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with Redefinition of *Atopospira* Jankowski, 1964
nov. stat. and *Brachonella* Jankowski, 1964
(Ciliophora, Armophorida)

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Redescription of *Atopospira galeata* (Kahl, 1927) nov. comb. and *A. violacea* (Kahl, 1926) nov. comb. with redefinition of *Atopospira* Jankowski, 1964 nov. stat. and *Brachonella* Jankowski, 1964 (Ciliophora, Armophorida)

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Abstract

The taxonomy of the Metopidae (Ciliophora, Armophorida) remains poorly understood since most of its members have not been studied by modern morphologic and molecular methods. Recent molecular investigations have indicated that the two most species-rich genera, *Metopus* and *Brachonella*, are likely nonmonophyletic with at least one well-supported 18S rDNA clade comprised of a species from each of these genera (*Brachonella galeata* and *Metopus violaceus*). We investigated these two species with silver impregnation and scanning electron microscopy. Both taxa share important morphologic characteristics not described in other species of *Metopus* or *Brachonella*. These synapomorphies include: (1) a diplostichomonad paroral membrane, (2) a bipartite adoral zone with a short buccal part composed of ordinary membranelles and a longer distal part composed of much smaller membranelles bearing a single cilium or none and extending the same length as the perzonal ciliary stripe. We transfer *Brachonella galeata* (Kahl, 1927) Jankowski, 1964 and *Metopus violaceus* Kahl, 1926 to genus *Atopospira* Jankowski, 1964 nov. stat. Pending detailed morphologic and molecular characterization, *Brachonella campanula*, *B. cydonia* and *B. pyriforma*, *B. intercedens*, and *B. lemani* remain in *Brachonella* Jankowski 1964.

Keywords: Adoral membranelle; Armophorida; *Brachonella*; *Metopus*; Paroral membrane; Phylogeny

Introduction

The ciliate family Metopidae Kahl, 1927 (order Armophorida Jankowski, 1964) consists mainly of free-living taxa inhabiting anaerobic or microaerobic biotopes (Lynn 2008). Over 90% of the species are classified in two of its nine genera, *Metopus* Claparède & Lachmann, 1858 and *Brachonella* Jankowski, 1964a (Lynn 2008). Since the revision of *Metopus* by Esteban et al. (1995), only a few detailed morphologic and molecular studies of metopid taxa have been published (Bourland et al. 2014; Çapar 2007; Foissner and Agatha 1999; Foissner et al. 2002; Vd'ačný 2007). Study of the Armophorida (i.e. caenomorphids and metopids) and its relationship to other orders is further hampered by the lack of corresponding morphologic data for most of the taxa with available gene sequences (Paiva et al. 2013). Consequently, the taxonomy and the phylogeny of the metopids remains unresolved and in need of much broader taxon sampling.

A recent 18S rDNA phylogeny of morphologically characterized armophorids, including eight new single-cell metopid sequences, provided strong evidence that, at least from a molecular standpoint, the genera *Metopus* and *Brachonella* are nonmonophyletic (Bourland et al. 2014). Ciliates identified as *Metopus violaceus* Kahl, 1926 and *Brachonella galeata* (Kahl, 1927) Jankowski, 1964b formed a clade with moderate to strong support in Maximum Likelihood and Bayesian analysis respectively. Monophyly of this clade was not rejected by the AU test (Shimodaira 2002). Despite a close molecular relationship no obvious morphologic synapomorphies for this clade were evident based on the previous descriptions (Jankowski 1964b; Kahl 1926, 1927). In this report we present a detailed morphologic and morphometric study of these two species, proposing at least two important morphologic synapomorphies, and

discussing the taxonomic implications of our findings. We place both taxa in *Atopospira* Jankowski, 1964a elevated here from subgenus to genus rank.

Material and Methods

Collection data. Populations of *Atopospira galeata* and *Atopospira violacea* were sampled from a garden tub mesocosm (Bourland et al. 2014). We originally found *A. galeata* in sulfidic bottom sediments of the outflow stream from Riverside Pond in Boise, Idaho (43°39'47.45"N; 116°16'57.49"W, elev. 796 m). We originally found *A. violacea* in the bottom sediments of a different eutrophic pond (43°40'57.20"N; 116°15'15.44"W, elev. 873 m) near Boise, Idaho (for details, see the occurrence and ecology section). The populations of both taxa from the mesocosm are indistinguishable from those from the original sites. Attempts to establish clonal cultures were unsuccessful.

Morphologic methods. Living cells were studied at magnifications of 40–1000× with brightfield, phase- and differential interference contrast illumination using a Zeiss Axioskop 2 plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA), a Flex digital camera, and calibrated Spot imaging software (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Video imaging was done using an Olympus BX53 microscope (Olympus America, Center Valley, PA, USA) and Canon 6D camera (Canon Inc., Tokyo, Japan). Attempts to induce formation of resting cysts by starvation in filtered (0.22 µm pore size) site water were unsuccessful as cells quickly died. Protargol and silver carbonate impregnation and scanning electron microscopy (SEM) were done according to Foissner (1991). Cells were fixed in 10% formalin for protargol impregnation and a 1:1 mixture of 2% osmium tetroxide and aqueous 2.5% glutaraldehyde for SEM. An acetone developer was used for protargol impregnations

(Vd'áčný and Foissner 2012). In vivo measurements were made from photomicrographs of freely swimming cells. Counts and measurements were made at magnifications of 630–1000×. Measurements were made directly with an ocular micrometer and also from microphotographs using calibrated software. Statistical analyses were performed using MedCalc for Windows, version 11.2 (MedCalc Software, Mariakerke, Belgium). Drawings were based on microphotographs.

Terminology. Terminology is according to Bourland et al. (2014), Foissner and Agatha (1999), and Lynn (2008). Suprageneric classification is according to Lynn (2008). We refer to the two parts of the adoral zone as (1) the “buccal part” (composed of ordinary membranelles and limited to the buccal cavity) and (2) the “distal part” (small membranelles with one or no cilia on the postoral somatic cortex).

Results and Discussion

Class Armophorea Lynn, 2004

Order Armophorida Jankowski, 1964

Family Metopidae Kahl, 1927

Genus *Brachonella* Jankowski, 1964a

Improved diagnosis. Medium-sized Metopidae, appearance bulky; usually with cortical granules; disproportionately large preoral dome overhanging elongated adoral zone of membranelles, adoral membranelles composed of long files of basal bodies, in deep groove spiraling in reverse S-shape around entire body, pitch of adoral zone spiral variable; paroral membrane a single file of basal bodies; cytostome displaced posteriorly.

Type species. *Metopus contortus* Levander, 1894 (now *Brachonella contorta* (Levander, 1894) Jankowski, 1964).

Species included. *Brachonella contorta* (Levander, 1894); *Brachonella caduca* (Kahl, 1927) Jankowski, 1964b; *B. campanula* (Kahl, 1932) Jankowski, 1964b; *B. cydonia* (Kahl, 1927) Jankowski, 1964b; *B. darwini* (Kahl, 1927) Jankowski, 1964b; *B. elongata* Jankowski, 1964b; *B. fastigata* (Kahl, 1927) Jankowski, 1964a; *B. intercedens* (Kahl, 1927) Jankowski, 1964a; *B. lemani* (Dragesco 1960) Jankowski, 1964a; *B. mitriformis* Alekperov, 1984; *B. pyriforma* (Levander, 1894) Jankowski, 1964a.

Etymology. Jankowski (1964a) named the genus in honor of French protistologist, Simone Villeneuve-Brachon, recognizing her important contributions to knowledge of the Heterotrichida.

Remarks. We consider Jankowski (1964a) as the place of publication for the genera *Atopospira* and *Brachonella* since they are described therein.

Genus *Atopospira* Jankowski 1964a nov. stat.

1964a *Atopospira* - Jankowski, Zool. Zh. 43, 506 (original description; established as subgenus of *Brachonella*).

1964b *Atopospira* - Jankowski, Arch. Protistenk. 107, 216 (taxonomic revision; published subsequent to [1964a]).

2001 *Atopospira* - Aescht, Denisia 1, 27 (listed as subgenus of *Brachonella* in “optional current usage”, p. 305).

2007 *Atopospira* - Jankowski, Phylum Ciliophora Doflein, 1901. In: Alimov, A. F. (Ed.), Protista. Part 2, 528 (brief taxonomic revision without illustration).

Improved diagnosis. Small to medium-sized Metopidae with diplostichomonad paroral

membrane and bipartite adoral zone of membranelles, a buccal part comprised of ordinary membranelles made of four long kineties, and a distal part composed of more numerous, smaller sparsely ciliated or even unciliated membranelles parallel to and extending nearly the length of the perizonal ciliary stripe. Elongated posterior cilia. Cortical granules absent. Degree of adoral zone spiraling varies from 180° to 360°. Division occurs in the free-swimming state. In lentic habitats, bacterivorous.

Type species. *Metopus galeatus* Kahl, 1927.

Species included. *Atopospira galeata* (basionym: *Metopus galeatus* Kahl, 1927), *A. violacea* (basionym: *Metopus violaceus* Kahl, 1926).

Etymology. Not given in the original or subsequent descriptions (Jankowski 1964a,b). A composite of the Greek “*atopos*” (misplaced/highly unusual) and the Latin “*spira*” (a coil or twist), possibly referring to the unusually short pitch of the adoral zone in the type species. Feminine gender.

***Atopospira galeata* (Kahl, 1927) nov. comb.**

1927 *Metopus galeatus* - Kahl, Arch. Protistenk. 57, 156, Fig. 18c-e (original description; no type material available).

1928 *Metopus galeatus* - Wetzel, Z. Morphol. Ökol. Tiere 13, 226 (included in the “uncertain” species of *Metopus* by the author; without illustration).

1931 *Metopus galeatus* - Kahl, Mikrokosmos 24, 11, Tafel 2, Bild 7 (brief redescription; more detailed illustration with elongated perizonal stripe cilia).

1932 *Metopus galeatus* - Kahl, Tierwelt Dtl. 25, 423, 424, Fig. 29, 30 (brief redescription of larger [80 µm] population; possible error in measurement).

1964 *Brachonella (Atopospira) galeata* - Jankowski, Zool. Zh. 43, 505 (taxonomic

revision, transfer to new genus *Brachonella* and fixation as type species of *Brachonella* (*Atopospira*); without description or illustration).

1964 *Brachonella* (*Atopospira*) *galeata* - Jankowski, Arch. Protistenk. 107, 192, Fig. 22a, b (taxonomic revision, brief description of Russian population).

1979 *Brachonella galeata* - Löffler, Monograph. biol. 37, 491 (list of Austrian ciliate species; live observation without illustration).

