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A huge diversity of metopids (Ciliophora, Armophorea) in soil from the Murray River floodplain, Australia. I. Description of five new species and redescription of *Metopus setosus* Kahl, 1927

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Abstract

Six metopid ciliates from soil of the Murray River floodplain in Australia were studied using live observation, various silver impregnation methods, scanning electron microscopy, and multivariate statistics. One of the species is affiliated with *M. setosus* while the others represent new taxa. *Metopus filum* nov. spec. is distinguished from most congeners by the slender body, the absence of cortical granules, and the low number of ciliary rows and adoral polykinetids. *Metopus palaeformides* nov. spec. most resembles *Heterometopus palaeformis* (Kahl, 1927) Foissner, 2016b but they can be distinguished by body size, the number of adoral polykinetids, and the oral area pattern. *Metopus murrayensis* nov. spec. is outstanding in having a globular macronucleus surrounded by innumerable refractive granules and a conspicuously thick preoral dome. *Metopus rex* nov. spec. and *M. magnus* nov. spec. are easily distinguished from most congeners by their large body size and the shape of the macronucleus. Moreover, *M. rex* displays up to 30 μm long endosymbiotic bacteria while the micronucleus of *M. magnus* is uniquely situated in a small macronuclear concavity. Multivariate statistics corroborates the distinctness of these six metopid populations.

Keywords: Metopidae; Oral area patterns; Morphology; Morphometry; Soil ciliates; Taxonomy

Introduction

Australian ciliate diversity is still a *terra incognita*. However, research over the past three decades has revealed many undescribed and peculiar ciliates in this outstanding biogeographic region. For instance, Blatterer and Foissner (1988) and Foissner (1988) discovered 23 new species belonging mainly to the class Spirotrichea Bütschli, 1889 in a variety of terrestrial habitats. Recently, Kumar and Foissner (2015, 2016) recognised seven additional new spirotrichean taxa, most of them being cryptic species. In sandy and floodplain soils of Australia, Foissner (1990, 2003) discovered two new remarkable members of the class Colpodea Small and Lynn, 1981: the large carnivorous *Kuehneltiella terricola* and *Pseudomaryna australiensis* with an envelope of clay particles embedded in a slimy matrix. This sheath makes the ciliate resemble inorganic soil particles, possibly protecting it from predators. As concerns the class Litostomatea Small and Lynn, 1981; Foissner (1994, 2016a) described *Spetazoon australiense* covered by a unique type of lepidosomes, i.e., organic scales of unknown function. In pools on top of

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Ayers Rock in the Red Centre of Australia, Gabilondo and Foissner (2009) discovered another peculiar litostomatean, *Fuscheria uluruensis*, differing from all congeners in having the macronucleus split into several oblong nodules. Finally, Foissner (2016b) recently discovered a new metopid, *Heterometopus meisterfeldi*, from the class Armophorea Lynn, 2004 in bottom soil of the dry Fogg Dam east of the town of Darwin, Northern Territory.

From a taxonomical and morphological viewpoint, metopids still represent an insufficiently explored ciliate group. Indeed, only few species have been thoroughly examined with modern methods (Bourland and Wendell 2014; Bourland et al., 2014; da Silva-Neto et al., 2015; Dragesco and Dragesco-Kernéis 1986; Foissner 1998, 2016a,b; Foissner and Agatha 1999; Foissner et al., 1992, 2002; Vďačný 2007). These studies documented that metopids are as diverse as proposed by Kahl (1927, 1932). Nevertheless, it was surprising to find over ten metopid species, seven of which are new taxa, in a single soil sample from the Murray River floodplain near the town of Albury, East Australia. Since we have obtained extensive morphological data, we shall report our findings on Australian metopids in a series of papers. In the present study, we describe six species, five represent new taxa and one can be affiliated with the insufficiently described *M. setosus* Kahl, 1927. In the following papers, we shall introduce the remaining two new species, with descriptions of their ontogenesis and conjugation. Finally, we shall attempt to unravel their evolutionary history.

Material and Methods

Material collection and taxonomic methods

The upper 5 cm soil layer with much organic debris was collected from the floodplain of the Murray River at the Landside of Ryans road near the town of Albury, Southeast Australia (S36°06′ E146°54′) in February 2006. It was air-dried for three weeks and sealed in a plastic bag. The sample had pH 5.2 in water and consisted of light brown, loamy soil and leaf litter mainly from red gum trees and *Myriophyllum*. It contained a rich ciliate community of almost 80 species. About 20 taxa were typical inhabitants of permanent and astatic water bodies while the others were edaphic species. All were reactivated from resting cysts in summer 2006, using the non-flooded Petri dish method, as described in Vďačný and Foissner (2012).

Metopids were investigated as described by Foissner (1991, 2014), i.e., using a combination of in vivo observation, silver impregnation, and scanning electron microscopy (SEM). Live ciliates were studied at low and high magnifications with bright field and differential interference contrast. The ciliature was revealed with protargol and silver carbonate impregnation. In vivo measurements were performed at 40–1000× while counts and measurements on protargol-impregnated specimens were conducted at a magnification of 1000×. Body size was calculated by some in vivo measurements and the protargol-impregnated specimens adding 15% preparation shrinkage (Foissner 2014). Illustrations of live specimens were based on free-hand sketches and microphotographs while those of impregnated cells were made with a drawing device.

Multivariate morphometric analyses

A multivariate approach was used to investigate the morphological variation, the taxonomic value of the morphometric features, and the species boundaries. Altogether, 24 features (16 quantitative and two qualitative binary characteristics as well as six derived ratios) were measured or scored in 106 protargol-impregnated interphase specimens. Because the number of macronuclei and micronuclei as well as of perizonal rows was the same in all taxa, they were excluded from the analyses. The oral area pattern was also excluded because it is a multistate qualitative feature for which the Euclidean distance and the Manhattan city block distance are not appropriate (Marhold 2011). All morphometric data were compiled in Supplementary Table S1.

Cluster analyses were carried out in the computer programme Syntax 2000 (Podani 2001), using a combination of four different algorithms (average linkage, complete linkage, single linkage and centroid method) with Euclidean distance and Manhattan city block distance as coefficients of distance (Marhold 2011). Principal Component Analysis (PCA) was conducted in Statgraphics, with standardisation option in effect (http://www.statgraphics.com).

Terminology

General terminology mainly follows Lynn (2008). Specific terminology is according to Kahl (1932), Jankowski (1964), Foissner and Agatha (1999), and Foissner (2016b). Classification of metopids follows Kahl (1932) because the revision of Esteban et al. (1995) has been widely criticised (e.g., Bourland et al., 2014; Dragesco 1996; Foissner 2016b; Foissner and Agatha 1999; Foissner et al., 2002).

Metopids are asymmetric, with more or less distinct anterior torsion, which makes the identification of body sides problematic. During divisional morphogenesis the sigmoid body becomes oblong (Foissner and Agatha 1999; Martin-Gonzalez et al., 1987) and body sides can be more easily distinguished. In late dividers, the newly formed adoral zone is not spiralled and curves to the left, resembling the situation in morphostatic cells of hypotrichs and heterotrichs. Consequently, the cell surface bearing the adoral zone and the cytostome could be designated as ventral side (Bourland et al., 2014; Lynn 2008). However, the matter is complex because the body is reshaped after division and the ends of the adoral zone become located on opposite sides in distinctly twisted species. Therefore, for the sake of simplicity, we sug-

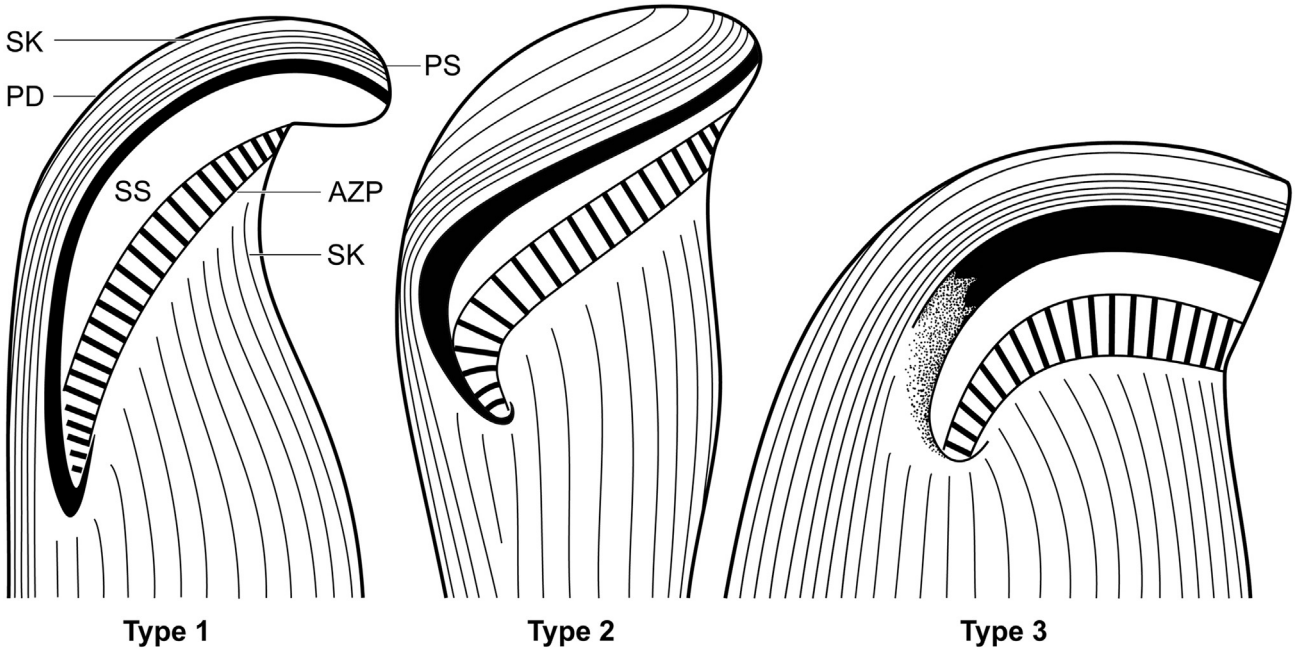

Fig. 1. Semischematic oral area patterns in metopids. AZP, Adoral zone of polykinetids; PD, preoral dome; PS, perizonal stripe; SK, somatic kineties; SS, side stripe.

gest to designate the surface with the proximal end and main portion of the adoral zone as ventral side and the opposite side as dorsal one.

