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Molecular phylogenetic and detailed morphological analyses reveal a new species of *Castrella* Fuhrmann, 1900 (Platyhelminthes, Dalytyphloplanida) from Switzerland

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Abstract. Rhabdocoel microturbellarian flatworms of the family Dalyelliidae are key components of freshwater ecosystems and represent one of their most taxonomically diverse groups. Recent molecular phylogenetic studies revealed two distinct genetic lineages within *Castrella truncata*, a species with a wide Nearctic–Palearctic distribution. In this study, we integrated phylogenetic and morphological analyses to describe a Swiss lineage as a new species: *Castrella schareri* sp. nov. Phylogenetic analysis reveals that *C. schareri* sp. nov. is closely allied with *C. alba*, while *C. truncata* groups with *C. pinguis*. Morphologically, the new species is characterised by a distally unbranched stylet, featuring a shorter proximal portion and a higher number of shorter distal spines compared to *C. truncata*. This finding significantly enhances our knowledge of *Castrella*, marking the first new species described in the genus in over seventy years.

Keywords. Dalyelliidae, freshwater, integrative taxonomy, microturbellarian, springs fauna.

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Introduction

Microturbellarian Rhabdocoela Ehrenberg, 1831 (Platyhelminthes Minot, 1876) are small, free-living flatworms that play a vital ecological role in freshwater ecosystems. As benthic meiofauna, they contribute significantly to nutrient cycling and energy flow through food webs (Young 1973, 1978; Schwartz & Hebert 1986; Tüzün *et al.* 2025). Despite their ecological importance and often high abundance, freshwater rhabdocoel microturbellarians remain understudied, particularly in terms of their taxonomy and phylogenetic relationships (Van Steenkiste *et al.* 2011, 2012, 2013). This lack of taxonomical knowledge, the so-called 'Linnean shortfall', represents a major impediment to species and ecosystems conservation (Lessa *et al.* 2024; Lima *et al.* 2024).

In freshwater ecosystems, rhabdocoel flatworms of the family Dalyelliidae Graff, 1905 are among the most taxonomically dominant groups, with over 160 described species. The majority of this diversity is concentrated in the genera *Gieysztoria* Ruebush & Hayes, 1939 (90 species) and *Microdalyellia* Gieysztor, 1938 (44 species) (Tyler *et al.* 2006–2025). Despite their diversity, most taxonomic studies on these microturbellarians date back to the 19th and early 20th centuries, and only limited taxonomic and phylogenetic advancements have been made in the past two decades.

Species of Dalyelliidae are predominantly known from the Nearctic and Palearctic regions (Graff 1882; Luther 1955), as well as South America (Marcus 1946; Reyes *et al.* 2021; Adami & Damborenea 2024). Fewer recent records come from Canada and the United States (Van Steenkiste *et al.* 2011), India, South Africa, and Australia (Van Steenkiste *et al.* 2012), and China (Wang & Wu 2005; Lu *et al.* 2013; Rong *et al.* 2016). Molecular phylogenetic research within the group has been relatively sparse, with a major contribution by Van Steenkiste *et al.* (2013) and more targeted studies by You *et al.* (2021) and Diez & Schmidt-Rhaesa (2024).

Within dalyelliids, the genus *Castrella* Fuhrmann, 1900 comprises seven recognised species. These are characterised by a sclerotised stylet that is not directly connected to the copulatory organ but instead housed in a separate pocket (Fuhrmann 1900; Luther 1955). The stylet consists of a proximal, spine-free 'handle' and a distal portion bearing multiple spines. Additional diagnostic traits of species of *Castrella* include short oviduct, a seminal receptacle in the form of a stalked vesicle, and stalked eggs (Luther 1955). Traditionally, species delineation within *Castrella* has relied on a narrow set of morphological features, primarily stylet shape.

Recent molecular studies have uncovered hidden diversity within nominal species. For example, *C. truncata* (Abildgaard, 1789) Sekera 1906, historically considered a widely distributed species across the Nearctic and Palearctic regions (Van Steenkiste *et al.* 2011), has been shown to comprise two divergent genetic lineages, indicative of cryptic speciation (Diez & Schmidt-Rhaesa 2024).

In the present study, I integrate molecular and morphological data to describe a previously unrecognised species of *Castrella* from Switzerland: *Castrella schareri* sp. nov.

Material and methods

Sampling and morphological study

The specimens studied here were collected in two localities of Switzerland: Röserental, Basel (April 12, 2024), and Centovalli, Ticino (September 8, 2024). The species description provides the geographic coordinates and ecological conditions of these localities. Specimens were extracted from vegetation using the oxygen depletion method (Schockaert 1996) and subsequently studied alive and whole mounted with lactophenol. The specimens used for molecular phylogenetic analyses were preserved in ethanol (99%) and stored at -20ºC. The drawings of the hard parts were made with a camera lucida on a Leica DM 2500 microscope, using Nomarski interference contrast. Measurements were taken along the central axis of the measured object. The holotype was deposited in the Museum of Nature Hamburg (ZMH), Leibniz Institute for the Analysis of Biodiversity Change, Germany, and the reference material is in the collection of Hasselt University (HU), Belgium.

In addition to the newly collected specimens, we studied the material of *C. truncata* deposited in the reference collection of HU: Ontario, Canada (V.4.09–V.4.10), Belgium (II.1.43–II.1.44), and Finland (VII.1.20–VII.1.32).

DNA extraction, PCR amplification, and sequencing

Complete specimens were used for total DNA extraction using a 'Salting out' DNA extraction method (Laumer 2023). Specimens were treated with 195 µl TNES buffer and 5 µl proteinase K (Invitrogen) at 55°C for ± 30 minutes. Subsequently, 65 µl 5M NaCl and 290 µl 96% EtOH were added to the lysate, using as coprecipitant 1.5 µl yeast tRNA (Invitrogen), and the resulting product was stored for 1 h at -20°C. Purification was performed with two EtOH (70%) wash steps and eluted overnight at 4°C in 0.1X TE buffer with 0.02% Tween™ 20 (Thermofisher).

PCR reactions were carried out using 0.2 ml PuReTaq Ready-To-Go PCR beads (GE Healthcare) on a Bio-Rad PCR T-100 Touch thermal cycler. Each reaction included 2.5 µl of each primer (5 µM), 1 µl of DNA, and 17 µl of purified water for a final volume of 25 µl. The primer pairs TimA (5' AMCTGGTTGATCCTGCCAG 3') and TimB (5' TGATCCATCTGCAGGTTCACCT 3'), and LSU5 (5' TAGGTCGACCCGCTGAAYTTA 3') and LSU6-3B (5' GAGAAGGGTTCCATGTGAACAGC 3') were used to amplify partial regions of 18S and 28S rDNA genes, consecutively (Van Steenkiste *et al.* 2013; Tessens *et al.* 2014). Both genes were amplified with the PCR conditions: 95°C for 3 min, followed by 9 cycles of 94°C for 30 s, 60°C for 30 s (-0.5°C/cycle), 72°C for 90 s, and 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 90 s, with a final extension of 72°C for 5 min. PCR products were verified on a 1% agarose gel, stained with HD Green Plus (Intas Science Imaging). PCR products were purified with ExoCleanUp FAST (Avantor) and sequenced by Macrogen Europe B.V. (Amsterdam) under BigDyeTM terminator cycling conditions on an ABI3730XL DNA Sequencer.