1995 *Brachonella galeata* - Esteban et al., Arch. Protistenk. 146, 139 (taxonomic revision of *Metopus*; without illustration).

2007 *Brachonella* (*Atopospira*) *galeata* - Jankowski, Protista. Part 2, Handbook on Zoology. p. 528 (taxonomic revision; without illustration).

2014 *Brachonella galeata* - Bourland et al., Eur. J. Protistol. 50, Figs. 47, 64 (molecular phylogeny).

Improved diagnosis based on Idaho population and original description. Body size about 45–60 × 45–55 μm in vivo. Outline pyriform. Preoral dome broadly helmet-shaped, forms anterior half, wider than mid-body, brim perpendicular to long axis. Posterior half obconical, rounded to truncate. About 16 ordinarily spaced ciliary rows about six of which extend onto preoral dome. Perizonal ciliary stripe composed of five rows of dikinetids forming about 45 false kineties, innermost row unciliated. Buccal part of adoral zone with about seven membranelles composed of four long rows, distal part on somatic cortex parallel to preoral dome brim, having about 19 small organelles, composed of four rows of two basal bodies, only single cilium on first basal body of posterior row. Adoral zone and perizonal ciliary stripe of approximately same length.

Etymology. A substantive adjective composed of the Latin primitive, *galeatus*, *i* masc. (helmet) and the Latin substantive suffix, *-atus*, *-a*, *-um* meaning (-ate, -like) i.e. “galeate” or “helmeted”, referring to the helmet-like shape.

Voucher material. We are unaware of any type material. Three protargol-impregnated voucher slides with many specimens are deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI, accession numbers 2014/22/1-3). Pertinent specimens are circled in black ink. An 18S rDNA sequence from the Idaho population has been deposited in GenBank (accession no. KF607084 as *Brachonella galeata*).

Description of Idaho population (Figs 1–19, 28–30; Tables 1, 2). Size in vivo 46–59 × 44–54 μm; protargol-impregnated specimens 37–58 × 35–59 μm; length:total width ratio 1:1, dorsoventrally compressed approximately 2:1. Cells dark under low magnification and violet under higher power due to contents of food vacuoles (Figs 28–30). Outline broadly pyriform; preoral dome with prominent brim spirals leftward 360° from mid-body to merge with body slightly anteriorly, dome brim overhangs postoral body; postoral half of body obconical, rounded to truncate depending on status of contractile vacuole. Macronucleus broadly ellipsoidal, in preoral dome, chromatin finely granular in vivo, scarce small peripheral nucleoli after protargol impregnation. Micronucleus difficult to see in vivo, ellipsoidal, often distant from macronucleus in silver-impregnated specimens. Contractile vacuole in posterior end. Neither cytopyge nor excretory pore identified. Cortex inconspicuously furrowed, without granules. Extrusomes absent. Cytoplasm colorless, contains scattered shiny approximately 2 μm globules, without crystals. Food vacuoles numerous, up to 15 μm across, invariably contain coccoid purple sulfur bacteria (Figs 29, 30). Anterior accumulation of cytoplasmic granules absent. Endosymbionts

usually absent (see occurrence and ecology section below). Movement slow, often appears suspended motionless.

Ordinary somatic cilia about 12 μm long in vivo, 10–15 approximately 20 μm long lank posterior cilia sparsely distributed over posterior end, immotile, never stiffen (Fig. 1). Somatic cilia fragile, thus many often missing in fixed specimens (Figs 14, 15). About 16 somatic kineties on average (including dome kineties), composed of dikinetids arranged slightly obliquely to long axis, only posterior basal bodies ciliated; kineties converge at posterior end; ten more or less meridional left somatic kineties, perpendicular to adoral zone. Six dome kineties, crowded to right of buccal vertex, spiral leftward onto dome in widely spaced furrows, small glabrous area right dorsal dome since dome kineties shortened anteriorly. Perizonal ciliary stripe on preoral dome brim, approximately same length as adoral zone, composed of five kineties. Perizonal stripe kinety 5 unciliated, thus not visible in SEM preparations (Figs 14–16, 18), at least one basal body in dikinetids of rows 2–4 bear 20 μm long cilia; rows 1–3 closely spaced, separated from rows 4 and 5, inclined posteriorly forming about 45 distinct “false kineties”. Wide space separates perizonal stripe kinety 5 and dome kinety 1 (Figs 2, 4, 10).

Fibrillar associates most prominent in perizonal stripe kinetids in silver carbonate preparations, consist of long right-projecting fibrils from kinetids of perizonal row 5 nearly reaching dome kinety 2 (probably kinetodesmal fibers), similar but much shorter fibril bundle from kinetids of rows 2–4, narrow transverse interkinetal fibrils between rows 1 and 2, 4 and 5 of perizonal stripe, denser bundles arise from rows 2 and 3, converge on dikinetids of row 4, probably transverse microtubular ribbons; faintly impregnating longitudinal interkinetal fibrils, probably postciliary microtubular ribbons (Fig. 10).

Adoral zone of membranelles parallels dome brim thus nearly equatorial; extends from cytostome to distal end of preoral dome brim. Bipartite, buccal part with five to seven membranelles composed of four long rows of basal bodies with approximately 8 μm long cilia in buccal cavity, five longest membranelles in center of buccal part; distal part of adoral zone on postoral somatic cortex, made up of about 19 (range 17–24) membranelles having four rows of only two basal bodies each, slightly oblique to long axis, only distal basal body of right row ciliated. Paroral membrane diplostichomonad (i.e. composed of parallel files of ciliated, non-zigzagging monokinetids); files separated by approximately 0.6 μm -wide cortical strip, inner file sometimes slightly longer, files extend from distal portion of buccal cavity on undersurface of preoral dome, curve toward cytostome under buccal lip proximally, usually one or both files with a few unciliated basal bodies distally; about 5 μm long cilia. Details of paroral difficult to resolve in silver impregnations due to superimposed of basal bodies and associated fibrillar structures. Paroral gives rise to inconspicuous anteriorly directed funnel of cytopharyngeal fibers. We found only a single silver-impregnated late divider indicating reproduction occurs in the free-swimming state and not in cysts (Fig. 49). The details of morphogenesis remain to be determined. Conjugants and cysts were not observed.

Occurrence and ecology. *Atopospira galeata* inhabits sulfidic bottom sediments in lentic habitats. Kahl (1927) discovered *A. galeata* in the sapropel of a stream feeding a bog in Germany during August. Jankowski (1964b) found it in a forest pond in Russia during June. *Atopospira galeata* has also been reported (without illustration) from Austria (Löffler 1979), but has not yet been reported from Gondwanan habitats. It has not been reported from soils. The Idaho population was originally collected from the nearly stagnant outflow stream of a pond in Boise Idaho (43°39'47.40" N 116°16'57.12" W; elev. 796 m) in April 2004. Since 2004 we have

maintained *A. galeata* in a garden tub mesocosm described in detail previously (Bourland et al. 2014). We have not found *A. galeata* elsewhere to date. Although found in anoxic/hypoxic habitats, *A. galeata* exhibits notable aerotolerance since it survives unscathed for up to seven days in open jars.

Atopospira galeata feeds exclusively on purple sulfur bacteria (probably *Lamprocystis roseopersicina*). We recently studied an abundant mesocosm population in early spring (March 2014). In addition to food vacuoles with the usual coccoid sulfur bacteria, cells contained long, thin rod-shaped cytoplasmic bacteria (Figs 47, 48). These appeared to be transient endosymbionts since they disappeared after several weeks. Prior to this observation we had never seen endosymbionts in this species during nearly ten years of careful study. The possibility that these were food organisms seems unlikely but cannot be excluded. Endosymbionts were not observed in the original stream population.

Comparison with original description and related species. The Idaho population matches the original and subsequent descriptions and illustrations by Kahl (1927, 1931, 1932) very closely in all respects. The size given in Kahl's last redescription differs significantly (80 μm vs. 40–60 μm) from the original description and second brief redescription. An error in measurement cannot be excluded (Foissner and Wenzel 2004). Kahl (1927) placed this species together in his "Gruppe V" with *M. fastigatus* and *M. violaceus*. *Atopospira galeata* is distinguished from *M. fastigatus* (Fig. 53) by shape (dorsoventral flattening absent vs. present) and the extent of the peristomial region (360° spiral vs. 180°). *Atopospira galeata* is easily distinguished from *Atopospira violacea* by length in vivo (46–59 μm vs. 95–133 μm), overall shape (dorsoventral flattening absent vs. present), outline of posterior margin (dentate processes absent vs. present), extent of peristomial region (360° spiral vs. 180°), and number of kineties

(15–22 vs. 35–48). Jankowski's (1964b) Russian population averaged about 65 μm . He also illustrates about 15 adoral membranelles vs. five to seven in the Idaho population, and the long posterior cilia as a discrete tuft vs. sparse and diffusely distributed. Jankowski (1964b) does not specify the number of kineties or adoral membranelles. It should be noted that neither Kahl (1927, 1931, 1932) nor Jankowski (1964b) studied this organism with silver impregnation. Kahl (1927) suggested *Caenomorpha heinrici* Blochmann, 1894 as a possible subjective synonym. Because Blochmann's (1894, his Fig. 3) description is somewhat vague and, curiously, only the posterior end of the organism is illustrated, we agree with Jankowski (1964b) that there is insufficient data to consider *C. heinrici* as a synonym of *A. galeata*. Furthermore, Corliss (1979) has designated *Caenomorphina* as a nomen oblitum.

***Atopospira violacea* (Kahl, 1926) nov. comb.**

1926 *Metopus violaceus* - Kahl, Arch. Protistenk. 55, 426, Textfig. B_{4 a,b} (original description; no type material available).

1927 *Metopus violaceus* - Kahl, Arch. Protistenk. 57, 157, Fig. 18g (redescription recognizing short proximal part of adoral zone and conjugation).

1929 *Metopus violaceus* - Wetzel, Z. Morphol. Ökol. Tiere 13, 226 (included in the “uncertain” species of *Metopus* by the author; without illustration).

1931 *Metopus violaceus* - Kahl, Mikrokosmos 24, 11, Tafel 2, Bild 5 (brief redescription; more detailed illustration with elongated perizonal stripe cilia).

1932 *Metopus violaceus* - Kahl, Tierwelt Dtl. 25, 422; 424, Figs 27, 28 (brief redescription; illustration of conjugants).

1964 *Metopus (Urostomides) violaceus* - Jankowski, Zool. Zh. 43, 506 (taxonomic revision; placement in new subgenus *Urostomides*; without description or illustration).

1964 *Metopus (Urostomides) violaceus* - Jankowski, Arch. Protistenk. 107, 204, Fig. 13 (taxonomic revision, description of Russian population).

1995 *Metopus violaceus* Kahl, 1927 - Esteban et al., Arch. Protistenk. 146, 139 (classified as junior synonym of *Metopus striatus*).

