

Marine Macrodasyida (Gastrotricha) from Hokkaido, Northern Japan

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Three new species of macrodasyidan gastrotrichs are described from the coasts of Hokkaido, northern Japan. *Cephalodasys mahoae* sp. nov. differs from congeners in having the oocytes developing from posterior to anterior. *Turbanella cuspidata* sp. nov. is characterized by a pair of small, ventrolateral projective organs at U06. *Turbanella lobata* sp. nov. is unique among congeners in having paired lateral lobes on the neck. We inferred the phylogenetic position of *C. mahoae* sp. nov. by maximum-likelihood analysis and Bayesian inference based on 18S rRNA, 28S rRNA, and COI gene sequences from 28 species of macrodasyids. In the resulting trees, *C. mahoae* sp. nov. formed a clade with an unidentified species of *Cephalodasys* Remane, 1926, but not with *C. turbanelloides* (Boaden, 1960).

Key Words: Interstitial, marine invertebrates, meiofauna, Pacific, Sea of Japan.

Introduction

The phylum Gastrotricha currently contains about 850 species of aquatic, microscopic animals (e.g., Todaro *et al.* 2014; Kolicka *et al.* 2017), which are classified into the two orders Chaetonotida and Macrodasyida. The former includes tenpin-shaped, hermaphroditic or parthenogenetic species found in marine, brackish, or freshwater habitats, whereas the latter includes vermiform, hermaphroditic species living interstitially in sand in marine ecosystems (Hummon and Todaro 2010; Kieneke and Schmidt-Rhaesa 2015), with exceptions of two freshwater species (Todaro *et al.* 2012). Macrodasyida currently comprises 365 species classified in 35 genera and 10 families (Hummon and Todaro 2018). Morphologically, macrodasyidans can be distinguished from chaetonotidans by having two pores on each side of the pharynx that allow excess water to be drained during feeding; macrodasyidans possess tubular adhesive glands on both anterior and posterior ends of the body as well as on the lateral surfaces, as opposed to chaetonotidans in which adhesive glands are present only on the posterior end of the body (e.g., Ruppert 1991).

Macrodasyidans have been poorly investigated in Japan, with seven named species so far reported, and only a few records of undetermined species exist for Hokkaido, northern Japan. Saito (1937) described the first macrodasyidan from Japan, *Tetranchyroderma dendricum* Saito, 1937, from Hiroshima. In an investigation of meiofauna around Kasado Island in the Seto Island Sea, Sudzuki (1976) detected five gastrotrich species, including undetermined species in the genera *Cephalodasys* Remane, 1926 and *Macrodasys* Remane,

1924, and described *Paradasys nipponensis* Sudzuki, 1976. Considering the rather inadequate description and illustration, seemingly based on juvenile specimens, however, there is little doubt that *P. nipponensis* must be regarded as *species inquirenda* (Chang *et al.* 2002). Chang *et al.* (2002) later described three species of macrodasyidans—*Tetranchyroderma schizocirratum* Chang, Kubota and Shirayama, 2002, *Thaumastoderma clandestinum* Chang, Kubota and Shirayama, 2002, and *Oregodasys itoi* Chang, Kubota and Shirayama, 2002—from Shirahama, middle Honshu, Japan. Hiruta (2002) mentioned undetermined macrodasyidans from Esashi, Hokkaido. More recently, Lee and Chang (2011) described two species—*Lepidodasys laeviacus* Lee and Chang, 2011 and *L. tsushimaensis* Lee and Chang, 2011—from Tsushima Island in the Korea Strait, between the Japanese mainland and the Korean Peninsula.

During the course of a faunal survey of marine interstitial animals around Hokkaido, we found three species of macrodasyidans that are new to science, one in *Cephalodasys* and the other two in *Turbanella* Schultze, 1853. We inferred the phylogenetic position of the new species of *Cephalodasys* within Macrodasyida by using partial 18S rRNA, 28S rRNA, and COI gene sequences. In this paper, we describe and illustrate these three species, and present the results of the molecular phylogenetic analysis.

Materials and methods

Specimen collection. Specimens were collected from 2012 to 2014 in the intertidal zone at low tide on three beaches around Hokkaido, Japan (Fig. 1). Sediments at the

ground-water level were sampled with a scoop and taken back to the laboratory. Animals were extracted by fresh-water-shock: the sediment was placed in a bucket with tap water, then agitated briefly (~10 sec) 2–3 times; the superna-

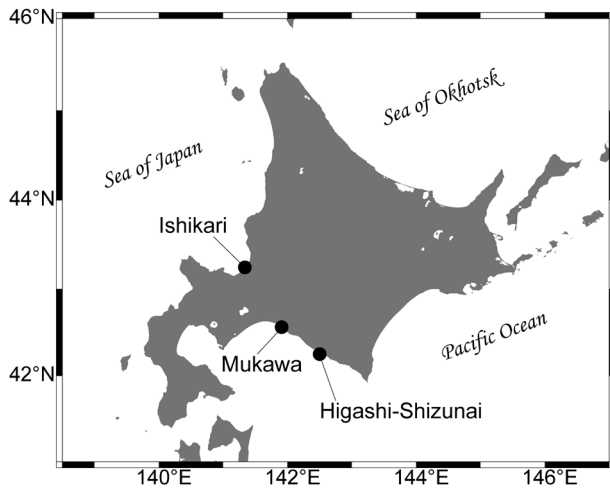


Fig. 1. Map of Hokkaido, showing three sampling localities in this study: Ishikari, Mukawa, and Higashi-Shizunai.

tant was sieved through 30- μ m mesh; the residue was immediately placed in seawater; and living macrodasyidans were collected under a Nikon SMZ-10 stereomicroscope and anaesthetized in a $MgCl_2$ solution isotonic with seawater (~30 psu). Some of the relaxed living specimens were mounted on a glass slide with a cover slip, examined under an Olympus BX51 light microscope, and photographed with a Nikon DS-5Mc digital camera system at several focal depths. Drawings were made from these images by using the GNU image manipulation program GIMP ver. 2.8. Some of the relaxed specimens were fixed in 10% formalin–seawater and mounted in glycerin on a glass slide, with the edges of the coverslip sealed with Canada balsam. Other specimens were observed by scanning electron microscopy (SEM). Material for SEM was fixed overnight in Bouin's solution. After dehydration in a graded ethanol series (50–100%; 30 min each concentration), the specimens were dried in a Hitachi HCP-2 CO_2 critical-point drier, mounted on stubs, coated with gold in a JEOL JFC-1100 ion sputter coater, and examined with a Hitachi S-3000N scanning electron microscope at 15 kV accelerating voltage. Type material has been deposited in the Invertebrate Collection at the Hokkaido University Museum (ICHUM), Sapporo, Japan.