Molecular phylogenetic analysis

An overview of all used sequences and corresponding accession numbers is presented in Table 1. Raw reads were quality-trimmed (error probability = 0.05) and assembled in Geneious Prime 2025.1.2 (Kearse *et al.* 2012). Consensus sequences were subjected to a BLAST search (Altschul *et al.* 1990) on the NCBI website (http://ncbi.nlm.nih.gov) to check for signs of contamination. All available sequences of Dalyelliidae and 'Typhloplanidae' were mined from GenBank (Benson *et al.* 2012), and a separate dataset was compiled for both 18S (84 sequences) and 28S rDNA (65 sequences). These datasets included an outgroup of four species of the sister families Jenseniidae Van Steenkiste, Rivlin, Kahn, Wakeman & Leander, 2021 and Temnocephalidae Monticelli, 1899, selected according to the phylogenetic work of Van Steenkiste *et al.* (2013) (Table 1).

Both datasets were aligned using MAFFT (Katoh & Standley 2013; Katoh *et al.* 2017), as implemented in Geneious, specifying the Q-INS-i algorithm to account for secondary structures. Ambiguously aligned regions were identified and eliminated on the Gblocks ver. 0.91b server (Castresana 2000), employing options for a less stringent selection. Resulting alignments were concatenated in Geneious. An initial partitioning scheme, defining gene boundaries, was constructed manually. The concatenated alignment and partition file were used as input for the ModelFinder tool (Kalyaanamoorthy *et al.* 2017) on the IQ-TREE webserver (Trifinopoulos *et al.* 2016). Model fit was evaluated using the Akaike Selection Criterion, and partition merging was enabled (Lanfear *et al.* 2012). The latter feature determines the best-fit partitioning scheme for a particular dataset, while also calculating the best-fitting evolutionary models for each selected subset.

Maximum likelihood (ML) analyses were conducted using the 'Tree Inference' tool on the IQ-TREE server (Nguyen *et al.* 2015), using edge-linked partitions, and specifying the model GTR+F+I+G4 for both genes. Branch support was assessed by ultrafast bootstrapping (UFBoot) (Hoang *et al.* 2017) and SH-aLRT branch tests (Guindon *et al.* 2010), both with 1000 replicates. Bayesian inference (BI) was carried out using the Metropolis-coupled Markov Chain Monte Carlo (MC3) algorithm, implemented in MrBayes ver. 3.2.6 (Ronquist *et al.* 2012) on the CIPRES Science Gateway (Miller *et al.* 2010). Two partitions, each under the GTR+F+I+G4 model, were specified (as inferred by ModelFinder). Two

independent runs were conducted simultaneously for 10 000 000 generations, each including one cold and three heated chains. Trees were sampled every 1000th generation, the first 25% being discarded as burn-in. Chain convergence was confirmed by the average standard deviation of split frequencies dropping below 0.01, the potential scale reduction factor approaching 1.0, and the log probability reaching a stationary distribution. The obtained topologies were summarised in a majority-rule consensus tree. Inferred posterior probabilities (pp) were employed as support values. ML and BI trees were visualised and rooted in FigTree ver. 1.4.4 (Rambaut 2006–2019). Weakly-supported clades (pp < 0.95, SH-aLRT < 80, and UFboot < 95) were collapsed.

Abbreviations

b	=	bursa
e	=	eyes
eg	=	egg
h	=	hook
od	=	oviduct
ov	=	ovary
ph	=	pharynx
pv	=	prostate vesicle
rod	=	rod-shaped proximal part of the stylet
sp, sp1, sp2	=	distal spiny plates of the stylet
sr	=	seminal reservoir
st	=	stylet
sv	=	seminal vesicle
vi	=	vitellaria

Institutional abbreviations

HU = Hasselt University
ZMH = Museum of Nature Hamburg

Results

Phylogenetic analyses

The analysed datasets had an extension of 1795 bp and 1757 bp for 18S and 28S rDNA partial genes, respectively (concatenated dataset of 3552 bp). The molecular phylogenetic analyses comprised sequences from 93 specimens, four of which were sequenced for this study (Table 1). The obtained topologies were convergent, and the BI tree is presented in Fig. 1. The phylogenetic result fully fits the one provided by Diez & Schmidt-Rhaesa (2024), and, therefore, we restrict the analysis to species of *Castrella*.

All species of *Castrella* form a fully supported clade (pp = 1; SH-aLRT = 100; UFboot = 100), which is sister to all other representatives of Dalyelliidae (pp = 1; SH-aLRT = 100; UFboot = 100) (Fig. 1). Within the genus, two clades are recovered, one including *C. pinguis* (Silliman, 1884) Fuhrmann, 1900 and *C. truncata* (pp = 0.99; SH-aLRT = 96.1; UFboot = 100), and the other including *C. alba* Luther, 1955 and *C. schareri* sp. nov. (pp = 0.95; SH-aLRT = 80.9; UFboot = 96). Five sequenced specimens of *C. schareri* sp. nov., including one previously identified as *C. truncata* by Diez & Schmidt-Rhaesa (2024), form a highly supported cluster (pp = 0.99; SH-aLRT = 96.1; UFboot = 96).

Table 1 (continued on next two pages). GenBank accession numbers of the specimens used in the phylogenetic analyses (*new sequences).

Species	18S rDNA	28S rDNA
Acrochordonoposthia conica	KC529487	KC529617
Bothromesostoma personatum	KC529501	–
Bothromesostoma personatum	M58347	–
Bothromesostoma sp.	D85098	–
Bryoplana xerophila	KC529489	KC529619
Carcharodopharynx sp.	KC529481	KC529612
Castrada hofmanni	KC529496	–
Castrada intermedia	KC529497	–
Castrada lanceola	AY775751	–
Castrada luteola	AY775752	–
Castrada neocomensis	KC529498	–
Castrada viridis	AY775753	–
Castrada sp.	AY775775	–
Castrella alba D-282	PQ722297	PQ722314
Castrella alba D-283	PQ722298	PQ722315
Castrella alba G-29	PQ722299	PQ722316
Castrella pinguis	KC529438	KC529569
Castrella schareri sp. nov. B-20*	–	PX577035
Castrella schareri sp. nov. B-21	PQ722300	PQ722317
Castrella schareri sp. nov. F-271*	–	PX577036
Castrella schareri sp. nov. F-274*	–	PX577037
Castrella schareri sp. nov. F-275*	–	PX577038
Castrella truncata	AY775777	KC529570
Dalyellia tatrica	KC529443	KC529574
Dalyellia viridis	KC529444	KC529575
Dalyelliidae indet.	KC529441	–
Dochmiotrema limicola	KC529495	KC529624
Dochmiotrema sp.	PP723168	PP723167
Gieysztoria acariaia	KC529470	KC529601
Gieysztoria ashokae	KC529466	KC529597
Gieysztoria beltrani	KC529475	KC529606
Gieysztoria cf. *billabongensi*s	KC529442	KC529573
Gieysztoria cf. *cuspidata*	KC529457	KC529588
Gieysztoria choctaw	KC529476	KC529607
Gieysztoria complicata	KC529473	KC529604
Gieysztoria cuspidata	KC529458	KC529589
Gieysztoria dodgei	KC529479	KC529610
Gieysztoria garudae	KC529467	KC529598

