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A new species of *Pompholyx* (Rotifera: Monogononta: Testudinellidae) from the United States

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ABSTRACT

Genus *Pompholyx* Gosse, 1851 (Rotifera; Monogononta; Testudinellidae) comprises three species described from freshwater plankton around the globe. Here we describe a new species of *Pompholyx* collected from a freshwater pond in Massachusetts, USA. The new species resembles its congeners with respect to the following characters: paired eyespots; a dorsally arched lorica with a dorsal occipital convexity behind the corona; lateral flared and rounded lorica surfaces; a ventral surface bearing an occipital concavity posterior of the mouth; a unique egg-gland system; and the absence of a foot. However, *P. faciemplarva* **sp. n.** differs from its congeners in possessing a transverse furrow on both the dorsal and ventral surfaces of the lorica. While the trophi of *P. faciemplarva* **sp. n.** generally resemble those of other species of Testudinellidae, they do have a symmetrical pattern of unci teeth (17/17) that differs from *Pompholyx sulcata* (17–20/18–21, right/left), the only other species in the genus with well-described trophi. The description of this new species enhances the floristic richness of freshwater in North America.

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Introduction

Genus *Pompholyx* (Rotifera: Monogononta: Testudinellidae) comprises three freshwater species that have been reported from six of the eight large biogeographical realms (Balian *et al.* 2008); it has not been recorded in Pacific and Antarctic regions (Segers 2007). The first two species, *Pompholyx complanata* Gosse, 1851 and *Pompholyx sulcata* Hudson, 1885, were described from freshwater ponds in the United Kingdom (Gosse 1851; Hudson and Gosse 1886), while the third species, *Pompholyx triloba* Pejler, 1957, was described from Swedish Lapland. These species were originally described using light microscopy, and because of this many characteristics were either not documented (e.g., trophi in *P. complanata* and *P. triloba*) or incompletely documented (egg shape of corona and internal anatomy of *P. triloba*). This, of course, makes species delimitation problematic. However, later morphological studies provided further insights into the general organisation of *P. sulcata* (Leissling 1924; Remane

1929–1933). In general, all species can be differentiated by body size and cross-sectional shape: it may be circular (*P. complanata*), may bear longitudinal furrows (*P. sulcata*), or may be somewhat flat with a dorsal arch (*P. triloba*) (see Ruttner-Kolisko 1974; Koste 1978). *Pompholyx complanata* and *P. sulcata* also possess a unique gland complex that appears to function in producing secretions for attaching and maneuvering/retracting oviposited eggs (Hudson and Gosse 1889; Leissling 1924; Ruttner-Kolisko 1974). De Smet (2005) provided the first detailed documentation of trophi in the genus (*P. sulcata*) and showed that they are very similar to other species of the family Testudinellidae, but with more rami scleropili and a different number of unci teeth that are asymmetrically arranged.

Here, we describe a fourth species belonging to the genus *Pompholyx* using scanning electron microscopy (SEM) to describe lorica morphology and trophi structure, and high-resolution light microscopy to document the internal anatomy.

Materials and methods

Zooplankton samples were collected with a 63- μ m mesh net from Flint Pond, a freshwater pond in Massachusetts, USA (42.675142°N, -71.425912°W) from July through September 2022 (Figure 1). Samples were placed in small open buckets and returned to the University of Massachusetts Lowell at ambient temperature. In the laboratory, small volumes of the pond water were decanted into Petri dishes (90 mm $\times$ 15 mm) and examined using Zeiss® Stemi stereomicroscopes. Specimens of *Pompholyx* were removed from the water using glass and