Table 1. List of gastrotrich taxa used in the molecular analysis with GenBank accession numbers.

Species	18S	28S	COI	Reference
<i>Acanthodasys aculeatus</i>	JF357639	JF357687	—	Todaro <i>et al.</i> (2011)
<i>Anandrodasys agadasys</i>	JN203487	—	—	Todaro <i>et al.</i> (2012)
<i>Cephalodasys mahoae</i> sp. nov.	LC018992	LC383934	LC383935	present study
<i>Cephalodasys turbanelloides</i>	AM231776	—	—	Todaro <i>et al.</i> (2006)
<i>Cephalodasys</i> sp. 1	AY963691	—	—	Petrov <i>et al.</i> (2007)
<i>Crasiella</i> sp. 1	JN203488	—	—	Todaro <i>et al.</i> (2012)
<i>Crasiella</i> sp. 2	JF970237	—	—	Papas and Riutort (2012)
<i>Dactylopodola typhle</i>	JF357653	JF357701	JF432038	Todaro <i>et al.</i> (2011)
<i>Diplodasys ankeli</i>	JF357667	—	JF432049	Todaro <i>et al.</i> (2011)
<i>Dolichodasys</i> sp.	AM231778	—	—	Todaro <i>et al.</i> (2006)
<i>Lepidodasys unicarenatus</i>	JF357665	—	JF432039	Todaro <i>et al.</i> (2011)
<i>Macrodasys buddenbrocki</i>	AY963692	KF921012	—	18S, Petrov <i>et al.</i> (2007); 28S, Petrov <i>et al.</i> (unpublished)
<i>Megadasys</i> sp. 1	JF357656	JF357704	—	Todaro <i>et al.</i> (2011)
<i>Megadasys</i> sp. 2	JF357655	JF357703	—	Todaro <i>et al.</i> (2011)
<i>Mesodasys littoralis</i>	JF357658	JF357706	JF432044	Todaro <i>et al.</i> (2011)
<i>Oregodasys tentaculatus</i>	JF357626	JF357674	JF432021	Todaro <i>et al.</i> (2011)
<i>Paradasys</i> sp.	AM231781	—	—	Todaro <i>et al.</i> (2006)
<i>Paraturbanella teissieri</i>	JF357661	JF357709	—	Todaro <i>et al.</i> (2011)
<i>Pleurodasys helgolandicus</i>	JF970236	—	—	Papas and Riutort (2012)
<i>Ptychostomella tyrrhenica</i>	JF357634	JF357682	JF432027	Todaro <i>et al.</i> (2011)
<i>Redudasys fornerise</i>	JN203489	—	KJ950122	Todaro <i>et al.</i> (2012)
<i>Tetranchyroderma thysanophorum</i>	JF357646	JF357694	JF432034	Todaro <i>et al.</i> (2011)
<i>Thaumastoderma moebjergi</i>	JF357671	JF357713	—	Todaro <i>et al.</i> (2011)
<i>Turbanella bocqueti</i>	JF357662	JF357710	JF432046	Todaro <i>et al.</i> (2011)
<i>Urodasys</i> sp. 1	DQ079912	—	—	Sørensen <i>et al.</i> (2006)
<i>Urodasys</i> sp. 2	AY218102	—	—	Giribet <i>et al.</i> (2004)
<i>Xenodasys riedli</i>	JN203490	—	—	Todaro <i>et al.</i> (2012)
<i>Xenodasys</i> sp.	AY228133	—	—	Giribet <i>et al.</i> (2004)
Outgroups				
<i>Xenotrichula intermedia</i>	JF357664	JF357712	JF432048	Todaro <i>et al.</i> (2011)
<i>Xenotrichula velox</i>	JN185488	JN185529	—	Känneby <i>et al.</i> (2012)

DNA extraction and amplification. DNA was extracted from 80 pooled specimens of *Cephalodasys mahoae* sp. nov. collected from Ishikari beach by using a DNeasy Blood & Tissue Kit (QIAGEN). Amplification of partial 18S rRNA, 16S rRNA, and COI genes was carried out using universal primers: 1F/9R (Giribet *et al.* 1996) for 18S; 16sar-L/16sbr-H (Palumbi *et al.* 1991) for 16S; LCO1490/HCO 2980 (Folmer *et al.* 1994) for COI. For 28S rRNA gene amplification, the primer pair 28Sa (Whiting *et al.* 1997) and 28S_3KR (Yamasaki *et al.* 2013) were used. Reactions were performed with an iCycler thermal cycler (Bio-Rad Laboratories) in final volumes of 10 μ l. PCR conditions were 0.5 min at 95°C; 30 cycles of 0.5 min at 95°C, 0.5 min at 45°C and 1.5 min (18S and 28S) or 0.5 min (16S and COI) at 72°C; and 7 min at 72°C. PCR products were purified with silica method (Boom *et al.* 1990). Direct sequencing was performed with BigDye Terminator ver. 3.1 (Life Technologies) reagents and a 3730 DNA analyzer (Life Technologies), using amplification primers, as well as internal primers for 18S [5R and 3F (Giribet *et al.* 1996); 18Sbi and S2.0 (Whiting *et al.* 1997)] and 28S [rd4.8a, rd5b, and rd7b1 (Schwendinger and Giribet 2005); F2012 (Giribet *et al.* 2010)]. Newly determined sequences have been deposited in DDBJ accession numbers LC018992 (18S), LC383934 (28S), LC383933 (16S), and LC383935 (COI).

