ABSTRACT

Acanthobdellida gnaw into the sides of salmonid fishes in frigid Arctic lakes and rivers, latching on with medieval facial hooks. Sister to leeches, they are an ancient lineage with two described species. Unfortunately, Acanthobdellida are rarely collected, leading to a paucity of literature despite their uniquely intermediate morphology between leeches and other clitellates. Populations range from Eurasia into Alaska (USA), but few specimens of *Acanthobdella peledina* are represented in molecular studies; no molecular data exist for *Paracanthobdella livanowi*, making its taxonomic position difficult to assess. We use phylogenetics and morphology to determine if allopatric populations of *A. peledina* are distinct species and assess the current classification scheme used for Acanthobdellida. We produce a new suborder — Acanthobdelliformes — to match Hirudinea taxonomy. Scanning electron micrographs indicate species-level differences in the anterior sucker and facial hooks: molecular phylogenetics mirrors this divergence between species. We assign both species to the single family Acanthobdellidae, and in so doing, abandon the monotypic family Paracanthobdellidae. Alaskan and European *A. peledina* populations are morphologically similar, but the former appear to be a distinctly divergent population. Our data strongly suggest the members of the order Acanthobdellida diverged relatively recently in their ancient history; however, based on genetic distance, this divergence appears to predate the most recent cycles of glaciation.

INTRODUCTION

Overview

Determining the evolutionary relationships between animals illuminates fundamental shifts in morphology, ecology, and behaviour. Accordingly, a foundational question in annelid systematics has been: What is the sister group to leeches? Mounting evidence points to Acanthobdellida Livanow, 1905 as the answer to this question (Tessler *et al.*, 2018a). These ectoparasites — primarily of salmonid fishes — are Arctic and sub-Arctic in distribution: found in remote boreal locations, and, accordingly, are rarely collected. Because of this, these ancient annelids are understudied, belying their importance in understanding the early evolution of leeches and their unique place on the tree of life, let alone their diverse suite of unique traits.

Acanthobdellida comprises two known species: *Acanthobdella peledina* Grube 1851, and *Paracanthobdella livanowi* (Epstein, 1966); see Figure 1 and Table 1. These each have their own monotypic family — Acanthobdellidae Livanow, 1905 and Paracanthobdellidae Epstein, 1987. The former species is by far the better studied of the two, appearing in a few molecular studies and a number of studies on the European populations in particular. However, it has a major disjunct population in Alaska, USA, which has not been studied since the first records in the 1970s (Holmquist, 1974; Hauck, Fallon, & Burger, 1979). The latter species, *P. livanowi*, has been in few studies and has yet to be incorporated into molecular phylogenetic analyses. Our paper uses new samples from a broad set of localities (Figure 2, Table 2) and seeks to better determine the evolutionary relationships between populations and species of Acanthobdellida, and learn about how molecularly and morphologically diverse the lineage really is.

Given the importance of these animals, and the fact that they form a unique order, we have decided to confer upon them the common name “hook-faced fish worms”. (The scanning electron micrograph in Figure 1 illustrates this charismatic feature.) They have been called “fish-worms” and “fish-lice” in Lappland (Dahm, 1962), but the former is not especially descriptive (e.g., various nematodes and trematodes also fit the description) and the latter is misleading (e.g., parasitic arthropods also bear this moniker). Accordingly, we have added specificity to one of the common names in referencing the hook-like chaetae on their anterior region.

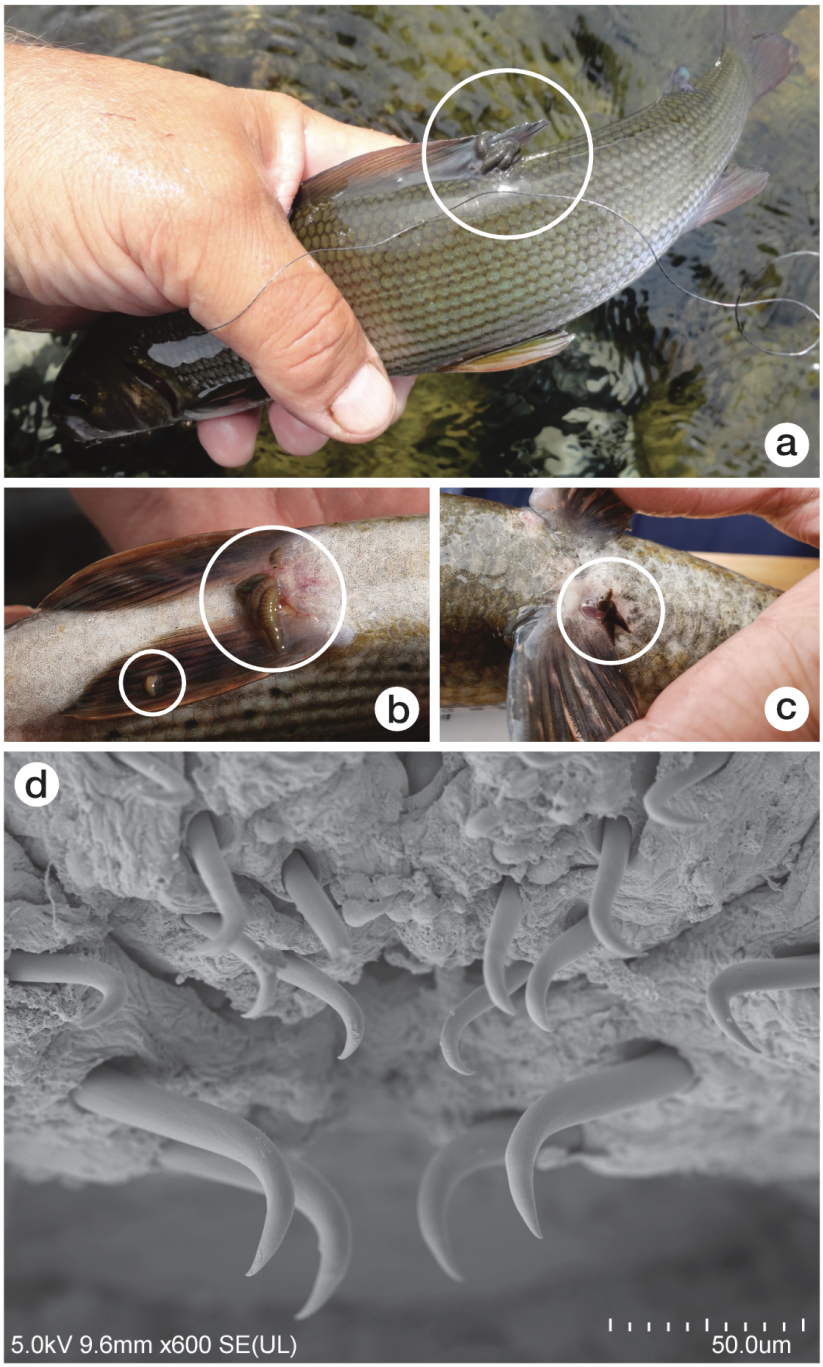

Figure 1. Photos of Acanthobdellida species: a-b) *Acanthobdella peledina* from two seperate grayling individuals [*Thymallus thymallus*] in Scandinavia; c) *Acanthobdella peledina* on Arctic grayling [*Thymallus arcticus*] from Alaska. Circles highlight *A. peledina* individuals on their hosts.The scanning electron micrograph of *P. livanowi* d) emphasizes the eponymous hooks we commemorate in the new common name: hook-faced fish worms.

Table 1. Comparison of Acanthobdellida species: *Acanthobdella peledina* and *Paracanthobdella livanowi*.