Table 1 (continued). GenBank accession numbers of the specimens used in the phylogenetic analyses

Species	18S rDNA	28S rDNA
Gieysztoria iberica	KC529461	KC529592
Gieysztoria infundibuliformis	KC529468	KC529599
Gieysztoria knipovici	KC529463	-
Gieysztoria ornata	KC529460	KC529591
Gieysztoria pavimentata	KC529472	KC529603
Gieysztoria rubra	KC529480	KC529611
Gieysztoria triquetra	KC529478	KC529609
Gieysztoria zuluensis	KC529465	KC529596
Krumbachia sp.	KC529488	KC529618
Krumbachia hiemalis D-159	PQ722301	PQ722322
Krumbachia hiemalis D-160	PQ722302	PQ722323
Mesocastrada sp.	U70081	-
Mesostoma jilinense	-	ON843458
Mesostoma lingua	AY775759	KC529626
Mesostoma lingua AY775759	AY775759	-
Mesostoma lingua AJ243682	AJ243682	-
Mesostoma thamagae	AY775760	-
Microdalyellia armigera Finland	KC529451	KC529582
Microdalyellia armigera Spain	KC529452	KC529583
Microdalyellia brevispina	KC529450	KC529581
Microdalyellia fairchildi	KC529447	KC529578
Microdalyellia fusca	KC529453	KC529584
Microdalyellia kupelwieseri Switzerland B-11	PQ722303	PQ722320
Microdalyellia kupelwieseri Switzerland B-12	PQ722304	PQ722321
Microdalyellia nanella	KC529449	KC529580
Microdalyellia picta	KC529446	KC529577
Microdalyellia rossi	KC529448	KC529579
Microdalyellia schmidtii Belgium	KC529445	KC529576
Microdalyellia schmidtii Hamburg D-156	PQ722305	PQ722318
Microdalyellia schmidtii Hamburg D-158	PQ722306	PQ722319
Microdalyellia sinensis	JF429837	-
Microdalyellia sp. ZXY 2011	HQ993095	-
Olisthanella truncula	KC529494	KC529623
Olisthanella truncula AY775761	AY775761	-
Opistomum arsenii	KC529491	KC529620
Phaenocora foliacea	KC529492	KC529621
Phaenocora shenda PHE1-18	ON843455	ON843453
Phaenocora shenda PHE2-18	ON843456	ON843454

Table 1 (continued). GenBank accession numbers of the specimens used in the phylogenetic analyses

Species	18S rDNA	28S rDNA
Phaenocora indet.	KC529493	KC529622
Phaenocora unipunctata	AY775762	-
Protoplanella simplex	KC529490	-
Pseudodalyellia alabamensis	KC529440	KC529571
Rhynchomesostoma rostratum	KC529499	KC529625
Rhynchomesostoma rostratum UH77.15	KC529500	-
Strongylostoma devleeschouweri	KC529486	-
Strongylostoma elongatum	AY775771	-
Strongylostoma elongatum spinosum	KC869830	KC869883
Strongylostoma radiatum	KC529485	KC529616
Strongylostoma sp. c_YC_2021	-	MW930399
Strongylostoma sp. h_YC_2021	-	MW930401
Typhloplana viridata	KC529484	KC529615
Outgroup		
Grappleria corona	MW052803	MW052802
Halammovortex sp.	KC529437	KC529567
Temnocephala fasciata	KC869834	KC869888
Temnosewellia minor	AY157183	AY157164

Taxonomy

Rhabdocoela Ehrenberg, 1831
Dalytyphloplanida Willems *et al.*, 2006
Neotyphloplanida Willems *et al.*, 2006
Limnotyphloplanida Van Steenkiste *et al.*, 2013
Dalyelliidae Graff, 1905
Castrella Fuhrmann, 1900

Castrella schareri sp. nov.
urn:lsid:zoobank.org:act:805206A8-C33D-4D74-9FF3-FD645A71E9CB
Figs 1, 2A–E, 3

Diagnosis

Species of *Castrella* with a ~66-µm long stylet. It shows a proximal rod-shaped part and distally, the stylet exhibits a single spiny plate, bearing numerous 5–9-µm-long, fine spines, and a lateral, triangular, 21–22 µm long hook.

Etymology

Species dedicated to my dear colleague Prof. Dr Lukas Schärer (Basel University, Switzerland) thanks to his enormous contribution to the study of microturbellarians, particularly Macrostomorpha. Lukas also collected and provided me with the material of the new species here described.

Material examined

Holotype

SWITZERLAND • whole mount; Basel, Röserental; 47°29′35.0″ N, 07°41′37.8″ E; type locality; 12 Apr. 2024; in mosses in a spring; ZMH V13888.

Other material

Switzerland • (Observations on live animals); 2 whole mounts; same data as for holotype; HU XXVI.3.23–XXVI.3.24. • 1 specimen used for molecular analyses; same data as for holotype. • 3 specimens used for molecular analyses; Ticino, Centovalli; 46°9′47.19″ N, 08°38′17.94″ E; 8 Sep. 2024; in mosses over rocks in a small river.

