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Research Article

Molecular phylogenetic position of a rare and enigmatic meiofaunal flatworm from the Pacific Ocean: *Retronectes hyacinthe* sp. nov. (Platyhelminthes: Catenulida)

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Catenulids comprise the earliest diverging major lineage of flatworms. Although the majority of catenulid species live in fresh water, a small number of taxa have been documented from marine interstitial environments and most of these belong to the genera *Retronectes* and *Paracatenula* within the family Retronectidae. Representatives of *Retronectes* are extremely uncommon and almost only found in detritus-rich sand, with the last formal description of a species of *Retronectes* dating back to 1977. Little is known about the biology of the seven known species in this genus despite the fact that a unique combination of characters makes them relatively straightforward to recognize. Moreover, previous molecular phylogenetic analyses have so far been unable to include any representatives of *Retronectes*, so the phylogenetic position of these rarely encountered marine catenulids remains unclear. Here we describe a new species of *Retronectes*, namely *R. hyacinthe* sp. nov., from subtidal seagrass meadows in British Columbia (Canada) and present an updated phylogeny inferred from 18S and 28S rDNA sequences, including data from the new species of *Retronectes* and a selection of other catenulids. Our molecular phylogenetic trees suggest that Retronectidae *sensu* Sterrer & Rieger, 1974 is not monophyletic, implying that the current taxonomic classification of the Catenulida and the importance of certain morphological characters on which this classification is based are in need of revision.

http://zoobank.org/urn:lsid:zoobank.org:pub:A38635FB-7A8A-4C11-B513-BDBF9EA780CB

Key Words: British Columbia, Catenulida, *Paracatenula*, Retronectidae, seagrass, Turbellaria

Introduction

The Catenulida is a major subgroup of flatworms with representatives most commonly found in freshwater environments around the globe (Balsamo et al., 2020). However, a small proportion of known catenulid species occur in marine interstitial habitats, such as members of the rarely encountered Retronectidae Sterrer & Rieger, 1974. This family was originally erected to accommodate species within the genera *Retronectes* and *Paracatenula* (Sterrer & Rieger, 1974), and it was considered exclusively marine until the discovery of two representatives in fresh water: an undetermined species of *Retronectes* from the Elbe river in Central Europe (Ax & Düren, 1993) and a new monotypic genus, *Myoretronectes*, from the Paraná river in South America (Noreña-Janssen & Faubel, 1996).

Marine retronectids remain enigmatic, mainly because they are rarely encountered. Members of *Paracatenula* have received attention over the past decade because they form interesting endosymbiotic relationships with chemosynthetic bacteria within a trophosome organ, providing the host with bulk nutrition (e.g., Gruber-Vodicka et al., 2011; Jäckle et al., 2019). However, very little is known about members of the genus *Retronectes*. Most recognized species were reported in a single publication by Sterrer and Rieger (1974), in which they describe five species of *Retronectes* and four species of *Paracatenula* based on compiled data from more than 10 years of collections in multiple locations around the Eastern and Western Atlantic Ocean. Several other species remained undetermined because of a lack of morphological data. At roughly the same time, two

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other species of *Retronectes* were described with light microscopy (Faubel, 1976) and light and electron microscopy (Doe & Rieger, 1977); however, since 1977, only a few records of *Retronectes* specimens have been mentioned in the literature (e.g., Armonies, 2017, 2023; Fegley et al., 2020; Smith et al., 2020).

Molecular phylogenetic analyses focusing on catenulid interrelationships have so far only included rDNA data from representatives of *Paracatenula* (Dirks et al., 2011; Larsson et al., 2008; Larsson & Jondelius, 2008). Here we present a novel representative of the genus *Retronectes*, namely *R. hyacinthe* sp. nov., collected from British Columbia (Canada), the first of its kind from the Pacific Ocean. In addition to morphological data, we provide a molecular phylogeny inferred from both 18S and 28S rDNA sequences of catenulids, including rDNA sequences from multiple isolates of the new species. The phylogenetic position of this representative of *Retronectes* suggests that the current family Retronectidae is not monophyletic and that a stable classification of the Catenulida has not yet been reached.