As concerns the oral area, we distinguish three types defined by the morphologies of the side stripe and the preoral dome (Fig. 1). Side stripe denotes the unciliated, channel-like area between the preoral dome and the adoral zone of polykinetids (Foissner 2016b; Kahl 1932). For the thickened and unciliated portion of the preoral dome, which lies between the perizonal and the side stripe, we coined the term dome lip. The proximal end of the dome lip typically displays a reverse J-pattern that gradually merges into the somatic cortex bordering the buccal entrance.

Type 1: The preoral dome is rather flat and distinctly projects from body proper. The dome lip is narrow. The side stripe forms a large and deep channel. This type occurs, for instance, in *M. palaeformides* nov. spec. (Fig. 8A, C, D), *M. magnus* nov. spec. (Figs 15A, B, 16A, C–E, 17B), *M. fuscus* (Bourland et al., 2014), *M. es* and *M. ovalis* (Foissner et al., 1992). Possibly, *Heterometopus meisterfeldi* Foissner, 2016b also belongs to this type.

Type 2: The preoral dome is rather thick and projects only slightly from body proper. The dome lip is narrow. The side stripe forms a moderately deep channel. This type has been as yet found in *M. setosus* (Figs 24F, 25B, D, 26A, B, E, F), *M. rostratus* and *Heterometopus palaeformis*, both described in Foissner (2016b).

Type 3: The preoral dome is conspicuously thick and projects indistinctly from body proper. The dome lip is broad. The side stripe forms a rather broad, almost flat channel. As yet, this pattern has been recorded only in *M. murrayensis* nov. spec. (Figs 21A, C, 22A, B).

Results and Discussion

Metopus filum nov. spec. Foissner and Vďačný (Figs 2A–S, 3A–J; Table 1)

Diagnosis: Size about 90 × 12 μm in vivo. Body narrowly oblong to cylindrical and slightly twisted anteriorly. Macronucleus between proximal end of adoral zone and second third of body, broadly to very narrowly ellipsoidal; one globular to ellipsoidal micronucleus. Contractile vacuole terminal. On average nine ciliary rows; elongated caudal cilia absent. Perizonal stripe composed of five kineties extending approximately 15% of body length and forming about nine false kineties. Type 2 oral area. Adoral zone slightly oblique, composed of an average of 14 polykinetids extending about 30% of body length.

Type locality: Loamy soil and leaf litter from the floodplain of the Murray River near to the town of Albury, Australia (S36°06′ E146°54′).

Type material: The holotype slide (reg. no. 2016/5) and two paratype slides (reg. nos 2016/6, 7) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. The holotype (Fig. 2D, E) and relevant paratype specimens have been marked by black ink circles on the coverslip.

Etymology: The Latin noun *filum* (thread, filament) refers to the narrow body, a main feature of this species. The name is

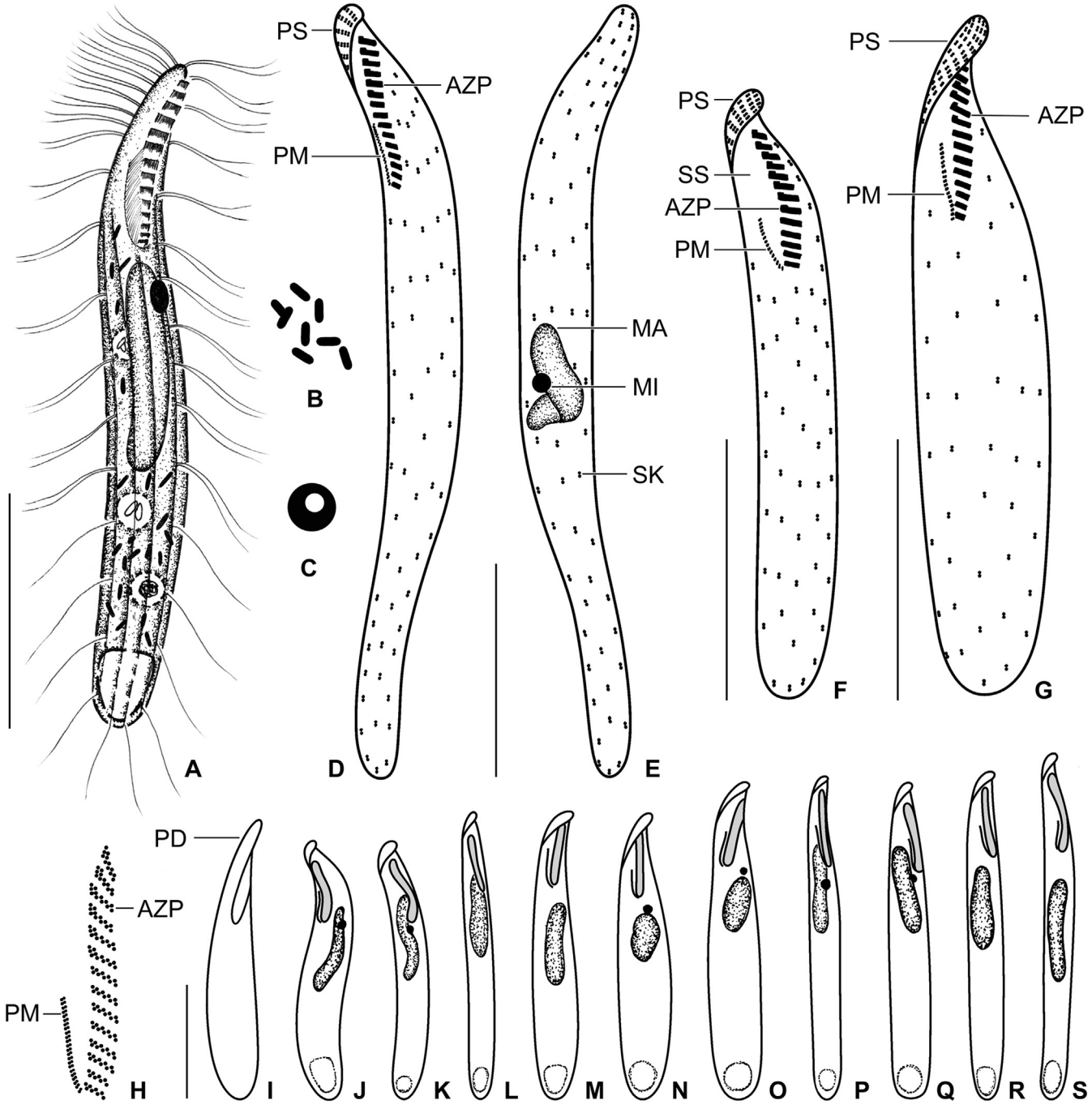

Fig. 2. A–S. *Metopus filum* nov. spec. from life (A–C, I) and after protargol impregnation (D–H, J–S). **A**: Ventral view of a representative specimen, length 85 μm. **B**: Symbiotic bacteria are ellipsoidal and about 2 μm long. **C**: The micronucleus is about 5 μm across and has sometimes a shining inclusion. **D, E**: Ciliary pattern of ventral and dorsal side as well as nuclear apparatus of holotype specimen, length 110 μm. **F, G**: Ventral view of ciliary pattern of paratype specimens, length 71 μm and 79 μm. **H**: Semi-schematic diagram of oral ciliature. The adoral zone of polykinetids extends slightly obliquely. It displays an indistinct sigmoid pattern and is composed of an average of 14 polykinetids. Distal polykinetids are made of two long rows of basal bodies and a very short row while proximal polykinetids are rectangular and consist only of two rows of basal bodies. The paroral membrane is dikinetal. **I–S**: Variability of body shape and size as well as of nuclear apparatus. The body is narrowly oblong to cylindrical with a length:width ratio of 5.3–10.6:1 in protargol preparations. The nuclear apparatus usually extends between the proximal end of the adoral zone and the second third of the body. The macronucleus is narrowly to very narrowly ellipsoidal, rarely broadly ellipsoidal. Drawn to scale. AZP, Adoral zone of polykinetids; MA, macronucleus; MI, micronucleus; PD, preoral dome; PM, paroral membrane, PS, perizonal stripe; SK, somatic kinety; SS, side stripe. Scale bars: 30 μm.