Category	***A. peledina***	***P. livanowi***
Anterior sucker [1] (Fig. 7)	Absent	Present (first five segments)
Chaetae (setae) [1, 2] (Fig. 5 & 8; Table 3)	One type: Similar for first five rows; angle of flexion (hooks) ~90°	Two types: Broader for fourth and fifth rows; angle of flexion ~150° (apart from chaetae on segment 3)
Crop and esophagus [2]	Not distinct from one another; crop undivided	Distinct from one another; crop divided
Somites [2]	31	31
Ovisac shape [2]	Twisted; 3-4.5 neurosomites long; extend posteriorly to ½ of testisac	Elongated with "horseshoe-shaped" ends; 7.5-9 neurosomites long; extend posteriorly to full length of testisac or farther
Testisacs [2]	Posterior end rounded; 7 neurosomites long	Posterior end curved; 8 neurosomites long
Distance between male and female gonopores [5] (Fig. 9)	3 complete annuli; however, male and female gonopores may be slightly anterior or posterior to their respective furrows	3 complete annuli; however, male and female gonopores may be slightly anterior or posterior to their respective furrows
Geography [3, 4] (Fig. 2)	Broadly across northern Eurasia and Alaska	Russian Far East
Free-living status [2, 5]	Only to reproduce (sometime between September and April)	Commonly observed in this state
Host records [2, 3, 4, 6 *]	Salmonidae[†]: *Brachymystax lenok*, *Coregonus autumnalis*, *Coregonus lavaretus*, *Coregonus muksun*, *Coregonus nasus*, *Coregonus peled*, *Coregonus pidschian*, *Coregonus sardinella*, *Coregonus tugun*, *Esox lucius*, *Hucho taimen*, *Prosopium cylindraceum*, *Salmo salar*, *Salmo trutta*, *Salvelinus alpinus*, *Salvelinus neiva*, *Stenodus leucichthys*, *Stenodus nelma*, and *Thymallus arcticus*, *Thymallus baicalensis*, *Thymallus pallasii*, *Thymallus thymallus* Lotidae: *Lota lota*	Salmonidae: *Oncorhynchus mykiss*, *Salvelinus leucomaenis*, *Salvelinus malma*[‡], and *Salvelinus taranetzi* Gasterosteidae: *Gasterosteus aculeatus*
Alternative prey species [2]	None known	Odonata and Chironomidae larvae
COI distance within species [7]	Average 0.42%; range 0% - 1.52% (please note this does not include Alaska samples)	0.11%
COI distance between species [7]	Average = 13.20%; range = 13.17% - 13.49%	

[1] (Sawyer, 1986), [2] (Bielecki *et al.*, 2014), [3] (Utevsky, Sokolov, & Shedko, 2013), [4] (Kaygorodova, Dzyuba, & Pronin, 2012), [5] (Epstein, 1987), [6] (Mitenev & Šul'man, 1999), [7] present study, and citations therein. * Multiple papers reference another paper that has a record of *A. peledina* being found on the marine fish *Scophthalmus maximus* (Scophthalmidae); however, the original source paper does not seem to reference this, and this seems highly unlikely as *A. peledina* is found in freshwater. [†]*A. peledina* has also been reported from *Salvelinus lepechni* which has been synonymized with *S. alpinus* (Makhrov et al., 2019). [‡] *P. livanowi* has been reported from *Salvelinus albus*, *Salvelinus kronocius*, and *Salvelinus schmidti*, all of which are now recognized as junior synonyms of *S. malma* per (Esin & Markevich, 2017), and *Salvelinus krogiusae* which has been synonymized with *S. taranetzi* by these authors.

Distribution and ecology

Of the two acanthobdellidans, *A. peledina* is clearly the more widespread species. We present a range map in Figure 2, which details the largely northern (often above the Arctic circle) and boreal span. Its distribution is most well-studied in Eurasia, where it is found in a variety of countries to the west and extends to Siberia and the Russian Far East (Kaygorodova *et al.*, 2012). In the 1970s, a notable range extension was documented as specimens were found in Alaska (Holmquist, 1974; Hauck *et al.*, 1979). It is unclear if the Alaska population is genetically distinct, or maybe a more recent transplant from Eurasia. There is at least one study that documents the distribution of *A. peledina* in Eastern Siberia around Lake Baikal, Russia, which is more moderate in climate than most other localities where this species is found (Kaygorodova & Dzyuba, 2018). In contrast, *P. livanowi* is restricted to the Russian Far East. Specifically, it is best documented in the Kamchatka Peninsula, but is also known from the Chukchi Peninsula and around Taui Bay (Utevsky *et al.*, 2013).

Acanthobdellida species parasitise freshwater fishes, most of which are from the family Salmonidae (Table 1). They are generally found around the base of fins (especially the dorsal fin); however, they are known to latch onto a variety of spots on a given fish's body. Ultimately, they feed on both fish blood and tissue (Kutschera & Epshtein, 2006; Bielecki *et al.*, 2014). Interestingly, a few specimens of *P. livanowi* have been found with insect larvae (Diptera and Odonata) in their stomach contents, suggesting they have a broader dietary range than just fish (Bielecki *et al.*, 2014). The fact that both acanthobdellidan species are — at least partially — sanguivorous has important implications for the evolution of blood-feeding within Hirudinea broadly. Since Acanthobdellida is sister to leeches (Hirudinida), and it has been posited that the ancestral leech was sanguivorous (Trontelj et al., 1999; Tessler et al., 2018a), it follows that the most recent common ancestor of leeches and acanthobdellidans may have been blood-feeding as well.

It is our experience in the field that *A. peledina* is highly variable in its abundance, which local fishermen have confirmed. This is further borne out by other empirical work where worms could be found in some seasons, but not others, and ranged in prevalence from exceedingly low numbers to over ⅔ of grayling examined (Kaygorodova *et al.*, 2012).

Both species are sometimes collected apart from their hosts. This type of free-living habit appears to be restricted to breeding and for juveniles in *A. peledina* (Andersson, 1988; Kaygorodova *et al.*, 2012).*P. livanowi* is more frequently found free-living (Utevsky *et al.*, 2013), which may have to do with its ability to feed on insect larvae as well as fish.

Figure 2. Map of Acanthobdellida collecting records across their known distribution. Circles: records for *Acanthobdella peledina*; triangles: records for *Paracanthobdella livanowi*. Red triangle and circles represent localities from the present study with numbers corresponding to those in Table 2. Dark grey triangles and circles represent previously-published records (from Kaygorodova, Dzyuba, & Pronin, 2012; Utevsky, Sokolov, & Shedko, 2013; Kaygorodova & Dzyuba, 2018 and references therein).

Prior phylogenetics research

The exact placement of *A. peledina* in the annelid tree was particularly controversial for over a century (Brinkhurst & Gelder, 1989; Purschke *et al.*, 1993). This controversy continued for roughly 20 years after the advent of molecular phylogenetic analysis, as DNA sequences attributed to this taxon (AY040701, AF115978, and AF003264) in a number of papers (Siddall & Burreson, 1998; Apakupakul, Siddall, & Burreson, 1999; Gelder & Siddall, 2001; Siddall *et al.*, 2001) were in fact sequences of contaminants; see Table 1 in our prior work (Tessler *et al.*, 2018a). We discovered and reconciled this error in a study that produced a large multilocus phylogeny using a broad suite of taxa (Tessler *et al.*, 2018a). Our results found that *A. peledina* seems to be sister to leeches, which reflects the early work on this taxon that was either done with morphology or did not have molecular contamination issues (Purschke *et al.*, 1993; Brinkhurst, 1999; Martin, 2001; Rota, Martin, & Erséus, 2001; Kaygorodova & Sherbakov, 2006; Marotta *et al.*, 2008; Świątek *et al.*, 2012). The placement of *A. peledina* as sister to leeches further supports a single origin of vertebrate parasitism for clitellates (Tessler *et al.*, 2018b). A number of morphological synapomorphies also support the phylogeny and link leeches with *A. peledina* (Purschke *et al.*, 1993). Unfortunately, prior to the present study, *P. livanowi* had not been examined in a molecular or phylogenetic context.

Most prior molecular phylogenetic work on *A. peledina* has focused on COI and 18S sequence data. However, more recent studies, such as our prior study, have incorporated other loci, such as 12S, 16S, and 28S (Tessler *et al.*, 2018a; Bolbat *et al.*, 2019). In addition to the Sanger data, a mitochondrial genome (Bolbat *et al.*, 2020), ultraconserved element (UCE) data (Phillips *et al.*, 2019a,b), and a transcriptome (Iwama *et al.*, 2020) have recently been sequenced.

Our findings (Tessler *et al.*, 2018a) have been confirmed by a corrected version of the study using the next generation UCE dataset on a reduced taxon set (Phillips *et al.*, 2019a,b). The mitogenome was incorporated into a phylogenetic tree, but it is unclear on the exact data sources used and it did not include any branchiobdellidans; still, it had *A. peledina* as sister to leeches (Bolbat *et al.*, 2020).

Not every study has found the same relationship of *A. peledina* as sister to leeches. However, the other studies have tended to use smaller datasets, have different foci, or are poorly supported or unresolved (Erseus & Kallersjo, 2004; Rousset *et al.*, 2008; James & Davidson, 2012; Bolbat *et al.*, 2019); see Table 1 of our prior work for more details (Tessler *et al.*, 2018a).

Only a few specimens of *Acanthobdella peledina* from a limited number of localities have been sampled for molecular studies. Although the documented range of *Acanthobdella* extends across Northern Eurasia, and into western North America, the vast majority of prior work has been conducted on specimens from Nordic countries. Fewer specimens, and no molecular data, are available for *Paracathobdella livanowi*, making its oft-debated taxonomic position difficult to assess.