Materials and methods

Two live individuals of *R. hyacinthe* sp. nov. were collected on 24 October and 26 October 2022, respectively, from sediments in subtidal seagrass meadows on Quadra Island, British Columbia, Canada (50°07′06″N, 125°13′16″W; 50°07′00″N, 125°13′22″W). Sand enriched with organic material at about 5 m and 6.1 m deep was sampled by scuba diving. Meiofauna were separated from the sediments using the $MgCl_2$ decantation method (Schockaert, 1996). The individual worms were isolated and observed under a Zeiss Stemi 508 stereoscope and subsequently whole mounted alive to be studied and photographed at the Quadra Island Ecological Observatory of the Hakai Institute under a Zeiss Axio Scope A1 compound microscope equipped with DIC using a Sony ILC-7RM4A digital camera. Measurements are given in micrometres (µm), while positions of anatomical structures are expressed in percentage of body length measured from the anterior tip (Ux).

Genomic DNA was extracted from both individuals using the DNeasy Blood & Tissue kit (Qiagen). Manufacturer's instructions were followed, with the exception that DNA was eluted in 60 µL of preheated AE elution buffer (60 °C). Fragments of the nearly complete 18S (1756 bp) and partial 28S rRNA (1753 bp) genes were PCR amplified using the primers and thermocycling conditions shown in Supplemental Table S1. Amplicons were visualized on 1.5% agarose gels stained with GelRed™ (Biotium), enzymatically cleaned with Illustra™ ExoProStar S (GE Healthcare), and subsequently sequenced by Genewiz (Azenta Life Sciences) through standard Sanger DNA sequencing using the amplification primers and internal sequencing primers (Supplemental Table S1). Resulting trace files were assembled into full sequences in Geneious v11.0.15 (Kearse et al., 2012) and subjected to a BLAST search on the NCBI website (http://blast.ncbi.nlm.nih.gov) to verify the specimens' taxonomic identity. Sequences were deposited in GenBank with the accession numbers provided in Supplemental Table S2.

The new 18S and 28S rDNA sequences of *R. hyacinthe* sp. nov. were aligned with rDNA sequences of 23 representatives of the Catenulida and seven representatives of the Macrostomorpha as an outgroup (Supplemental Table S2) using the E-INSI algorithm in MAFFT (Katoh & Toh, 2008). Taxa were only selected when both 18S and 28S rDNA sequences were available. These alignments were subsequently trimmed with ClipKIT using the default settings (Steenwyk et al., 2020). The trimmed 18S rDNA and 28S rDNA alignments were concatenated in Geneious v11.0.15.

The best-fit partition and model of molecular evolution corresponding to the 18S and 28S rDNA datasets and GTR + GAMMA + I, respectively, were recovered for the concatenated dataset (18S + 28S) in PartitionFinder v.2.1.1 using a greedy search and all three model selection criteria (AIC, AICc, BIC) (Lanfear et al., 2017). These partition scheme and models were subsequently used in the phylogenetic analyses of the concatenated dataset. A maximum likelihood (ML) analysis was performed with the RAxML v8.2.11 plugin (Stamatakis, 2014) in Geneious v11.0.15 selecting the algorithm for best-scoring ML tree search and non-parametric bootstrapping (1000 replicates). Seven macrostomorphs were selected as the outgroup based on current knowledge of the phylogenetic relationships within flatworms (Egger et al., 2015; Laumer et al., 2015).

A Bayesian analysis was performed on the same dataset in MrBayes v3.2.7a (Ronquist & Huelsenbeck, 2003) through XSEDE in the CIPRES Science Gateway v3.3 (https://www.phylo.org), using default prior and mcmc settings, in two independent simultaneous runs for 40 million generations. Trees were sampled every 400th generation after a 25% burn-in. Convergence was assessed through the LogL values, ESS values (estimated sample size) and the average deviation of split frequencies. The remaining 75,000 trees were summarized in a 50% majority-rule consensus tree. Branch support was evaluated with the ML bootstrap values (bs) and Bayesian posterior probabilities (pp) for the ML and Bayesian trees, respectively. To further investigate

our bootstrap support values regarding the phylogenetic position of *R. hyacinthe* sp. nov., a ML bootstrap consensus network was constructed in SplitsTrees v4.17.0 (Huson & Bryant, 2006) based on the 1000 bootstrap pseudoreplicates from the RAxML analysis. An approximately unbiased (AU) test was conducted in IQ-TREE v1.6.12 (Nguyen et al., 2015) to test alternative placements of *Retronectes* within the Catenulida. The AU test compared the log-likelihood scores among a set of trees generated in four different partitioned IQ-TREE ML analyses based on the same dataset, including an unconstrained tree search and three constrained tree searches (Supplemental Table S3). The number of RELL replicates was specified to 100,000.