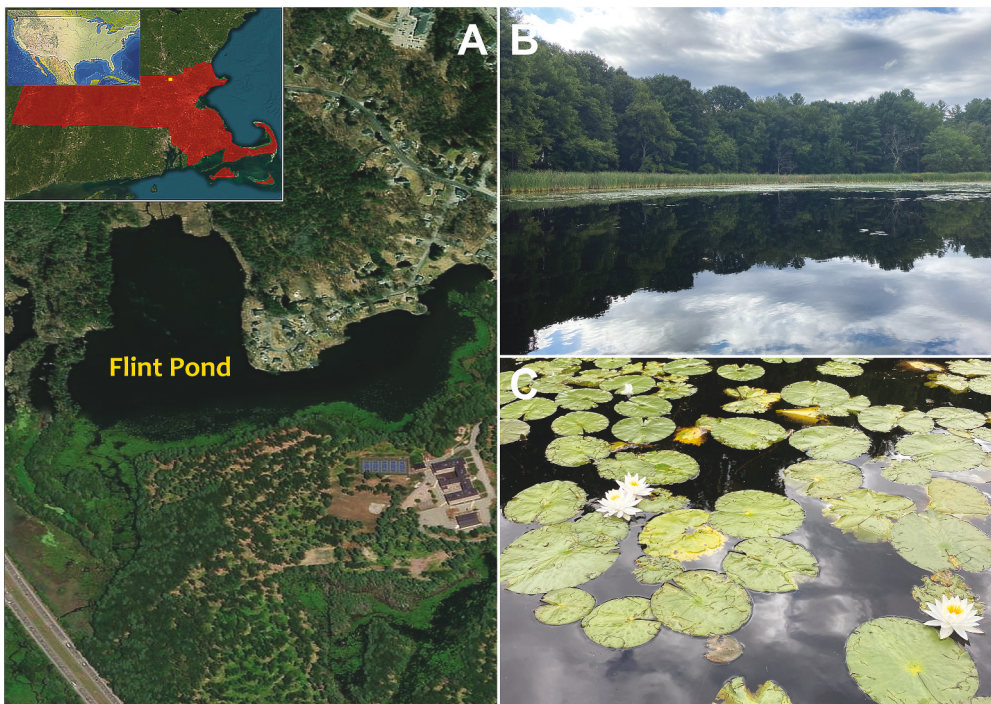


Figure 1. (A) Map of Flint Pond, Massachusetts, United States of America; (B) photo of sampling location on Flint Pond; (C) photo of floating plants present on the pond.

mechanical micropipettes and placed in a small glass bowl (3 mL) with 1 mL of carbonated water to anesthetize the rotifers. Rotifers were placed on glass microscope slides and examined at high magnification with a Zeiss Axio A1 compound microscope equipped with Differential Interference Contrast (DIC); photomicrographs were taken using a camera JENOPTIK GRYPHAX® AVIOR and photomicrographs were processed in JENOPTIK GRYPHAX® image analysis software. The holotype specimen was destroyed during the documentation of internal anatomy, but several paratypes were prepared (below). Trophi were isolated on a circular cover slip by sequentially dissolving tissues in a 2% NaOCl solution and rinsing with distilled water (Segers *et al.* 1993; Segers 1995). Trophi were imaged on a JEOL JSM 6390 scanning electron microscope at 15 kV.

We prepared some paratypes for SEM by flooding the slide-mounted specimens with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). The specimens ($n = 11$) were moved to a microcentrifuge tube and stored at 4°C. After approximately 1 week in the cold, the tubes were placed at room temperature, rinsed in 0.1 M cacodylate buffer containing sucrose (4×15 min), postfixed in OsO₄ for 1 h, and then rinsed in buffer again (4×15 min). Rotifers were next dehydrated in a graded ethanol series (20 min at 70, 90, 100, 100%) while in mesh-covered BEEM® capsules and then critically point dried with a Tousimis SAMDRI-795 CPD. Rotifers were mounted on carbon tape on aluminium stubs and then coated with gold in a Denton Vacuum Desk IV sputter coater. Animals were imaged on a JEOL JSM 6390 scanning electron microscope at 10 kV.

Specimens were also prepared in resin for paratype deposition ($N = 2$) and others for eventual transmission electron microscopy. Anaesthetized specimens on glass slides were flooded with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and placed in a microcentrifuge tube and stored at 4°C. After approximately 1 week in the cold, the tubes were placed at room temperature, rinsed in 0.1 M cacodylate buffer containing sucrose (4×15 min), postfixed in OsO₄ for 1 h, and then rinsed in buffer again (4×15 min). Rotifers were next dehydrated in a graded ethanol series (20 min at 70% and 90% and 30 min at 100% (2X)) and then transferred to a microcentrifuge tube with a 3:1 ratio of ethanol:Spurr's low-viscosity resin (Electron Microscopy Sciences) for 2 h on a slow rotator. This was followed by a 1:1 ratio of ethanol:resin for 2 h on a rotator overnight and then 1:3 ethanol: Spurr's for 2 h the following day. Two paratypes were embedded in pure resin on glass slides and a cover glass was placed on each. Both specimens were then placed in an oven at 60°C overnight.

Digital photographs were edited using Adobe Photoshop® (Release 22.5, 1990–2021) and schematic drawings were created using Adobe Illustrator®.

Results

Order FLOSCULARIACEAE Harring, 1913

Family TESTUDINELLIDAE Harring, 1913

Genus *Pompholyx* Gosse, 1851

Pompholyx faciemlarva Araújo, Walsh, Wallace and Hochberg **sp. n.**

(Figures 2–6)

ZooBank: urn:lsid:zoobank.org:act:99CBE73C-C91C-42E2-9C98-1CAEB5C30387.