Alignment and phylogenetic analyses. To assess the phylogenetic affinities of *Cephalodasys mahoae* sp. nov., our analyses included 28 species of macrodasyidans, and the chaetonotidan *Xenotrichula intermedia* Remane, 1934 and *X. velox* Remane, 1934 as an outgroup (Table 1). For 18S and 28S, alignment of the sequences was performed by using MAFFT ver. 7 (Katoh and Standley 2013), employing the *E-INS-i* strategy. For COI, sequences were aligned by MUSCLE (Edgar 2004) implemented in MEGA ver. 6.0 (Tamura *et al.* 2013), using the *Align Codons* option. Alignment-ambiguous areas were removed by using Gblocks ver. 0.91b (Castresana 2000), allowing smaller final blocks, gap positions within the final blocks, and less strict flanking positions, but not allowing many contiguous non-conserved positions. After eliminating ambiguous sites, each data set was 1617bp (18S), 1996bp (28S), and 624bp (COI) long, respectively. To determine the best partition scheme for maximum-likelihood (ML) analysis and Bayesian inference (BI), PartitionFinder ver. 2.1.1 (Lanfear *et al.* 2017) was used employing the *greedy* algorithm. For BI, the most suitable substitution model for each partition selected by PartitionFinder ver. 2.1.1 (Lanfear *et al.* 2017) was GTR+I+G for 18S+28S and COI (1st codon); GTR+G for COI (2nd codon); and HKY+I+G for COI (3rd codon). ML analysis was performed by using RAxML ver. 8.0.0 (Stamatakis 2014) with GTR+G model of nucleotide substitution for all partitions consisting of 1000 rapid bootstraps. BI was carried out using MrBayes ver. 3.2.3 (Ronquist and Huelsenbeck 2003; Altekar *et al.* 2004) with two independent Metropolis-coupled analyses (four Markov chains of 50,000,000 generations for each analysis). Trees were sampled every 100 generations. Values of run convergence indicated that sufficient amounts of trees and parameters were sampled (average standard deviation of split frequencies=0.001120; minimum effective sample size

of tree lengths=340.20; potential scale reduction factor of tree lengths=1.009). Run convergence was also assessed with Tracer ver. 1.7 (Rambaut *et al.* 2018); effective sample sizes for all parameters were above 200.

Abbreviations and conventions. Morphological symbols and conventions follow those of Hummon (2010): L, length; H, height; Lt, total body length from anterior tip of head to posterior tip of caudum and its adhesive tubes; PhJIn, junction between pharynx and intestine; LPh, pharyngeal length from anterior tip of head to PhJIn; TbA/TbL/TbD/TbDL/TbV/TbVL/TbP, adhesive tubes of the anterior, lateral, dorsal, dorso-lateral, ventral, ventro-lateral, and posterior (caudal) groups; U, percentage units of Lt from anterior to posterior.

Results

Taxonomy

Order **Macrodasysida** Remane, 1925

Family **Cephalodasyidae** Hummon and Todaro, 2010

Genus ***Cephalodasys*** Remane, 1926

Cephalodasys mahoae sp. nov.

(Figs 2, 3)

Material examined. Holotype, ICHUM 4977 (adult), mounted on glass slide, Mukawa, Hokkaido, Japan (42°33.417'N, 141°55.724'E), surface layer of medium-grained sand at water's edge, 28 June 2012. Two paratypes (mounted on glass slides): ICHUM 4978 (adult), same collection data for holotype; ICHUM 4979 (subadult), Ishikari beach, Hokkaido, Japan (43°15.420'N, 141°21.438'E), medium-grained sand, 55 cm depth, 5 m landward from high-water line, 27 August 2013. Three additional specimens (two adults, one subadult) collected together with holotype, lost after observation. About 10 specimens from Ishikari beach, 20 November 2011, lost after observation.

Etymology. The specific name is after Ms Maho Ikoma, who assisted in sampling.

Description. *Habitus.* Adult Lt 410–460 μ m (435 μ m in holotype); L of anterior end to PhJIn (at U31–36) 140–160 μ m (157 μ m in holotype); body transparent, flattened ventrally, transversely convex dorsally; head bearing shallow lateral lobes at U06 (Figs 2A, 3A); neck constriction inconspicuous at U09–11; trunk widest in mid-body region, tapering gradually to caudal base; caudum blunt. Cuticle finely granular, without epidermal glands.

Adhesive tubes. TbA two per side (L 5 μ m), occurring on lobe inserted at U08–09 (Figs 2B, 3C); TbVL 10–14 per side (L 4–6 μ m), insertions obscure, irregularly spaced, often asymmetrically arranged, present only along intestine (U38–80), none in pharyngeal region or behind anal opening (Figs 2A, B, 3A); TbD/TbDL absent; TbP 4–6 per side (L 8–11 μ m), inserting on tapered posterior end of trunk and directed posteriorly (Figs 2A, B, 3A–C).

Ciliation. Mouth surrounded by short sensory cilia (L 6 μ m) (Figs 2A, B, 3A, B), with longer cilia (L 13 μ m) inserted on each side at points of head sculpting (Figs 2A, B, 3A, B); ciliary hairs (L 11 μ m) forming circum-cephalic band at

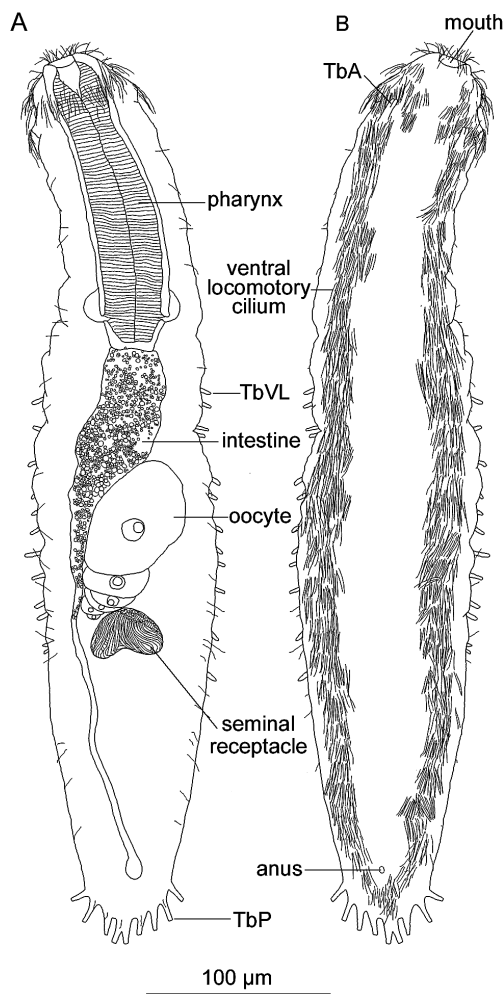


Fig. 2. *Cephalodasys mahoae* sp. nov., line drawings of holotype (ICHUM 4977). A, Dorsal view showing the digestive and reproductive tracts, glands, adhesive tube, and tactile body ciliation; B, ventral view showing the adhesive tubes and locomotory ciliation. Abbreviations: TbA, anterior adhesive tube; TbP, posterior adhesive tube; TbVL, ventro-lateral adhesive tube.