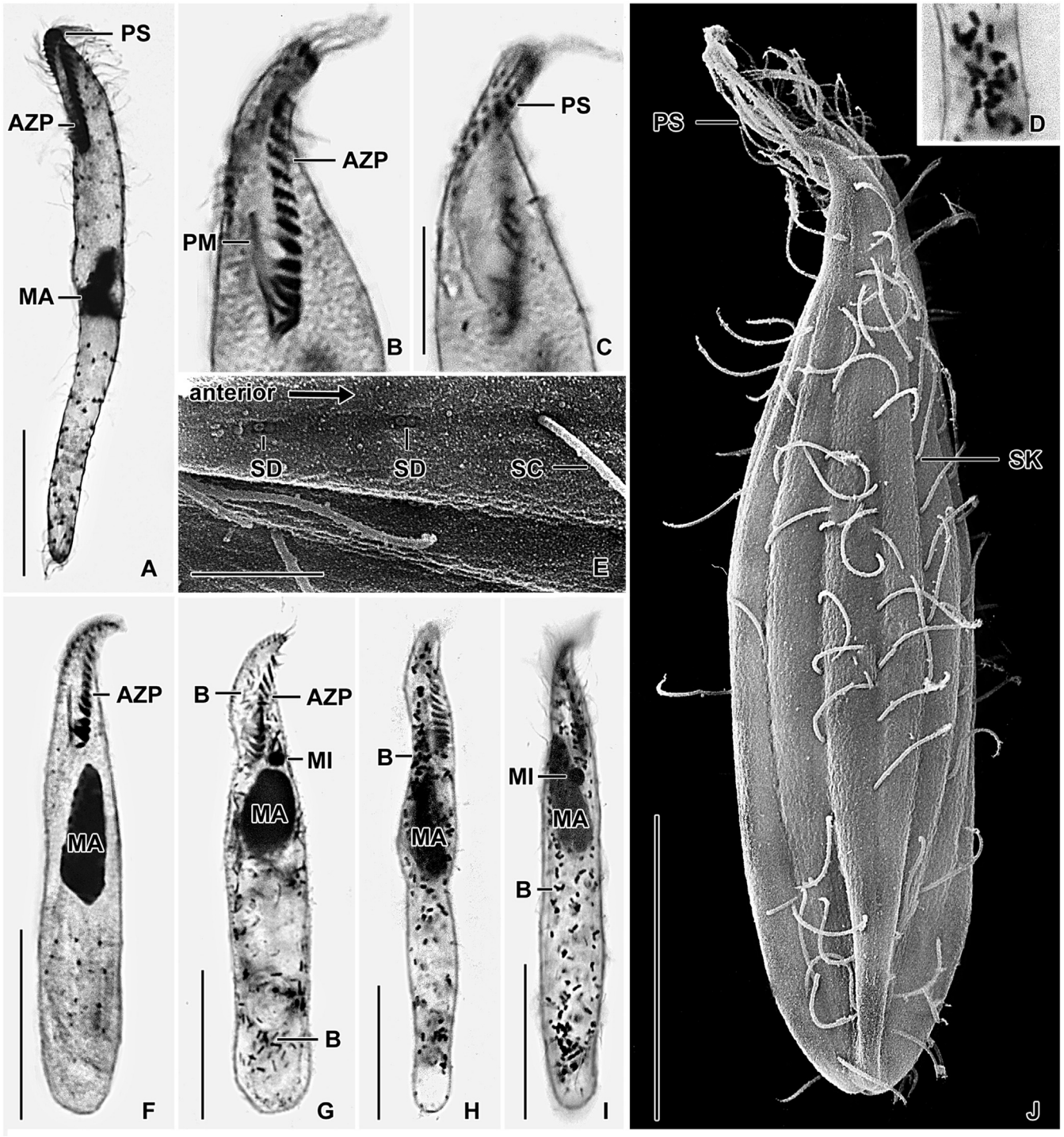

Fig. 3. A–J. *Metopus filum* nov. spec. after protargol impregnation (A–D, F–I) and in the scanning electron microscope (E, J). **A**: Ventral view of holotype specimen, showing the cylindrical body that is only slightly twisted anteriorly. The preoral dome is inconspicuous, narrower than mid-body and carries the perizonal stripe. The posterior body end is narrowly rounded. **B, C**: Detail of oral body portion, showing the dikinetal paroral membrane, the slightly oblique and indistinctly sigmoid adoral zone of polykinetids, and the perizonal stripe. **D**: Symbiotic bacteria are ellipsoidal and about 2 μm long. **E**: The somatic ciliature is composed of dikinetids but only a single basal body is ciliated in the posterior body region. **F–I**: Variability of body shape and size as well as of nuclear apparatus. Numerous symbiotic bacteria are scattered throughout the body. **J**: Overview showing the oblong and loosely ciliated body. AZP, Adoral zone of polykinetids; B, symbiotic bacteria; MA, macronucleus; MI, micronucleus; PM, paroral membrane, PS, perizonal stripe; SC, somatic cilium; SD, somatic dikinetids; SK, somatic kinety. Scale bars: 5 μm (E), 10 μm (B, C), 20 μm (J), and 30 μm (A, F–I).

Table 1. Morphometric data on *Metopus filum* nov. spec.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	77.8	76.0	12.1	2.6	15.5	62.0	110.0	21
Body, maximum width of preoral dome	7.7	8.0	1.7	0.4	22.1	5.0	12.0	21
Body, width at cytostome	8.9	9.0	2.0	0.4	23.0	6.0	13.0	21
Body, maximum postoral width	10.8	10.0	2.2	0.5	20.3	8.0	15.0	21
Body, length:width ratio	7.4	7.0	1.6	0.3	21.1	5.3	10.6	21
Anterior body end to proximal end of PS, distance	11.4	11.0	2.0	0.5	17.4	8.0	15.0	19
Perizonal stripe, percentage of body length	14.7	14.1	2.4	0.5	16.3	9.8	18.8	19
Anterior body end to distal end of AZP, distance	4.2	4.0	0.7	0.2	16.5	3.0	6.0	21
Anterior body end to proximal end of AZP, distance	22.2	22.0	1.9	0.4	8.8	19.0	25.0	21
Adoral zone of polykinetids, percentage of body length	28.8	29.1	2.5	0.5	8.7	22.7	33.9	21
Anterior body end to distal end of PM, distance	14.0	14.0	1.5	0.4	10.8	10.0	16.0	16
Anterior body end to macronucleus, distance	21.0	16.0	9.2	2.0	43.5	11.0	44.0	21
Macronucleus, length	25.0	25.0	6.2	1.4	25.0	14.0	38.0	21
Macronucleus, width	6.1	6.0	1.8	0.4	28.8	4.0	11.0	21
Macronucleus, length:width ratio	4.4	4.5	1.5	0.3	34.0	1.3	6.5	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length	3.4	3.0	0.8	0.2	23.4	2.0	5.0	17
Micronucleus, width	3.0	3.0	0.8	0.2	25.1	2.0	5.0	17
Micronucleus, length:width ratio	1.1	1.0	–	–	–	1.0	2.0	17
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	17
Somatic ciliary rows, total number	9.4	9.0	1.4	0.3	14.5	8.0	13.0	21
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	20
False kineties in perizonal stripe, number	9.5	9.0	1.9	0.4	19.7	7.0	13.0	19
Adoral polykinetids, number	13.3	14.0	1.2	0.3	8.7	11.0	15.0	21
Paroral membrane, length	9.3	9.0	0.8	0.2	8.5	8.0	11.0	16

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZP – Adoral zone of polykinetids; CV – coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; PM – paroral membrane; PS – perizonal stripe; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

treated as a noun in the nominative singular standing in apposition to the generic name [Art. 11.9.1.2 of the International Commission on Zoological Nomenclature (1999)].

Description: Size in vivo 70–125 × 9–17 μm, usually about 90 × 12 μm, as calculated from some in vivo measurements and the morphometric data adding 15% preparation shrinkage. Body narrowly oblong to cylindrical, length:width ratio 5.3–10.6:1, near 7:1 both in vivo and in protargol preparations (Table 1), slightly twisted anteriorly; preoral dome inconspicuous, flattened, indistinctly or slightly projecting from body proper; posterior end rounded (Figs 2A, D–G, I–S, 3A, F–J). Nuclear apparatus usually between proximal end of adoral zone and second third of body. Macronucleus narrowly to very narrowly ellipsoidal, rarely broadly ellipsoidal or coiled, length:width ratio 1.3–6.5:1 and size about 25 × 6 μm after protargol impregnation; nucleoli small and globular to ellipsoidal, well recognizable only in weakly impregnated specimens. Micronucleus attached or near to macronucleus; shape and size rather variable both in vivo and after protargol impregnation, i.e., globular to ellipsoidal, 4–8 × 3–5 μm in size in vivo and 2–5 μm after protargol impregnation; sometimes with a shining inclusion in vivo (Figs 2A, C, E, J–S, 3A, F–I; Table 1). Contractile vacuole in posterior body end (Fig. 2A, J–S). Cortex flexible, distinctly furrowed by ciliary rows (Fig. 3E, J); no cortical granules recognizable. Cytoplasm colourless; contains some 5 μm-sized food vacuoles with bacterial spores; usually studded with ellipsoidal, 2 μm-long bacteria deeply impregnating with the protargol method used (Figs 2B, 3D, G–I).

Somatic ciliature composed of dikinetids; cilia about 10 μm long in vivo, paired except for posterior body portion, loosely spaced and arranged in an average of nine meridional rows; elongated caudal cilia absent. Perizonal stripe inconspicuous because extending only approximately 15% of body length; invariably composed of five ciliary rows having both basal bodies ciliated; segmented into nine false kineties on average (Fig. 2D–G; Table 1).

Type 2 oral area. Adoral zone extends slightly obliquely on ventral side, indistinctly J-shaped in some obliquely oriented cells; occupies only about 30% of body length; composed of an average of 14 polykinetids: anterior polykinetids made of two long rows of basal bodies and a very short row recognizable when the cell is observed under a certain angle, posterior polykinetids rectangular and consisting of only two rows of basal bodies. Paroral membrane dikinetal and only 9 μm long after protargol impregnation; begins on average 14 μm posterior to anterior body end and extends slightly obliquely to proximal end of adoral zone (Figs 2D, F–H, 3B; Table 1). Cytopharyngeal fibres not recognizable in vivo or

after protargol impregnation. Side stripe flat and comparatively narrow.