Material examined

Eleven specimens were examined using light microscopy; measurements were taken with an ocular micrometer. All specimens were mounted and photographed and/or digitally recorded. The type specimen was documented, as is permitted digitally recorded: digital type documentation is allowed by The International Code of Zoological Nomenclature, namely in article 73.1.4 (The International Commission on Zoological Nomenclature 1999), even when the documented specimen has been lost. The type photomicrographs are available at the Smithsonian National Museum of Natural History under accession numbers USNM 1606876 (holotype) and USNM 1606877, USNM 1606878, USNM 1606879, USNM 1606880, USNM 1606881 (paratypes). Ten other specimens were examined using SEM.

Differential diagnosis

Pompholyx faciemlarva **sp. n.** can be distinguished from *P. complanata*, *P. sulcata* and *P. triloba* by the presence of transverse furrows on its lorica, the structure of the stalk gland (3-lobed), and the number and symmetrical arrangement of unci teeth in the trophi. There are 17 teeth on each side in the new species, compared to 17–20/18–21 (right/left) in *P. sulcata* (the only other species with a complete description).

Etymology

The species name is an adjectival name derived from the form of the rotifer's corona, which resembles the mask used by scuba divers (∞ -shaped) (Figure 3B): face (Latin: *faciem*) and mask (Latin: *larva*).

Description

The description is based on an adult specimen of 110 μm (95–125 μm) body length. The body shape is ellipsoid, but with a flat anterior margin and rounded triangular posterior end. The corona is complete, ∞ -shaped, and bears two dorsolateral red eyes (Figures 2A, 3B, 4D, 6A). Coronal cilia are approximately 8–20 μm long. In cross section, the smooth and mostly featureless lorica is arched dorsally and with flared lateral edges (Figures 2A, 3B, 6). SEM reveals a fine network of fibres across parts of the lorica, but these may be artefacts of fixation (Figure 6). A large transverse furrow extends laterally across the body on both dorsal and ventral sides around mid-body length. The furrow is present in all living specimens when swimming and does not change when animals are anesthetized; the furrow remains in preserved animals. An occipital convexity (edge) is present just posterior of the corona on the dorsal side. The ventral lorica surface is smooth, but narrower in width than the dorsal surface: it is 80 μm long and has an inverted triangle shape with a 53 μm wide anterior border and a 19 μm wide posterior tip. A pectoral concavity (notch) just posterior to the corona is present on the ventral surface (Figures 2B, 3C). A ciliated sub-labium extends from the pectoral concavity during locomotion. The posterior end has a terminal 6 μm diameter cavity that leads to the cloaca (Figure 6B–D), which is rectangular in shape and ~ 20 μm long (Figures 2C, 4B). The cloaca has three openings to the blastocoel: one for the stalk gland, one to the anus, and one for oviposition (Figure 2C).