Results

Description

Catenulida Graff, 1905
Retronectidae Sterrer & Rieger, 1974

We update the status of the family Retronectidae by excluding the genus *Paracatenula* Sterrer & Rieger, 1974, based on the results of the molecular phylogenetic analyses (see Discussion). Sterrer and Rieger (1974) did not specifically designate a type genus for the family. However, based on eponymy, we reserve the name Retronectidae for the family including the genera *Retronectes* Sterrer & Rieger, 1974 and *Myoretronectes* Noreña-Janssen & Faubel, 1996 (see Discussion).

Emended diagnosis. Based on Noreña-Janssen and Faubel (1996): Catenulida without ciliated pits or furrows, and without refractile bodies. Posterior and anterior gangliar lobes may be lacking as well as paired granular strands of parenchymous nature usually present between gut and body wall. Mixed gonads distinct and elongated, usually hourglass-shaped: anterior part in male condition containing sperm cells; posterior part often with large germinative cells. Sperm nucleus usually of distinct shape (conglomerate, rod, ribbon, spicule, banana). Statocyst with single or several statoliths. With mouth, pharynx and gut.

Retronectes Sterrer & Rieger, 1974

Type species. *Retronectes thalia* Sterrer & Rieger, 1974.

Diagnosis. See Noreña-Janssen and Faubel (1996).

Retronectes hyacinthe Van Steenkiste & Leander, sp. nov.

Type material. Two specimens were used for genomic DNA extraction; therefore, one of the DNA vouchers becomes the holotype (BBM MI4943, extracted DNA in buffer stored at −80 °C).

Other material. Extracted DNA from the other specimen. Images of the live specimens.

Type locality. Hyacinthe Bay, Quadra Island, BC (50°07′06.0″N, 125°13′16.0″W; 50°07′00.0″N, 125°13′22.0″W): detritus-rich sand from seagrass meadows at about 5 m and 6 m deep, respectively.

Etymology. The species epithet refers to the type locality. Hyacinth (Greek: Ύακινθος or Hyakinthos) is also a Greek mythological character, lover of Apollo, and according to some sources the son of the muse Clio.

Diagnosis. Species of *Retronectes* with granular strands, a statocyst containing one statolith, a wrinkled epidermis and constriction at the level of the statocyst; 30–37 µm long sperm cells with 6–11 µm long nuclei of the conglomerate type.

Description and remarks. The smaller, complete animal was about 1500 µm long, while the larger animal missing the posterior part behind the gonads (Fig. 1) measured 2200 µm. It is therefore likely that the latter individual was much longer in length, possibly in excess of 3000 µm. The sparsely ciliated epidermis (ep, Fig. 5) is wrinkled along the entire length of the animal. Live animals appear white under the stereoscope and curl up into a spiral.

Internal anatomy typical of representatives of *Retronectes* as described by Sterrer and Rieger (1974). Granular strands (gs, Figs 1–3) extend throughout the whole length of the body. These strands are yellowish giving the rostrum a more intense colouration where the strands converge. An oval statocyst (sc, Figs. 1, 3) with one round statolith is present in the head region. The statocyst is 11–14 µm long and 7–8 µm wide; the diameter of the statolith is 5–7 µm. The statocyst is associated with the brain, which extends into the rostrum. The rostrum is clearly demarcated by a constriction (white arrowheads, Figs 1–3) at the level of the statocyst and measures 75 µm in the smaller specimen (U5) and 185 µm in the larger specimen (U6).

Immediately posterior to the statocyst and constriction, the mouth (m, Fig. 2) can be discerned as an opening on one side of the body (U7). It is also marked by the presence of glands with dense rod-like secretions (rg, Figs 2–3) exiting around the mouth opening. The pharynx (ph, Figs 2–3) is poorly visible and corresponds with a clear transparent zone behind the mouth opening. The intestine fills almost the entire body posterior to the pharynx. Rod-like secretions are also present in or around the intestinal tissue. A second type of glands with coarse granular secretions (cg, Figs 2–3) surround the transition zone between the pharynx and intestine.