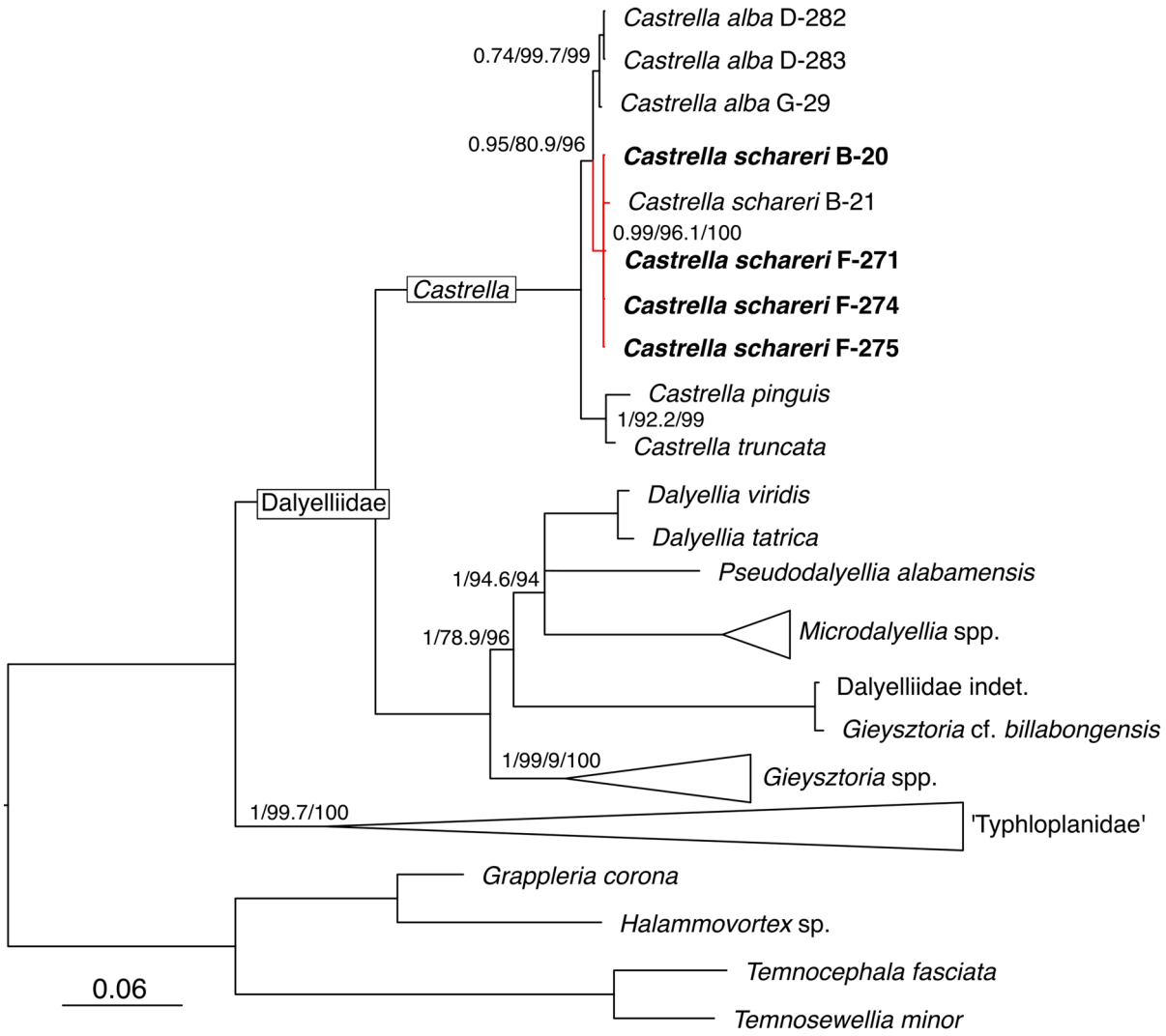

Fig. 1. Majority-rule consensus tree from the Bayesian analysis of the concatenated 18S + 28S rDNA dataset. Branches with support values below the thresholds in the legend of the three analyses were collapsed. Support values are represented in the order of posterior probabilities / SH-aLRT / ultrafast bootstrap. Branches without symbols have pp = 1, SH-aLRT = 100, and UFboot = 100. New sequences obtained for this study are highlighted in bold.

Description

Live animals (Fig. 2A–B) measuring 0.46 – 0.69 mm long ($\bar{x}$ = 0.58 mm; n = 5), the anterior end is broad and the posterior pointy. They are brownish pigmented due to the presence of fine pigment granules in the epidermis. A pair of eyes (Fig. 2A–B: e) is located posterior to the anterior body's margin. The eyes are bilobed and give the appearance of four eyes; however, both pigmented spots are connected, and they probably seem more separate because of the squeezing. Posterior to the eyes, the doliiform pharynx is located (Fig. 2A–B: ph). The gut contains diatoms.

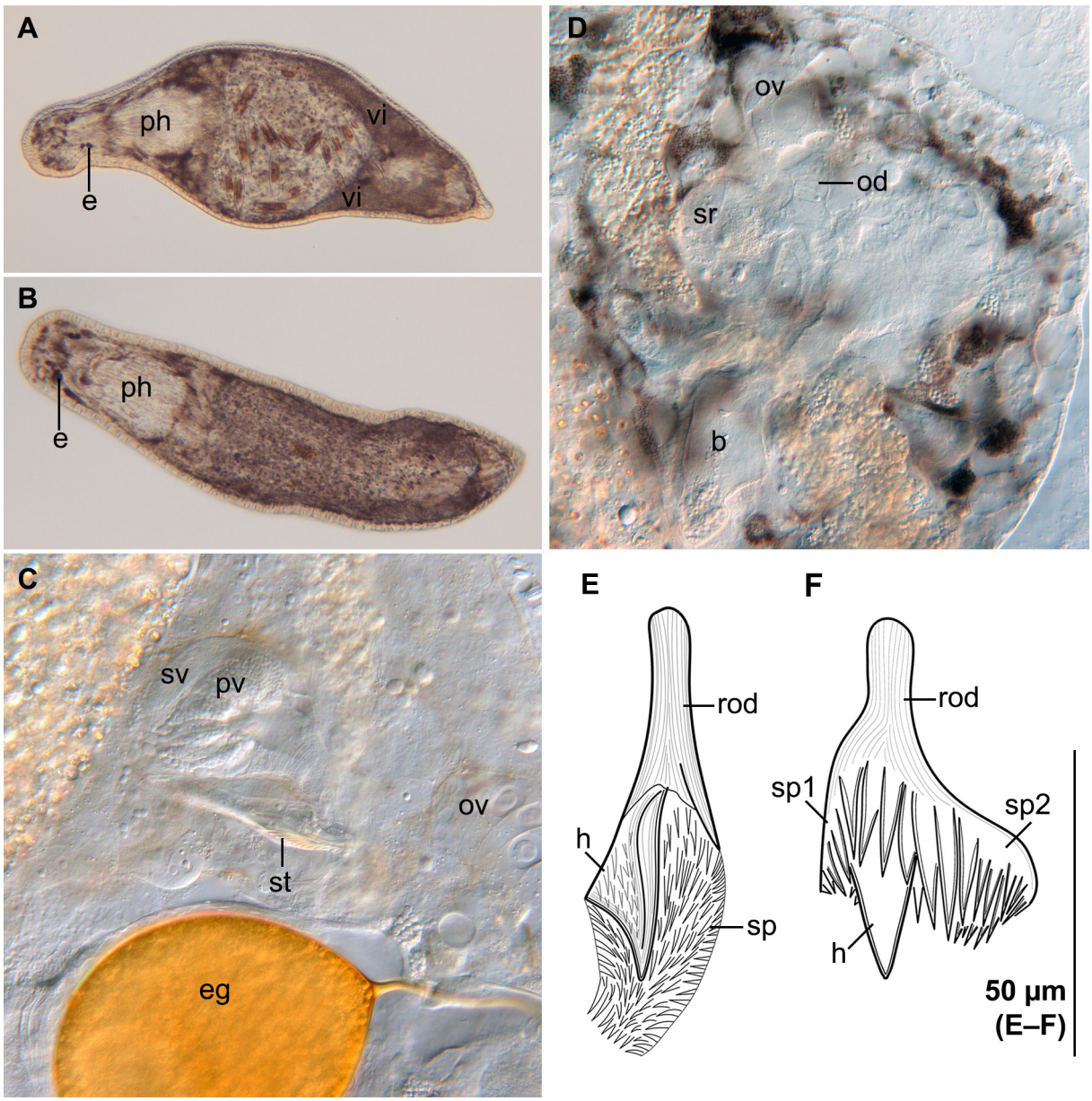

Fig. 2. A–E. *Castrella schareri* sp. nov., holotype (ZMH V13888). **A–B**. Habitus of live specimens. **C–D**. Micrographs of atrial organs. **E**. Stylet. – **F**. *Castrella truncata* (Abildgaard, 1789) Sekera 1906, specimen from Ontario (HU V.4.10), stylet. Abbreviations: see Material and methods.