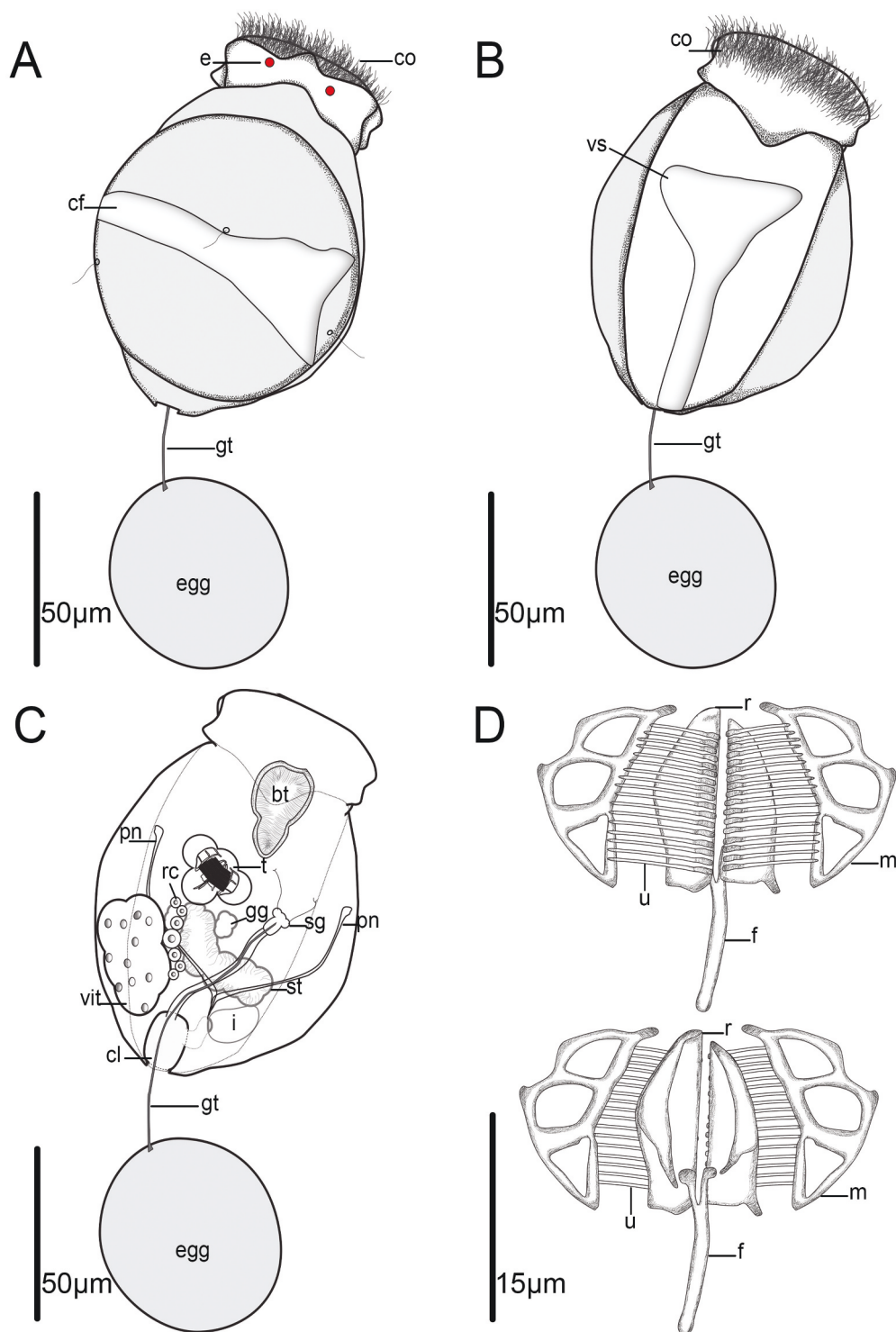


Figure 2. Schematic illustrations of *Pompholyx faciellarva* sp. n. (A) Dorsal view; (B) ventral view; (C) internal view; (D) trophus. Abbreviations: bt, buccal tube; cf, lorica furrow; cl, cloaca; co, corona; e, eye; f, fulcrum; gg, gastric glands; gt, Gossesche thread; i, intestine; m, manubrium; mo, mouth; pn, protonephridia; r, rami; rc, round (unidentified) cells; sg, stalk gland; st, stomach; t, trophi; vit, vitellarium; vs, ventral surface; u, unci.