Prior morphological research

The fundamental early works on *A. peledina* were written near the beginning of the 20th Century (Kowalewsky, 1896; Livanow, 1906, 1931). Members of the order Acanthobdellida have been referred to as a "missing link" between leeches and other clitellates. Certain plesiomorphic features of Acanthobdellida hint at a relationship with non-hirudinid clitellates, such as the presence of chaetae (also referred to as setae or bristles) and an oligochaete-like male reproductive system (Purschke *et al.*, 1993). Somewhat similarly they have less developed suckers (Bielecki *et al.*, 2014). At the same time, they exhibit features that link them to leeches, such as subdivided segments, eyes, reduced or absent internal segmentation, certain digestive enzymes, no visible clitellum, and several reproductive and nervous system characters (Sawyer, 1986; Brinkhurst & Gelder, 1989; Purschke *et al.*, 1993; Westheide, 1997; Cichocka *et al.*, 2021).

The acanthobdellidan feeding apparatus has been called a rudimentary proboscis, linking them to leeches (Bielecki *et al.*, 2014). However, we would also like to note that their feeding apparatus is similar to the eversible pharynx found in other non-hirudinid clitellates, and the leech proboscis is likely a modified pharynx itself (Brinkhurst, 1982, 1999). This has led others to suggest that probosces are plesiomorphic for leeches, rather than a synapomorphy for the defunct order Rhynchobdellida (Trontelj, Sket, & Steinbrück, 1999; Tessler *et al.*, 2018a).

A number of important morphological features have been compared between *A. peledina* and *P. livanowi*. We have highlighted some of the more notable differences in Table 1, many of which are described in further detail by Bielecki *et al.* (2014). Of the two studies to include specimens of *A. peledina* from Alaska, only one went into detail about the morphology of the specimen found and it appeared to be a juvenile (Holmquist, 1974).

Current research goals

In this study, we build a framework from molecular phylogeny and scanning electron-based morphology to fill gaps in the knowledge of hook-faced fish worms. Our specific goals were to: 1) place *P. livanowi* in a phylogenetic tree to determine its relationship to *A.*

peledina, and determine how recently the two acanthobdellidan species diverged; 2) determine if *A. peledina* in general is truly one extremely widespread species or if it is made up of multiple cryptic species, especially focusing on the allopatric populations of *A. peledina* in Alaska, and 3) assess whether or not *P. livanowi* is most accurately placed within its monotypic genus and family.

MATERIAL AND METHODS

Specimen acquisition

The specimens used in this study were from a variety of countries and localities and are listed in Table 2. Acanthobdellidans preserved in ethanol were used for molecular analysis. Other specimens were fixed in 96% ethanol or in 2.5% glutaraldehyde in a 0.1M phosphate buffer (pH 7.4) for examination using a stereo (dissection) microscope and compound microscope, and scanning electron microscopy (SEM) analysis. Alaskan specimens are housed in the Invertebrate Zoology collection at the American Museum of Natural History, European specimens are housed at the Institute of Biology at the University of Silesia in Katowice, and Asian specimens are stored at .

Table 2. Localities and GenBank accessions for Acanthobdellida individuals used in this study.

ID	Country	Locality	COI (mtDNA)	12S (mtDNA)	16S (mtDNA)	18S (nuclear)	28S (nuclear)
Acanthobdella peledina							
AN1	Norway	1) Lille Rostavatn Lake 68°59'27.60"N, 019°38'25.08"E	XXXX	XXXX	–	XXXX	–
AN2	Norway	1) Lille Rostavatn Lake 68°59'27.60"N, 019°38'25.08"E	XXXX	XXXX	–	XXXX	–
AN3	Norway	1) Lille Rostavatn Lake 68°59'27.60"N, 019°38'25.08"E	XXXX	XXXX	–	XXXX	XXXX
AN4 ‡	Norway	2) Moskanjavri Lake 68°55'18.33"N, 020°11'56.65"E	XXXX	XXXX	–	XXXX	XXXX
AN5 ‡	Norway	2) Moskanjavri Lake 68°55'18.33"N, 020°11'56.65"E	XXXX	XXXX	–	XXXX	XXXX
AN6 †	Norway	2) Moskanjavri Lake 68°55'18.33"N, 020°11'56.65"E	XXXX	XXXX	–	XXXX	XXXX
as1	Sweden	3) Skuppe, Pite River 66°260'59.2"N 18°20'23.2"E	MH351651	–	MH351635	MH351628	MH351642
as2	Sweden	3) Skuppe, Pite River 66°260'59.2"N 18°20'23.2"E	MH351652	–	MH351636	MH351629	MH351643
Mitogenome	Sweden	3) Skuppe, Pite River 66°260'59.2"N 18°20'23.2"E	MT741802			–	–

AP1 §	Sweden	4) Pite River, 65°21’52’’N, 21°19’22’’E	XXXX	XXXX	–	XXXX	–
AP2	Sweden	4) Pite River, 65°21’52’’N, 21°19’22’’E	XXXX	XXXX	–	XXXX	XXXX
AP3	Sweden	4) Pite River, 65°21’52’’N, 21°19’22’’E	XXXX	XXXX	–	XXXX	XXXX
AP4 §	Sweden	4) Pite River, 65°21’52’’N, 21°19’22’’E	XXXX	XXXX	–	XXXX	–
AP5 §	Sweden	4) Pite River, 65°21’52’’N, 21°19’22’’E	XXXX	XXXX	–	XXXX	–
AL1 *	Sweden	5) Lainio River 68°17'02.5224"N, 21°26'19.7412"E	XXXX	XXXX	–	XXXX	XXXX
AL3 *	Sweden	5) Lainio River 68°17'02.5224"N, 21°26'19.7412"E	XXXX	XXXX	–	XXXX	XXXX
AL4 †	Sweden	5) Lainio River 68°17'02.5224"N, 21°26'19.7412"E	XXXX	XXXX	–	XXXX	XXXX
AL5 *	Sweden	5) Lainio River 68°17'02.5224"N, 21°26'19.7412"E	XXXX	XXXX	–	XXXX	XXXX
F1	Finland	6) Lapland 68°23’6.72’’N, 24°9’10.08’’E	XXXX	XXXX	–	XXXX	XXXX
F2 ¶	Finland	6) Lapland 68°23’6.72’’N, 24°9’10.08’’E	XXXX	XXXX	–	XXXX	XXXX
F3 §	Finland	6) Lapland 68°23’6.72’’N, 24°9’10.08’’E	XXXX	XXXX	–	XXXX	–
F4 ¶	Finland	6) Lapland 68°23’6.72’’N, 24°9’10.08’’E	XXXX	XXXX	–	XXXX	XXXX
F5	Finland	6) Lapland 68°23’6.72’’N, 24°9’10.08’’E	XXXX	XXXX	–	XXXX	XXXX
IMO33	USA	7) Alaska, I-Minus Lake Outlet 68°33'24.00"N, 149°34'29.27"W	–	–	XXXX	–	XXXX
IMO9	USA	7) Alaska, I-Minus Lake Outlet 68°33'24.00"N, 149°34'29.27"W	–	XXXX	–	–	XXXX
AlaskaB	USA	8) Alaska, Chandler Lake 68°14'57.94"N 152°42'40.30"W	–	–	XXXX	XXXX	XXXX
Paracanthobdella livanowi							
64	Russia	9) Kamchatka, Lake Kronotskoye 54°43'1.20"N, 160°21' 36.00"E	XXXX	XXXX	–	–	XXXX
PL11	Russia	9) Kamchatka, Lake Kronotskoye 54°43'1.20"N, 160°21' 36.00"E	XXXX	XXXX	–	XXXX	XXXX
Only one was used for phylogenetic analyses When specimens had identical sequences for all loci, only one specimen was used for phylogenetic analyses. These are demarcated as follows: *, †, ‡, §, ¶.							

DNA amplification and sequencing

DNA from Alaskan specimens was amplified following our prior work (Tessler *et al.*, 2018a) for 28S, 18S, 16S, and COI; 12S amplification followed protocols from our prior work (de Carle *et al.*, 2017). Throughout the course of our research on these animals, we have noticed that acanthobdellidan DNA is rather difficult to amplify and sequence. These specimens were no exception: amplification of COI was attempted for the Alaskan specimens using a primer set that amplifies a larger stretch of COI (Tessler, Siddall, & Oceguera-Figueroa, 2018c) — and was used successfully in (Tessler *et al.*, 2018a) for a Swedish *A. peledina* — as well as LCO and HCO (Folmer *et al.*, 1994), but none of the resulting PCR products produced usable sequences for the Alaska samples. The authors also endeavored to amplify additional nuclear loci without success. In spite of several concerted efforts, we have never been able to amplify ITS and repeated attempts to sequence Histone H3 yielded only host DNA.

For the European samples, amplification of 12S followed the same methods as above. In order to generate sequences for COI, 28S, and 18S from Swedish, Finnish, and Norwegian specimens of *Acanthobdella peledina* — particularly those contaminated by material from fishes, *Salmo trutta* and *Thymallus thymallus* — a series of acanthobdellidan–specific primers was designed. In a few cases, these primers were used in combination with primers from other studies (see Table S1 and citations therein).