U07; sensory cilia (L 13 µm) in lateral and dorsal columns on trunk; ventral locomotory cilia (L 13 µm) running posteriorly from circum-cephalic ring in two longitudinal bands along lateral body margins, remaining separate throughout, but with post-anal ciliary patch.

Digestive tract. Mouth terminal, of medium width (18 µm); buccal cavity cup-shaped, shallow; walls lightly cuticular; pharynx medium throughout, with basal pharyngeal pores at U28–32; intestine divided into broad, anterior, granular region with refractive granules at anterior and posterior ends, and narrower, posterior, non-granular region; anus at U91.

Reproductive tract. Hermaphroditic, probably protogynous; testes not seen; oocytes lying along anterior part of intestine (U50–70), developing from posterior to anterior, largest (67×44 µm) anteriorly (Figs 2A, 3C); seminal receptacle present behind oocytes (Figs 2A, 3C).

Remarks. Among the specimens examined, only one had multiple eggs, thus indicating the direction of oocyte development, from posterior to anterior. This orientation,

however, is opposite that in the diagnosis of *Cephalodasys* (Hummon and Todaro 2010; Kieneke *et al.* 2015). Notwithstanding, we placed the new species in this genus because all characters except the orientation of oocyte development agree with the generic diagnosis in Hummon and Todaro (2010) and Kieneke *et al.* (2015). That *C. mahoae* sp. nov. and *Cephalodasys* sp. of Petrov *et al.* (2007) were sister taxa in the resulting phylogenetic tree (Fig. 4) may justify our generic assignment (see also Phylogeny section below). Alternatively, *C. mahoae* sp. nov. could have been placed in *Paradasys* Remane, 1934, because of its resemblance with the type species, *P. subterraneus* Remane, 1934. However, species in *Paradasys* differ from *C. mahoae* sp. nov. by lacking lateral adhesive tubes (Remane 1934; Karling 1954; Rao and Ganapati 1968; Schmidt 1974; Rao 1980; Hummon 2008).

Among 13 species of congeners (Hummon and Todaro 2010; Hummon 2011; Kieneke *et al.* 2015), *C. mahoae* sp. nov. most closely resembles *C. miniceraus* Hummon, 1974 from the Caribbean Sea. Because the latter species was described based only on subadults, the orientation of oocyte development is unknown. *Cephalodasys mahoae* sp. nov. differs from *C. miniceraus* in the arrangement of TbL/TbVL (U38–80 in *C. mahoae* sp. nov., U26–90 in *C. miniceraus*).

Family **Turbanellidae** Remane, 1926

Genus ***Turbanella*** Schultze, 1853

Turbanella cuspidata sp. nov.

(Figs 5, 6)

Material examined. Twelve adults. Holotype, ICHUM 4980, mounted on glass slide, Higashi-Shizunai, Hokkaido, Japan (42°17.333'N, 142°27.590'E), medium-grained sand, surface layer at water's edge, 19 May 2012. Six paratypes, same collection data as for holotype: ICHUM 4981, mounted on glass slide; ICHUM 4991–4995, on SEM stubs. Four additional specimens destroyed after observation.

Etymology. The new specific name is an adjective from the Latin *cuspidatus* (made pointed), indicating the pair of small, ventrolateral, projective organs.

Description. *Habitus.* Adult Lt 570–670 µm (640 µm in holotype); L of anterior end to PhJIn (at U32–27) 160–180 µm (177 µm in holotype). Body medium in length; head sculpted, with lateral cones at U07 (Figs 5A, 6A, B) and small ventrolateral projective organ at U06 on each side (Figs 5B, 6A, B); neck constriction at U09; trunk widest in mid-body region, tapering gradually to caudal base; caudum slightly cleft, incised from tips to U98, bearing a medial cone (L 5–10 µm) (Figs 5B, 6D). Glands 30–40 per side, medium size (6 µm in diameter), scattered along lateral and medial columns.

Adhesive tubes. TbA 7–8 per side (L 4–12 µm), occurring on lobe inserted at U09–11 (Figs 5B, 6A, B), most medial tube on hand always set lower than others; TbL/TbVL 18–25 per side (L 10–16 µm, insertions difficult to distinguish), irregularly spaced and often asymmetrically arranged, with five in pharyngeal region, one at PhJIn, and others along intestine, but none behind anal opening, most bearing cilia; TbDL 8–13 per side, evenly spaced and sym-