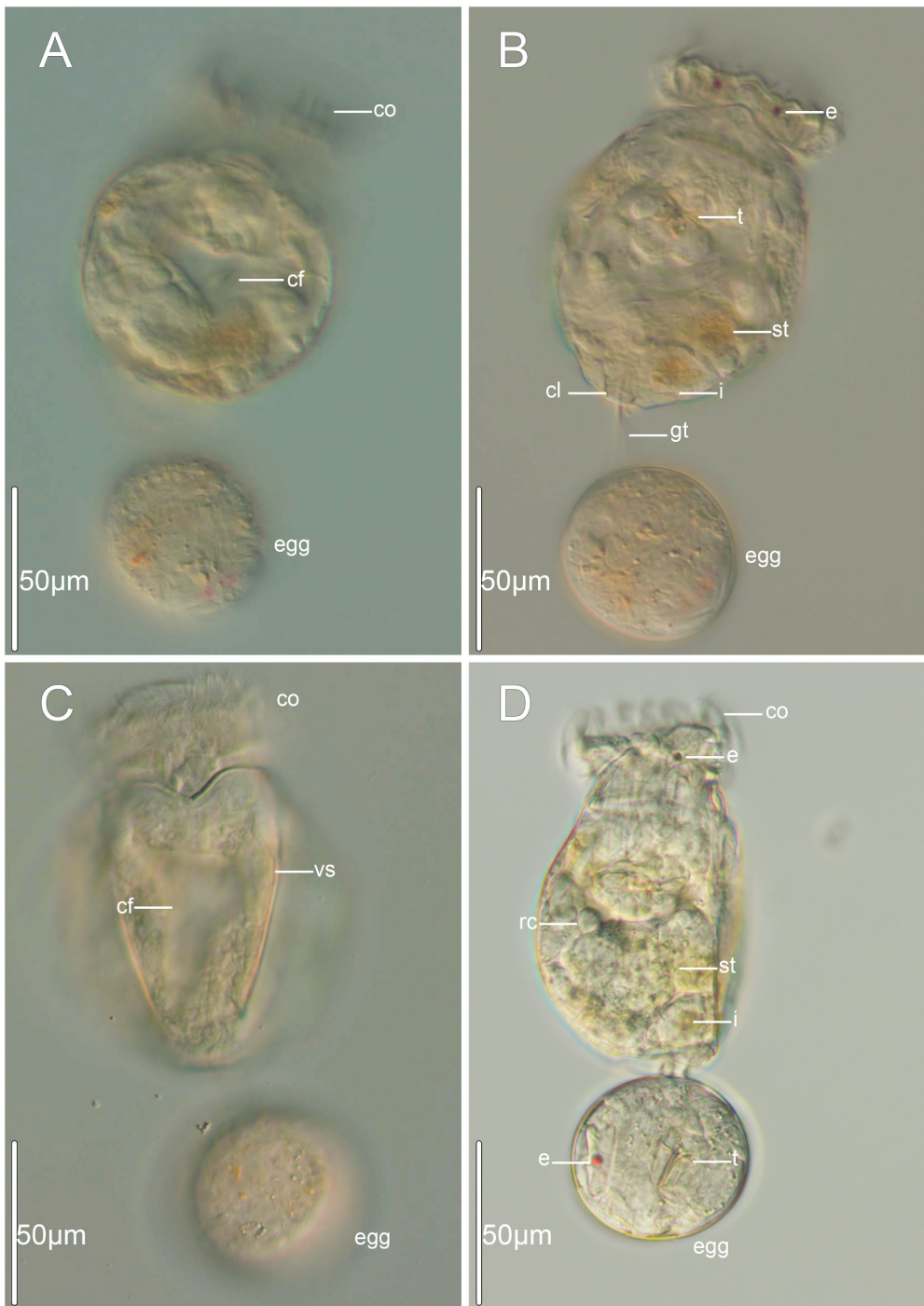


Figure 3. DIC microphotograph of *Pompholyx faciellarva* sp. n. (A) Dorsal arch view; (B) middle dorsal view; (C) ventral view; (D) lateral view. Abbreviations: cf, lorica furrow; cl, cloaca; co, corona; e, eye; gt, Gossesche thread; i, intestine; mo, mouth; pn, protonephridia; rc, round (unidentified) cells; st, stomach; t, trophi; vs, ventral surface.

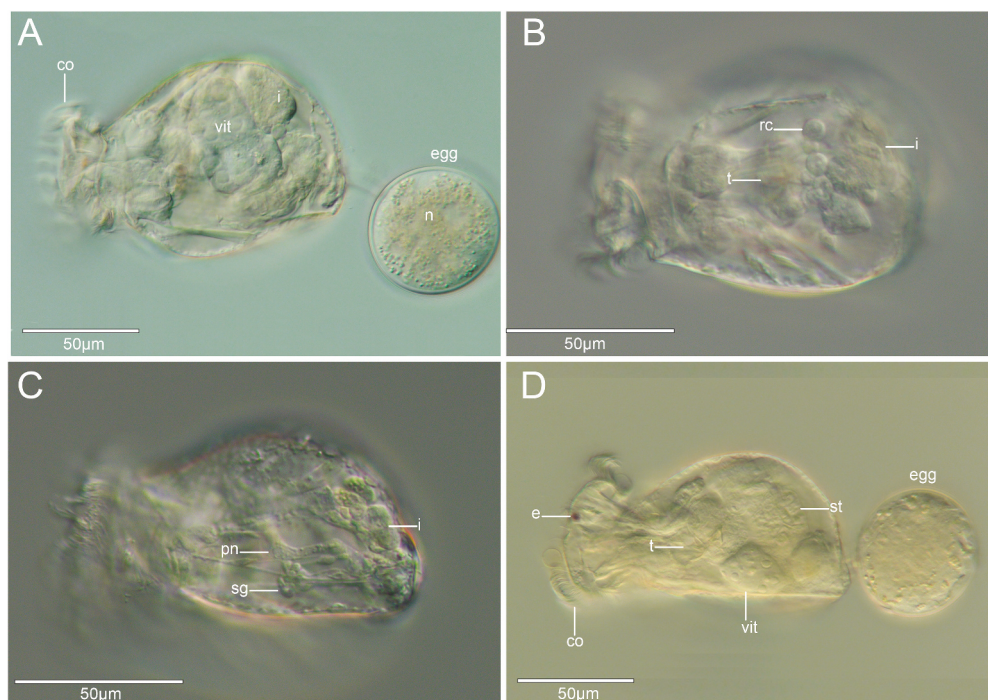


Figure 4. DIC microphotograph of internal anatomy of *Pompholyx faciellarva* **sp. n.** (A–D) Different focal planes of ventrolateral body view. Abbreviations: co, corona; e, eye; i, intestine; n, nucleus; pn, protonephridia; rc, round (unidentified) cells; sg, stalk gland; st, stomach; t, trophi; vit, vitellarium.