The anterior part of the genital organ and genital pore are on the opposite side of the mouth behind the pharynx and coarse granular glands. The entire genital organ seems to have mixed gametes and stretches from the genital pore (U13) to slightly less than two-thirds of the body length (U60). The genital pore is surrounded by glands with a fine granular secretion (fg, Fig. 4). In the anterior part, the sperm cells (sp, Fig. 4) in the seminal vesicle are elongate, irregular or triangular depending on the surrounding tissue and measure 30–37 µm. The sperm cells contain large granules and the 6–11 µm long sperm nuclei are of the conglomerate type (see Sterrer & Rieger, 1974). Behind the seminal vesicle, the gonad narrows but still contains a few sperm cells (sp, Fig. 3). In the posterior part of the body, the mixed gonad broadens again and contains large gametes or germinative cells – potentially representing egg cells (but see Discussion) – stacked in a single row (gc, Fig. 6). In one of the two animals, a group of four sperm cells can be observed immediately anterior to these large cells (sp, Fig. 6). In the posterior part of the gonad, some of these larger cells seem to gradually develop into possibly four sperm cells with granules and conglomerate-type nuclei (sp, Fig. 5), supporting Sterrer & Rieger's suggestion that the gonad might have a mixed production of eggs and sperm. In the complete animal, the mixed gonad fills the body up to about two-thirds of the body length, while the posterior third of the body is only filled with intestinal tissue.

Paracatenulidae Van Steenkiste & Leander, fam. nov.

We propose this new family which includes all representatives of the genus *Paracatenula* Sterrer & Rieger, 1974 to align the family-level classification within the Catenulida with the results of our molecular phylogenetic analyses (Figs 7, 8).

Diagnosis. Catenulids without ciliated pits or furrows, and without refractile bodies. Posterior and anterior gangliar lobes present as well as paired granular strands of parenchymous nature. Distinct gonads lacking, instead genital tissue composed of 'dorsal chord' with sperm cells. Sperm nucleus usually of distinct shape (rod, ribbon, spindle, spicule, banana). Statocyst present or absent, with single or several statoliths. Without mouth, pharynx and gut. Trophosome with endosymbiotic bacteria present.

Type genus. *Paracatenula* Sterrer & Rieger, 1974.

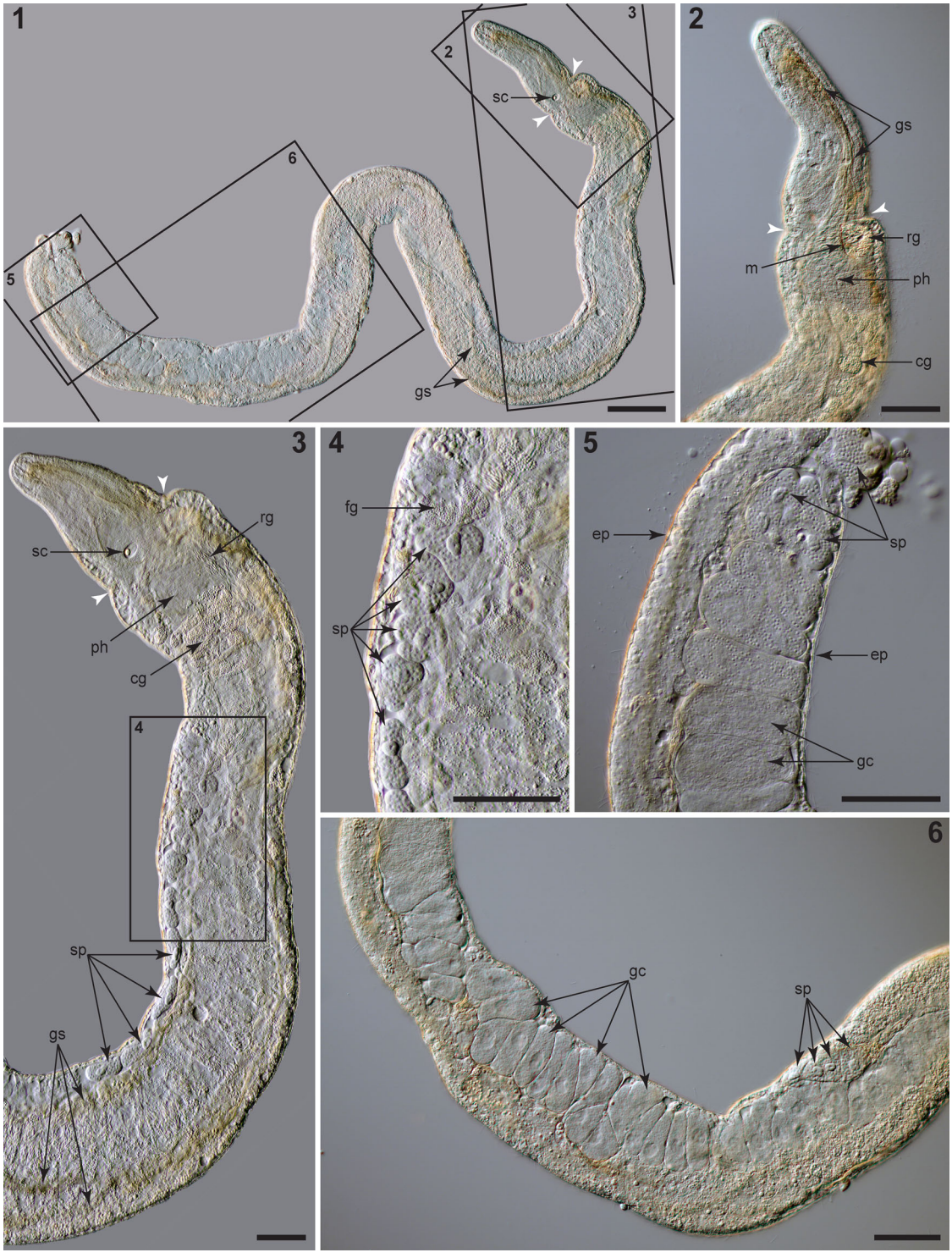
The molecular phylogenetic position of *Retronectes*