The atrial organs, testes, and ovaries are located in the posterior body's third. The vitellaria are brownish and run along the body's sides (Fig. 2A: vi), between the pharynx and the posterior end. Posteriorly, they fuse before entering the oviduct. The stylet (Fig. 2C: st, 2E, 3) has the typical structure of that in known species of *Castrella*; it shows a proximal rod-shaped part (Figs 2E, 3: rod) and a distal spiny plate (Figs 2E, 3: sp). It is 56–80 µm ($\bar{x}$ = 66 µm; n = 3). The proximal part is 20–30 µm ($\bar{x}$ = 24 µm; n = 3), representing about 36% of the total stylet length. The distal part carries more than 90 fine spines measuring 5–6 µm in the holotype to 7–9 µm in the other specimens. On one lateral side of the spiny plate, there is a strong triangular hook measuring 21–22 µm (n = 2) (Figs 2E, 3B–D: h). The copulatory bulb receives proximally the vas deferens and the necks of extracapsular prostatic glands. The ejaculatory duct forms an internal seminal vesicle (Fig. 2C: sv) in the proximal part of the bulb, which is followed by the prostate vesicle (Fig. 2C: pv). The ejaculatory duct opens into the male atrium at the level of the spiny plate of the stylet.

The structure of the female system does not differ from that in other species of *Castrella*. The ovary (Fig. 2C–D: ov) has the oocytes organised in a row, and increasing in size from proximal to distal; the most anterior part of the ovary exhibits a proliferation area without differentiated oocytes. Distally, the ovary connects to the oviduct (Fig. 2D: od), and close to it opens the common vitelloduct. A seminal receptacle (Fig. 2D: sr) also empties in the anterior part of the oviduct. Both the oviduct and the bursa (Fig. 2D: b) open into the common atrium.

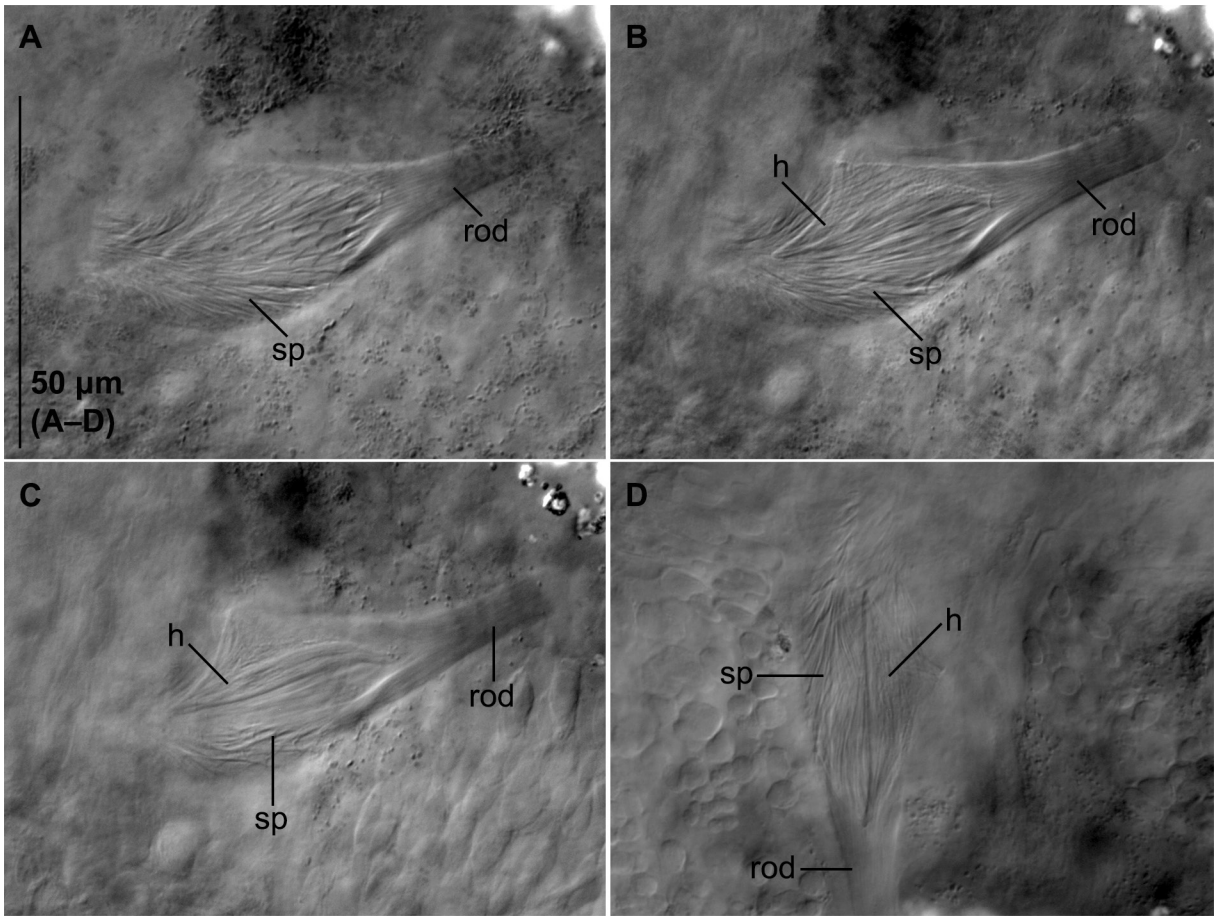

Fig. 3. Micrographs of the stylet in *Castrella schareri* sp. nov. **A–C**. Holotype (ZMH V13888). **D**. Reference specimen (HU XXVI.3.23). Abbreviations: see Material and methods.

Discussion

The genus *Castrella* currently comprises seven valid species (Tyler *et al.* 2006–2025), six of which are assigned to the subgenus *Castrella* and one to *Nasonoviella* Luther, 1955. In this study, we describe an eighth species within the genus. Our phylogenetic analysis confirms the monophyly of *Castrella* and reveals two distinct subclades: one comprising the Nearctic species *C. pinguis*, and the circum-boreal *C. truncata*, and the other consisting of Palearctic species (*C. alba* and *C. schareri* sp. nov.). Despite the wide distribution of *C. truncata* (see Van Steenkiste *et al.* 2011 for a summary), the variation in the morphology of its stylet has not been interpreted as evidence of cryptic diversity (see further discussion).

Diez & Schmidt-Rhaesa (2024) identified two distinct phylogenetic lineages within *C. truncata*: one based on a specimen from Ontario, Canada (included in the phylogeny by Van Steenkiste *et al.* 2013), and another from Basel, Switzerland. Although their study focused on microturbellarians from Hamburg, Germany, they included the Swiss specimen in their molecular analysis, but its morphological assignment remained tentative.