Corona. The corona is ∞-shaped (i.e., it resembles a number 8 lying on its side or a scuba diving mask) and ciliation is complete. Cilia appear to be of a mostly uniform length of 6–18 µm. Small patches of presumable sensory cilia are present in the apical field. A ciliated sub-labium is present on the ventral surface just posterior of the mouth within the pectoral notch.

Digestive tract. Terminal mouth is somewhat ventral in position, but still within the coronal field. The mouth leads to a 30 µm long ciliated buccal cavity that has an inverted pear-shape. The ciliated stomach has two salivary/gastric glands connected to the anterior part of the stomach. The intestine is 20 µm in total length and has two sphincters: one sphincter demarcates the transition from the stomach to the intestine; the second sphincter demarcates the transition from the intestine to the cloaca (Figures 2C,D, 3B, 4B,E, 6B,C,D). No bladder was observed.

Trophi. Trophi malleoramate. The rami are 13 µm long and have an elongate-triangular shape with rounded latero-ventral margins, delimiting large latero-ventral fenestrae (Figure 5D). The fulcrum is elongate and straight (11 µm). The unci plates consist of 16 right and 17 left (frontal view) weakly curved and strongly webbed asymmetrical teeth (lengths: 5–7 µm). The crescent-shaped manubria are 15 µm long and composed

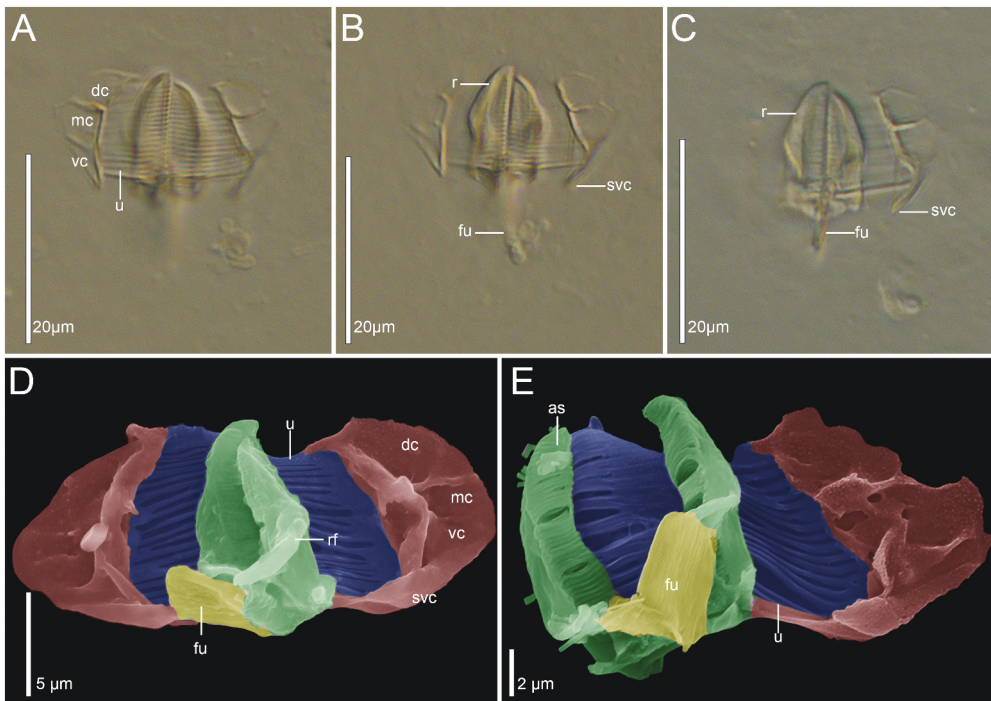


Figure 5. Trophi of *Pompholyx faciemplarva* sp. n. (A–C) DIC microphotographs. (A) Frontal view; (B) focal plane between frontal and caudal view; (C) caudal view. (D,E) SEM false colour-coded microphotographs. (D) Caudal view; (E) slightly internal view of the ramus. Abbreviations: as, arched rami scleropili; dc, dorsal chamber; fu, fulcrum; mc, median chamber; r, ramus; rf, ramus fenestra; svc, sub-ventral chambers; u, uncus; vc, ventral chamber.

of superimposed dorsal, median, ventral, and small sub-ventral chambers (Figures 2C, D, 5).

Female reproductive system. Vitellarium is syncytial with at least 12 nuclei (Figures 2C, 4D). Germarium not observed. Amictic eggs are oviposited one at a time and retained by the female at her posterior end. Each egg is approximately 50 µm in diameter and connected to an adhesive string that is secreted internally by a stalk gland and protrudes out the cloaca (Figures 2, 3, 4A,D).