Amplification of 18S and COI from a single specimen of *Paracanthobdella livanowi* was achieved using the same methods as for the European *A. peledina* specimens. Additional primers (ACA873Rev, ACA940Rev, and COI–E [Bely & Wray, 2004]) have also proven suitable for COI amplification in this species; in contrast, primers used to amplify COI for hirudinids in other studies (Williams *et al.*, 2013; Tessler *et al.*, 2018c) did not produce any bands for *P. livanowi*. To amplify 28S rDNA from P. livanowi contaminated by fish material, we designed another set of acanthobdellidan-specific primers: 28SFrw390 and 28SRev1217 (Table S1).

Phylogenetic analyses

The phylogenetic matrix includes 74 terminals (of which, 20 are acanthobdellidans; see Table 2), each has sequences for ≥ 3 of the 5 loci. Outgroup sequences include taxa from the remaining hirudinean orders — Branchiobdellida (n = 24) and Hirudinida (n = 28) — and two lumbriculid species (*Eremidrilus coyote* and *Lumbriculus variegatus*). Following the results of previous studies (e.g. Erséus and Källersjö 2004, Tessler *et al.*, 2018a), the tree was rooted on the branch leading to Lumbriculidae. A complete list of sequences used for phylogenetic

analysis is available in Table S2. We did not include three sequences that have been used in prior studies but are actually from contaminants (AY040701, AF115978, and AF003264). Three other sequences (AY040680, AF099948, and AF099953) were also excluded, as they had peculiarities that indicated possible quality issues and did not meet the minimum matrix occupancy requirement. Specimens that had identical sequences for all loci (COI, 12S, 16S, 18S, and 28S) were treated as a single tip for the purposes of phylogenetic analyses (see Table 2).

Sequences were aligned with MAFFT ver. 7.453 (Katoh & Standley, 2013), using automatic choice of search strategy for COI, 12S, 16S, and 18S. To account for long gaps caused by the sequencing of varying regions across different studies, the E-INS-i strategy was used for 28S. Uncorrected pairwise distance within and between acanthobdellidan species was calculated using MEGAX (Kumar *et al.*, 2018). Phylogenetic analyses were performed on three datasets: one with only mitochondrial loci (COI, 12S, and 16S); one with nuclear loci (18S and 28S); and one with all five loci concatenated. Model testing, maximum likelihood tree inference, and bootstrapping (1,000 pseudoreplicates) were performed using IQ-TREE 2 (Minh *et al.*, 2020). IQ-TREE was called as follows: “iqtree2 -s <matrix> -spp <partitions> -m TESTMERGE -mset mrbayes -ninit 10000 -bb 10000 -wbtl”. The data matrix and the resulting tree files can be found as Supplementary Files.

Morphological methods

To compare the two species we focused on external morphology, especially on the anterior body part bearing chaetae, the clitellar region with gonopores and, to a lesser extent, on the posterior sucker. Additionally, the chaetal dimensions (length, breadth, and flexion angle) were measured. All comparisons were made between specimens of similar size. For scanning electron microscopy (SEM) and stereo microscope analysis, specimens were divided into three size categories: small specimens (3-5 mm long with a maximum width of 0.8 mm), medium-sized (6 to 10 mm with a maximum width of 2 mm), and larger specimens (11 to 13 mm long wiht a maximum width of 3 mm). Additionally, in the case of *A. peledina* we analyzed two very large specimens — 25 mm long with a maximum width of 9 mm — using a stereo microscope. These specimens were collected during winter: they were found in a fishing net, but were not attached to any host. Most probably, they were free-living at the time of collection.

For analyses of the chaetae, fully grown specimens were chosen (Table 3). The anterior body fragments were incubated with 0.1% trypsin solution to digest the body tissues and release the chaetae. The isolated chaetae were mounted onto microscope slides, covered with

coverslips, and incubated for about 24 hours at the temperature of 30-40°C. Then, chaetae were analyzed under a Olympus U-DA 1M17005 microscope using Cell^B software. Measurements of the chaetae were made under a magnification of 10 x 0.25. The pictures of chaetae were taken under magnification of 4 x 0.10 and 10 x 0.25. Chaetae from all five segments were measured, with five measurements recorded for each chaeta: chaetal length, chaetal breadth at each of three points — a) in the place where the chaeta is bent (flexion point); b) at the midpoint of the chaeta (the distal part which extends outside the body); and c) at the proximal part (the part of chaeta hidden within body) — and the angle of chaeta flexion. For the latter measurement, two artificial lines were created to measure the angle of chaeta flexion: one along the middle of the chaeta and the second along the chaeta tip. Examples of each measurement are displayed in Figure 5a-c. A total of 640 chaetae were measured: 2 chaetae from each segment (segments 1-5) in 32 individuals of *A. peledina* and 32 individuals of *P. livanowi* (Table. 3).

For stereo microscope analysis, fixed specimens were washed in PBS buffer and placed on Petri dishes. An Olympus ZX81 camera and Leica M205C stereo microscope were used. For SEM analysis, both ethanol and glutaraldehyde fixed specimens were washed in PBS buffer, then postfixed in 1% OsO_4 in a 0.1M phosphate buffer (pH 7.4) for two hours. After osmium postfixation, samples were dehydrated in a series of ethanol washes from 30% to 99.9%. Then, samples were dried in a Leica CPD 300 critical point dryer (Leica Microsystems, Vienna, Austria) and mounted on aluminium stubs with double-sided adhesive carbon tape and sputter coated with gold in a Pelco SC-6 sputter coater (Ted Pella Inc., Redding, CA, USA) to obtain a layer approximately 25 nm thick. Specimens were analyzed with a Hitachi SU8010 field emission scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) at 5.0 or 10 kV accelerating voltage with a secondary electron detector.

RESULTS

Molecular results

All three phylogenetic analyses (mitochondrial loci, nuclear loci, and all loci concatenated) agree that Acanthobdellida is monophyletic and sister to leeches (Figure 3a). *Paracanthobdella livanowi* is sister to *A. peledina* (Figure 3). These species are genetically distinct, with a COI distance of over 13% (Table 1). However, they are on a very long branch that diverged from leeches.

Within *A. peledina*, the Alaska specimens appear as sister to the other populations in all three trees (Figure 3b; Supplementary Files). Unfortunately, COI was not available for the Alaskan specimens, so a consistent genetic distance does not seem worth calculating. Furthermore, there is a notable amount of missing data for different samples, and a different region of 28S was sampled for the Alaskan and European specimens. Still, a number of synapomorphies exist for the Alaska specimens. 12S, 16S, and 28S all appear to have single nucleotide differences for the Alaska population; however 12S only has one sequence of the proper length to determine this. ND1, which was not used in our phylogenetic matrix, was compared between one Alaska specimen and ND1 from the only mitogenome of *A. peledina*; this gene appears to have multiple differences between individuals, but cannot be generalized without further sampling.

Figure 3. Maximum likelihood phylogeny of Acanthobdellida (starred node). Majority rule consensus of 1,000 bootstraps (lnL = -70595.750). The entire phylogeny is present in subplot a), which shows Acanthobdellida as sister to Hirudinida (leeches). Taxa with multiple representative taxa are collapsed to triangles, with the length of the triangle corresponding to the maximum branch length from the base of the clade to the tips. In subplot b), the starred node from subplot a is expanded to show all Acanthobdellida individuals. The Alaska and Nordic *A. peledina* populations form distinct clades. Inset images show c) photographs of overall morphology of medium-sized specimens; d) scanning electron micrographs of the fifth row of chaetae; and e) silhouettes of chaetae from the first chaetal row for *Acanthobdella peledina* (A) and *Paracanthobdella livanowi* (P).

Within the Nordic *A. peledina* samples, genetic variability was limited. Many samples had near-identical genetic information (see Table 2). The furthest genetic divergence between samples was about 1.5% for COI, while most were less divergent or identical.

General morphology, A. peledina *versus* P. livanowi

The preserved specimens of both species are white or yellowish (Figure 4), except for the large *A. peledina* specimens filled with blood which are much darker (Figure 4c). The natural coloration pattern and eye pigmentation are not preserved [other work has discussed this color change (Bielecki *et al.*, 2014)], except for the chaetae which are brownish along their length, darkening to black at the distal end (Figure 4). We did not find prominent differences in external morphology between populations of *A. peledina* collected in different Nordic localities (see Table 2) and in Alaska. Accordingly, the following descriptions of *A. peledina* refer to all analysed *A. peledina* specimens in aggregate.