The trimmed concatenated 18S + 28S rDNA sequence alignment has 32 sequences belonging to 29 taxa and contains 3162 bp (18S: 1732 bp; 28S: 1430 bp). The phylogenetic analyses and the ML consensus network are summarized in Figs 7 and 8. Topologies were congruent for both the ML and Bayesian tree after collapsing branches below the thresholds (pp <0.95 and bs <70). The results can be summarized as follows: (a) the two basal clades of catenulids as defined by Larsson and Jondelius (2008) and Larsson et al. (2008) – one clade consisting of representatives of Catenulidae and Retronectidae, and one clade consisting of representatives of Stenostomidae – are recovered in our trees; (b) the genus *Retronectes* (Retronectidae), represented by *Retronectes hyacinthe* sp. nov., emerges as the sister lineage to a clade consisting of the genus *Paracatenula* (Retronectidae) and the genus *Catenula* (Catenulidae) (pp = 1; bs = 79), rendering Retronectidae *sensu* Sterrer & Rieger, 1974 non-monophyletic (Fig. 7); (c) the latter scenario is confirmed by the AU test, which rejected the topology with a constrained monophyly of Retronectidae *sensu* Sterrer & Rieger, 1974 ($P = 0.039$, Supplemental Table S3); (d) the statistical support values in the consensus network based on ML bootstrap pseudo-replicates (Fig. 8) show low pseudo-replicate counts for the two alternative scenarios in the Retronectidae–Catenulidae clade: bs = 18.1 for the scenario (*Paracatenula*, (*Retronectes*, *Catenula*)), and bs = 2.6 for the scenario (*Catenula*, (*Retronectes*, *Paracatenula*)).