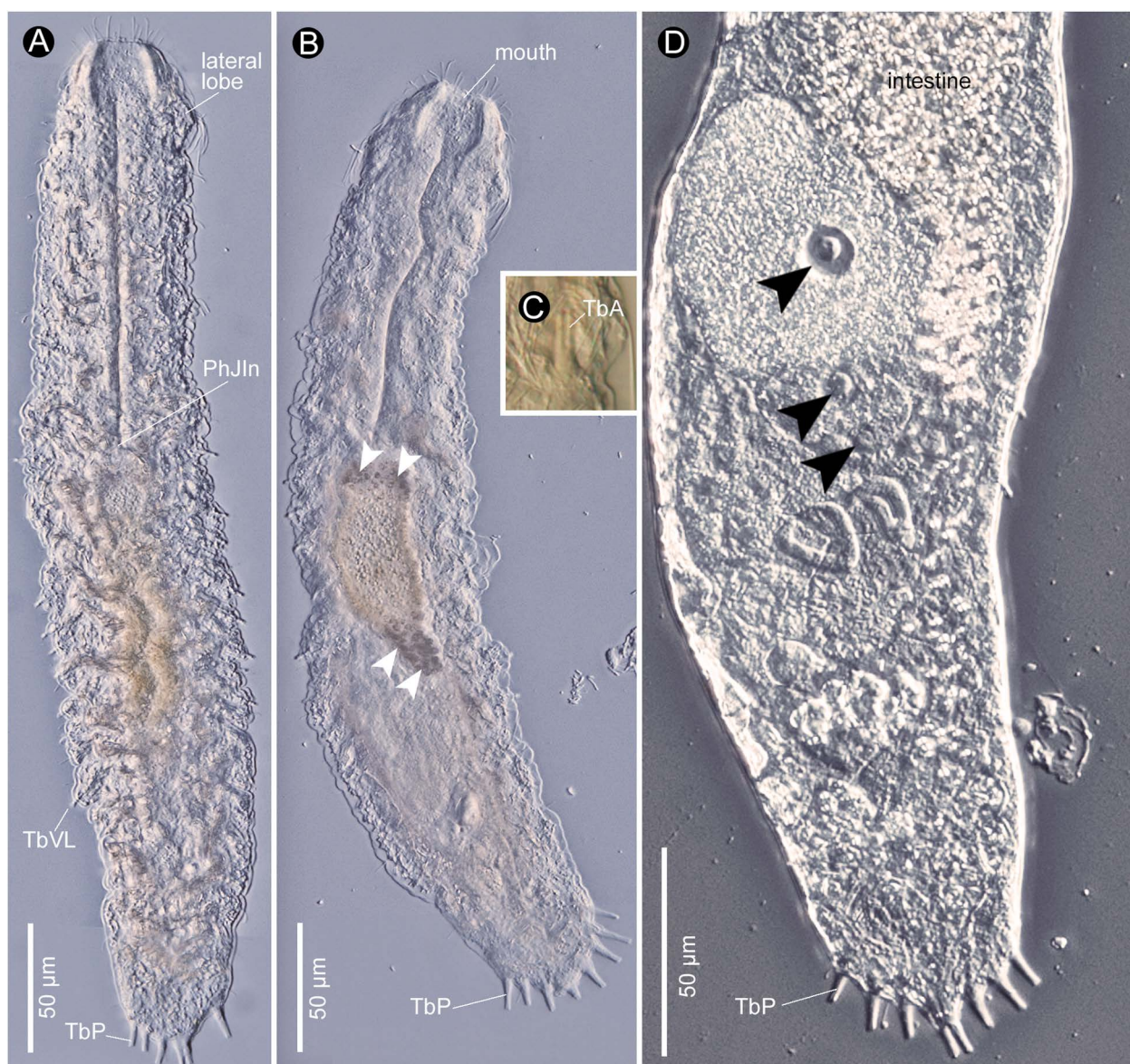


Fig. 3. Differential interference contrast photomicrographs of *Cephalodasys mahoae* sp. nov. A, Subadult specimen from Ishikari beach, paratype ICHUM 497; B, subadult specimen from Mukawa (lost after being photographed), showing refractive granules at anterior and posterior ends of anterior intestine (indicated by arrowheads); C, anterior adhesive tube (TbA), subadult specimen from Ishikari beach, lost after observation; D, posterior region of body, showing three oocytes (arrowheads) developing from posterior to anterior; holotype (ICHUM 4977). Abbreviations: PhJIn, junction between pharynx and intestine; TbVL, ventro-lateral adhesive tube; TbP, posterior adhesive tube.

metrically arranged, with three in pharyngeal region and remainder along intestine, most bearing cilia; TbD 15–20 per side, with three in pharyngeal region and remainder along intestine, most bearing cilia; ‘cirrata’ [Seitenfüsschen] tubes occurring at U39; TbP 10–12 per side, arrayed along rear edge of each lobe, lengthening medial to lateral (L 4–13 µm).

Ciliation. Mouth surrounded by short sensory cilia (L 6 µm), with longer cilia (L 11 µm) inserted at points of head sculpting on each side; ciliary hairs (L 11 µm) forming circum-cephalic band at U07; sensory cilia (L 7 µm) occurring on trunk in lateral and dorsal columns; each Tb on trunk bearing cilium (L 13 µm) arising from rear apex of tube support; ventral locomotory cilia (L 15 µm) running in two longitudinal bands along lateral body margins to anus, separate except beneath (*i.e.*, ventrally in) pharyngeal region (Fig.

5B).

Digestive tract. Mouth terminal, of medium width (18 µm); buccal cavity cup-shaped, shallow; walls lightly cuticular; pharynx of medium width throughout, with basal pharyngeal pores at U29–24; intestine narrows anterior to posterior; anus at U94.

Reproductive tract. Hermaphroditic; paired testes extending posteriorly from U32, their vasa deferentia recurving anteriorly and exiting at U38; bilateral oocytes developing in posterior to anterior direction, largest (125×57 µm) in anterior region of intestine (Figs 5A, 6C).

Remarks. Among approximately 30 species in the genus *Turbanella*, four species (*T. amphiatlantica* Hummon and Kelly, 2011; *T. bocqueti* Kaplan, 1958 *sensu* Boaden (1974); *T. varians* Maguire, 1976; and *T. wieseri* Hummon,

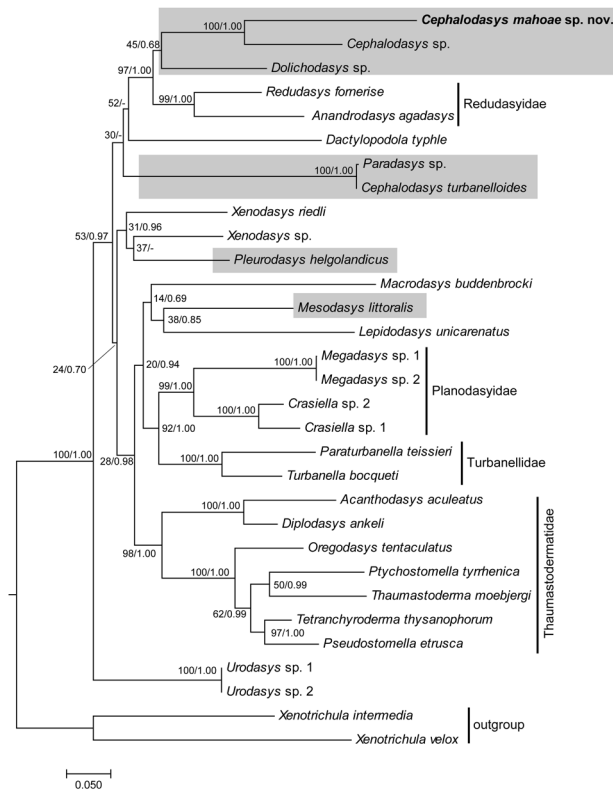


Fig. 4. Maximum-likelihood tree ($\ln L = -37094.311007$) showing phylogenetic relationships among *Cephalodasys mahoae* sp. nov. and 28 other macrodasyidans, inferred from concatenated 18S, 28S, and COI sequences. *Xenotrichula intermedia* and *X. velox* (Chaetodontida: Xenotrichulidae) represent the outgroup. The scale indicates branch length in number of substitutions per site. Numbers at nodes are bootstrap value and Bayesian posterior probability. Planodasyidae, Redudasyidae, Thaumastodermatidae, and Turbanellidae were recovered monophyletic, while Cephalodasyidae (shaded) was non-monophyletic.