The overall morphology of small specimens representing both species is very similar, except for small differences in the shape of the anterior part of the body (see below, Figure 4). The body is narrow and elongated, worm-like (Figure 4). In both species, the anterior body bears the mouth opening and five rows of chaetae (Figures 4, 6 & 7), whereas the posterior end forms an inconspicuous sucker (Figures 4 & 6). The number and distribution of chaetae are the same in both species, i.e., five rows of chaetae in five subsequent segments (Figures 4f, 7 & 8). There are four separated pairs of chaetae in each row; thus, eight chaetae per segment (Figures 4f, 7 & 8). In total, 40 chaetae are present. The anterior body region differs in shape: in *A. peledina*, it is cone-shaped, whereas it is cup-shaped in *P. livanowi* (Figures 6 & 7). The mouth opening is narrow, cleft-like, and surrounded by the 1st row of chaetae (Figures 7 & 8). In close vicinity to the mouth, some receptors — preliminarily identified as chemoreceptors — occur (Figure 8a, b). Between the 3rd and 5th rows of chaetae, an inconspicuous deepening can be observed, which is better developed in *P. livanowi* (Figures 7 & 8). Gonopores in small specimens are hardly visible (Figure 9a). They form narrow clefts, with the male pore laying in the furrow between segments XI and XII, and the female pore located three complete annuli below on the last annulus of segment XII. Below the female pore, the entrance to the spermatheca (area copulatrix) occurs in the furrow between segments XII and XIII (Figure 9a). The unique patterns of genital openings are specific to each species. In both species, the posterior end bears the sucker in the form of a rounded depression (Figure 6).

Figure 4. General morphology of *Acanthobdella peledina* (a-c, e) and *Paracanthobdella livanowi* (d, f-g) visualized by stereo microscope. *A. peledina*: a) medium-sized specimen [7 mm; Sweden], b) large specimen [12 mm; Norway], c) very large specimen [25 mm; Finland], and e) anterior body region of specimen figured in b [note higher magnification]. *P. livanowi*: d) small specimen [5mm; Kamchatka], f) anterior body region of specimen figured in d [note higher magnification], g) anterior body region of a large specimen [12 mm; Kamchatka]. Arrow: anterior sucker; double arrows: posterior sucker; d: deepening between pairs of chaetae; Arabic numerals mark rows of chaetae.

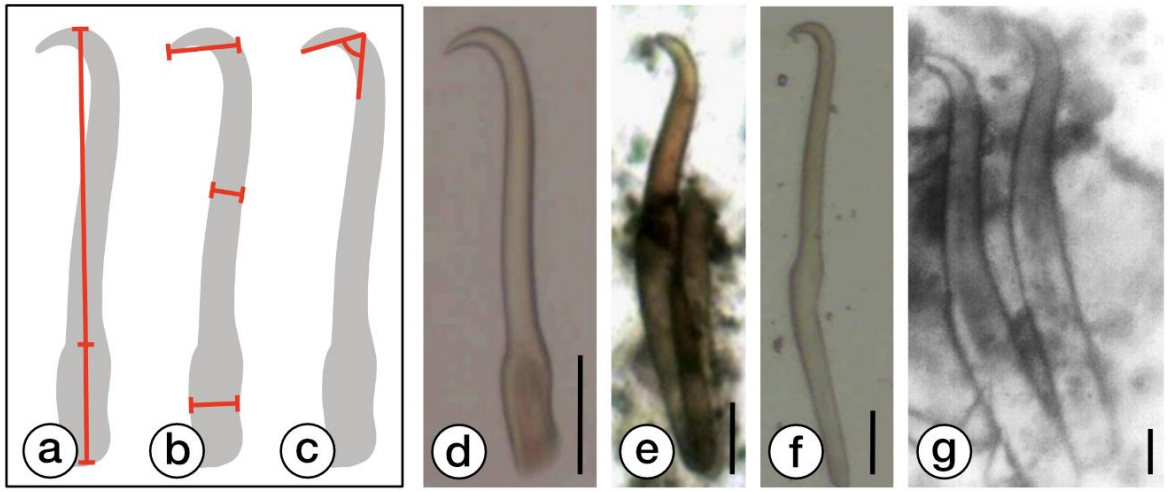

Figure 5. The chaetae of Acanthobdellida. Silhouettes show exemplary measurements of a) chaetal length; b) chaetal breadth (from top to bottom) at the point of flexion, midsection (distal), and midsection (proximal); and c) flexion angle. Photographs, taken using a compound microscope, show d) the chaetae of *Acanthobdella peledina* in the first segment; e) the chaetae of *Paracanthobdella livanowi* in the first segment; f) chaetae of *A. peledina* in the fifth segment; g) chaetae of *P. livanowi* in the fifth segment. Scale bar = 35 µm.

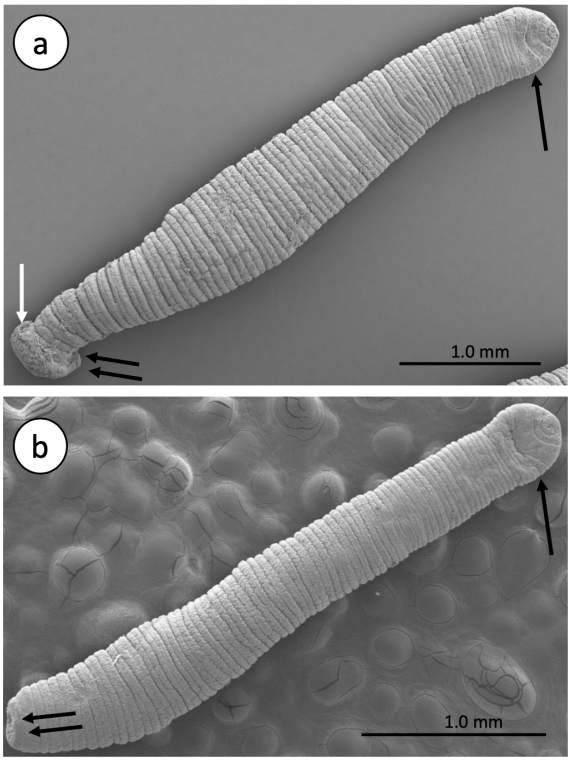

Figure 6. Scanning electron micrographs of general morphology in small specimens of a) *Acanthobdella peledina* [4mm; Alaska] and b) *Paracanthobdella livanowi* [3.5mm; Kamchatka]. Single black arrows: anterior body region; double black arrows: posterior sucker. In the *A. peledina* specimen, some fragments of host tissue (white arrow) are still attached to the sucker.

In medium-sized and larger specimens of both species, the difference in the form of the anterior part of the body is clear. In *A. peledina*, this region is still cone-shaped; however, the deepening between rows of chaetae becomes more pronounced (Figures 4a,b, e; 7c; 8c). In *P. livanowi*, the anterior body end is cup-shaped with a conspicuous deepening between the chaetae (Figures 4g, ; 7d; 8d). In *A. peledina* there is no clear demarcation between the chaetiferous segments and the rest of the body, whereas in *P. livanowi* this region forms the anterior sucker and is clearly separated from the rest of body by a constriction (Figures 4a, b, g; 7c, d). In larger specimens of *P. livanowi* — in contrast to *A. peledina* — the segment limits in the vicinity of the anterior sucker are hardly visible (Figures 4g; 7d). The posterior sucker in larger specimens of both species is still inconspicuous: it is narrower than the rest of the body and cone-like (Figures 4; 9 c,d). The sucker itself is in the form of shallow, crater-like depression (Figure 9 c,d).

In larger specimens of both species, the distribution of chaetae is the same as in small specimens (Figure 8). However, the external portions of the chaetae in *P. livanowi* are longer

and hook-like (Figure 8 c-f). It should be mentioned that, in a few specimens, some chaetae were damaged or completely absent (Figure 8 c); most probably they were damaged during material collection. In both species, there is a significant correlation between body size and the chaetal length for each segment (in *A. peledina* r = 0.83-0.97, $p < 0.001$; in *P. livanowi* r = 0.96-0.99, $p < 0.001$).

All chaetae (n = 20) were measured for 32 individuals of *A. peledina* and *P. livanowi*, for a total of 640 chaetae per species: the detailed results of this analysis are summarized in Table 3. The average length of chaetae increases from segment 1 to 5 in both species (Table 3). In both species, chaetae located in the first three segments (1-3) are shorter than those from segments 4 and 5; additionally, the breadth of chaetae is maximal in the middle, whereas both ends are thinner (Table 3). The recorded differences between species constituted chaetal breadth and flexion angle (Figure 5). In *A. peledina,* chaetae from all five segments are of similar breadth, whereas in *P. livanowi,* chaetae from segments 1-3 are distinctly thinner, with the chaetae in segments 4 and 5 being almost twice as broad (Table 3). In *A. peledina,* chaetae are flexed at a right angle, while in *P. livanowi* the angle is usually obtuse and varies from 97° to 160° (Figure 5; Table 3).