Comparison with similar species: *Metopus filum* resembles *M. tenuis* Kahl, 1927 in body shape and size, the nuclear pattern, and the low number of somatic kineties. However, they distinctly differ by several features of the oral ciliature. *Metopus tenuis* displays a prominent paroral membrane and only six adoral polykinetids, extending merely to the level of the distal end of the paroral membrane. On the other hand, *M. filum* possesses an ordinary paroral membrane and 11–15 adoral polykinetids, extending almost to the anterior body end. Moreover, *M. filum* has a comparatively loose ciliature while it is rather dense in *M. tenuis* (Kahl 1927, 1932). *Metopus tenuis* sensu Jankowski (1964) resembles *M. filum* in the number of ciliary rows (10–12 vs. 8–13) and adoral polykinetids (12–15 vs. 11–15) but differs by the dense (vs. loose) ciliature. However, the density of the ciliature in Jankowski's drawing must be taken with caution because nearly all of his *Metopus* illustrations show identical dense ciliation, a fact already recognized for *M. inversus* by Foissner and Agatha (1999) and for *Atopospira violacea* by Bourland and Wendell (2014). Therefore, identity of *M. tenuis* sensu Jankowski (1964) remains questionable and its conspecificity with *M. filum* cannot be excluded.

Metopus palaeformides nov. spec. Foissner and Vďačný (Figs 4A–G, 5A–H, 6A–D, 7A–D, 8A–D; Table 2)

Diagnosis: Size about 200 × 40 μm in vivo. Body narrowly oblong and slightly twisted anteriorly. Macronucleus between proximal portion of adoral zone and second third of body, very narrowly oblong to cylindrical; one globular to broadly ellipsoidal micronucleus. Contractile vacuole terminal. Cortical granules about 0.5 μm across, colourless, narrowly spaced forming about five rows between adjacent kineties. On average 19 ciliary rows; elongated caudal cilia absent. Perizonal stripe composed of five kineties extending approximately 30% of body length and forming about 44 false kineties. Type 1 oral area. Adoral zone oblique, composed of an average of 31 polykinetids extending about 38% of body length.

Type locality: Loamy soil and leaf litter from the floodplain of the Murray River near to the town of Albury, Australia (S36°06′ E146°54′).

Type material: The holotype slide (reg. no. 2016/8) and two paratype slides (reg. nos 2016/9, 10) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. The holotype (Fig. 4F, G) and relevant paratype specimens have been marked by black ink circles on the coverslip.

Etymology: A composite of the stem of the species-group name *palaeform·is*, *-is*, *-e* [m, f, n] and the Latin suffix *-ides* (like, resembling), referring to the similarity with *Heterometopus palaeformis*.

Description: Size in vivo 165–255 × 30–55 μm, usually about 200 × 40 μm, as calculated from some in vivo measurements and the morphometric data adding 15% preparation shrinkage. Body narrowly oblong, i.e., length:width ratio near 5:1 on average, ranging from 4.0:1 to 6.0:1 in vivo, after protargol impregnation, and in SEM (Table 2), not flattened, slightly twisted anteriorly; preoral dome rostrate, flattened, distinctly projecting from body proper; postoral body portion oblong, sometimes wrinkled posteriorly by longitudinal folds, rear end quite variable, narrowly to broadly rounded, depending on state of contractile vacuole (Figs 4A, B, F, G, 5A–E, 8C, D). Nuclear apparatus usually between proximal portion of adoral zone and second third of body. Macronucleus very narrowly oblong to cylindrical and up to 100 μm long in vivo while only up to 67 μm after protargol impregnation, indicating considerable preparation shrinkage; nucleoli small and globular to ellipsoidal, well recognizable both in vivo and in some protargol-impregnated specimens. Micronucleus attached to or near to macronucleus, displaced posteriorly in some squashed silver carbonate preparations (Fig. 6B); shape and size similar both in vivo and after protargol impregnation, i.e., globular to broadly ellipsoidal and 5–6 μm across in vivo and 4–7 μm after protargol impregnation; surrounded by a hyaline membrane in vivo (Figs 4A, B, G, 5A–E, 6A, B, 7A, B; Table 2). Contractile vacuole in posterior body end (Figs 4A, F, 5A–E). Cortex flexible, furrowed by ciliary rows in SEM (Fig. 8A, C, D); cortical granules about 0.5 μm in size, colourless, narrowly spaced forming about five rows between adjacent kineties, faintly to deeply impregnated with silver carbonate (Figs 4, 6C). Cytoplasm colourless, sometimes appearing dark at low magnification (40×) because studded with many globular to ellipsoidal lipid (?) droplets 2–3 μm in diameter and 5–10 μm-sized food vacuoles containing flagellates and about 2 μm long bacteria; symbiotic bacteria not recognisable in vivo and after protargol impregnation. Swims slowly; dies quickly on microscope slides, possibly due to presence of oxygen.

Somatic ciliature composed of dikinetids, usually both basal bodies ciliated not only in oral portion of cell but also postorally, an unusual feature observed in several specimens in vivo and confirmed in SEM (Fig. 8A–D); cilia about 15 μm long in vivo; elongated caudal cilia absent. On average 19 meridional to slightly sigmoidal ciliary rows; dome kineties gradually shortened and curved slightly to distinctly leftwards anteriorly (Figs 4F, G, 5H, 6B, 7A, 8A, C, D; Table 2). Perizonal stripe distinct in vivo, shorter than adoral zone, i.e., occupies about 30% (vs. 38%) of body length; invariably composed of five rows: rows 1–3 more narrowly spaced than rows 4 and 5; segmented into 44 false kineties on average; posterior end usually separated from ordinary somatic kineties by a gap; sometimes a monokinetid at anterior or posterior end of some perizonal rows (Figs 4F, 5F–H, 6A, B, D, 7B–D; Table 2).

Somatic dikinetids associated with four fibres after silver carbonate impregnation (Fig. 4D). Fibre "a" associated with