Building on their findings, I re-examined morphological vouchers from the Basel specimens and sequenced a second specimen from the same locality. Additionally, three more specimens were collected in Centovalli, Switzerland. All were photo-vouchered and used for molecular analyses. The inclusion of these new sequences clarified internal relationships within *Castrella* and confirmed that the Swiss specimens represent a species distinct from *C. truncata*, a conclusion also supported by detailed morphological assessment.

Morphological characterization within *Castrella* has historically been limited. Species were described between 1789 and 1955, often based on incomplete documentation of the stylet, and type material is unavailable. However, recent contributions by Van Steenkiste *et al.* (2011) and Diez & Schmidt-Rhaesa (2024) have provided high-quality morphological data for three species: *C. alba*, *C. pinguis*, and *C. truncata*. In his monograph on dalyelliids, Luther (1955) offered illustrations and descriptions for all known species of *Castrella*. However, identifying *C. truncata* is particularly challenging due to its broad distribution, extensive synonymy, and high variability in stylet morphology (Hofsten 1910). Original descriptions often lack measurements of the stylet, and interpretations of its structure vary considerably across authors.

Castrella (*Nasonoviella*) *lutheri* (Nasonov, 1917) Luther, 1955 is distinguishable from other species of *Castrella* by a stylet with two proximal parts connected distally to a spiny plate (Nasonov 1917, 1925; Luther 1955). *Castrella alba*, the closest phylogenetic relative of *C. schareri* sp. nov., possesses a markedly different stylet: delicate, small, and with a reduced proximal part (Luther 1955; Diez & Schmidt-Rhaesa 2024). A specimen from Brandenburg, Germany, misidentified as *C. alba* by Diez & Schmidt-Rhaesa (2024: fig. 7a–c), in fact, bears a stylet more consistent with *C. truncata*—an error likely due to the specimen being studied only alive prior to DNA extraction and unsuccessful PCR amplification. Reexamination of the German specimens of *C. alba* (excluding the misidentified one) led to an updated stylet size: 43–60 µm in length ($\bar{x}$ = 52 µm; n = 2), rod-shaped portion measuring 14–20 µm ($\bar{x}$ = 17 µm; n = 2), and distal hook 14–22 µm long ($\bar{x}$ = 18 µm; n = 2). *Castrella alba* is thus distinguished from *C. schareri* sp. nov. by its shorter stylet proximal part (26% vs 36% of the total stylet length) and its ~18-µm-long, claw-shaped distal hook (triangular and 21–22 µm long in *C. schareri* sp. nov.). As noted by Luther (1955) and Diez & Schmidt-Rhaesa (2024), the stylet in *C. alba* is extremely delicate and vanishes after fixation, in contrast to the well-preserved stylet in whole-mounted specimens of *C. schareri* sp. nov.

Castrella cilindrica Riedel, 1932 is primarily identified by its body length, which is about nine times its width, while its sclerotised organ is simply described as a long tube ending in a fan-shaped structure

(Riedel 1932; Luther 1955). *Castrella groenlandica* Riedel, 1932 features a stylet with two distal arms, each bearing four spines, fewer than in other species (Riedel 1932; Luther 1955). The largest stylet among known species is found in *C. pinguis* (152–172 µm), with its distal spines arranged in three groups: two with fine spines (one significantly larger), and one with strong, broad, triangular spines (Van Steenkiste *et al.* 2011). *Castrella vernalis* Beklemischev, 1921 is characterised by a proximally broad stylet with 15–17 fine distal spines (Beklemischev 1921). Given the above, the morphological comparison of *C. schareri* sp. nov. should focus specifically on its distinction from *C. truncata*.

The stylet of *C. truncata* has variously been described as having two spiny distal branches and one or two strong hooks (Fuhrmann 1900; Hofsten 1907, 1910; Van Steenkiste *et al.* 2011). Other accounts mention configurations with one spiny branch and two hooks (Dorner 1902; Plotnikow 1905), or with two (Vejdovsky 1895) or four (Graff 1882) spiny branches. Reviewing previous descriptions, Hofsten (1910) attributed much of this variation to interpretative differences and proposed that his earlier depiction of *C. truncata* stylet (Hofsten 1907: fig. 16) should be considered definitive. He described the stylet as having two spiny branches, one larger than the other, bearing 12 and 12–13 spines respectively, along with a complex central hook, illustrated as two strong triangular spines.