Protonephridia. One pair of protonephridia is present in each lateral body margin; these extend to the region around the posterior intestinal sphincter (Figures 2C, 4C).

Other structures. An interconnected chain of large cells (6 µm) is located close to the stomach and appears to attach to the stomach wall (Figures 2C, 3D, 4B). The cells are not ova; their function is undetermined.

A stalk gland is present on the ventral side. The gland has at least three apical lobes (total length: 8 µm) and secretes a sticky thread ('Gossesche thread') approximately 80 µm long that passes through the cloaca to attach to a single amictic egg. At least two muscles insert on or close to the stalk gland and function to release or retract the adhesive thread,

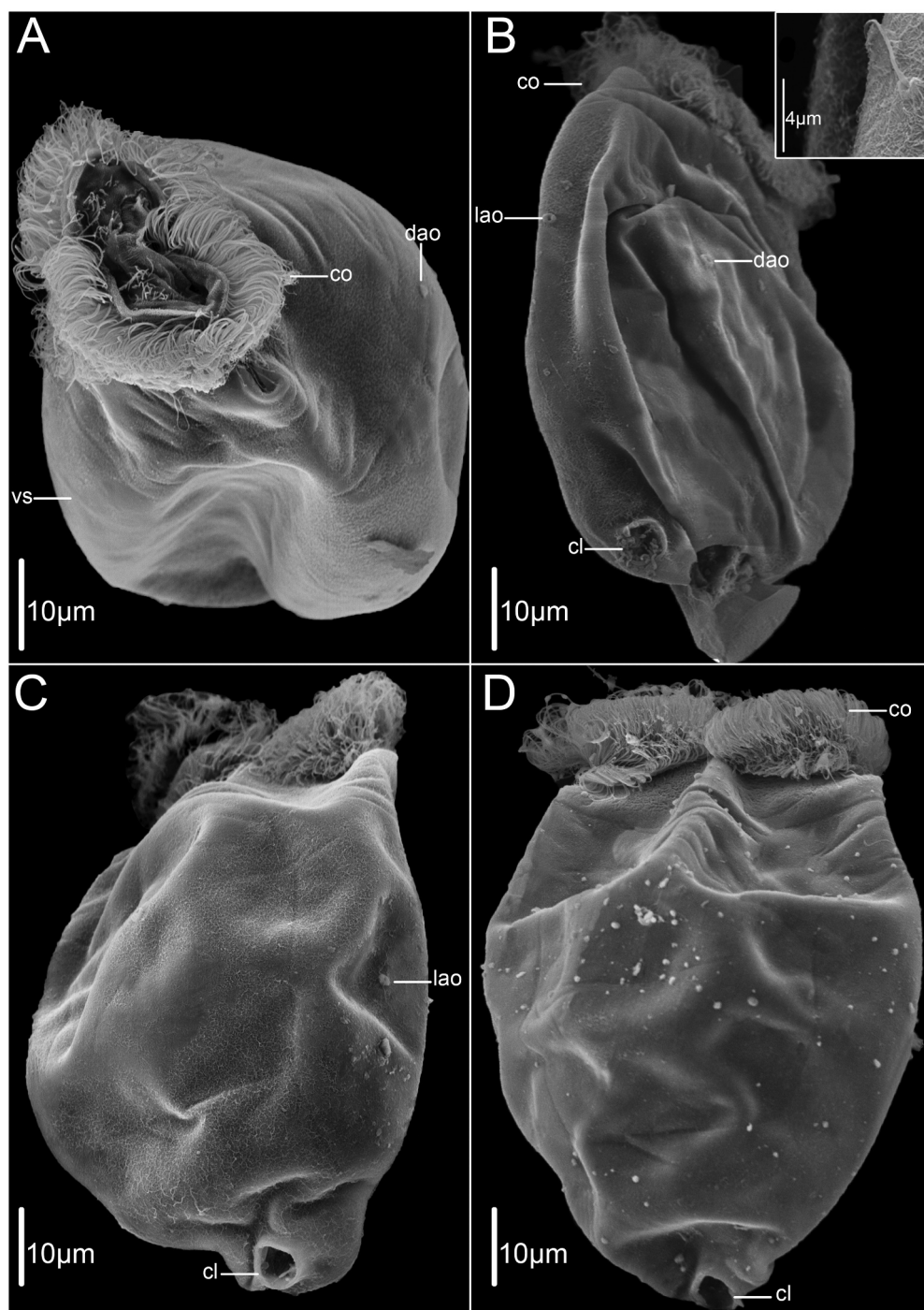


Figure 6. SEM microphotographs of *Pompholyx faciellarva* sp. n. (A,B) Dorsolateral view; (C) ventrolateral view; (D) ventral view. Abbreviations: cl, cloaca; co, corona; dao, dorsal antennae opening; lao, lateral antennae opening.

thereby moving the egg farther from or closer to the cloaca (Figures 2C, 3B, 4A,D). No more than one amictic egg was ever observed being carried by an adult female.