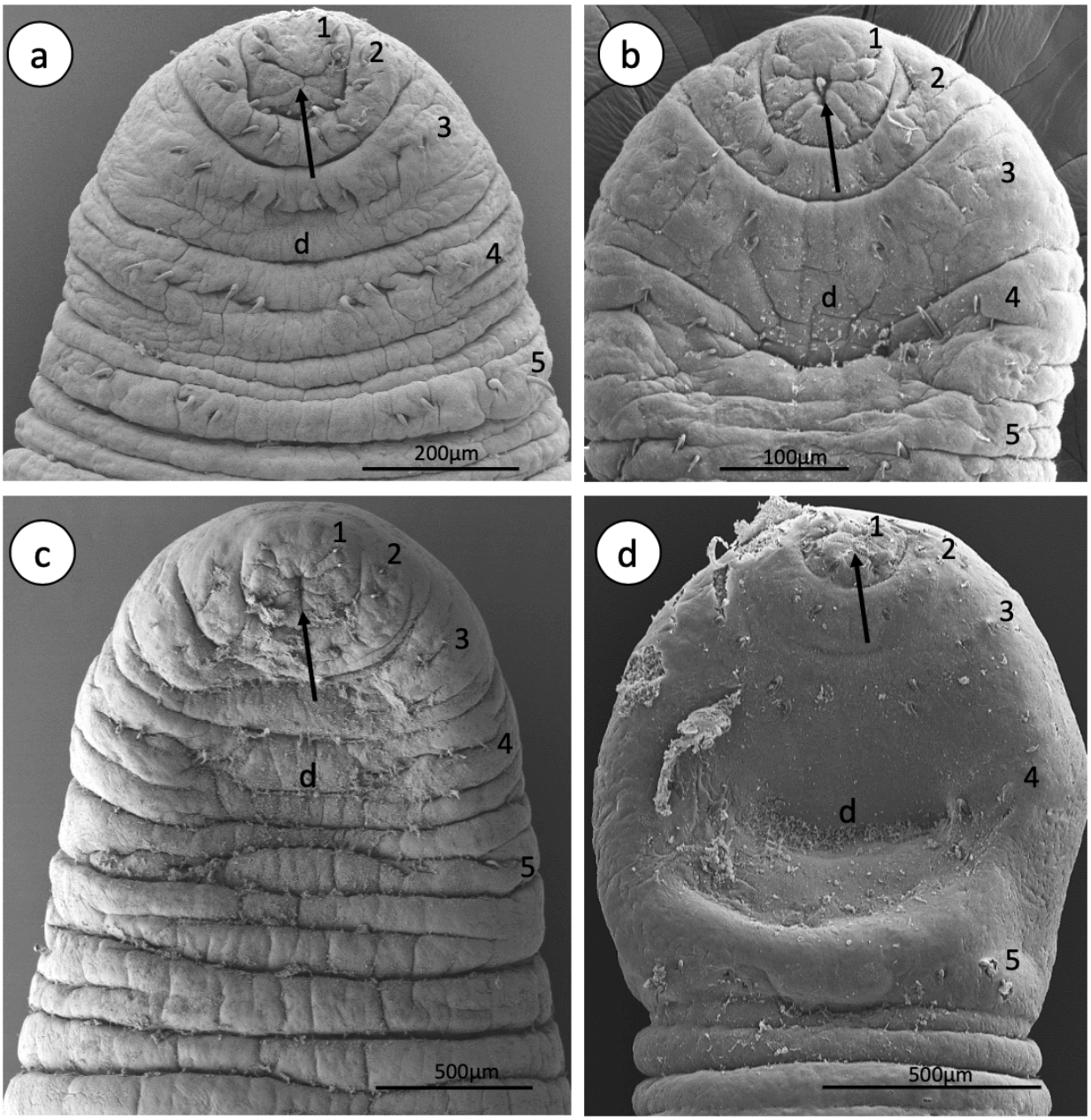

Figure 7. Scanning electron micrographs of the anterior body region. Small specimens of a) *Acanthobdella peledina* [5 mm; Sweden] and b) *Paracanthobdella livanowi* [3.5 mm]; large specimens of c) *A. peledina* [12 mm; Norway], and d) *P. livanowi* [11 mm; Kamchatka]. In large *P. livanowi* specimen, body segmentation and chaetae in anterior sucker are barely visible. Arrow: mouth opening; Arabic numerals 1-5 indicate rows of chaetae; d: deepening between pairs of chaetae.

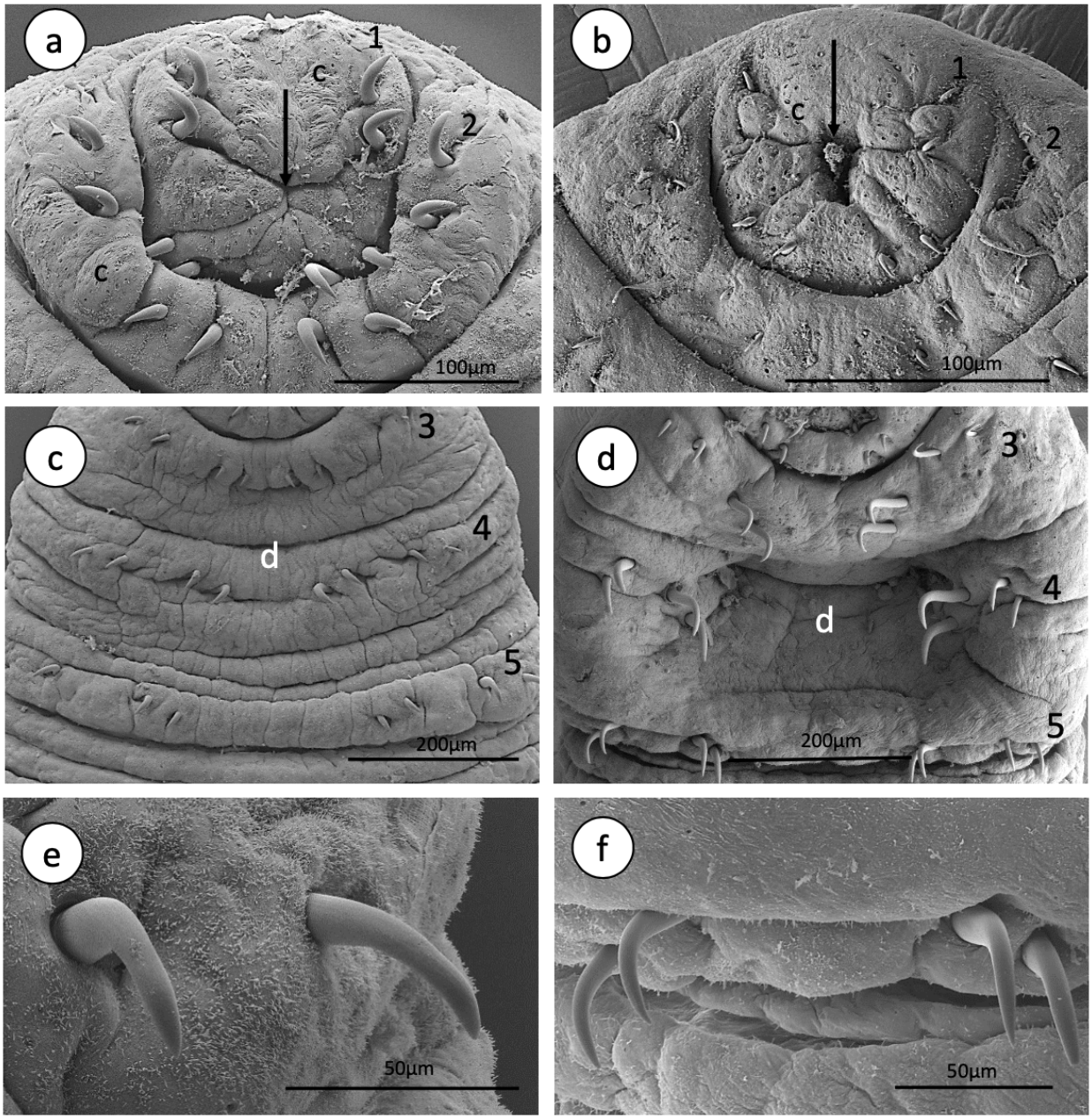

Figure 8. Scanning electron micrographs showing details of the anterior body region. First and second rows of chaetae in a) small *Acanthobdella peledina* [5 mm; Sweden] and b) small *Paracanthobdella livanowi* [3.5 mm; Kamchatka]. Third, fourth, and fifth rows of chaetae in: c) medium-sized *A. peledina* [7 mm; Sweden] and d) *P. livanowi* [6 mm; Kamchatka] Chaetae from the fifth row of e) medium-sized *A. peledina* [6 mm; Sweden] and f) medium-sized *P. livanowi* [6 mm; Kamchatka]. Arrow: mouth opening; Arabic numerals 1-5 indicate rows of chetae; c: putative chemoreceptors; d: deepening between pairs of chaetae.