2007 *Metopus (Urostomides) violaceus* - Jankowski, Protista. Part 2, Handbook on Zoology. p. 526 (taxonomic revision; without illustration).

2014 *Metopus violaceus* - Bourland et al., Eur. J. Protistol. (in press), (molecular phylogeny).

Improved diagnosis based on Idaho population and original description. Body size about 95–135 × 60–85 µm in vivo. Outline broadly pyriform. Dorsoventrally flattened 2:1. Preoral dome two thirds of body length. Posterior margin obliquely truncate with short right and left tooth-like cortical projections. Cortex inflexible, fluted by prominent longitudinal ridges. About 41 ordinarily spaced ciliary rows about 14 of which extend onto preoral dome. Perizonal ciliary stripe composed of five rows of dikinetids forming about 50 false kineties. Buccal part of adoral zone composed of about ten membranelles with four long rows, distal part about 22 small unciliated membranelles consisting of two to eight basal bodies.

Etymology. From the Latin adjective *violaceus*, *-a*, *-um* (violet colored), referring to the color imparted to cells by Rhodobacteria in food vacuoles. Mandatory change of gender ending necessary (ICZN 1999, Article 31.2) because *Atopospira* is feminine: *Atopospira violacea* (Kahl, 1926) nom. corr.

Voucher material. There is no record of original type material for *A. violacea*. Two protargol-impregnated slides with multiple specimens from the Idaho mesocosm population are deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI, accession numbers

2014/22/4-6). Pertinent specimens are circled in black ink. An 18S rDNA sequence from the Idaho mesocosm population has been deposited in GenBank (accession no. KF607086 as *Metopus violaceus*).

Description of Idaho population (Figs 20–27, 31–46). Size in vivo 95–133 × 60–84 µm; protargol-impregnated specimens 63–115 × 47–77 µm; length:total width ratio 1.5:1, dorsoventrally compressed approximately 2:1. Cells violet due to contents of food vacuoles. Outline broadly pyriform; preoral dome with prominent brim spirals leftward 180° from posterior body third to merge with body at right margin of dorsal surface; postoral body third obconical, obliquely truncate, bicuspid with right and left margins ending in short tooth-like projections ventral view, posterior end acuminate in lateral view due to dorsoventral flattening. Macronucleus broadly ellipsoidal, in preoral dome, chromatin finely granular in vivo, scattered approximately 5 µm nucleoli in protargol-impregnated specimens. Micronucleus ellipsoidal, near macronucleus. Contractile vacuole in posterior end. Cytopyge and excretory pore not identified. Cortex inflexible, hyaline, fluted with sharp longitudinal ridges, granules and extrusomes absent. Cytoplasm colorless, contains scattered shiny approximately 2 µm globules; crystals and endosymbionts not seen by light microscopy. Food vacuoles numerous, up to 35 µm across, invariably contain coccoid purple sulfur bacteria (Figs 31, 32, 36, 37). Anterior accumulation of cytoplasmic granules absent. Movement very slow, often appears suspended motionless over detritus.

Ordinary somatic cilia about 12 µm long in vivo, 10–20 approximately 25 µm long lank posterior cilia sparsely distributed over posterior end, immotile, never stiffen. Somatic cilia fragile, thus many missing in fixed specimens. About 41 meridional somatic kineties on average (including dome kineties) in furrows between pellicular ribs, composed of dikinetids arranged

slightly obliquely to long axis in silver preparations, only posterior basal bodies ciliated. Kineties slightly shortened posteriorly leaving small bare area along posterior margin; on average 14 dome kineties, narrowly spaced with irregularly arranged dikinetids to right of buccal vertex extend onto dome in widely spaced furrows without spiraling, dome kineties shortened anteriorly leaving small glabrous area right dorsal dome. Perizonal ciliary stripe on brim of preoral dome, same length as adoral zone, composed of five kineties made of dikinetids, both basal bodies with approximately 20 μm long cilia; rows 1–3 closely spaced and separated from rows 4 and 5, inclined posteriorly forming about 50 “false kineties”.

Fibrillar associates of dikinetids most prominent in perizonal stripe in silver carbonate preparations, consist of very long slender right-projecting fibrils from dikinetids of perizonal row 4 and 5 overlapping dome kinety 1, similar but much shorter structures arise from rows 3 and 4; short posteriorly projecting fibrils from dikinetids of rows 4 and 5; narrow transverse interkinetal fibrils rows 1 through 5 (Figs 38, 39). Silverline pattern not studied.

Adoral zone of membranelles parallels dome brim; extends from cytostome to distal end of preoral dome brim. Bipartite, with eight to twelve (usually ten) rectangular buccal membranelles composed of four long rows of basal bodies with approximately 8 μm long cilia, membranelles decrease in length from proximal to distal, fibrillar associates of left-most row extend horizontally to overlap the adjacent adoral membranelle in silver carbonate impregnations (Figs 39, 40); distal part of adoral zone with about 22 (range 16–30) unciliated obliquely oriented membranelles composed of only two to eight basal bodies. Paroral membrane diplostichomonad (i.e. composed of parallel files of ciliated, non-zigzagging monokinetids); files separated by cortical strip, extends from distal buccal cavity on undersurface of preoral dome, curve toward cytostome under buccal lip proximally, usually one or both files with a few unciliated basal

bodies distally; about 5 μm long stiff cilia. Paroral gives rise to funnel of cytopharyngeal fibers, curves into preoral dome on right. We found only one late divider in silver-impregnated specimens, confirming that division occurs in the free-swimming state as in *A. galeata* (Fig. 50). The details of earlier stages of morphogenesis are unknown. We found no conjugants or cysts .

Occurrence and ecology. Like *Atopospira galeata*, *A. violacea* inhabits sulfidic bottom sediments in lentic habitats and very slow-flowing streams. Kahl (1926) discovered *A. violacea* during spring in a pond with abundant Rhodobacteria near Hamburg, Germany. A report in a species list from the Potomac River, U.S.A. is not accompanied by a description or illustrations (Patrick 1996). The type locality for the Idaho population is a eutrophic pond near Boise, Idaho (43°40'57.20" N 116°15'15.44" W; elev. 873 m) where we originally collected it in June 2006. We have not found *A. violacea* elsewhere to date. Since 2006 we have maintained *A. violacea* in the same garden tub mesocosm as *A. galeata*. Like *A. galeata*, *A. violacea* feeds exclusively on purple sulfur bacteria (probably *Lamprocystis roseopersicina*). We have never observed endosymbionts in this species. *Atopospira violacea* also exhibits a significant degree of aerotolerance since it survives, along with *A. galeata*, for several days in open jars.

Comparison with original description and related species. The Idaho population matches Kahl's (1926) original description and subsequent redescrptions (Kahl 1927, 1931, 1932) very closely. The illustrations show slightly elongated posterior cilia (Kahl 1931, 1932) but they are mentioned only in the last redescription (Kahl 1932). Kahl (1926, 1927, 1931, 1932) depicted a serrated posterior margin, supposing that each pellicular rib extended posteriorly as a short projection, (Figs 50a,b, 51–53a). However, study of the Idaho population by DIC (in vivo), silver impregnation and SEM shows that the right and left side ribs coalesce into two slightly offset dentate processes separated by a smooth pellicular concavity giving the truncate posterior

margin a bicuspid outline in dorsal and ventral views (Figs 23, 27a–d, f, 35, 41–43). The Idaho population differs significantly from Jankowski's (1964b) Russian population in the following respects: number of somatic kineties (35–48 vs. 26); morphology of adoral zone (bipartite vs. monomorphic); number of large adoral membranelles (8–12 vs. >21 [Jankowski 1964b, his Fig. 13]); aggregation of refractive granules at anterior end (absent vs. present). Although Jankowski's observations were based on live material and mercuric chloride-fixed specimens, these significant differences make conspecificity doubtful. Jankowski's population likely belongs to a different genus, possibly *Metopus*. Esteban et al. (1995) proposed synonymy of a large number of *Metopus* species, including *A. violacea*, and *M. fastigatus* as junior synonyms of *M. striatus* McMurrich, 1884. The synonymy of *A. violacea* and *M. striatus* must be rejected for many reasons including: (1) absence vs. presence of extrusomes and cortical granules; (2) bipartite vs. monomorphic adoral zone; (3) 35–48 somatic kineties vs. 18–24; (4) presence vs. absence of pellicular ribs; (5) presence vs. absence of bicuspid posterior margin; (6) a 7.4% pairwise difference between their 18S rDNA sequences (Bourland et al 2014; Jankowski 1964b; Kahl 1926, 1927, 1931, 1932). *Atopospira violacea* differs from *M. fastigatus* in size (95–133 vs. 40–50 μ) and outline (presence vs. absence of bicuspid posterior margin).

Molecular characterization and phylogeny of metopids (Fig. 60). The 18S rDNA gene sequence for *A. galeata* is 1670 nucleotide pairs in length and that of *A. violacea* is 1671 bp long. These two taxa form a well-supported clade in phylogenetic analyses (Bourland et al. 2014). The pair wise distance between the 18S rDNA sequences of *A. galeata* and *A. violacea* and *Brachonella spiralis* is 10.5% and 10.6% respectively. The distance between *A. galeata* and *A. violacea* is 2.4%. The mean distance between four *Metopus* species (*M. laminarius*, *M. setosus*, *M. striatus* and *M. fuscus*) is 6.3%. The distances between these *Metopus* species and *B. spiralis*

range from 9.9–10.9%. Phylogenetic studies at the suprageneric level support a sister group relationship to the Litostomatea (Paiva et al. 2013; Vd'áčný et al. 2010).

Generic classification of *Atopospira galeata* and *A. violacea*. Jankowski (1964a) erected the genus *Brachonella* and split it into two subgenera, *Brachonella* (*Brachonella*) (type species: *Metopus contortus* Levander, 1894) and *Brachonella* (*Atopospira*) (type species: *Metopus galeatus* Kahl, 1927). In that same year (1964b) he provided the following diagnoses: “Subgenus 1. *Brachonella* s. str. - The anterior body part much exceeds the posterior one in length; [buccal cavity] is long, spiraling, with beginning and ending edges near the anterior and posterior body ends respectively; [cytostome] is shifted to the posterior pole, dorsally”; “Subgenus 2. *Atopospira* Jankowski, 1964a - Body ovoid to pyriform, with upper and lower parts more or less coinciding in length; the lower body part is narrow, the upper one is wide, especially in the equatorial zone. [Adoral zone of membranelles] is almost ring-like, equatorial with beginning and ending parts located on the same level. [Cytostome] is equatorial or posteriorly displaced”.