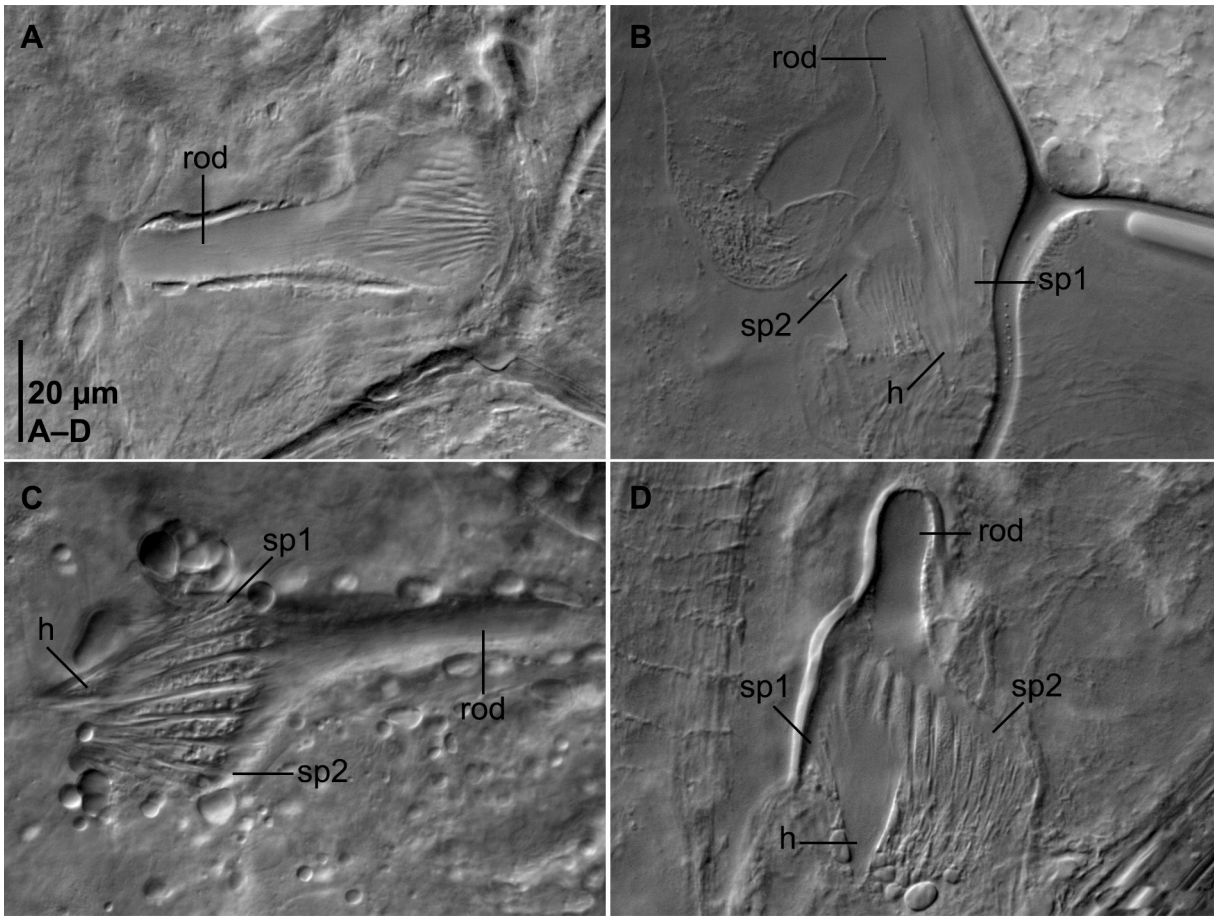

Fig. 4. Stylet micrographs in different specimens of *Castrella truncata* (Abildgaard, 1789) Sekera 1906. **A**. Specimen from Belgium (HU II.1.44). **B**. Specimen from Finland (HU VII.1.29). **C–D**. Specimens from Ontario (HU V.4.09 and HU V.4.10, respectively). Abbreviations: see Material and methods.

The examination of specimens of *C. truncata* from Canada, Belgium, and Finland reveals a stylet with two distal spiny branches and a central, strong, triangular hook (Figs 2F, 4). In contrast, *C. schareri* sp. nov. lacks differentiated spiny branches and instead has a broad spiny plate. Moreover, in *C. schareri* sp. nov., the hook is attached asymmetrically to one side of the spiny plate, whereas in *C. truncata*, it is more centrally positioned. The hook in *C. truncata* typically extends beyond the distal spines, while in *C. schareri* sp. nov., the longest extension corresponds to the spines, with the hook remaining shorter.

Further distinguishing features include the proportion of the proximal stylet segment relative to the spiny portion, which is significantly shorter in *C. schareri* sp. nov. ($\bar{x}$ = 36%; n = 3) compared to *C. truncata* ($\bar{x}$ = 47%; n = 5). Additionally, the spines of *C. schareri* sp. nov. are shorter (5–9 µm) than those in *C. truncata*, which range from 10–11 µm in the Belgium population (Fig. 4A), 12–23 µm in the specimen from Finland (Fig. 4B), and 11–26 µm in the specimen from Ontario (Figs 2F, 4C–D). Spine arrangement also differs; in *C. truncata*, spines are aligned in a row along the spiny branches, while in *C. schareri* sp. nov., they are dispersed across the entire spiny plate without forming rows.

Together, the phylogenetic and morphological evidence confirms the distinctiveness of *C. schareri* sp. nov. and underscores the need for intensified sampling efforts, even in well-studied regions such as Europe. This study highlights the importance of combining morphological and molecular data in species delimitation. It offers a revised perspective on *Castrella* and demonstrates that Europe, despite being considered well-documented, still harbours undescribed microturbellarian diversity. Comprehensive surveys and molecular phylogenetics are critical to advancing our understanding of these invertebrates.

Finally, the considerable variation in stylet morphology observed in *C. truncata* suggests the potential presence of additional cryptic species. Unfortunately, due to inadequate historical descriptions and the absence of type material, it is currently not feasible to reassess the species previously synonymised under *C. truncata*. As such, this study represents an important milestone in the revitalization of microturbellarian taxonomy in Europe, culminating in the first description of a new species of *Castrella* in over 70 years.

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References

Adami M.L. & Damborenea,C. 2024. A new species of *Gieysztoria* Ruebush & Hayes, 1939 (Platyhelminthes, Rhabdocoela, Dalyelliidae) from Argentina with comments on geographical distribution of the genus in the Neotropical region. *Zoosystema* 46 (28): 723–732. https://doi.org/10.5252/zoosystema2024v46a28

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Beklemischev V.N. 1921. Matériaux concernant la systématique et la faunistique des Turbellaria de la Russie de l'Est. *Bulletin de l'Academie de Sciences de Russie* 6 (15): 631–656. [In Russian.]

Benson D.A., Karsch-Mizrachi I., Clark K., Lipman D.J., Ostell J. & Sayers E.W. 2012. GenBank. *Nucleic Acids Research* 40: D48–D53. https://doi.org/10.1093/nar/gks1195

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17 (4): 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Diez Y.L. & Schmidt-Rhaesa A. 2024. Little neighbours in Hamburg: free-living aquatic flatworms (Platyhelminthes). *Evolutionary Systematics* 8: 279–310. https://doi.org/10.3897/evolsyst.8.139468

Dorner G. 1902. Über die Turbellarienfauna Ostpreussens. *Zoologischer Anzeiger* 25: 491–493.

Fuhrmann O. 1900. Note sur les Turbellariés des environs de Genève. *Revue suisse de Zoologie* 7: 717–731.

Graff L. v. 1882. *Monographie der Turbellarien. I. Rhabdocoelida*. Verlag von Wilhelm Engelmann, Leipzig.

Guindon S., Dufayard J.-F., Lefort V., Anisimova M., Hordijk W. & Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59 (3): 307–321. https://doi.org/10.1093/sysbio/syq010

Hoang D.T., Chernomor O., Von Haeseler A., Minh B.Q. & Vinh L.S. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35 (2): 518–522. https://doi.org/10.1093/molbev/msx281

Hofsten N. v. 1907. Studien über Turbellarien aus dem Berner Oberland. *Zeitschrift für wissenschaftliche Zoologie* 85: 391–654.

Hofsten N. v. 1910. Zur Synonymik und systematischen Stellung von *Castrella truncata*. *Zoologischer Anzeiger* 35(21): 652–669.

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermiin L.S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14 (6): 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K. & Standley D.M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30 (4): 772–780. https://doi.org/10.1093/molbev/mst010

Katoh K., Rozewicki J. & Yamada K.D. 2017. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings Bioinformatics* 20 (4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. & Drummond A.A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647–2164. https://doi.org/10.1093/bioinformatics/bts199

Lanfear R., Calcott B., Ho S.Y.W. & Guindon S. 2012. Partition Finder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. https://doi.org/10.1093/molbev/mss020

Laumer C. 2023. Picogram input multimodal sequencing (PiMmS) V.1. https://doi.org/10.17504/protocols.io.rm7vzywy5lx1/v1

Lessa T., Stropp J., Hortal J. & Ladle R.J. 2024. How taxonomic change influences forecasts of the Linnean shortfall (and what we can do about it)? *Journal of Biogeography* 51 (8): 1365–1373. https://doi.org/10.1111/jbi.14829

Lima M., Salles F.F., de Andrade A.F.A., Dias-Silva K., Juen L. & Brasil L.S. 2024. Linnean and Wallacean shortfalls of Leptophlebiidae (Ephemeroptera: Insecta) in the Brazilian Amazon. *Aquatic Conservation: Marine and Freshwater Ecosystems* 34(5): e4163. https://doi.org/10.1002/aqc.4163

Lu Y.-H., Wu C.-C., Xia X.-J., Wang A.-T. 2013. Two new species of *Gieysztoria* (Platyhelminthes, Rhabdocoela, Dalyelliidae) from a freshwater artificial lake in Shenzhen, China. *Zootaxa* 3745 (5): 569–578. https://doi.org/10.11646/zootaxa.3745.5.5

Luther A. 1955. Die dalyelliiden (Turbellaria Neorhabdocoela), eine Monographie. *Acta Zoologica Fennica* 87: 1–337.