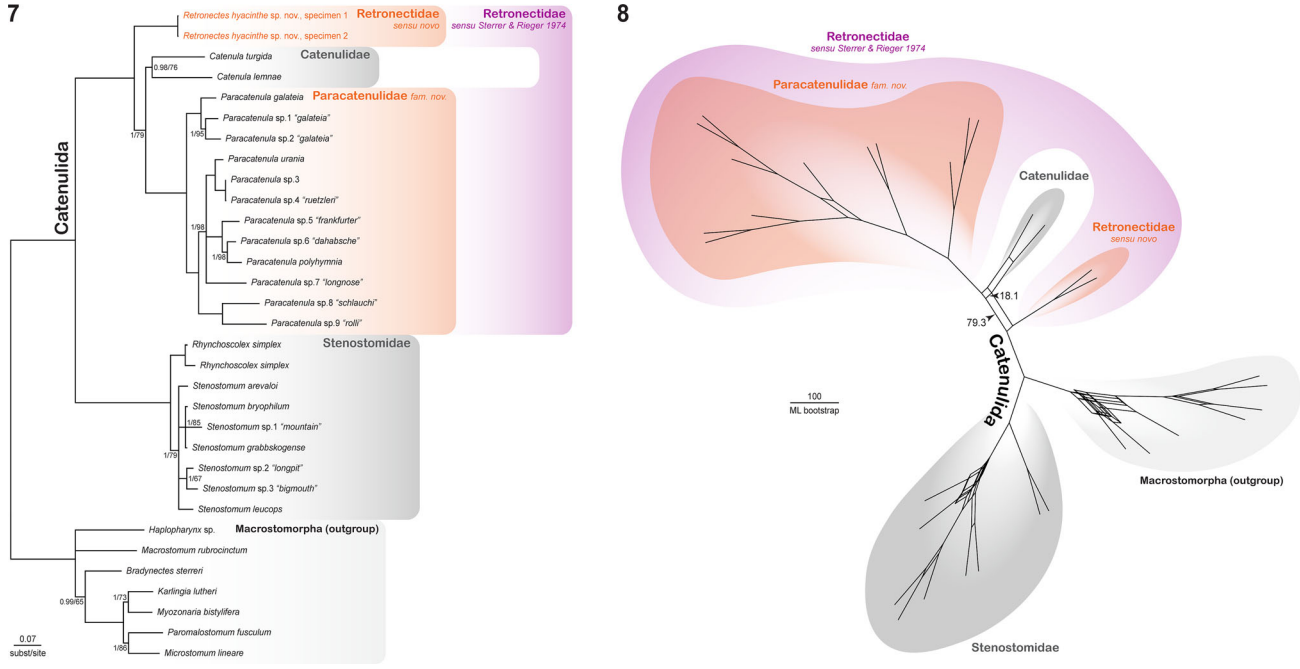
Discussion

The first species of *Retronectes* from the Pacific Ocean

The two live specimens were unmistakably recognized as marine catenulids and representatives of *Retronectes* by the presence of a statocyst, granular strands, a digestive system with mouth, pharynx and intestine, and a distinct genital organ with sperm cells and large germinative cells. Species of *Retronectes* are mostly



Figs 1–6. DIC micrographs of a live specimen of *Retronectes hyacinthe* sp. nov. from British Columbia. (**1**) Composite image of specimen (with the tail part broken off) showing the statocyst (sc), granular strands (gs) and a constriction at the base of the head region (white arrowheads). (**2**) Detail of the head region showing the constriction (white arrowheads), granular strands (gs), mouth (m), pharynx (ph), coarse granular glands (cg) and glands with rod-like secretions (rg). (**3**) Detail of the anterior part of the body including the head and anterior part of the mixed genital organ. (**4**) Detail of the seminal vesicle containing a number of sperm cells (sp) and fine granular glands (fg). (**5**) Detail of the posterior part showing the sparsely ciliated epidermis (ep) and the development of large germinative cells (gc) into sperm cells (sp). (**6**) Detail of the enlarged part of the mixed genital organ showing large germinative cells (gc) in a single row. Scale bars: (**1**) = 100 µm; (**2**–**6**) = 50 µm.



Figs 7–8. Phylogenetic position of *Retronectes hyacinthe* sp. nov. within Catenulida inferred from 18S and 28S rDNA sequences. (**7**) Bayesian majority-rule consensus tree of the MrBayes analysis based on the trimmed concatenated alignment. Branch support values of the Bayesian analysis (pp, posterior probabilities) and the topologically congruent ML analysis (bs, bootstrap) are not shown when pp = 1 and bs = 100. Branches with support values of pp <0.95 or bs <70 are collapsed. All other branches with annotations have support values of pp ≥0.95 and/or bs ≥70. (**8**) Consensus network of the RAxML bootstrap analysis based on the trimmed concatenated alignment. Pseudo-replicate counts (∼bs) pertaining to the interrelationships of *Retronectes*, *Paracatenula* and *Catenula* are shown with a threshold value of 5. Retronectidae *sensu* Sterrer & Rieger, 1974 refers to the traditional family containing the genera *Retronectes*, *Paracatenula* and *Myoretronectes*, while Retronectidae *sensu novo* and Paracatenulidae fam. nov. are proposed as new taxa to bring the family-level classification of the Catenulida in line with the phylogenetic results.

distinguished from each other based on the morphology of the statocyst and sperm cells (Sterrer & Rieger, 1974). Only one other species of *Retronectes*, *R. clio* Sterrer & Rieger, 1974 from the Northeast Atlantic (Skagerrak, Sweden), has sperm nuclei of the conglomerate type. The new species from British Columbia resembles *R. clio* in many respects; however, a close comparison between the two species brings several differences to light, which are summarized in Table 1. *Retronectes clio* is generally smaller than *R. hyacinthe* sp. nov., but has a relatively larger head region. Other differences are found in the morphology of the statocyst, which is round in *R. clio* and oval in *R. hyacinthe* sp. nov., the length of the mixed gonad relative to body size, which is shorter in *R. hyacinthe* sp. nov. than in *R. clio*, and the larger size of the sperm cells and sperm nuclei in *R. hyacinthe* sp. nov. (Table 1).