In larger specimens, the unpaired gonopores are clearly visible (Figure 9b). In *A. peledina*, the male gonopore is located ⅔ of the way down the length of the fourth annulus of segment XI, and the female gonopore is located three complete annuli below, ⅓ of the way down the length of the last annulus of segment XII (not shown). The spermathecal opening is located on the first annulus of segment XIII, close to the furrow separating it from the previous segment (not shown). In *P. livanowi*, the male gonopore is located ⅔ of the way down the length of the fourth annulus of segment XI; the female gonopore is located three complete annuli below, in the middle of the fourth annulus of segment XII (Figure 9b). The opening of the spermatheca is located on the next annulus below the female gonopore, which is the first annulus of segment XIII (Figure 9b).

Table 3. Morphometricsof chaetae in *Acanthobdella peledina* and *Paracanthobdella livanowi.* Values are averaged across all specimens measured. See Figure 5a-c for measurement specifications.

Segment number	Specimen length	Chaetal length	Breadth at flexion point	Breadth at distal midsection	Breadth at proximal midsection	Flexion angle
A. peledina - 32 specimens examined						
1	11 – 15 mm	128 µm	6 µm	12 µm	8 µm	90 °
2		146 µm	6 µm	12 µm	8 µm	85 °
3		160 µm	6 µm	12 µm	7 µm	90 °
4		209 µm	6 µm	11 µm	8 µm	90 °
5		262 µm	6 µm	13 µm	8 µm	90 °
P. livanowi - 32 specimens examined						
1	15 – 23 mm	227 µm	8 µm	15 µm	9 µm	160 °
2		254 µm	8 µm	17 µm	9 µm	150 °
3		276 µm	8 µm	18 µm	9 µm	97 °
4		310 µm	14 µm	27 µm	17 µm	140 °
5		380 µm	15 µm	25 µm	15 µm	138 °

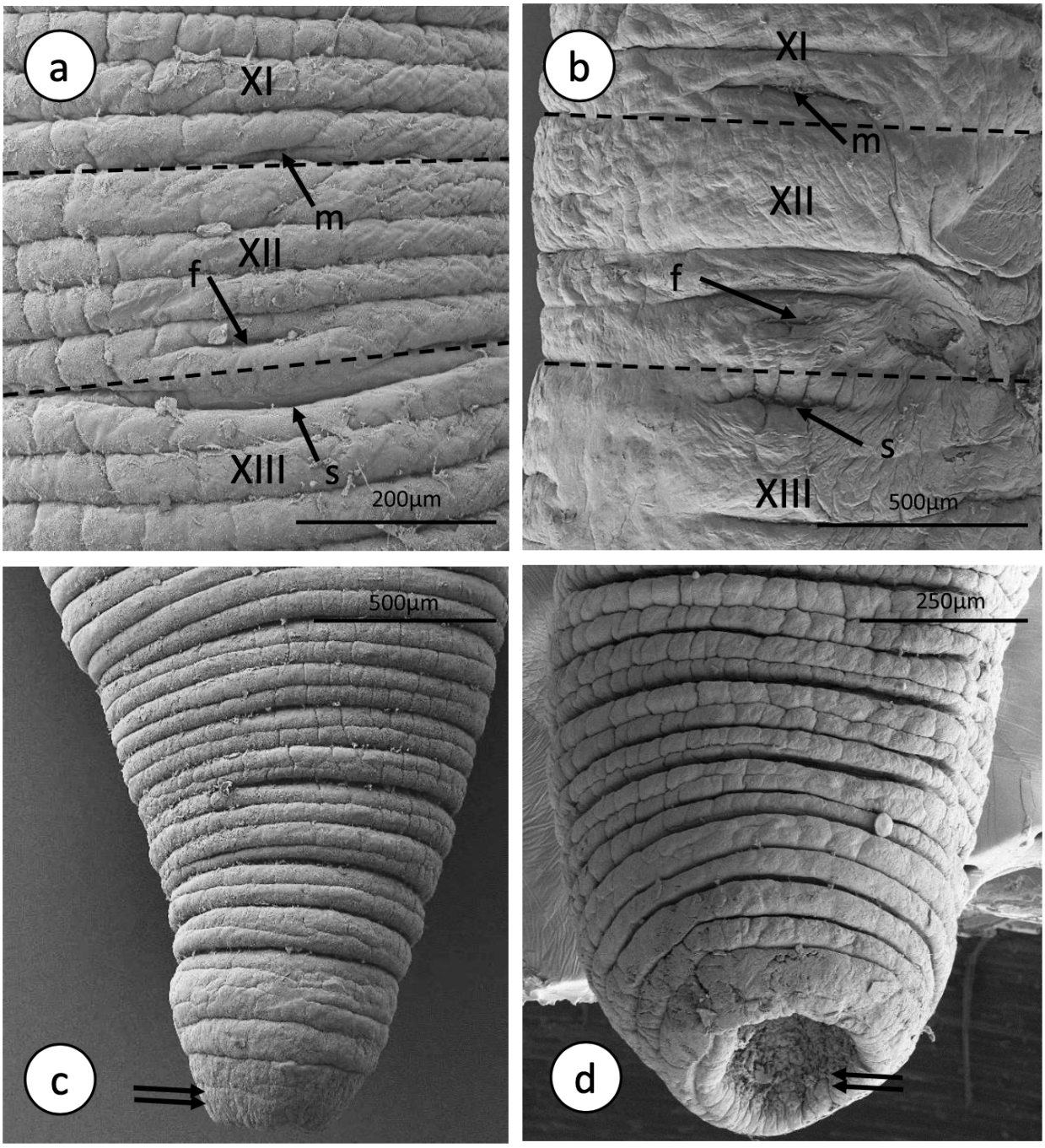

Figure 9. Scanning electron micrographs of gonopores and posterior body regions. Gonopores in: a) small *Acanthobdella peledina* [4 mm; Alaska] and b) large *Paracanthobella livanowi* [11 mm; Kamchatka]. The posterior body region for c) medium-sized *A. peledina* [7 mm; Sweden] and d) medium-sized *P. livanowi* [6 mm; Kamchatka]. Double arrows: posterior sucker; f: female gonopore; m: male gonopore; s: opening of the spermatheca. Dotted lines and Roman numerals denote segments.

TAXONOMY

Taxonomic scheme

Class: Clitellata Michaelsen, 1919

Subclass: Hirudinea Lamarck 1818

Order: Acanthobdellida Livanow, 1905

Suborder: Acanthobdelliformes, nov.

Family: Acanthobdellidae Livanow, 1905

Genus: *Acanthobdella* Grube, 1851

Species: *Acanthobdella peledina* Grube, 1851

Genus: *Paracanthobdella* Epstein, 1987

Species: *Paracanthobdella livanowi* (Epstein, 1966)

Notes on our taxonomic scheme

Here we present a taxonomic scheme for hook-faced fish worms (Acanthobdellida). In spite of its historical significance, we do not retain the family Paracanthobdellidae for *Paracanthobdella livanowi* because both morphological and molecular analyses suggest that the two acanthobdellidan species share many characteristics. Initially, Acanthobdellidae and Paracanthobdellidae were differentiated based on a few morphological differences that were viewed as taxonomically-important (Epstein, 1987). Specifically, members of Paracanthobdellidae were said to possess a primitive prostomium and well-developed anterior sucker. Although the two-family system seemed well-supported at the time, we have found that the prostomium of *P. livanowi* is not more conspicuous or developed than the anterior region of *A. peledina*. Moreover, the area between rows of chaetae in the cephalic extremity of the latter can be deepened such that it resembles a shallow sucker. It should also be noted that juvenile individuals of *P. livanowi* do not bear well-developed anterior suckers: this character is only common to large-bodied individuals of the species. The sum of this evidence suggests to us that the morphological differences between the two acanthobdellidan species, while pronounced, are not sufficient to warrant two families. We therefore classify both *Acanthobdella* and *Paracanthobdella* under the single family Acanthobdellidae.

We establish a new suborder (Acanthobdelliformes) to better match the taxonomy we erected for Hirudinea in our prior work (Tessler *et al.*, 2018a), which divided Hirudinida (leeches) into five suborders (Americobdelliformes, Erpobdelliformes, Hirudiniformes, Glossiphoniiformes, Oceanobdelliformes). Acanthobdelliformes is defined by the presence of chaetae on each of five

contiguous segments in the anterior body region, and 31 somites — mid-body ones are quadrannulate (with annulus a3 being subdivided) (Sawyer, 1986; Purschke *et al.*, 1993; Bielecki *et al.*, 2014). While previous studies have reported different numbers of segments for each acanthobdellidan species, e.g. 29 for *Acanthobdella* and 30 for *Paracanthobdella* (Bielecki *et al.*, 2014), this discrepancy is due to presumed differences in the number of the segments that comprise the posterior sucker, which should be substantiated by a careful morphological analysis in the future.

