. Clarendon Press, Oxford, UK.
- Siddall, M.E., Apakupakul, K., Bureson, E.M., Coates, K.A., Erséus, C., Gelder, S.R., Källersjö, M., Trapido-Rosenthal, H., 2001. Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Mol. Phylogenet. Evol.* 21, 346–351.
- Siddall, M.E., Brugler, M.R., Kvist, S., 2015. Comparative transcriptomic analyses of three species of *Placobdella* (Rhynchobdellida: Glossiphoniidae) confirms a single origin of blood feeding in leeches. *J. Parasitol.* 102, 143–150.
- Siddall, M.E., Bureson, E.M., 1998. Phylogeny of leeches (Hirudinea) based on mitochondrial cytochrome c oxidase subunit I. *Mol. Phylogenet. Evol.* 9, 156–162.

- Skelton, J., Farrell, K.J., Creed, R.P., Williams, B.W., Ames, C., Helms, B.S., Stoekel, J., Brown, B.L., 2013. Servants, scoundrels, and hitchhikers: current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida). *Freshw. Sci.* 32, 1345–1357.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post analysis of large phylogenies. *Bioinformatics* 30.
- Tessler, M., Marancik, D., Champagne, D., Dove, A., Camus, A., Siddall, M.E., Kvist, S., 2018a. Marine leech anticoagulant diversity and evolution. *J. Parasitol.* <http://dx.doi.org/10.1645/17-64>.
- Tessler, M., Siddall, M.E., Ocegüera-Figueroa, A., 2018b. Leeches from Chiapas, Mexico, with a new species of *Erpobdella* (Hirudinida, Erpobdellidae). *Am. Mus. Novit.* 3895, 1–15.
- Trontelj, P., Sket, B., Steinbrück, G., 1999. Molecular phylogeny of leeches: Congruence of nuclear and mitochondrial rDNA data sets and the origin of bloodsucking. *J. Zoolog. Syst. Evol. Res.* 37, 141–147.
- Williams, B.W., Gelder, S.R., Proctor, H.C., Coltman, D.W., 2013. Molecular phylogeny of North American Branchiobdellida (Annelida: Clitellata). *Mol. Phylogenet. Evol.* 66, 30–42.