The type locality of *R. hyacinthe* sp. nov. is characterized by a patchwork of shallow water seagrass meadows and sediments enriched with organic material fringing a rocky shoreline. Marine retronectids are typically found in sheltered subtidal sandy sediments with high amounts of organic detritus. In addition, Sterrer and Rieger (1974) affirmed that retronectids occur within the redox discontinuity layer and therefore are almost always found together with gnathostomulids, which are also typically associated with this specific habitat. These observations correspond with our findings that the sampled seagrass meadows on Quadra Island are also particularly rich in gnathostomulids, both in terms of abundance and species diversity (unpublished data). However, not only is *R. hyacinthe* sp. nov. the first species of *Retronectes* from a seagrass meadow, it is also the first record of this genus and family from the Pacific Ocean.

Ongoing sampling efforts since the summer of 2021 to collect and study meiofaunal biodiversity in Hyacinthe Bay, Quadra Island, BC on a regular basis (monthly to bimonthly), have only yielded two specimens of *R. hyacinthe* sp. nov. This suggests that this species is either rare and/or that its occurrence and abundance depend on specific environmental conditions with a limited time window. While additional specimens of *R. hyacinthe* sp. nov would have allowed for a more detailed assessment of its anatomy and the preservation of morphological vouchers, the unique combination of

Table 1. Comparison of morphological characters between *Retronectes clio* and *Retronectes hyacinthe* sp. nov.

Character	*Retronectes clio* Sterrer & Rieger, 1974	*Retronectes hyacinthe* sp. nov.
Body length	1250 µm (up to 2800 µm in some specimens)	1500–3000 µm
Rostrum	130 µm (U10)	75–185 µm (U5–6)
Mouth	U15	U7
Statocyst	U10; round with diameter 10 µm	U5–6; oval with dimensions 11–14 µm × 7–8 µm
Statolith	round with diameter 5 µm	round with diameter 5–7 µm
Gonopore	U18	U13
Mixed gonad	extends between U18 and U89	extends between U13 and U60
Sperm cells	irregular to triangular; 10–15 µm	irregular to triangular; 30–37 µm
Sperm nuclei	conglomerate-type; 3 µm	conglomerate-type; 6–11 µm

morphological characters distinguishing it from other congeners, its type locality in the Pacific Ocean, and its 18S and 28S rDNA sequences, make future identification and comparison easily possible; therefore, the specimens from British Columbia are formally described as a new species.

Taxonomic and phylogenetic implications

To date, only a few studies have addressed the phylogenetic interrelationships of the Catenulida with molecular data (Dirks et al., 2011; Larsson et al., 2008; Larsson & Jondelius, 2008). These phylogenetic frameworks based on rDNA and cytochrome oxidase I sequences showed that catenulids consisted of a freshwater clade including representatives of Stenostomidae (*Rhynchoscolex* and *Stenostomum*), and a mixed clade with marine representatives of Retronectidae (*Paracatenula*) and freshwater representatives of Catenulidae (*Catenula*). This contrasted with earlier phylogenetic hypotheses based on morphological characters, which placed the marine Retronectidae as a sister group to a freshwater clade including Catenulidae and Stenostomidae (Ehlers, 1994). Based on molecular phylogenetic results and a reassessment of the diagnostic morphological characters, Larsson et al. (2008) synonymized the freshwater genera *Suomina* and *Anokkostenostomum* with *Catenula* and *Stenostomum*, respectively.

While the traditional families Retronectidae, Catenulidae and Stenostomidae corresponded to monophyletic groups in the molecular phylogenetic trees of Larsson and Jondelius (2008), Larsson et al. (2008) and Dirks et al. (2011), synapomorphies for the clade uniting *Paracatenula* (Retronectidae *sensu* Sterrer & Rieger, 1974) and *Catenula* (Catenulidae) remained unclear. With the inclusion of rDNA sequences of *Retronectes*, our analyses show that *Retronectes* is the sister lineage to the *Paracatenula–Catenula* clade (Figs 7, 8); the alternative scenarios, *Paracatenula* as the sister lineage to a *Retronectes–Catenula* clade or *Catenula* as the sister lineage to a *Retronectes–Paracatenula* clade – i.e., the scenario in which Retronectidae *sensu* Sterrer & Rieger, 1974 is monophyletic – are not supported. As a result, the current classification of Retronectidae *sensu* Sterrer & Rieger, 1974 as a family uniting the representatives of *Retronectes* and *Paracatenula* should be reconsidered.