Silver impregnation studies demonstrating a bipartite adoral zone first suggested that *M. violaceus* Kahl, 1926 might belong to a new genus. This impression was strengthened when SEM findings showed a diplostichomonad paroral instead of the stichomonad morphology found in *Metopus* species and *Brachonella spiralis* (Bourland et al. 2014; Dragesco and Dragesco-Kernéis 1986; Foissner and Agatha 1999; Foissner et al. 2002). Molecular studies confirmed that *M. violaceus* had a large (9.9%) pairwise distance from the most closely related *Metopus*, *M. laminarius* (Bourland et al. 2014). The molecular phylogeny showed an unexpected affinity between *M. violaceus* and *Brachonella galeata* (Kahl, 1927) Jankowski, 1964. Further silver impregnation and SEM studies showed this species to share the morphologic characters of a

diplostichomonad paroral and a bipartite adoral zone like that of *M. violaceus*. Furthermore, the type species, *Brachonella contorta* (Levander, 1894), lacks these morphologic characters and also lacks a close molecular relationship with *B. galeata* (Bourland et al. 2014; Dragesco and Dragesco-Kernéis 1986; Jankowski 1964b). The molecular and morphologic evidence warrants placement of both species in a separate genus, *Atopospira* Jankowski, 1964a nov. stat.

Atopospira galeata nov. comb. is the type species (ICZN 1999, Article 61.2.2). We propose that *Brachonella mitriformis*, *B. campanula*, *B. cydonia*, *B. fastigata*, *B. pyriforme*, *B. lemani*, and *B. intercedens* remain in the genus *Brachonella* Jankowski, 1964a pending further molecular and morphologic characterization of these species and other metopids (e.g. the 18s rDNA of *Metopus* sp., type species of *Metopus*, has not yet been sequenced). In addition to broader taxon sampling for morphologic and molecular studies, future investigations of morphogenesis are needed to shed light on the relationships among the Metopidae. As yet, few such studies have been done due, in part, to the rarity of dividers even in well-populated raw cultures. Foissner and Agatha (1999) speculated that division in cysts might account for the rarity of free-swimming dividers in raw cultures of metopids. Division in the free-swimming state is likely a plesiomorphy of the Metopidae (Bourland et al. 2014, Esteban et al. 1995; Foissner and Agatha 1999; Martin-Gonzales et al. 1988) .

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Figure legends

Figs 1–8. *Atopospira galeata*, Idaho population from life (1) and after silver carbonate impregnation (2–8). **1.** Ventral view of a representative specimen. **2, 3.** Left dorsolateral and right ventrolateral views (same specimen) showing paroral and buccal adoral zone (white and black arrowhead respectively and the crowded, disorganized dikinetids of proximal part of dome kineties (black arrow). Structures on opposite aspect shown in gray. **4, 5.** Anterior and posterior polar views of same specimen. Margin of buccal cavity (black arrowhead) obscures buccal adoral zone and proximal portion of paroral (shown in grey). **6.** Semi-schematic of perizonal stripe kinety fibrillar associates. Black arrow indicates anterior end. **7.** Semi-schematic of adoral zone membranelles. Black arrow indicates anterior end. **8.** Oral structures, black arrow marks distalmost membranelle of buccal portion of adoral zone; white arrow denotes proximalmost membranelle of distal portion of adoral zone. AB, anterior basal body; BAZ, buccal part of adoral zone; D, dorsal; DAZ, distal part of adoral zone; DK1, preoral dome kinety 1; F, fiber; FK, false kineties; IF, interkinetal fiber; KD, kinetodesmal fibers; Ma, macronucleus; Mi, micronucleus; PB, posterior basal body; POM, paroral membrane; PR, pellicular ridge; PS, perizonal ciliary stripe; PS1, 5, perizonal ciliary stripe kineties 1 and 5; R, right; V, ventral. Scale bars: 25 μm , (1–5), 5 μm (8).

Figs 9–13. *Atopospira galeata*, Idaho population after silver carbonate impregnation. **9.** Left posterolateral view. Perizonal ciliary stripe structures appear reversed because they are viewed through the thin preoral dome rim. **10.** Left anterolateral view showing postciliary microtubular ribbons in the wide space separating perizonal stripe row 5 and dome kinety 1 (white arrowhead) and the long kinetodesmal fibers of perizonal stripe row 5 (black arrowhead). **11.** Posteroventral view showing proximal and distal ends of the perizonal ciliary stripe (black and white asterisks

respectively). The distal part of the adoral zone (white arrowheads) extends nearly to the distal end of the perizonal stripe. The posterior ends of dome kineties form an area of crowded, slightly disorganized dikinetids right of the buccal lip (black arrowhead). **12.** Posterior pole (asterisk) view showing buccal (white arrowhead) and reduced distal (black arrowheads) adoral zone membranelles, the proximal end of the perizonal stripe and the paroral membrane (black arrow). **13.** Posterior view showing perizonal stripe extending to the proximal margin of the buccal lip (black arrow). The paroral membrane (black arrowhead) extends out of the buccal cavity onto the undersurface of the preoral dome. Postoral somatic kineties (white arrow) extend to the level of the distal part of the adoral zone (proximal and distal ends marked by white and black asterisks respectively). BAZ, buccal part of the adoral zone; DAZ, distal part of the adoral zone; DK1, preoral dome kinety 1; FK, false kineties; Ma, macronucleus; Mi, micronucleus; POM, paroral membrane; PS, perizonal ciliary stripe; SK, somatic kineties. Scale bars: 25 μm .

Figs 14–19. *Atopospira galeata*, Idaho population in SEM. **14.** Ventral view showing buccal adoral membranelles (white arrowhead), diplostichomonad paroral (black arrowhead) and elongated posterior cilium. Fragile somatic cilia are often lost in preparation (asterisk). **15.** Left dorsolateral view showing paroral membranelle cilia (white arrow) and single cilium (arrowhead) of membranelles of the distal part of the adoral zone. The perizonal ciliary stripe is indicated by the black arrow. Most of the postoral somatic cilia have been lost in preparation (asterisk). **16.** Anterior view showing proximal (black asterisk) and distal (white asterisk) ends of the perizonal stripe. The arrow marks elongated perizonal stripe cilia. Arrowheads mark preoral dome kineties. **17.** Posterior pole (asterisk) view showing inner (white arrowhead) and outer (black arrowhead) paroral membrane cilia with intervening pellicular ridge (black arrow). **18.** Ventral view showing inner (white arrowhead) and outer (black arrowhead) files of the paroral

membranelle, the latter with a few unciliated basal bodies. The white arrow marks the distal-most membranelle of the buccal part of the adoral zone and the black arrow marks the single cilium of the first membranelle of the distal part of the adoral zone. **19.** Detail view of the basal bodies (white arrow) and single cilium (black arrow) of the membranelles of the distal part of the adoral zone. Scale bars: 10 μm (14–18), 2.5 μm (19).

Figs 20–27. *Atopospira violacea*, Idaho population from life (20, 27a–d), after protargol (21–25, 27e–h) and silver carbonate impregnation (26). **20.** Ventral view of a representative specimen. **21, 22.** Ventral and dorsal view of the same specimen. **23.** Ventral view showing crowded disorganized dikinetids in posterior dome kineties (cf. Figs 3, 11) and the left (black arrow) and right dentate posterior marginal projections (cf. Fig. 43). **24.** Left lateral view showing the small glabrous area on the dorsal region of the preoral dome (asterisk), the paroral membrane (black arrowhead), and the right dentate projection. **25.** Oral structures showing buccal (white arrowhead) and distal part of the adoral zone membranelles (black arrowhead), and paroral membrane (black arrow). The white arrow marks perizonal stripe kinety 5. **26.** Semi-schematic of fibrillar associates of perizonal stripe kineties. Black arrow indicates anterior end (cf. Fig. 6). **27.** Body shape variants. BAZ, buccal part of the adoral zone; DAZ, distal part of the adoral zone; DK1, preoral dome kinety 1; IF, transverse microtubular ribbons; KD, kinetodesmal fibers; Ma, macronucleus; Mi, micronucleus; PC, postciliary microtubular ribbon, POM, paroral membrane; PS, perizonal ciliary stripe; SK, somatic kineties. Scale bars: 25 μm (20–24, 27a–h), 10 μm (25, 26).

Figs 28–34. *Atopospira galeata* (28–30) and *A. violacea* (31–34), Idaho populations from life (DIC). **28.** Posterior view showing margin of preoral dome brim (black arrowhead) and elongated perizonal stripe cilia (white arrowhead). **29.** Lateral view (optical section) showing

showing margin of preoral dome brim (white arrowheads) and elongated posterior cilia. **30.** Left lateral view Showing paroral membrane cilia (black arrowhead), elongated perizonal stripe cilia (white arrowhead), contractile vacuole (black asterisk), elongated posterior cilium, and food vacuoles containing purple sulfur bacteria (white asterisks). **31.** Left dorsolateral view showing pellicular ribs (black arrowheads), food vacuoles containing purple sulfur bacteria (asterisks) and elongated posterior cilia (white arrowheads). **32.** Right lateral view showing food vacuoles (white asterisks), elongated posterior cilia (white arrowheads), contractile vacuole (black asterisk), and the right dentate posterior marginal projection (black arrowhead). **33.** Ventral view (strongly squashed specimen), showing paroral membrane (white arrowhead), buccal part of the adoral zone membranelles (black arrowheads) and contractile vacuole (asterisk). **34.** Ventral view (strongly squashed specimen) showing outer (white arrowhead) and inner (black arrowhead) cilia and superimposed basal bodies (white arrow) of paroral membrane, and cilia of buccal part of the adoral zone (black arrow). Scale bars: 25 μm (28–32), 10 μm (33), 5 μm (34).