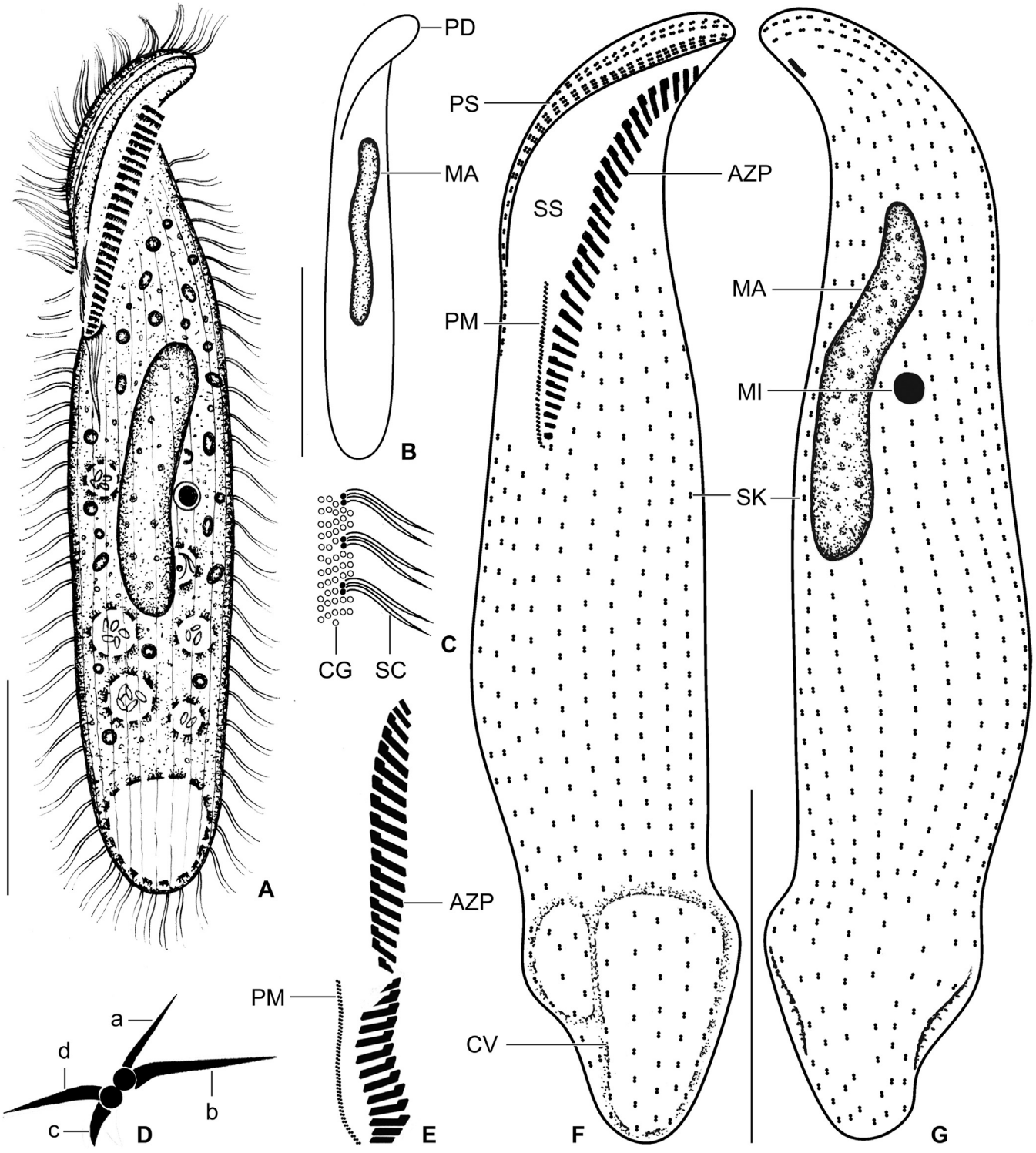

Fig. 4. A–G. *Metopus palaeformides* nov. spec. from life (A–C) and after silver carbonate (D) and protargol (E–G) impregnation. **A**: Ventral view of a representative specimen, length 200 μm. **B**: Shape variant. Note the macronucleus up to 100 μm long in vivo. **C**: Surface view showing the cortical granulation. **D**: Fibrillar associates of a somatic dikinetid. **E**: Semi-schematic diagram of oral ciliature. The adoral zone of polykinetids extends slightly obliquely and is twisted in the proximal third. Anterior- and posterior most polykinetids are rectangular while the others are L-shaped. The paroral membrane is dikinetal. **F, G**: Ciliary pattern of ventral and dorsal side as well as nuclear apparatus of holotype specimen, length 161 μm. a–d, Fibrillar associates; AZP, adoral zone of polykinetids; CG, cortical granules; CV, contractile vacuole; MA, macronucleus; MI, micronucleus; PM, paroral membrane, PD, preoral dome; PS, perizonal stripe; SC, somatic cilia; SK, somatic kineties; SS, side stripe. Scale bars: 50 μm (A, F, G) and 100 μm (B).

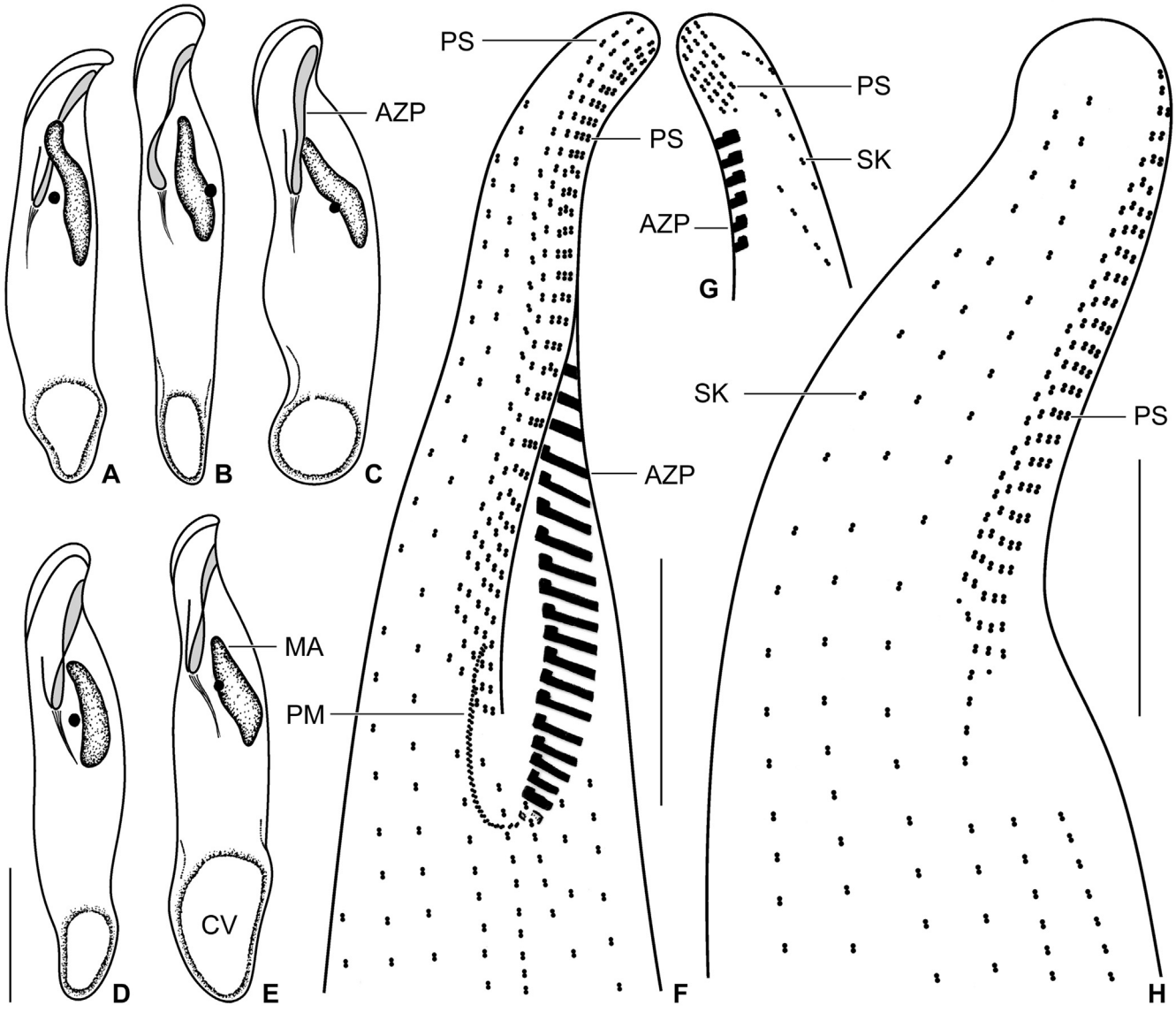

Fig. 5. A–H. *Metopus palaeformides* nov. spec. after protargol impregnation. **A–E**: Variability of body shape and size as well as of nuclear apparatus. Drawn to scale. **F, G**: Ventrolateral and dorsolateral view of ciliary pattern in anterior body portion of a paratype specimen. **H**: Dorsolateral view of ciliary pattern in anterior body portion. AZP, Adoral zone of polykinetids; CV, contractile vacuole; MA, macronucleus; PM, paroral membrane, PS, perizonal stripe; SK, somatic kineties. Scale bars: 20 μm (F–H) and 50 μm (A–E).

anterior basal body, extends leftwards in a slightly anterior direction, sometimes difficult to recognise because comparatively faintly impregnated. Fibre "b" associated with anterior basal body, runs to the left and slightly posteriorly, conspicuous because comparatively long and usually deeply impregnated. Fibre "c" connected to posterior basal body, directed posteriorly to the right, inconspicuous because shortest amongst the four basal body associates. Fibre "d" attached to posterior basal body, extends anteriorly to the right, well recognizable because comparatively long and usually deeply impregnated (Figs 4D, 6D, 7A, B). All fibres very short or not impregnated with silver carbonate in perizonal stripe rows, except for fibre "d" which deeply impregnates in perizonal row 5 (Figs 6D, 7B).

Type 1 oral area. Adoral zone extends obliquely on ventral side; occupies about 38% of body length; composed of an average of 31 polykinetids up to 10 μm wide and with cilia about 7 μm long in vivo; proximal- and distalmost polykinetids rectangular, others L-shaped and composed of at least two long rows of basal bodies and a short row. Paroral membrane dikinetal and about 23 μm long after protargol impregnation; begins about 45 μm posterior to anterior body end and extends vertically to proximal end of adoral zone; paroral cilia about 10 μm long in vivo, form a nice membrane well recognizable in vivo (Figs 4E–G, 5F, 6B, 8A; Table 2). Cytopharyngeal fibres originate at proximal end of adoral zone and paroral membrane, extend backwards forming a slender funnel about 20 μm long after protargol impregna-