Based on our results, we establish the new family Paracatenulidae fam. nov. to accommodate the genus *Paracatenula.* While Sterrer and Rieger (1974) did not designate a type genus for the family Retronectidae, we prefer to reserve the name Retronectidae *sensu novo* for the family now including the genera *Retronectes* and *Myoretronectes* based on eponymy. No molecular data are currently available for *Myoretronectes* and, as such, the inclusion of this genus should be considered provisional. The proposed changes will bring the classification of the Catenulida in line with our current understanding of the phylogenetic relationships within the group and will still allow for proper diagnosis of each taxon based on morphological characters.

The phylogenetic position of *Retronectes* and the resulting non-monophyly of Retronectidae *sensu* Sterrer & Rieger, 1974 has implications for our understanding of trait and character evolution within the Catenulida. Currently, our inferences about ancestral, homologous or homoplasious traits in catenulids are limited. For instance, as a result of Larsson et al.'s (2008) phylogenetic framework, it was clear that the supposedly close relationship between Catenulidae and Stenostomidae based on shared ultrastructural characters of the protonephridia (Ehlers, 1994) had to be abandoned. The presence of a gonad with characteristic sperm cells uniting Retronectidae *sensu* Sterrer & Rieger, 1974 contrasts with the common habitus of representatives of Catenulidae forming chains of zooids for asexual reproduction. However, reproductive structures have been reported in a few specimens belonging to the genus *Catenula* (Marcus, 1945; Nuttycombe, 1956; Sekera, 1924), while zooid formation and paratomy was also reported for the undetermined freshwater species of *Retronectes* (Ax & Düren, 1993). In addition, more recent findings suggest that asexual fragmentation is

probably the most common mode of reproduction among representatives of *Paracatenula* (Dirks et al., 2012). Based on the basal position of *Retronectes* in the *Retronectes–Paracatenula–Catenula* clade in our trees, we hypothesize that the presence of a distinct gonad is the plesiomorphic condition in this clade, with asexual reproduction through paratomy becoming the dominant mode of reproduction among representatives of Paracatenulidae fam. nov. and Catenulidae, accompanied by the reduction or loss of the mixed gonad in most representatives of Paracatenulidae fam. nov. and Catenulidae, respectively. It remains unclear what the dominant mode of reproduction is for representatives of Retronectidae *sensu novo*. In fact, Sterrer and Rieger (1974) even suggested the possibility of a new mode of reproduction involving parthenogenesis from the large 'oocyte-like' germinative/sperm cells. Whether or not the common ancestor of catenulids predominantly reproduced sexually or asexually, it seems like the ability for paratomy remains a basic feature for the group as a whole as pointed out by Ax and Düren (1993).

Large-scale comparative morphological studies centred on traits associated with the nervous system, protonephridia, musculature and digestive system focus on well-known lineages (e.g., Hooge, 2001; Leisch et al., 2011; Moraczewski, 1981; Moraczewski et al., 1977; Moraczewski & Czubaj, 1974; Schuchert & Rieger, 1990; Silveira, 1998; Soltynska et al., 1976). To gain a better comprehension of key innovations among the different groups of catenulids such studies should also include representatives of *Retronectes* in addition to representatives of the other major catenulid lineages. Future molecular phylogenetic studies of Catenulida should also aim to comprise more representatives of Retronectidae *sensu novo*, including those from freshwater environments (e.g., *Myoretronectes* and perhaps yet undetermined freshwater species of *Retronectes*) in order to elucidate habitat shifts and associated adaptations within this early-diverging group of flatworms. Field-based surveys and collections of live animals remain the cornerstone to study these marine catenulids. Our results clearly demonstrate how species discovery of such rare taxa can have profound implications on our understanding of diversity and evolutionary history of an otherwise abundant group of animals.

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Disclosure statement

The authors have no conflict of interest to declare that is relevant to the content of this article.

Availability of data and material

GenBank accession numbers of the sequences generated for this study are included in the Supplemental Material.

Authors' contributions

Conceptualization: NWLVS, BSL; Methodology: NWLVS, AC, TF; Formal analysis and investigation: NWLVS; Writing – original draft preparation: NWLVS, BSL; Writing – review and editing: NWLVS, BSL; Funding acquisition: BSL; Resources: AC, TF, BSL; Supervision: BSL

Sampling

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Supplemental data

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