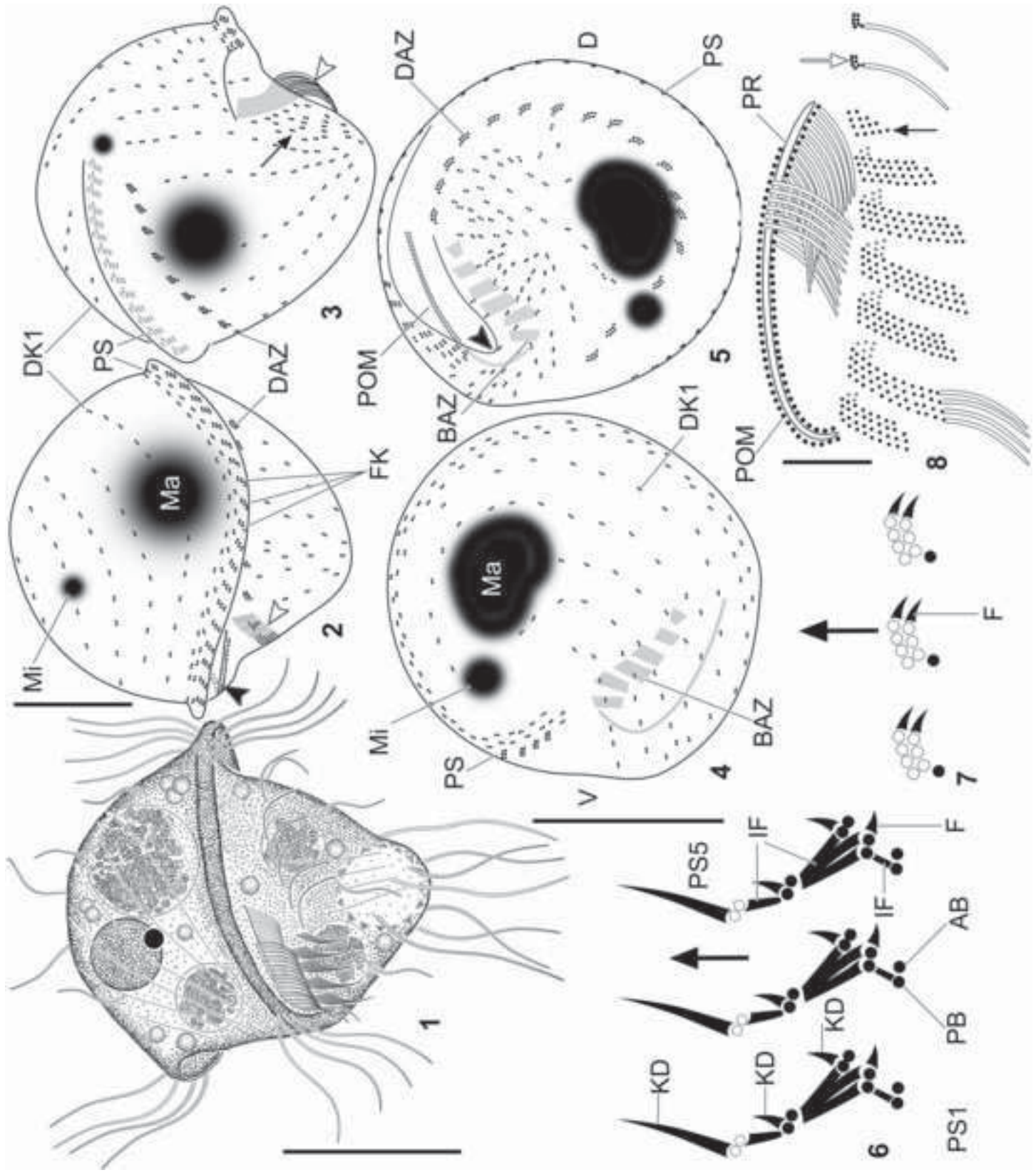
Figs 35–41. *Atopospira violacea*, Idaho population after protargol (35–37, 40, 41) and silver carbonate impregnation (38, 39). **35.** Ventral view showing cytopharyngeal fibers (black arrowheads), the paroral membrane (white arrowhead), perizonal ciliary stripe (black arrow), and preoral dome kinety 1 (white arrow). **36.** Dorsal view showing the distal part of the adoral zone membranelles (black arrowhead), distal end of the perizonal ciliary stripe (white arrowhead), a preoral dome kinety (black arrow), elongated posterior cilia (white arrow), food vacuoles (white asterisks), and small glabrous area of preoral dome (black asterisk). **37.** Left lateral view showing the buccal part of the adoral zone, the termination of the distal part of the adoral zone (white arrowhead), the paroral membrane (black arrow), perizonal ciliary stripe (white arrow), and food vacuoles (asterisks). **38.** Ventral view showing fibrillar associates of the perizonal

stripe and dome kineties. **39.** Detail of perizonal stripe fibrillar associates. Very long kinetodesmal fibers of perizonal stripe kinety 5 (white arrows) project right anteriorly (white arrowheads) across the wide space between perizonal stripe kinety 5 and dome kinety 1 (black arrowheads). Posterior ciliary microtubular ribbons (black arrows) course through the interkinetal space. **40.** Ventral view showing fibers (black arrow) of paroral membrane (black arrowhead), membranelles of the buccal part of the adoral zone (white arrowheads) and food vacuoles (asterisks). **41.** Ventral view of oral structures showing paroral membrane (white arrows), buccal adoral membranelles (black arrowheads) and leftward-projecting fibrillar associates of distal row. DK1, dome kinety 1; FK, false kineties; Ma, macronucleus; PS5, perizonal stripe kinety 5; PZS, perizonal ciliary stripe; SK, somatic kineties. Scale bars: 25 μm (35–38), 10 μm (40, 41), 5 μm (39).

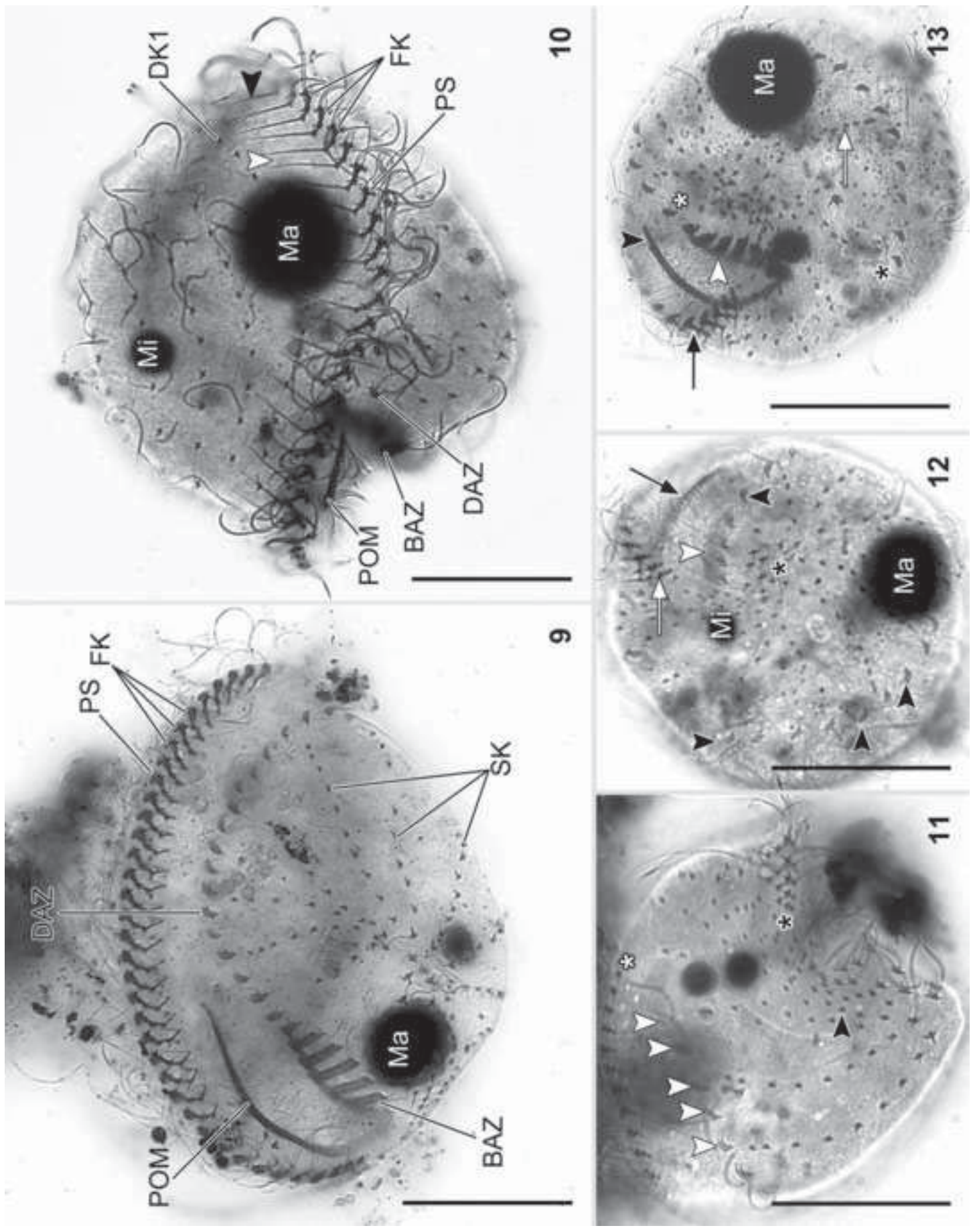
Figs 42–46. *Atopospira violacea*, Idaho population in SEM. **42.** Ventral view showing proximal margin of the buccal cavity (black arrow), dome kinety 1 (white arrowhead), perizonal stripe cilia (black arrowhead), and cortical area (white arrow) where cilia have been lost in processing. **43.** Left lateral view showing perizonal ciliary stripe (black arrow), paroral membrane cilia (black arrowhead), and left posterior marginal dentate process (white arrow). **44.** Posterodorsal view showing left (white arrow) and right (white arrowhead) dentate processes and the area where barren distal adoral zone basal bodies are hidden beneath cortex (asterisks). **45.** Detail of oral area showing outer (black arrowhead) and inner (white arrowhead) paroral membrane cilia, and perizonal stripe cilia (white arrow). **46.** View of paroral membrane showing outer (black arrow) and inner (white arrow) files separated by a pellicular ridge (black arrowhead). Scale bars: 25 μm (42–44), 10 μm (45), 5 μm (46).

Figs 47–50. *Atopospira galeata* (47–49) and *A. violacea* (50) from life (48) and after protargol impregnation (47, 49, 50). **47.** Left lateral view showing rod-shaped cytoplasmic bacteria (white arrowheads) and coccoid sulfur bacteria in food vacuoles (black arrowheads). **48.** Ventral view showing rod-shaped cytoplasmic bacteria (black arrowheads) and buccal part of the adoral zone (black arrow). **49.** Anterior view of late divider showing postoral somatic kinety (white arrow), dome kinety (black arrow) and perizonal ciliary stripe (black arrowheads). **50.** Dorsal view of late divider showing distal part of the adoral zone of the proter (black arrow) and the perizonal ciliary stripe of the proter (black arrowhead) and opisthe (white arrowhead), strand connecting dividing micronuclei (white arrows) and food vacuoles (asterisks). Ma, macronucleus. Scale bars: 10 μm (47–49), 25 μm (50).