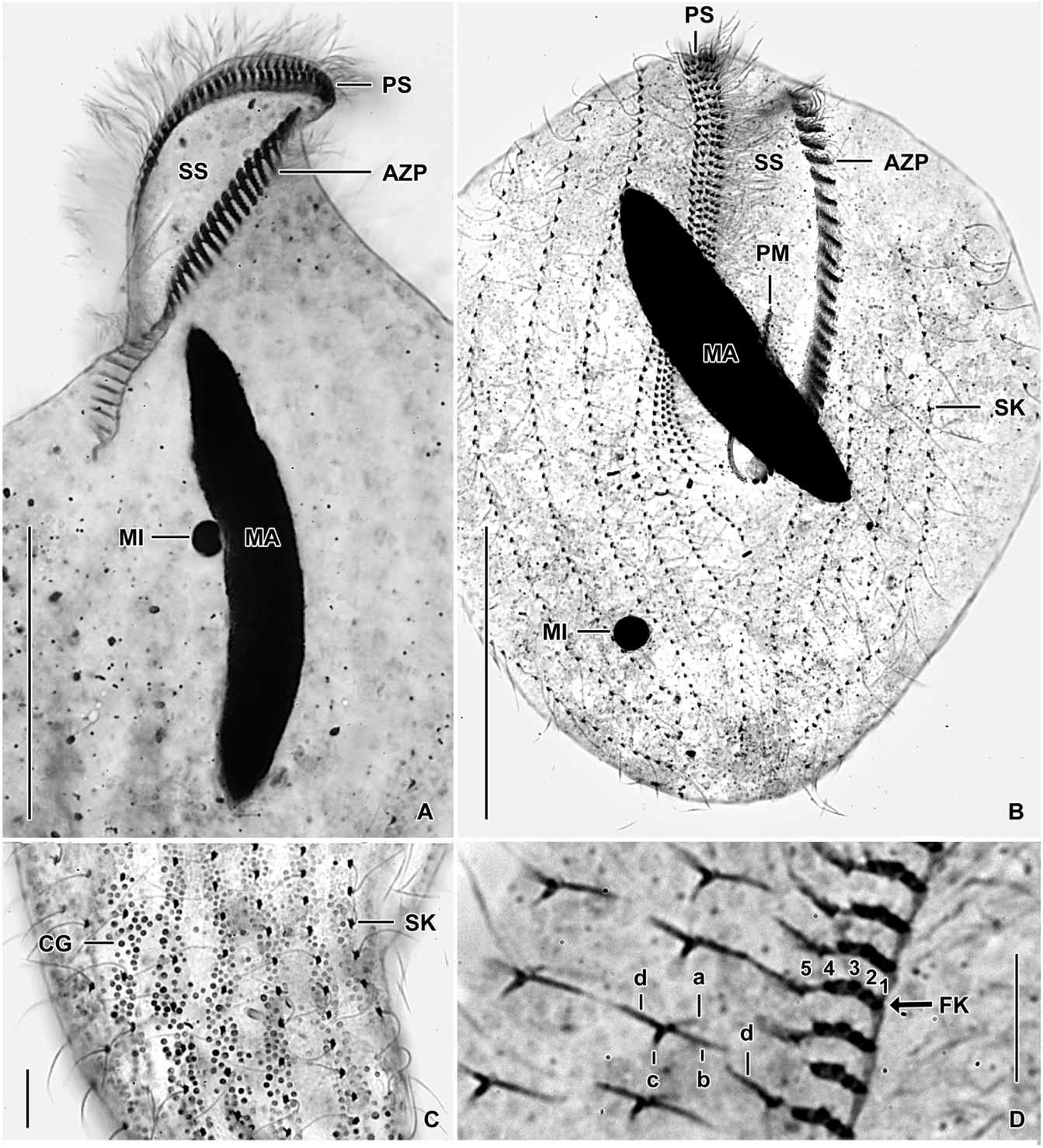

Fig. 6. A–D. *Metopus palaeformides* nov. spec. after silver carbonate impregnation (pressed specimens). **A**: Ventral view of anterior body portion, showing the perizonal stripe, the obliquely extending adoral zone of polykinetids and the long macronucleus. **B**: Overview of ventral side, showing the oral and somatic ciliary pattern as well as the nuclear apparatus. The micronucleus was displaced posteriorly by coverslip pressure. **C**: Surface view showing the dense cortical granulation. **D**: Detail of perizonal stripe and adjacent somatic kineties. The perizonal stripe consists of five rows that are segmented into false kineties (arrow). In contrast to the somatic dikinetids, the fibrilar associates are very short or not impregnated except for structure "d" which deeply impregnates in row 5. 1–5, Perizonal stripe rows; a–d, fibrilar associates; AZP, adoral zone of polykinetids; CG, cortical granules; FK, false kinety; MA, macronucleus; MI, micronucleus; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 5 μm (C, D) and 50 μm (A, B).

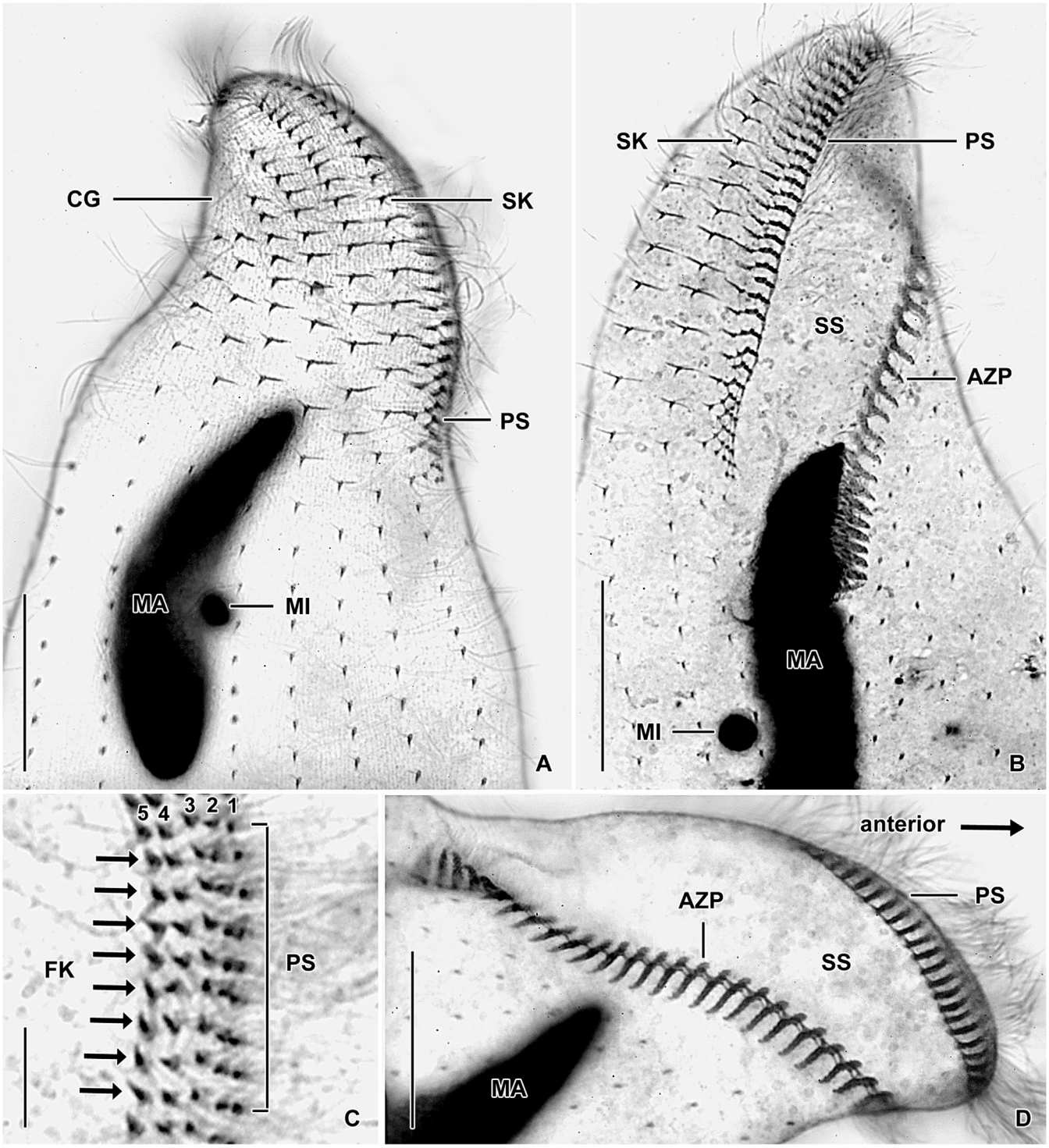

Fig. 7. A–D. *Metopus palaeformides* nov. spec. after silver carbonate impregnation. **A**: Dorsal view, showing ciliary pattern and nuclear apparatus. **B**: Ventrolateral view, showing oral and somatic ciliary pattern as well as the micronucleus surrounded by a distinct membrane. Note the wide side stripe that is an unciliated area between adoral zone of polykinetids and preoral dome. **C**: Detail of perizonal stripe. There are invariably five perizonal rows that are segmented into many false kineties (arrows). Stripe rows 1–3 are arranged more closely than rows 4 and 5. **D**: Ventral view of oral body portion, showing the perizonal stripe segmented into false kineties, the obliquely extending adoral zone of polykinetids, and the wide side stripe between perizonal stripe and adoral zone. 1–5, Perizonal stripe rows; AZP, adoral zone of polykinetids; CG, cortical granules; FK, false kineties; MA, macronucleus; MI, micronucleus; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 5 μm (C) and 30 μm (A, B, D).