The higher taxonomy — class through order — follows our prior classification scheme (Tessler *et al.*, 2018a). Others have constructed alternative schemes, but we feel ours to be internally more consistent and phylogenetically appropriate. Alternative classifications include subclass Acanthobdellidea Livanow, 1905 (Archihirudinea Lukin, 1956 is an equivalent synonym) and subclass Acanthobdelliones (Epstein, 1987).

It is important to note that the two acanthobdellidan species were originally in the same genus (Epstein, 1966). However, in the late 1980s, *P. livanowi* was given its own genus and even family (Epstein, 1987). Ultimately, the decision to classify a monophyletic lineage of two species into one vs. two genera and/or families is subjective. We have decided to retain the genus-level classification proposed by Dr. Epstein (rather than lump them) to honour his contributions to the study of Hirudinea and accentuate the differentiation of the anterior sucker, chaetae, and internal anatomy that separates these species. This is also in concordance with the suggestions in the most recent, broad morphological comparison paper (Bielecki *et al.*, 2014); see Table 1 for a number of the differences between these species.

DISCUSSION

Our results indicate that the hook-faced fish worms (Acanthobdellida) diverged from leeches a long time ago, and that this order has many presumably plesiomorphic features, yet the extant species, and populations thereof, diverged recently. This is the first time *Paracanthobdella livanowi* has been included in a molecular phylogenetic study, helping to ascertain the aforementioned patterns. Furthermore, this is the first time American populations of *Acanthobdella peledina* have been incorporated into a molecular phylogenetic study, helping to indicate that this population is genetically distinct from the Nordic populations. Still, these populations appear to be morphologically indistinguishable based on scanning electron micrographs. While Acanthobdellida has recently received more attention over the last decade, our results help to fill in important understanding of the evolution of this fish-parasitizing clade.

Systematics of Acanthobdellida

Acanthobdellida is clearly monophyletic and sister to leeches. Prior problems with placement of *A. peledina* based on sequences from contaminants caused a lot of problems for past studies, which we helped sort out more recently (Tessler *et al.*, 2018a). These species, in our experience, are very hard to work with molecularly. It is easy to sequence contaminants from the environment or the host tissue. Even in recent studies, this has been an issue (Phillips *et al.*, 2019a,b). This paper, however, bolsters the claim that Acanthobdellida is sister to leeches, helping to substantiate it as a unique order within Hirudinea. To make this order better match the rankings found in the sister order (leeches), we have erected a new suborder called Acanthobdelliformes. Leeches comprise five suborders, in comparison.

Paracanthobdella livanowi is sister to *A. peledina* (Figure 3; Supplemental Files), as was expected based on morphology. The genetic distance between the two species at the COI locus (13.20%) is higher than values for other Hirudinean species pairs, which has been reported at roughly 8% (Oceguera-Figueroa, Léon-Règagnon, & Siddall, 2010; de Carle *et al*., 2017; Iwama *et al*., 2019). Interestingly, while the divergence between *A. peledina* and *P. livanowi* is substantial, the split is relatively recent, especially in comparison with the long branch length for Acanthobdellida, and the level of variability found in the closely related branchiobdellidans and leeches. The result is consistent for the mitochondrial, nuclear, and concatenated datasets (Figure 3; Supplemental Files). Although patterns of glaciation are often invoked to explain recent divergences in northern species, estimates for the rate of COI divergence between species pairs of annelids have been estimated at less than one percent per million years (Chevaldonné *et al*., 2002). Therefore, although the divergence between the two acanthobdellidan species is a relatively recent event in the history of the lineage, it likely predates the most recent glacial cycles. It bears mentioning that the ranges of both species most likely overlap (Table 1; Figure 2) in the Kamchatka region (Kaygorodova *et al.*, 2012), and that the known hosts of both species include salmonid fishes. Unfortunately, without fossils it is difficult to have any sense of this, or to even attempt a molecular clock analysis that would provide much confidence.

Populations of **Acanthobdella peledina**

The present evidence suggests *Acanthobdella peledina* from Alaska is distinct, to some degree, from European samples. However, Siberia and the Russian Far East have not been adequately sampled genetically for *A. peledina*. Accordingly, it is hard to fully know the genetic variability and population structuring of this species. Coupled with increased taxon sampling,

additional genetic sampling of Alaskan populations could possibly indicate that they are a unique species or population. Sampling of quickly evolving nuclear loci, or ideally, RADSeq data, would be useful for determining whether gene flow exists between the Alaskan and Nordic localities. COI – the most common marker for determining differences between leech species and populations (de Carle *et al.*, 2017; Tessler *et al.*, 2018d; Mack, de Carle, & Kvist, 2019) – and additional nuclear loci unfortunately did not amplify for these samples, potentially leading to some issues with missing data. Furthermore, as no external morphological differences were noted between samples of Nordic and Alaskan *A. peledina*, we refrain from formal species or population delimitation analyses at this time. Still, the fact that the Alaskan population is sister to, and genetically divergent from, the Nordic samples at minimum indicates that this is not an invasive or non-native species that was only recently translocated by humans, which would have been plausible given that the first records of this species in Alaska came from the 1970s (Holmquist, 1974; Hauck *et al.*, 1979) and it has not officially been reported since then, despite the clear importance of these American animals.

The Nordic populations are genetically fairly similar, despite being sampled from multiple countries. Maximum genetic distance at the COI locus is 1.52%, which is below the average value (~2.4%) typically reported for species of Hirudinea (de Carle et al., 2017; Anderson, Braoudakis, & Kvist, 2020; Mack *et al.*, 2019; Iwama *et al*., 2019; Kvist, 2015). However, the countries sampled are all in relatively close proximity. It would be most useful to add samples from central and eastern Russia. Unfortunately, a 12S sequence for *A. peledina* in the Baikal region of Russia from a recent publication was not made publicly available (Bolbat *et al.*, 2019).

Morphology

Our morphological examination and comparison of *Paracanthobdella* and populations of *Acanthobdella* help to further characterize these species. The scanning electron micrographs (Figures 6-9) and morphometry of facial hooks (n=1,280) help to accentuate the main external differences between the two species: 1) the presence or absence of a cup-shaped depression between rows of chaetae (anterior sucker), and 2) the chaetal dimensions and shape (Figure 5; Table 3). The differences in both of these characteristics become more notable as the species mature. The deep cup-shaped anterior sucker, which is viewed as the most important distinguishing feature of *P. livanowi,* gradually develops through ontogeny from a rather flat state characteristic of juvenile individuals of the species. In *A. peledina*, the anterior end does not form a clearly separated sucker even in fully grown specimens; however, a deep cavity appears between chaetae concomitantly with the animal growth. The chaete shape differs between

species: in *A. peledina* chaetae are bent at right angle and the breadth of the chaetae is similar in all rows; whereas in *P. livanowi*, the angle is obtuse and chaetae in rows 4-5 have substantially larger breadth (Figure 5). The well-developed prostomium, which has been considered as another distinguishing feature of the species and the genus, was found less prominent and conspicuous than presented in the previous studies (Epstein, 1987).

Other studies have examined the internal morphology of these species (Bielecki *et al.*, 2014); known differences from this work and others are summarized in Table 1.

Acanthobdella peledina has the same morphology across the Nordic and Alaskan populations examined here, and seemingly is indistinguishable from Siberian populations (Kaygorodova & Świątek, unpublished SEM data) and in other studies (Kaygorodova *et al.*, 2012). Still, while we did not find differences between *A. peledina* from Alaska and Eurasia, it is entirely possible that detailed internal examinations may unearth differences, given that these populations appear to be genetically divergent.

CONCLUSION AND FUTURE DIRECTIONS

Our results help shed light on the fact that hook-faced fish worms (Acanthobdellida) constitute an ancient lineage that is most closely related to leeches, and that its species and populations appear to have more recently diverged. It is even possible that there are multiple species within *A. peledina*; specifically, the American and Nordic populations appear to be genetically distinct and are likely reproductively isolated. However, there are important gaps to fill in the knowledge of the populations of this species before definitive action is taken on determining whether or not they actually represent the same species. Those gaps are: 1) adding specimens from localities for central and eastern Russia, 2) getting additional genetic data (i.e., COI and additional nuclear data) for Alaskan samples, and 3) looking for internal morphological differences between populations.

CONFLICT OF INTEREST

We do not have any conflict of interest.

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SUPPORTING INFORMATION

Table S1. PCR Primers and Thermal Profiles.

Table S2. Sequences Used in Phylogenetic Analysis.

File S1. Data Matrix and Maximum Likelihood Tree in NEXUS Format.