Ecology and distribution

Pompholyx faciemplarva **sp. n.** was found in Flint Pond, Tyngsborough, MA, USA, during the summer months (June–September 2022). This pond has a rich submerged macrophyte community and the plankton was abundant, comprising at least 11 genera of rotifers and numerous other zooplanktonic taxa. The new species is common, but not abundant. Its collection from the surface waters (< 1 m depth) of the pond suggests it is fully planktonic. Other common rotifers from the same collections include the following: *Asplanchna priodonta* Gosse, 1850, *Asplanchnopus multiceps* (Schrank, 1793), *Brachionus angularis* Gosse, 1851, *Hexarthra* sp., *Keratella cochlearis* (Gosse, 1851), *Kellicottia* sp., and *Polyarthra* spp.

Discussion

This new species represents the fourth valid species of *Pompholyx* Gosse, 1851 (Segers, 2007), but a lack of detail on two of the described species, *P. complanata* Gosse, 1851 and *P. triloba* Pejler, 1957, makes a differential diagnosis complicated. Microscopic details of the lorica and a description of the trophi are two important taxonomic features that are absent from the original descriptions of both species. For this reason, the new species can only be compared in detail to *P. sulcata* Hudson, 1885, which has received attention in several studies (Hudson and Gosse 1889; Leissling 1924; Remane 1929–1933) including a detailed description of its trophi via SEM (De Smet 2005).

The ellipsoid body shape, presence of a lorica and stalk gland system, and absence of a foot are characteristics of species of *Pompholyx* (Ruttner-Kolisko, 1974). To date, the only species with well-described trophi is *P. sulcata*. According to De Smet (2005), the trophi of *P. sulcata* follows that of other species of Testudinellidae and includes paired manubria, rami and unci, and an unpaired fulcrum. The number of unci teeth, which may be symmetrical or asymmetrical, is described as 17–20 (right)/18–21 (left) for 20 measured specimens. By contrast, the trophi of the new species appear to only possess a symmetrical number (17) of unci teeth (11 specimens), but this is based solely on DIC microscopy. We recognise that a limitation of our study is the lack of SEM analyses of the trophi, which may provide additional details (e.g., precise shape of unci teeth, number of rami scleropili) that can be used in current and future taxonomic assessments. Nevertheless, the size of the trophi elements and the consistency in our observations of the unci and other characteristics argues against synonymy with *P. sulcata*.

The new species has a smooth and mostly featureless lorica. SEM reveals a fibrous texture to the lorica in some regions, and occasionally small drop-like features, but their inconsistent presence leads us to conclude that these are artefacts of fixation. The lorica possesses an anterior edge (convexity) on the dorsal (occipital) side and an anterior notch (concavity) on the ventral (pectoral) side just below the corona. The cross-sectional profile of the new species shares qualities with the three described species. In *P. faciemplarva* **sp. n.**, the lorica is dorsally convex (arched) with two longitudinal furrows demarcating the flared lateral edges; the ventrum is somewhat convex and in the shape of an inverted triangle. By comparison, the lorica of *P. complanata* is mostly circular; the lorica of *P. sulcata* has four convex lobes,

each demarcated by a longitudinal furrow; and the lorica of *P. triloba* has an arched dorsal surface, flared lateral edges, and a somewhat concave ventrum. In addition to these differences, the new species possesses transverse furrows on both dorsal and lateral sides. These furrows are consistent in living and preserved specimens and do not appear to be the result of muscle contraction in specific body regions, nor are they artefacts of preservation.

Various sensory organs are noted for *P. faciementarum* sp. n. It appears to possess a sublabium, which is a ventral, ciliated, tongue-shaped extension below the mouth and positioned in the ventral concavity of the lorica. To our knowledge, a sublabium has not been reported in any other species of *Pompholyx*, but that structure might have been misinterpreted as a ventral antenna in *P. complanata* (Hudson and Gosse, 1889). In our studies, the sublabium was only observed during locomotion as the animal rotated about its long axis during ciliary gliding. Other sensory organs include a pair of lateral antennae and a single, dorsal antenna around mid-body length on the lorica. Both *P. sulcata* and *P. complanata* also possess antennae in similar positions.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data in the form of videos are available from the authors upon request.

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