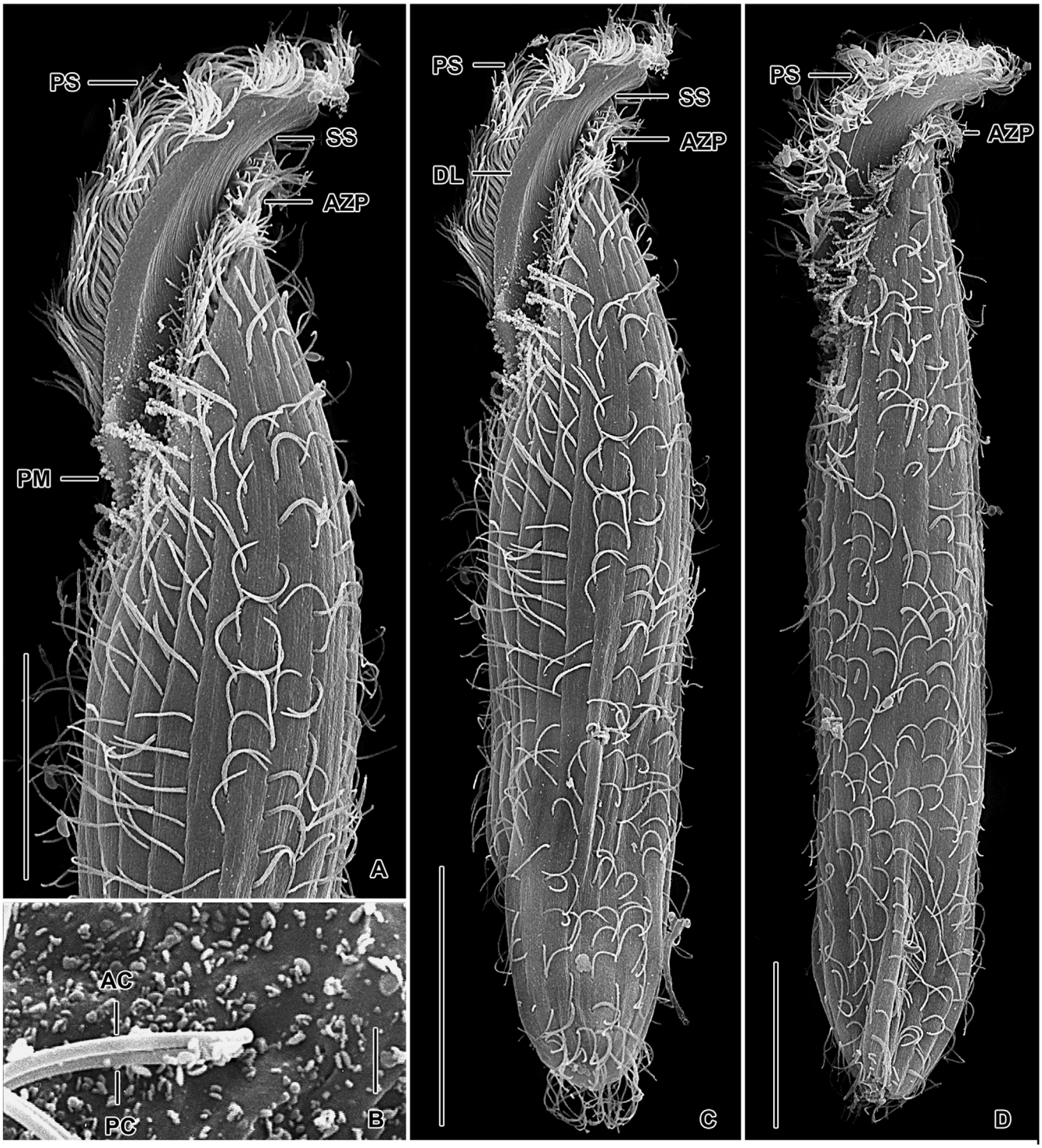

Fig. 8. A–D. *Metopus palaeformides* in the scanning electron microscope. **A**: Detail from anterior body portion of the specimen shown in (C). Note the beautiful metachronal waves produced by the narrowly spaced perizonal cilia. The side stripe is smooth to finely ribbed and completely overhangs the slightly obliquely extending adoral zone of polykinetids. **B**: Surface view in postoral body region, showing both basal bodies of the somatic dikinetids ciliated. This is an unusual feature in metopids because typically only the posterior basal body is ciliated. **C, D**: Overviews showing the oblong body only slightly twisted anteriorly. The preoral dome is rostrate, broader than mid-body, and overhangs the left body margin. The posterior body end is moderately rounded, sometimes it is wrinkled by longitudinal folds. AC, Anterior cilium; AZP, adoral zone of polykinetids; DL, dome lip; PC, posterior cilium; PM, paroral membrane; PS, perizonal stripe; SS, side stripe. Scale bars: 1 μm (B), 20 μm (A) and 30 μm (C, D).

Table 2. Morphometric data on *Metopus palaeformides* nov. spec.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	174.8	174.0	19.7	4.3	11.3	143.0	220.0	21
Body, maximum width of preoral dome	30.6	28.0	6.7	1.5	21.9	20.0	46.0	21
Body, width at cytostome	34.0	32.0	5.5	1.2	16.1	26.0	45.0	21
Body, maximum postoral width	36.3	35.0	6.4	1.4	17.5	27.0	50.0	21
Body, length:width ratio	4.9	4.9	0.6	0.1	11.4	4.0	5.9	21
Anterior body end to proximal end of PS, distance	52.4	52.0	4.4	1.0	8.5	42.0	59.0	21
Perizonal stripe, percentage of body length	30.3	30.6	4.0	0.9	13.1	23.5	36.9	21
Anterior body end to distal end of AZP, distance	11.0	11.0	2.6	0.6	23.5	6.0	15.0	21
Anterior body end to proximal end of AZP, distance	65.3	65.0	4.4	1.0	6.7	58.0	75.0	21
Adoral zone of polykinetids, percentage of body length	37.7	38.0	3.9	0.8	10.2	31.8	43.6	21
Anterior body end to distal end of PM, distance	44.7	44.0	4.6	1.0	10.3	35.0	57.0	21
Anterior body end to macronucleus, distance	39.8	42.0	8.6	1.9	21.7	18.0	55.0	21
Macronucleus, length	49.4	47.0	8.2	1.8	16.6	38.0	67.0	21
Macronucleus, width	11.2	11.0	1.4	0.3	12.5	9.0	15.0	21
Macronucleus, length:width ratio	4.4	4.6	0.8	0.2	16.9	3.2	5.7	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length	5.0	5.0	0.7	0.2	14.9	4.0	7.0	21
Micronucleus, width	5.0	5.0	0.7	0.2	14.9	4.0	7.0	21
Micronucleus, length:width ratio	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Somatic ciliary rows, total number	19.4	19.0	1.8	0.4	9.2	17.0	24.0	21
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	21
False kineties in perizonal stripe, number	44.0	45.0	2.6	0.6	6.0	40.0	47.0	21
Adoral polykinetids, number	31.1	31.0	1.7	0.4	5.6	28.0	35.0	21
Paroral membrane, length	22.8	22.0	2.3	0.5	9.9	20.0	27.0	21

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZP – Adoral zone of polykinetids; CV – coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; PM – paroral membrane; PS – perizonal stripe; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

tion (Fig. 5A–E). Dome lip smooth and 1.3–3.5 μm wide (mean = 2.5 μm, $n = 4$) in SEM. Side stripe a deep, smooth or finely ribbed channel, 3.8–6.2 μm wide (mean = 5.0 μm, $n = 4$) in SEM (Fig. 8A, C, D), well recognizable also in vivo as well as after silver carbonate (Figs 6A, B, 7B, D) and protargol impregnation where about 11 μm wide (Fig. 4F).

Comparison with similar species: *Metopus palaeformides* most resembles *Heterometopus palaeformis* (Kahl, 1927) Foissner, 2016b. Comparison of both species is, however, complex because *H. palaeformis* is considered highly polymorphic in the revision of Esteban et al. (1995). Although Kahl (1927, 1932) observed some shape variation in nature and recognized forma *typica*, *ovalis*, and *attenuatus*, he stated that their body length varies only between 70 and 80 μm. An ordinary size variation was also detected in the neotype Madagascan and the voucher Dominican specimens from non-flooded Petri dish cultures studied by Foissner et al. (2002) and Foissner (2016b), respectively. On the other hand, Esteban et al. (1995) noted a huge variability in body size (70–200 × 8–31 μm) in specimens kept for several years in cultures. However, according to their Fig. 3 and Table 1, the high variation can be related mainly to starvation, and "typical" trophic cells were ordinarily variable (70–132 × 8–31 μm, $n = 403$). Likewise, the number of adoral polykinetids and ciliary rows is of usual variability across all *H. palaeformis* populations studied in detail (for review, see Foissner 2016b).

Metopus palaeformides differs from *H. palaeformis* in several quantitative (body size, number of adoral polykinetids and ciliary rows) and qualitative (cortical granulation and oral area pattern) features. First of all, *M. palaeformides* is distinctly larger than *H. palaeformis*: 165–255 μm vs. 70–80 μm in Kahl's (1927, 1932) specimens, 80–120 μm in Madagascan cells (Foissner et al., 2002), 70–80 μm in Dominican individuals (Foissner 2016b), and 70–132 μm in "typical" English specimens (Esteban et al., 1995). Further, *M. palaeformides* possesses a higher number of adoral polykinetids: 28–35 vs. 17–24 in Madagascan cells, 18–21 in Dominican individuals, and 10–20 in English specimens. Moreover, *M. palaeformides* has slightly more ciliary rows: 17–24 vs. 15–20 in the Madagascan population, 10–15 in Dominican cells, and 8–14 in English specimens. As concerns qualitative features, cortical granules are narrowly spaced forming about five rows between adjacent kineties in *M. palaeformides*, while granule rows are loosely spaced within and between kineties in *H. palaeformis* (Foissner et al., 2002). Finally, *M. palaeformides* differs from *H. palaeformis* in the somatic ciliation (both basal bodies of postoral somatic dikinetids ciliated vs. only posterior basal body ciliated) and in the oral area pattern (type 1 vs. 2).