2010) share many features with *T. cuspidata* sp. nov., but differ from the latter in the following ways. *Turbanella amphiatlantica* lacks the slight neck constriction; *T. bocqueti* has larger body size (Lt 800–1320) (Hummon 2008); *T. varians* lacks lateral head cones; and *T. wieseri* bears nine TbD per side. In addition, the ventrolateral projective organ on each side the head is characteristic of *T. cuspidata* sp. nov.; the organ is a simple, cylindrical projection, about 45 μm in length and 20 μm in width, sticking out ventrally from a portion slightly anterior to the base of the lateral cone (Fig. 6A, B).

***Turbanella lobata* sp. nov.**
(Figs 7, 8)

Material examined. Holotype, ICHUM 4982 (adult), Ishikari beach, Hokkaido, Japan (43°15.420'N, 141°21.438'E), medium-grained sand, 58 cm depth, 5 m landward from high-water level, 14 May 2014. Paratype: ICHUM 4983 (subadult), same collection data as for holotype.

Etymology. The specific name is an adjective from the Latin *lobatus* (lobed), referring to the lateral lobes at U11.

Description. *Habitus.* Adult Lt 390 μm ; L of anterior

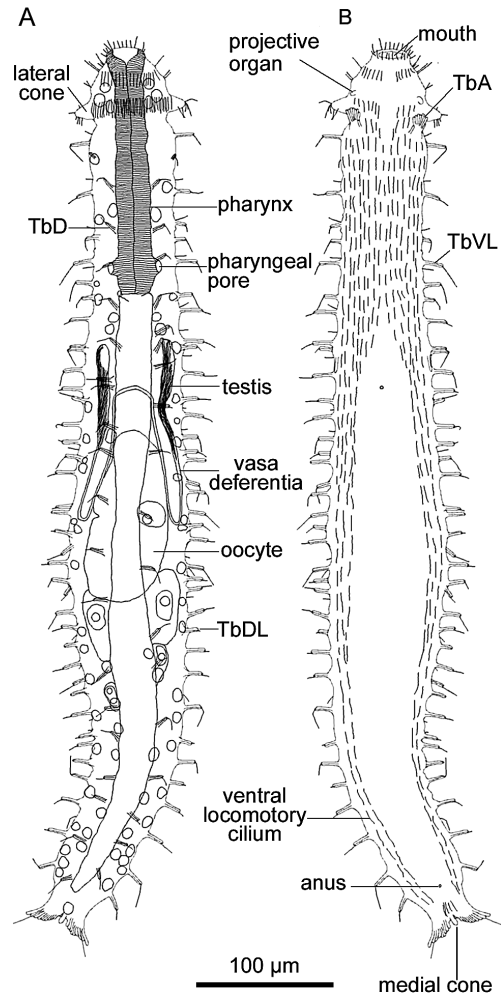


Fig. 5. *Turbanella cuspidata* sp. nov., line drawings of holotype (ICHUM 4980). A, Dorsal view, showing digestive and reproductive tracts, glands, adhesive tubes, and tactile body ciliation; B, ventral view, showing adhesive tubes and locomotory ciliation. Abbreviations: TbA, anterior adhesive tube; TbD, dorsal adhesive tube; TbDL, dorso-lateral adhesive tuber; TbVL, ventro-lateral adhesive tube.

end to PhJIn (at U30) 132 μm . Body short; head slightly sculpted, with lateral cones at U07 and additional lateral lobes at U11 (Figs 7A, 8A); neck constriction at U11; trunk widest in mid-body region, tapering gradually to caudal base; caudum moderately cleft, incised from its tips to U97, medial cone absent. Glands 35–40 per side, medium in size (6 μm in diameter), scattered in lateral and medial columns.

Adhesive tubes. TbA four per side (L 5–7 μm), occurring on lobe inserted at U11 (Fig. 7B); TbL 10–12 per side (L 8–12 μm), some bearing cilia, irregularly spaced and often asymmetrically arranged, with two in pharyngeal region, one behind anal opening, and others along intestine; TbD 7–8 per side, with one in pharyngeal region and remainder along intestine; ‘cirrata’ [Seitenfüßchen] tubes occurring at U38; TbP four per side, arrayed along rear edge of each lobe, lengthening medial to lateral (L 3–9 μm) (Figs 7A, B, 8C).

Ciliation. Mouth surrounded by short sensory cilia (L 3 μm), with longer cilia (L 5 μm) inserted at points of head sculpting on each side; ciliary hairs (L 11 μm) forming cir-