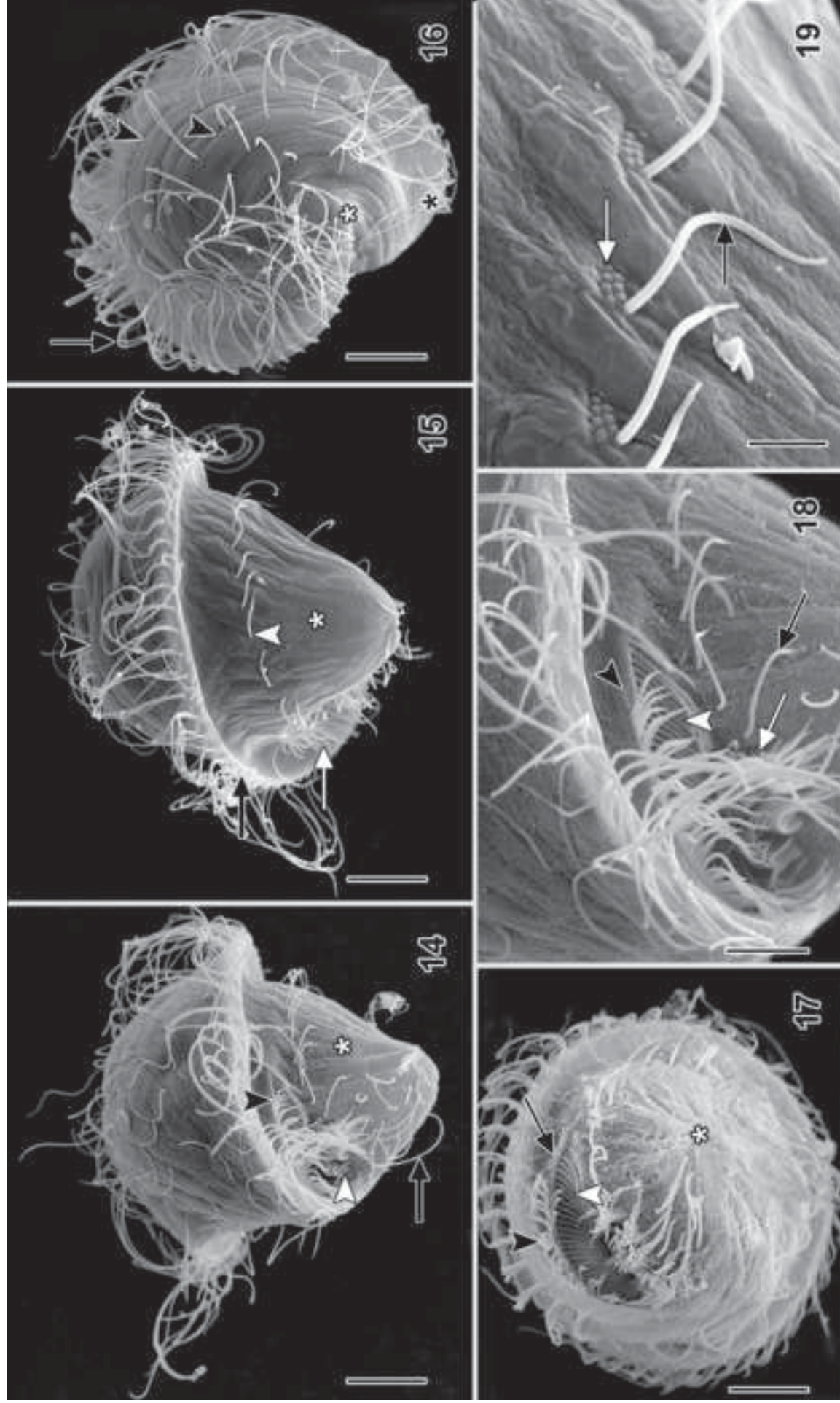
Figs 51–59. *Atopospira galeata* (51, 52, 54), *A. violacea* (55–59), and *Metopus fastigatus* (53) in life (51–53, 55–58) and after mercuric chloride fixation (54, 59). **51.** “Ventral” (a), “dorsal” (b, 60 μm), and posterior (c) views, and optical section of preoral dome brim (from Kahl 1927). **52.** General aspect, 40 μm (from Kahl 1931). **53.** “Dorsal” (a, about 40 μm) and ventral view (b; both from Kahl 1927). **54.** Dorsal (a, about 65 μm) and anterodorsal (b) views (from Jankowski 1964b). **55.** Ventral (a) and dorsal (b) views, about 100 μm (from Kahl 1926). **56–58.** General aspect, 80–100 μm (56, 57, from Kahl 1927, 1931) 130 μm (a), and (b) conjugation (58, from Kahl 1927). **59.** “Lateroventral” view, 115 μm (from Jankowski 1964b).