Marcus E. 1946. Sobre Turbellaria Brasileiros. *Boletim Faculdade Filosofia, Ciencias e Letras Universidade Sao Paulo, Zoologia* 11: 5–254.

Miller M.A., Pfeiffer W. & Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop 2010*: 1–8. https://doi.org/10.1109/GCE.2010.5676129

Nasonov N.V. 1917. Sur la faune des Turbellaria de Finlande I. *Bulletin de l'Académie des Sciences de Russie* 14: 1095–1112. [In Russian.]

Nasonov N.V. 1925. La faune des Turbellaria de la presquîle de Kola. *Bulletin de l'Académie des Sciences de Russie* 19 (1–5): 53–74. [In Russian.]

Nguyen L.-T., Schmidt H.A., von Haeseler A. & Minh B. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32 (1): 268–274. https://doi.org/10.1093/molbev/msu300

Plotnikow W. 1905. Uber einige rhabdocöle Turbellarien Sibiriens. *Zoologische Jahrbücher, Abteilung für Systematik, Geographie und Biologie der Tiere* 21: 479–490

Rambaut A. 2006–2019. FigTree: Tree Figure Drawing Tool. Available from http://tree.bio.ed.ac.uk/software/figtree/ [accessed 1 Jun. 2025].

Reyes J., Binow D., Vianna R.T., Brusa S. & Martins S.E. 2021. Free-living microturbellarians (Platyhelminthes) from wetlands in Southern Brazil, with the description of three new species. *Zoological Studies* 60: 221–233. https://doi.org/10.6620/ZS.2021.60-22

Riedel G. 1932. Ergebnisse einer von E. Reisinger & O. Steinböck mit Hilfe das Rask-Orsted Fonds durchgeführten zoologischen Reise in Grönland 1926. 3. Macrostomida. 4. Dalyelliidae. *Videnskabelige Meddelelser fra Dansk naturhistorisk Forening* 94: 33–90.

Rong C., Liu X., Zhao J. & Wang A. 2016. Two new planarian species of Dalyelliidae (Platyhelminthes: Rhabdocoela). *Sichuan Journal of Zoology* 35(1): 97–103. [In Chinese.]

Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Hohna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2 efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schockaert E.R. 1996. Turbellarians. *In*: Hall G.S. (ed.) *Methods for the Examination of Organismal Diversity in Soils and Sediments*: 211–225. CAB International, Wallingford.

Schwartz S.S. & Hebert P.D. 1986. Prey preference and utilization by *Mesostoma lingua* (Turbellaria, Rhabdocoela) at a low arctic site. *Hydrobiologia* 135: 251–257. https://doi.org/10.1007/BF00006537

Sekera E. 1906. Über die Verbreitung der Selbstbefruchtung bei den Rhabdocoeliden. *Zoologischer Anzeiger* 30: 142–153.

Tessens B., Janssen T. & Artois T. 2014. Molecular phylogeny of Kalyptorhynchia (Rhabdocoela, Platyhelminthes) inferred from ribosomal sequence data. *Zoologica Scripta* 43 (5): 519–530. https://doi.org/10.1111/zsc.12066

Trifinopoulos J., Nguyen L.-T., von Haeseler A. & Minh B.Q. 2016. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W232–W235. https://doi.org/10.1093/nar/gkw256

Tüzün N., Lemke N., Diez Y.L., Artois T. & Monnens M. 2025. Tiny killers: first record of Rhabdocoel flatworms feeding on water flea embryos. *Ecology and Evolution* 15: e71277. https://doi.org/10.1002/ece3.71277

Tyler S., Artois T., Schilling S., Hooge M. & Bush L.F. (eds). 2006–2025. World list of turbellarian worms: Acoelomorpha, Catenulida, Rhabditophora. Dalyelliidae Graff, 1905. Available from https://www.marinespecies.org/aphia.php?p=taxdetails&id=142081 [accessed 1 Jun. 2025].

Van Steenkiste N.W.L., Gobert S., Davison P., Kolasa J. & Artois T. 2011. Freshwater Dalyelliidae from the Nearctic (Platyhelminthes, Rhabdocoela): new taxa and records from Ontario, Canada and Michigan and Alabama, USA. *Zootaxa* 3091 (1): 1–32. https://doi.org/10.11646/zootaxa.3091.1.1

Van Steenkiste N.W.L., Tessens B., Willems W., Van Mulken E. & Artois T. 2012. The "Falcatae", a new Gondwanan species group of *Gieysztoria* (Platyhelminthes: Dalyelliidae), with the description of five new species. *Zoologischer Anzeiger* 251 (4): 344–356. https://doi.org/10.1016/j.jcz.2011.12.001

Van Steenkiste N.W.L., Tessens B., Willems W., Backeljau T., Jondelius U. & Artois T. 2013. A comprehensive molecular phylogeny of Dalytyphloplanida (Platyhelminthes: Rhabdocoela) reveals multiple escapes from the marine environment and origins of symbiotic relationships. *PLoS ONE* 8 (3): e59917. https://doi.org/10.1371/journal.pone.0059917

Vejdovsky F. 1895. Zur vergleichenden Anatomie der Turbellarien (Zugleiche ein Beitrag zur Turbellarienfauna Bohemens.) I Über die Gattung Opistoma O. Schm. II. Der Geschlechtsapparat der Derostomeen. III. Über zwei Vortex-Arten mit Berücksichtigung deren Geschlechtsorgane. IV. Über die Prorhynchiden Böhmens. V. Zur Kenntnis der Macrostomiden. *Zeitschrift für wissenschaftliche Zoologie* 60 (1): 90–163.

Wang A.-T., Wu H.-L. 2005. A new record genus and three new species of Dalyelliidae (Platyhelminthes, Rhabdocoela, Dalyellioida) from China. *Acta Zootaxonomica Sinica* 30 (2): 300–308. [In Chinese.]

You G.Y., Huang S.X., Xia M.Y., Wang A.T., Zhang Y. & Li S.F. 2021. A New species and new record of freshwater turbellarians of the genus *Gieysztoria* (Platyhelminthes: Rhabdocoela) from China. *Zoological Science* 39 (2): 206–214. https://doi.org/10.2108/zs200103

Young J.O. 1973. The prey and predators of *Phaenocora typhlops* (Vejdovsky) (Turbellaria: Neorhabdocoela) living in a small pond. *The Journal of Animal Ecology* 42 (3): 637–643. https://www.jstor.org/stable/3129

Young J.O. 1978. An ecological study of *Phaenocora unipunctata* (Örsted) (Turbellaria; Rhabdocoela): predator-prey interrelationships. *Hydrobiologia* 60: 145–149. https://doi.org/10.1007/BF00163180

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