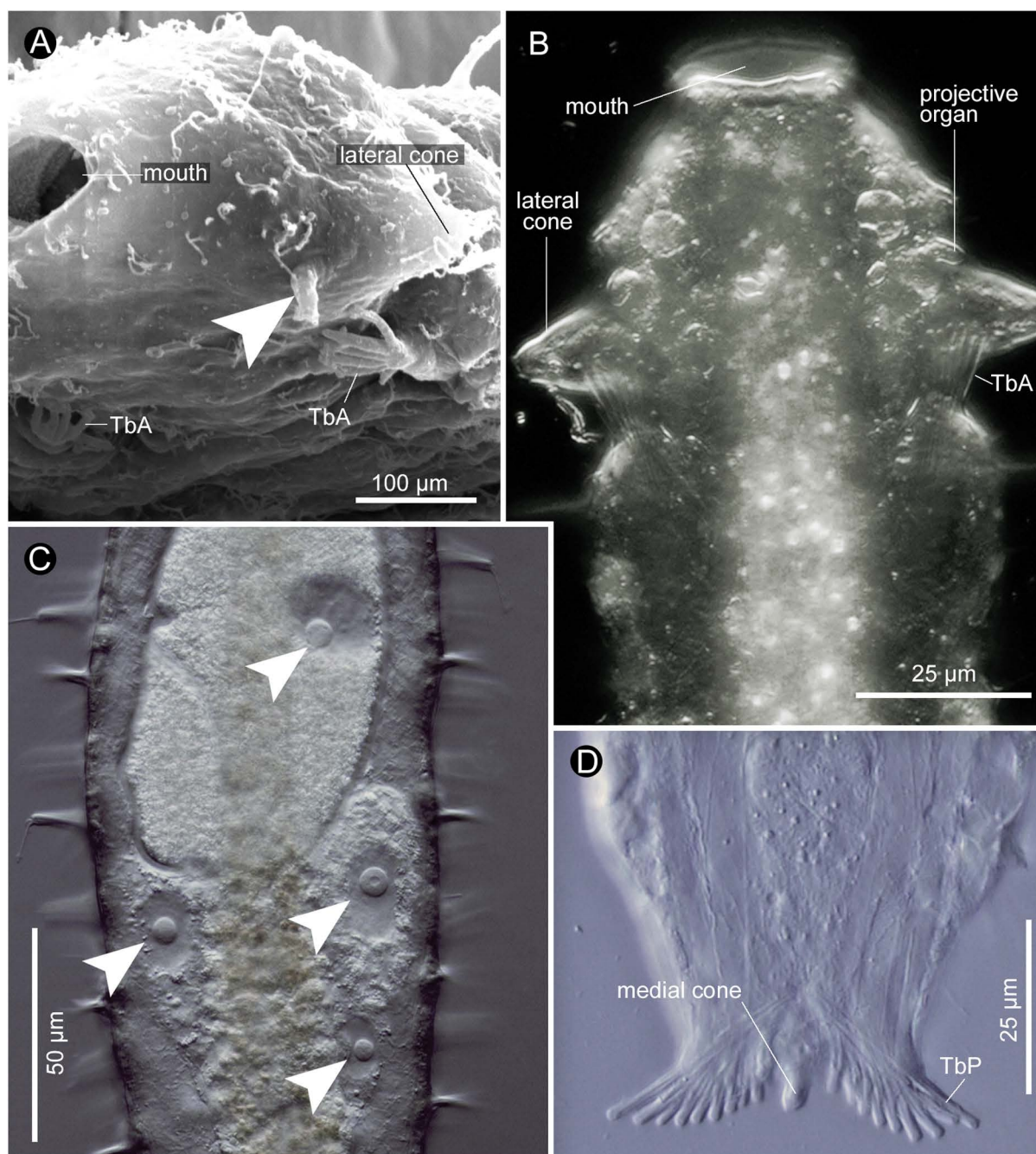


Fig. 6. *Turbanella cuspidata* sp. nov. A, SEM image of head and neck region; arrowhead indicates projective organ, left anteroventral view, paratype ICHUM 4991. B–D, Differential interference contrast photomicrographs of holotype (ICHUM 4980); B, head and neck region, ventral view; C, mid-body region showing oocytes; D, caudal part, ventral view, showing medial caudal cone and arrangement of posterior adhesive tubes (TbP). Abbreviation: TbA, anterior adhesive tube.

cum-cephalic band at U07; sensory cilia (L 9 μm) occurring on trunk in lateral columns; each Tb inserted on trunk, bearing cilium (L 11 μm) arising from rear apex of tube support; ventral locomotory cilia (L 12 μm) running in two longitudinal bands along lateral body margins to anus (Fig. 7B).

Digestive tract. Mouth terminal, of medium width (11 μm); buccal cavity conical-shaped; walls lightly cuticular; pharynx of medium width throughout, with basal pharyngeal pores at U30; intestine narrowing anterior to posterior; anus at U91.

Reproductive tract. Hermaphroditic; paired testes ex-

tending posteriorly from U59, their vasa deferentia recurving anteriorly, but terminal not seen; bilateral oocytes developing posterior to anterior, largest (53×23 μm) in anterior region of intestine (Figs 7A, 8B).

Remarks. Among approximately 30 species in the genus *Turbanella*, five species share many features with *Turbanella lobata* sp. nov.: *T. caledoniensis* Hummon, 2008; *T. lutheri* Remane, 1952; *T. otti* Schrom, 1972; *T. pacifica* Schmidt, 1974; and *T. subterranea* Remane, 1934. These species differ from *T. lobata* sp. nov. as follows: *T. caledoniensis* lacks lateral head lobes and the neck constriction; *T. lutheri*

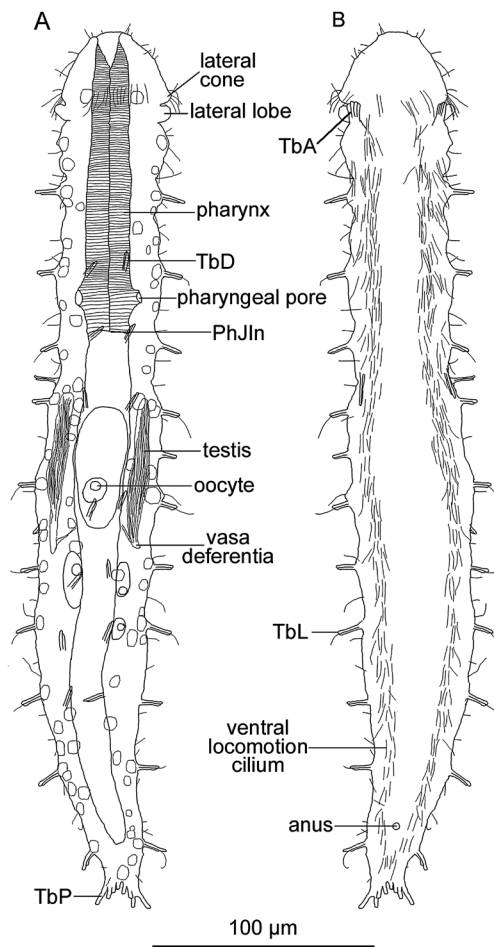


Fig. 7. *Tulbanella lobata* sp. nov., line drawings of holotype (ICHUM 4982). A, Dorsal view, showing digestive and reproductive tracts, glands, adhesive tubes, and tactile body ciliation. B, ventral view, showing adhesive tubes and locomotory ciliation. Abbreviations: PhJIn, junction between pharynx and intestine; TbA, anterior adhesive tube; TbD, dorsal adhesive tube; TbL, lateral adhesive tube; TbP, posterior adhesive tube.

bears TbP from the outside edge; *T. otti* bears a prominent medial caudal cone; *T. pacifica* lacks TbD; and *T. subterranea* has only a slight neck constriction. The dorsolateral lobes at the neck constriction in *T. lobata* sp. nov. (Fig. 8A) are unique among members of the genus. However, this character should be treated with caution, because it might be as a result of freshwater osmotic shock during extraction.