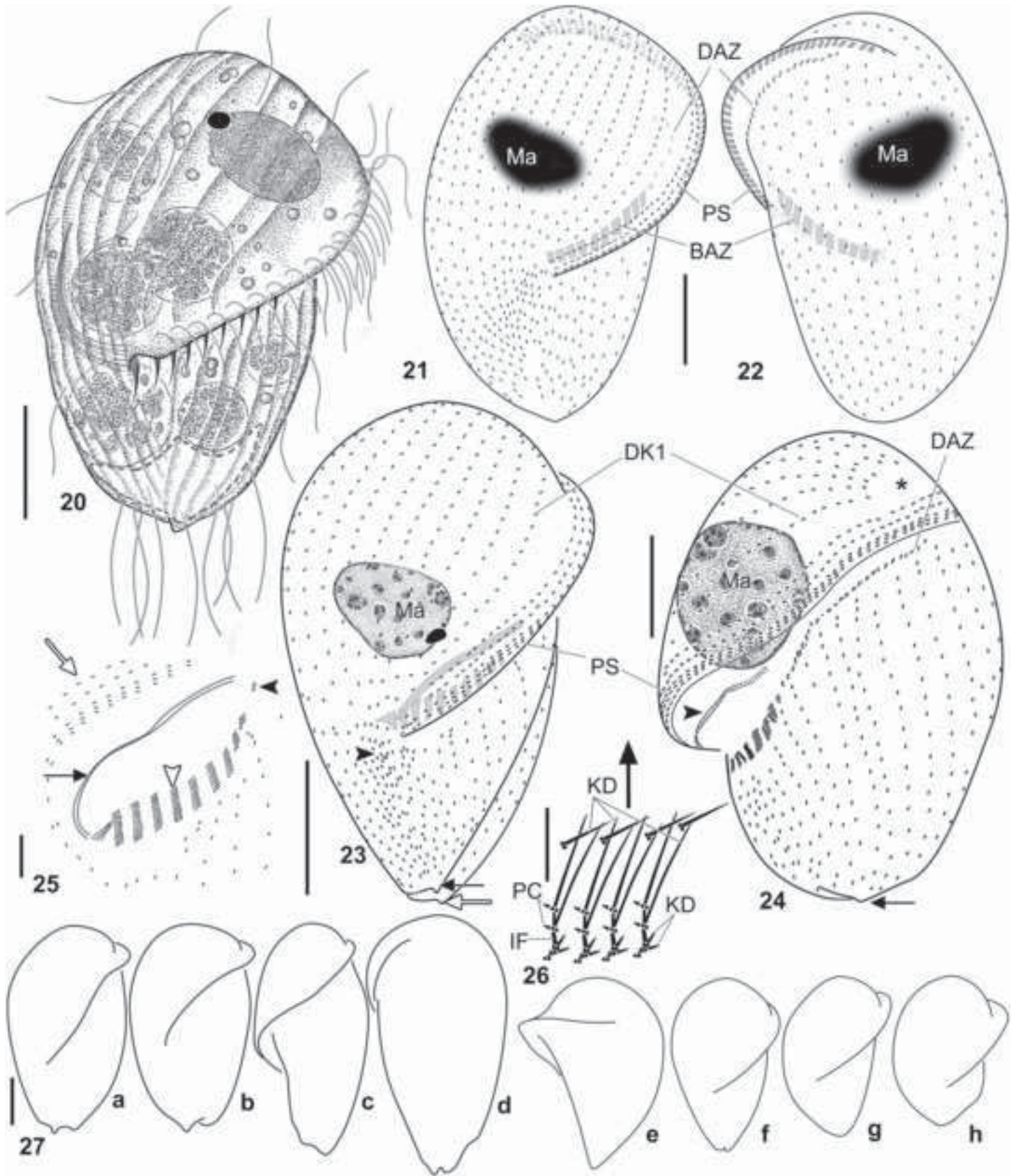


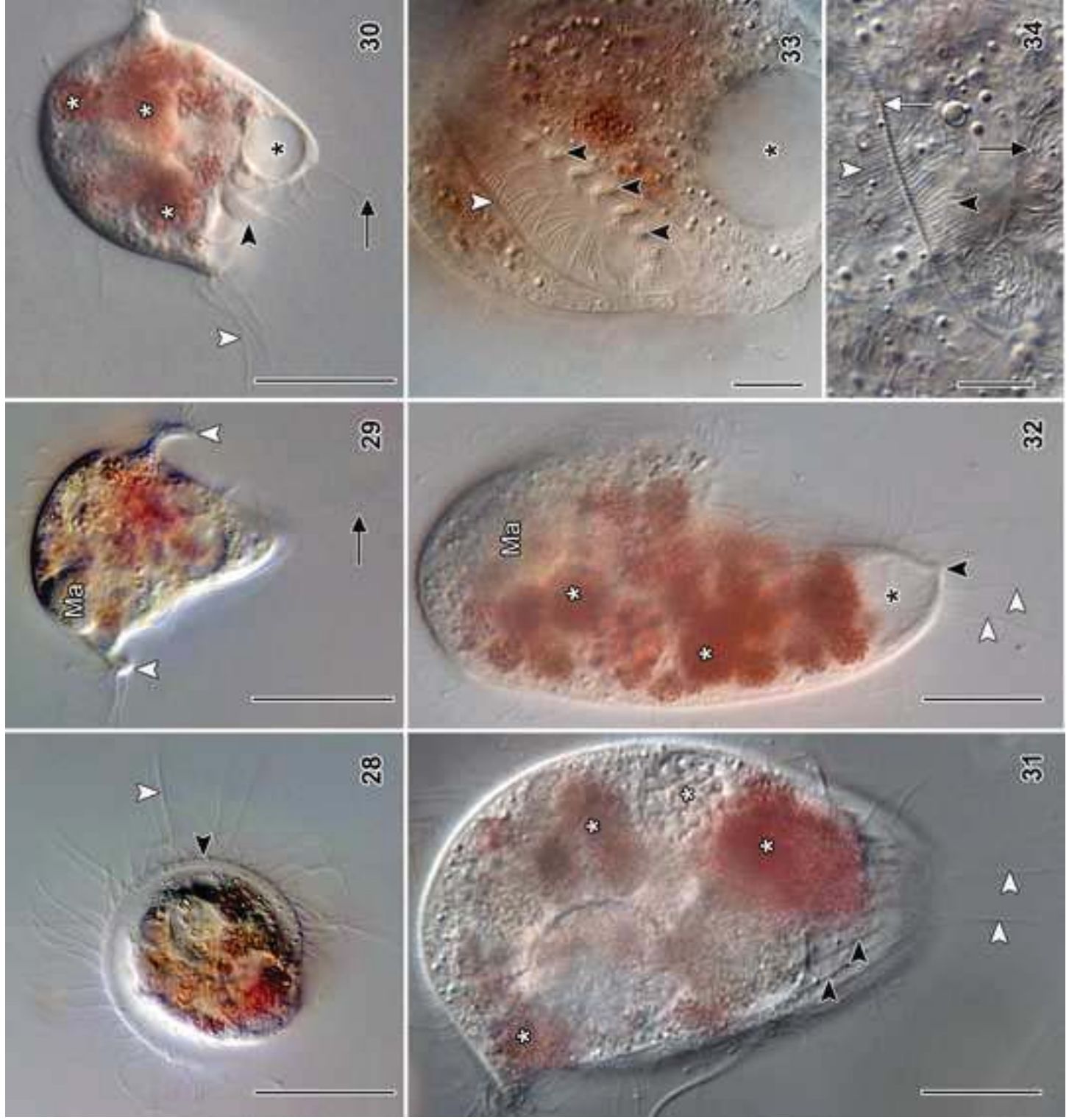
Figs 1-8

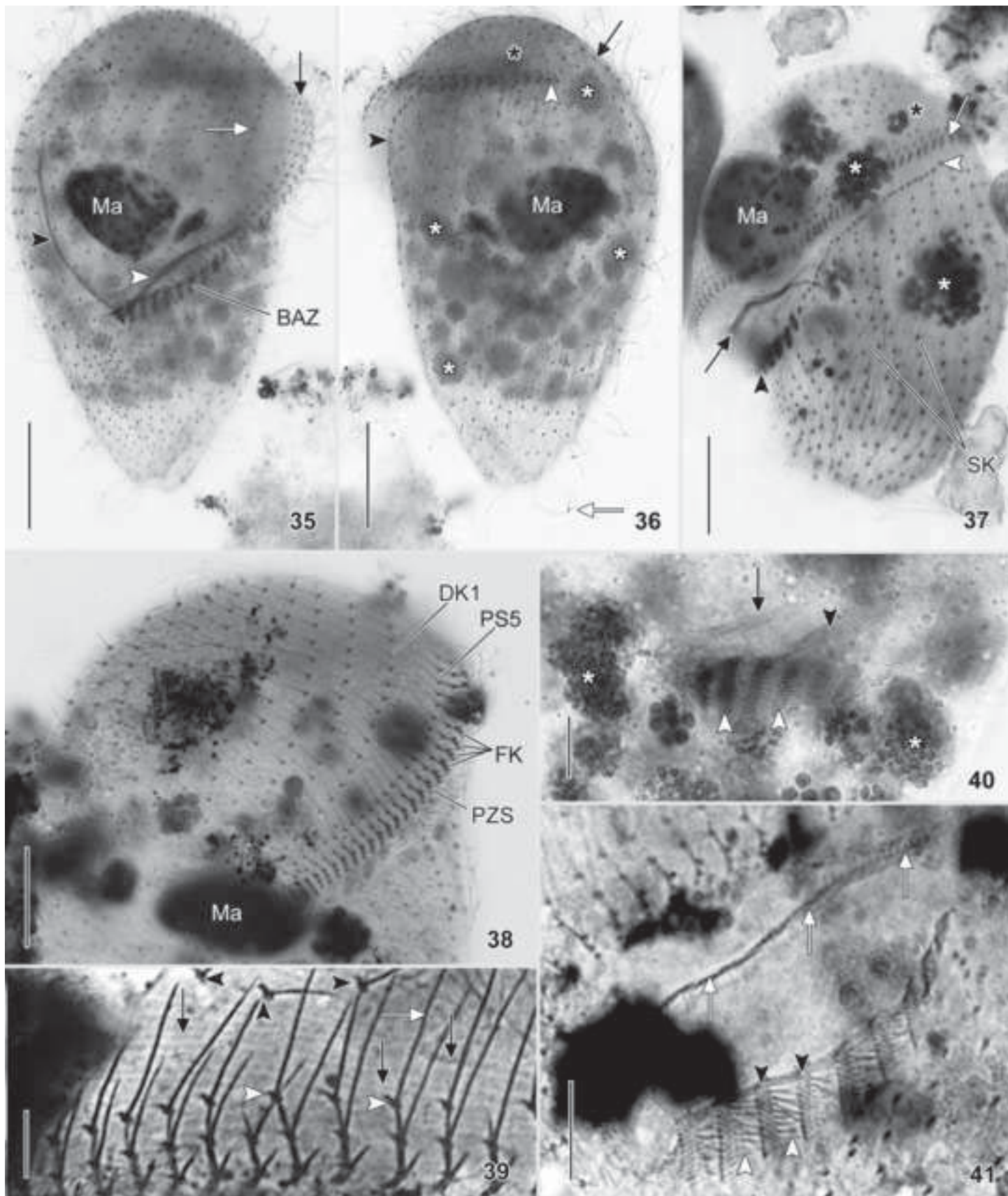


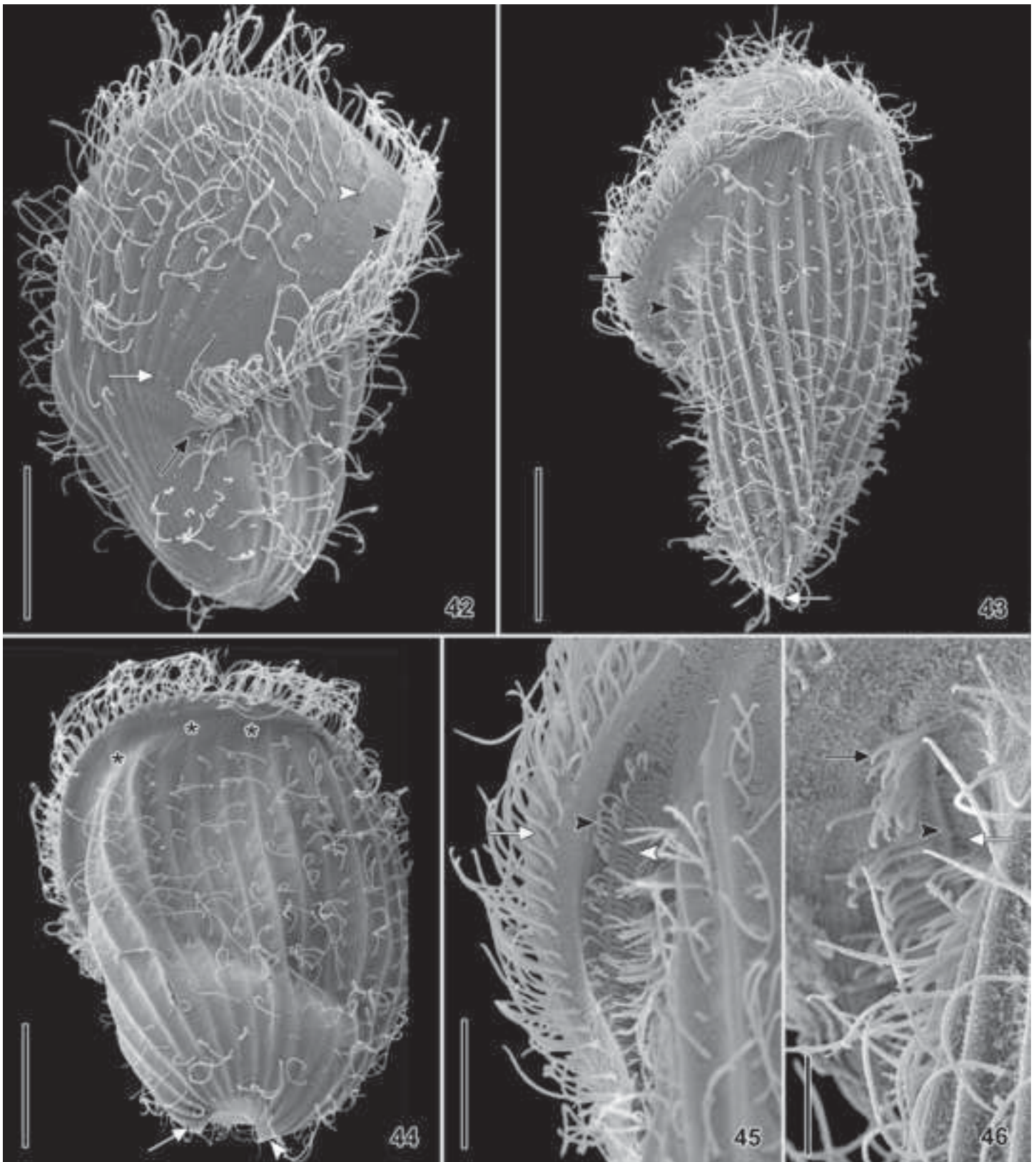
Figs 9-13

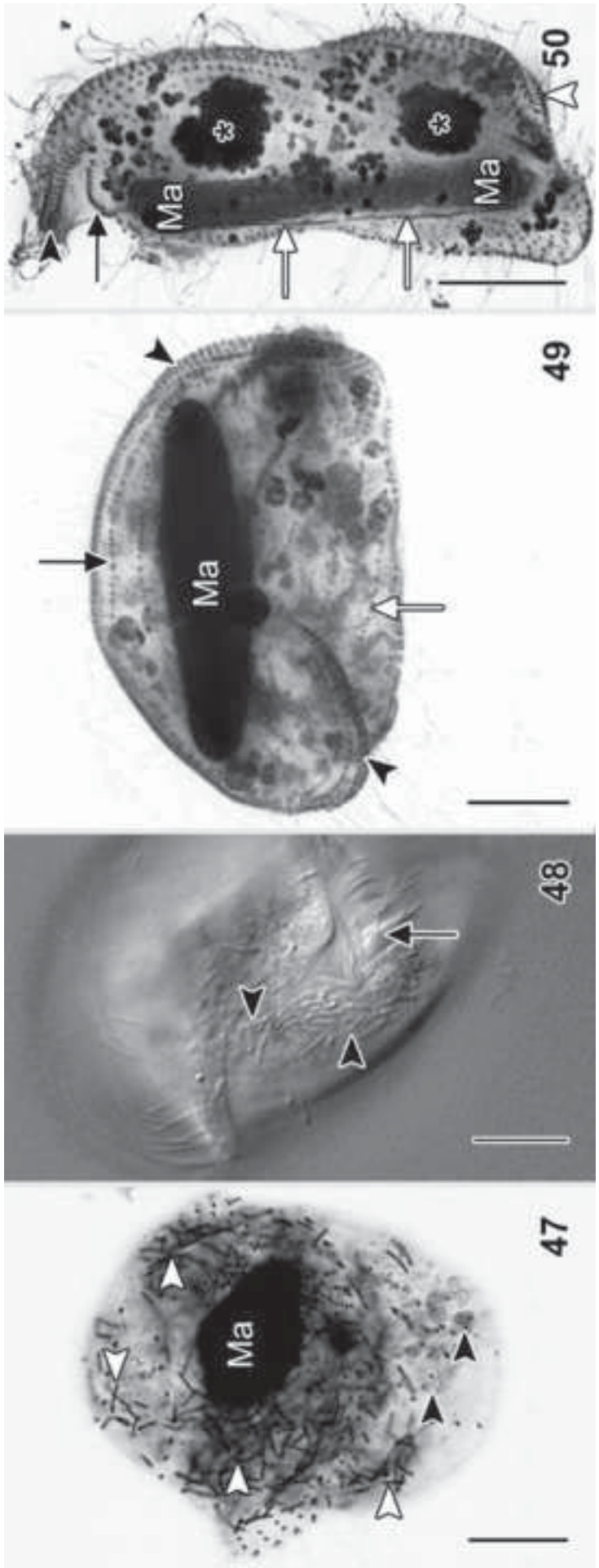












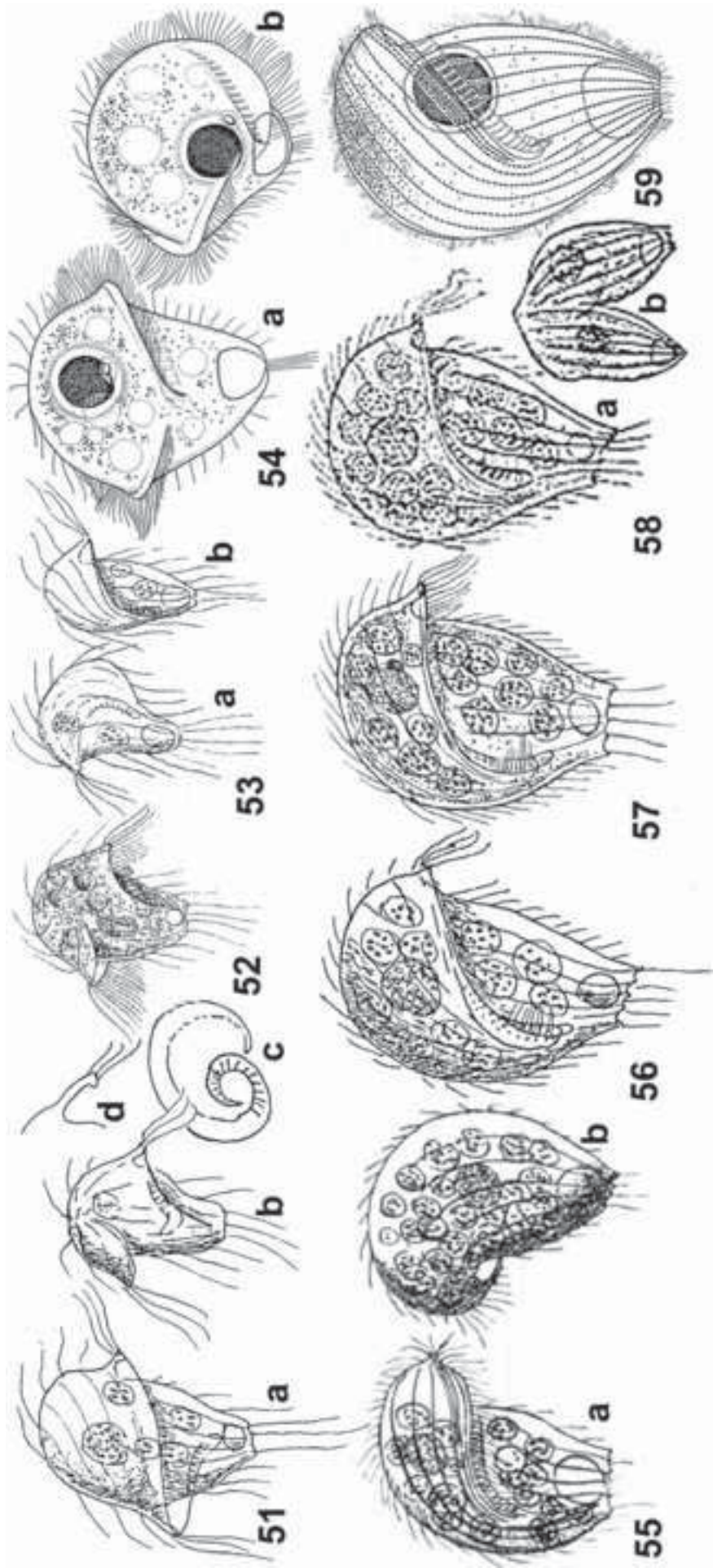


Table 1. Morphometric data from Idaho populations of *Atopospira galeata* (upper line) and *Atopospira violacea* (lower line) in vivo.

Characteristic ^a	Mean	M	SD	CV	Min	Max	n
Body, length	51.7	51.0	3.24	6.3	46.0	59.0	14
	106.5	103.0	10.66	10.1	95.0	133.0	13
Body, total width ^b	49.2	49.5	2.89	5.9	44.0	54.0	14
	73.0	74.0	8.70	11.9	60.0	84.0	13
Body, width at cytostome ^c	37.2	38.0	2.83	7.6	30.0	40.0	14
	62.8	61.0	8.54	13.6	50.0	77.0	13
Body, length : total width, ratio	1.0	1.1	0.07	6.7	0.9	1.2	14
	1.5	1.5	0.17	11.8	1.2	1.8	13
Body, thickness ^d	–	–	–	–	–	–	–
	38.1	36.0	8.36	21.5	29.0	51.0	9
Preoral dome, height ^e	20.7	21.5	3.32	16.0	15.0	26.0	14
	44.8	42.8	13.31	29.7	30.0	72.0	13
Macronucleus, length	15.3	16.0	1.68	11.0	11.2	16.6	9
	25.8	26.0	2.94	11.4	21.0	30.0	11
Macronucleus, width	12.2	12.6	1.15	13.5	9.8	14.1	9
	18.8	19.0	2.83	15.1	14.0	23.7	11
Anterior end to posterior end of macronucleus, distance	20.8	18.1	6.39	30.6	15.4	33.8	7
	39.8	40.4	7.22	18.1	28.3	49.0	11

^a Based on uncompressed cells. All measurements in μm . CV, coefficient of variation (%); M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of individuals investigated; SD, standard deviation.

^b Width of preoral dome brim.

^c Ventral view.

^d Width in lateral view.

^e Corresponds approximately to distance from anterior end to cytostome.

Table 2. Morphometric data from Idaho populations of *Atopospira galeata* (upper line) and *Atopospira violacea* (lower line).

Characteristic ^a	Method	Mean	M	SD	CV	Min	Max	n
Body, length	P	47.8	47.5	6.50	13.6	37.0	58.0	18
		91.4	90.0	12.92	14.1	63.0	115.0	41
Body, total width ^b	P	43.3	43.0	6.39	14.8	35.0	59.0	18
		60.3	61.5	7.84	13.0	47.0	77.0	41
Body, width at cytostome	P	24.8	24.0	4.73	19.1	16.0	33.0	16
		48.6	48.0	6.08	12.5	38.0	61.0	40
Body, length : total width, ratio	P	1.1	1.1	0.12	10.5	0.9	1.3	18
		1.5	1.5	0.14	9.2	1.2	1.8	43
Anterior cell end to proximal end of adoral zone, distance	P	32.1	31.0	4.37	13.6	26.0	39.0	11
		57.3	56.0	8.37	14.6	41.0	74.0	40
Anterior cell end to posterior end of macronucleus, distance	P	23.1	23.5	7.05	30.5	10.0	31.0	12
		40.0	38.0	11.84	29.6	16.0	64.0	39
Distance anterior cell end to proximal end of buccal part of adoral zone:body length, ratio in %	P	67.0	69.6	7.80	11.7	55.0	79.5	11
		63.0	63.0	5.04	8.0	51.0	76.0	41
Distance anterior cell end to posterior end macronucleus:body length, ratio in %	P	50.0	49.6	17.16	34.4	21.0	76.2	12
		43.9	46.0	11.27	25.6	19.0	72.0	39
Macronucleus, length	P	12.1	12.0	1.53	12.6	9.0	15.0	20
		22.9	22.0	3.38	14.8	18.0	34.0	38
Macronucleus, width	P	10.6	10.2	1.69	15.9	8.0	14.0	20
		15.2	15.0	2.27	14.9	11.0	22.0	38

Micronucleus, length	P	3.9	4.0	0.52	13.3	3.0	5.0	18
		4.0	34.0	0.656	16.5	3.0	5.0	19
Micronucleus, width	P	3.6	3.5	0.60	16.0	3.0	5.0	18
		3.3	3.5	0.56	17.0	2.0	4.0	19
Membranelles in buccal part of adoral zone, number ^b	P, SC	6.7	7.0	0.56	8.3	5.0	7.0	27
		10.1	10.0	0.72	7.1	8.0	12.0	40
Membranelles in distal part of adoral zone, number ^c	P, SC	18.9	18.5	1.68	8.9	17.0	24.0	14
		22.0	22.0	2.85	13.0	16.0	30.0	27
Preoral dome kineties, number	P, SC	6.1	6.0	0.67	11.0	5.0	8.0	12
		13.7	14.0	0.89	6.5	12.0	15.0	32
Somatic kineties, number ^d	P, SC	16.6	16.0	2.03	12.3	15.0	22.0	16
		41.0	41.0	3.46	8.4	35.0	48.0	31
Paroral membrane, length ^e	P	18.0	18.0	2.47	13.7	12.0	22.0	17
		33.8	35.5	6.00	17.7	23.0	47.0	34
False kineties, number	P, SC	45.1	44.0	3.60	8.0	40.0	50.0	12
		50.0	50.0	3.94	7.9	42.0	56.0	23
Perizonal ciliary stripe rows, number	P, SC	5.0	5.0	0.00	0.0	5.0	5.0	17
		5.0	5.0	0.00	0.0	5.0	5.0	34

^a All measurements in μm , made only from permanent mounts of protargol-impregnated cells.

CV, coefficient of variation (%); M, median; Min, minimum; Max, maximum; n, number of individuals studied; P, protargol; SC, silver carbonate; SD, standard deviation.

^b Width of of preoral dome brim.

^c See Figs 2–5, 8.

^d Includes dome kineties. Approximate due to disordered kineties right of oral apparatus (see Figs 3, 5).

^e Longest of the two paroral membranes. Measured as chord of arc.