Phylogeny

In the resulting tree, *Cephalodasys mahoae* sp. nov. was sister to *Cephalodasys* sp. of Petrov *et al.* (2007) with full support of bootstrap (BS) value and Bayesian posterior probability (PP) (Fig. 4). These two OTUs were nested within a rather well-supported subclade (97% BS, 1.00 PP) that contains *Dolichodasys* sp. (Cephalodasyidae), *Redudasys fornerise* Kisielewski, 1987 (Redudasyidae), and *Anandrodasys agadasys* (Hochberg, 2003) (Redudasyidae). *Cephalodasys turbanelloides* (Boaden, 1960) *sensu* Todaro *et al.* (2006) was sister to *Paradasys* sp. *sensu* Todaro *et al.* (2006), not to the *C. mahoae*+*Cephalodasys* sp. clade. *Cephalodasys tur-*

banelloides was originally established in *Paradasys* (Boaden 1960), suggesting that transfer of the species to *Cephalodasys* (Hummon 1974) may require a revision. Our analysis indicates *Cephalodasys* and Cephalodasyidae as currently diagnosed (Hummon and Todaro 2010; Kienek *et al.* 2015) are not monophyletic (Fig. 4). However, additional gene markers may recover them as monophyletic, because support values for basal nodes are generally low (Fig. 4). In any case, inclusion of *C. maximus* Remane, 1926, the type species of *Cephalodasys*, as well as *P. subterraneu*, the type species of *Paradasys* (see Remarks for *C. mahoae* sp. nov. above), in molecular phylogenetic context is indispensable to test the appropriateness of the generic placement of *C. mahoae* sp. nov., as well as for taxonomic revision of the family.

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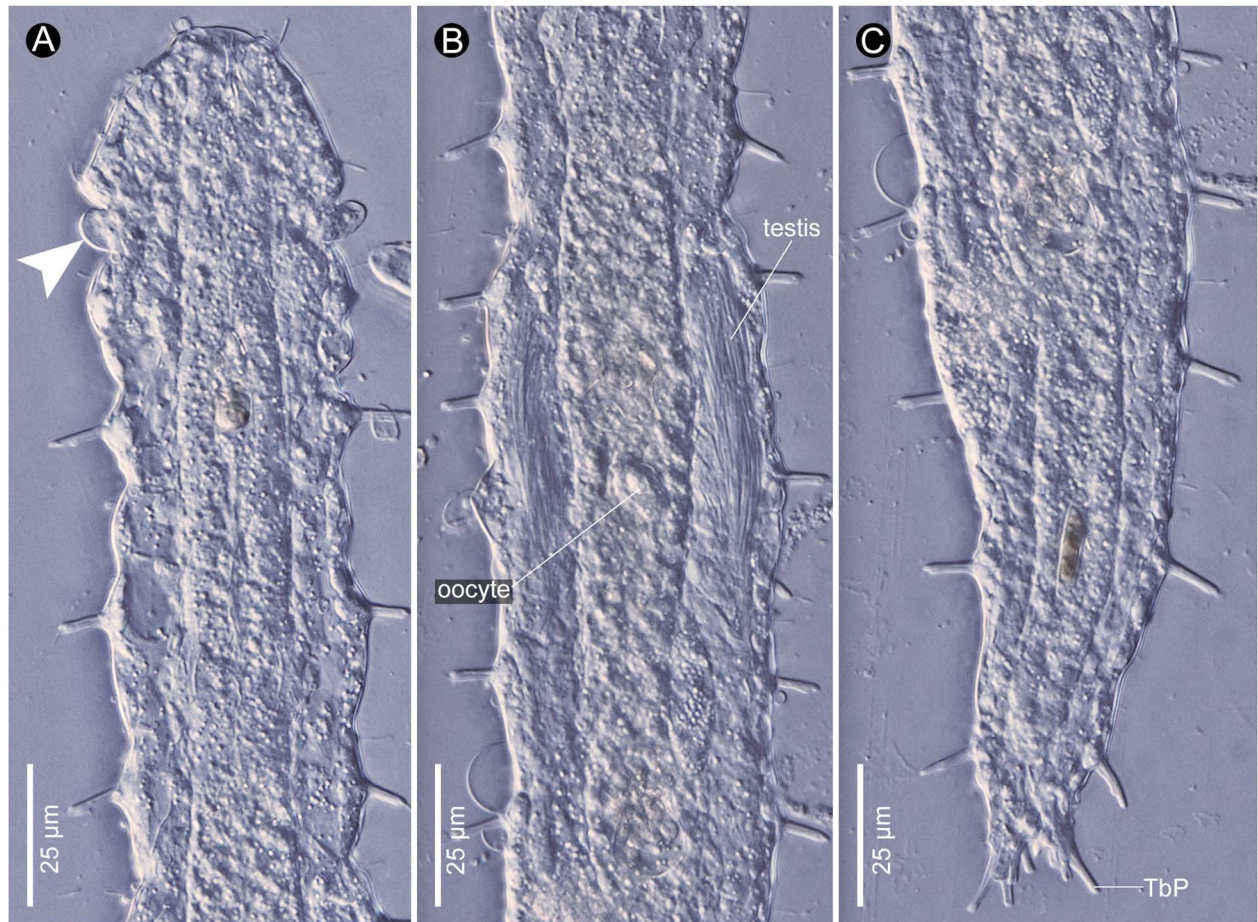


Fig. 8. Differential interference contrast photomicrographs of *Turbanella lobata* sp. nov., holotype (ICHUM 4982). A, Head and neck region; arrowhead indicates lateral lobe on the neck; B, mid-body region, showing oocytes and testes; C, caudal part, showing the arrangement of the posterior adhesive tubes (TbP).

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