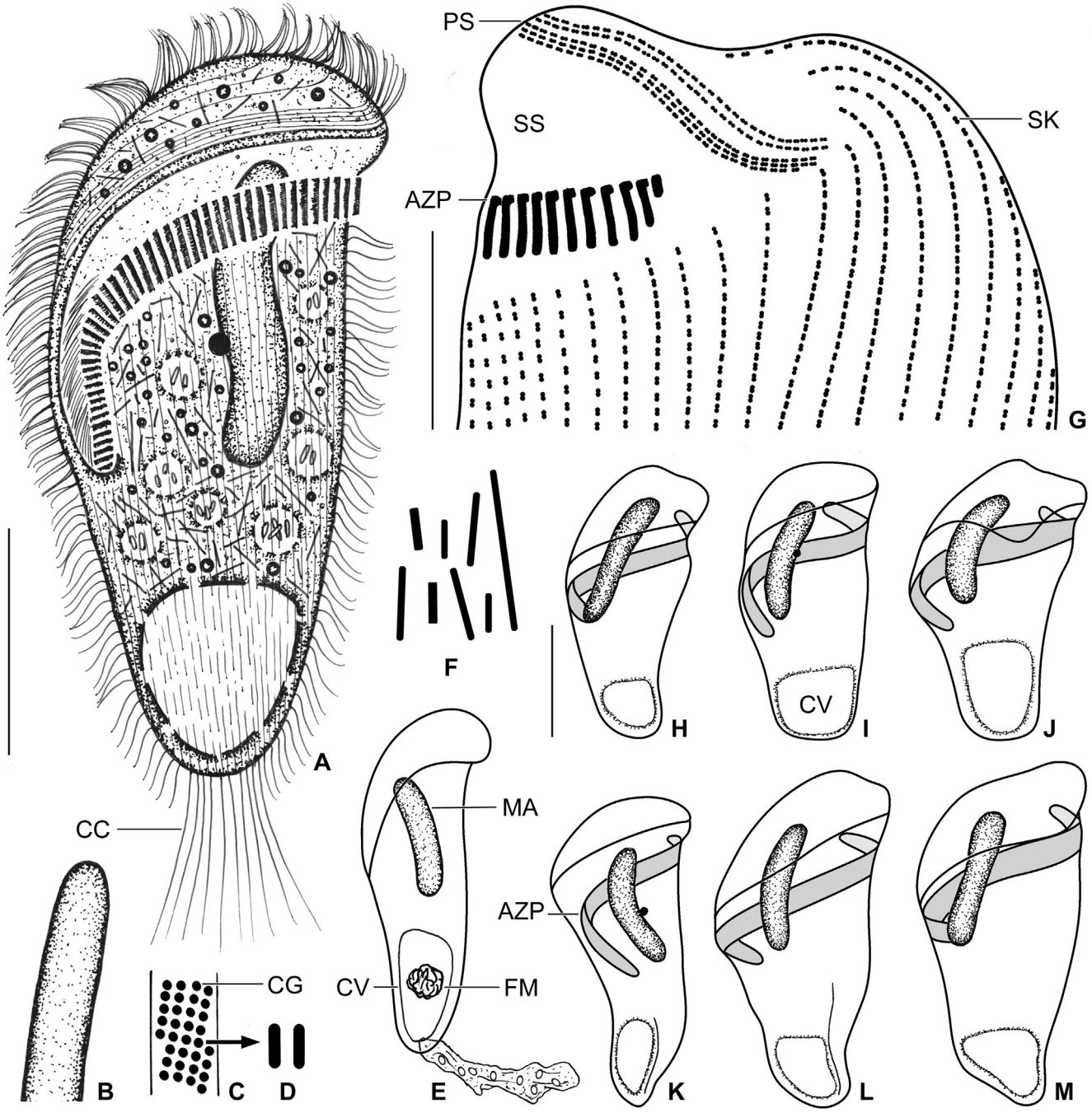

Fig. 9. A–M. *Metopus rex* nov. spec. from life (A–F) and after protargol impregnation (G–M). **A**: Ventral view of a representative specimen, length 160 μm. **B**: The preoral dome is comparatively narrow in dorsal view. **C**: Surface view showing cortical granulation. The granules are narrowly spaced, forming about five oblique rows between adjacent kineties. **D**: The cortical granules are about 2.0 × 0.7 μm in size. They are colourless, highly refractive, and oriented perpendicularly to the cell surface. **E**: Shape variant. The macronucleus is oblong and slightly curved. The faecal mass is slimy, contains many bacterial spores, and is expelled through the cytopyge. **F**: There are innumerable symbiotic bacteria scattered throughout the cytoplasm. They are oblong to filiform and 5–30 × 1–2 μm in size. **G**: Dorsolateral view of ciliary pattern in anterior body portion, showing the distal end of the perizonal stripe and of the adoral zone of polykinetids. **H–M**: Variability of body shape and size as well as of nuclear apparatus. The body is ovate to bluntly cuneate and nicely spiralled anteriorly. The posterior body end is quite variable, i.e., narrowly to broadly rounded, rectangular, and sometimes even irregular, depending on the state of the contractile vacuole. Drawn to scale. AZP, Adoral zone of polykinetids; CC, caudal cilia; CG, cortical granules; CV, contractile vacuole; FM, faecal mass; MA, macronucleus; PS, perizonal stripe; SK, somatic kinety; SS, side stripe. Scale bars: 20 μm (G) and 50 μm (A, H–M).

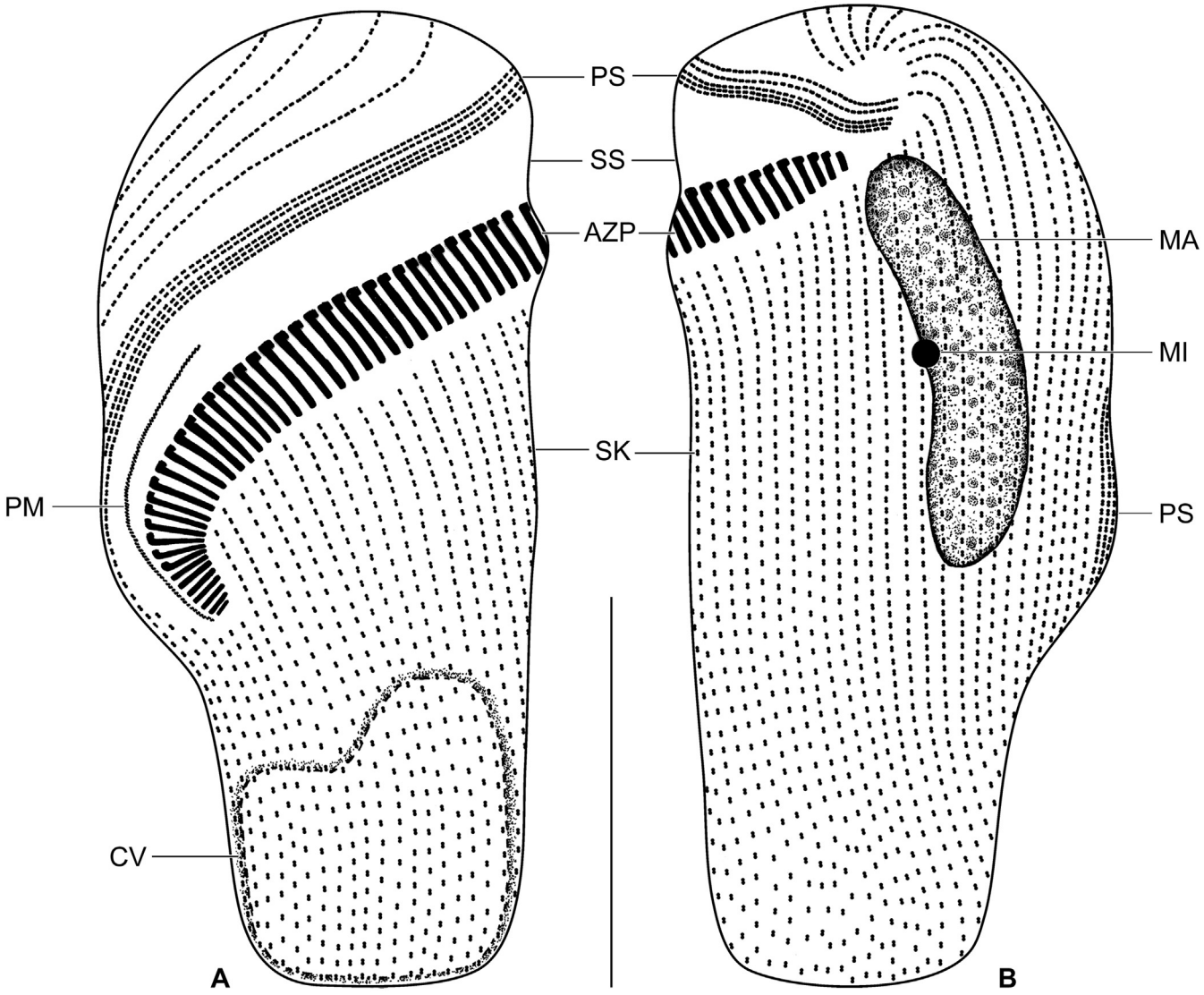

Fig. 10. A, B. *Metopus rex* nov. spec. after protargol impregnation. Ventral (A) and dorsal (B) view of ciliary pattern as well as of nuclear apparatus and contractile vacuole in the holotype specimen, length 126 μm. AZP, Adoral zone of polykinetids; CV, contractile vacuole; MA, macronucleus; MI, micronucleus; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bar: 50 μm.

Metopus rex nov. spec. Foissner and Vďačný (Figs 9A–M, 10A, B, 11A–H; Table 3)

Diagnosis: Size about 160 × 65 μm in vivo. Body obovate to bluntly cuneate and twisted anteriorly. Macronucleus between anterior and posterior end of adoral zone, oblong and slightly curved; one globular to broadly ellipsoidal micronucleus. Contractile vacuole terminal. Cortical granules highly refractive, about 2.0 × 0.7 μm in size, colourless, narrowly spaced forming about five rows between adjacent kineties. On average 60 ciliary rows; caudal cilia about 30 μm long. Perizonal stripe composed of five kineties extending approximately 52% of body length and forming more than 100 false kineties. Type 1 oral area. Adoral zone distinctly spiralled, composed of an average of 62 polykinetids extending about 60% of body length.

Type locality: Loamy soil and leaf litter from the floodplain of the Murray River near to the town of Albury, Australia (S36°06′ E146°54′).

Type material: The holotype slide (reg. no. 2016/11) and four paratype slides (reg. nos 2016/12–15) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. The holotype (Fig. 10A, B) and relevant paratype specimens have been marked by black ink circles on the coverslip.

Etymology: The Latin noun *rex* (king) refers to the majestic appearance of this ciliate. The species name is treated as a noun in the nominative singular standing in apposition to the generic name (Art. 11.9.1.2 of the International Commission on Zoological Nomenclature, 1999).

Description: Size in vivo 140–180 × 45–85 μm, usually about 160 × 65 μm, as calculated from some in vivo measure-

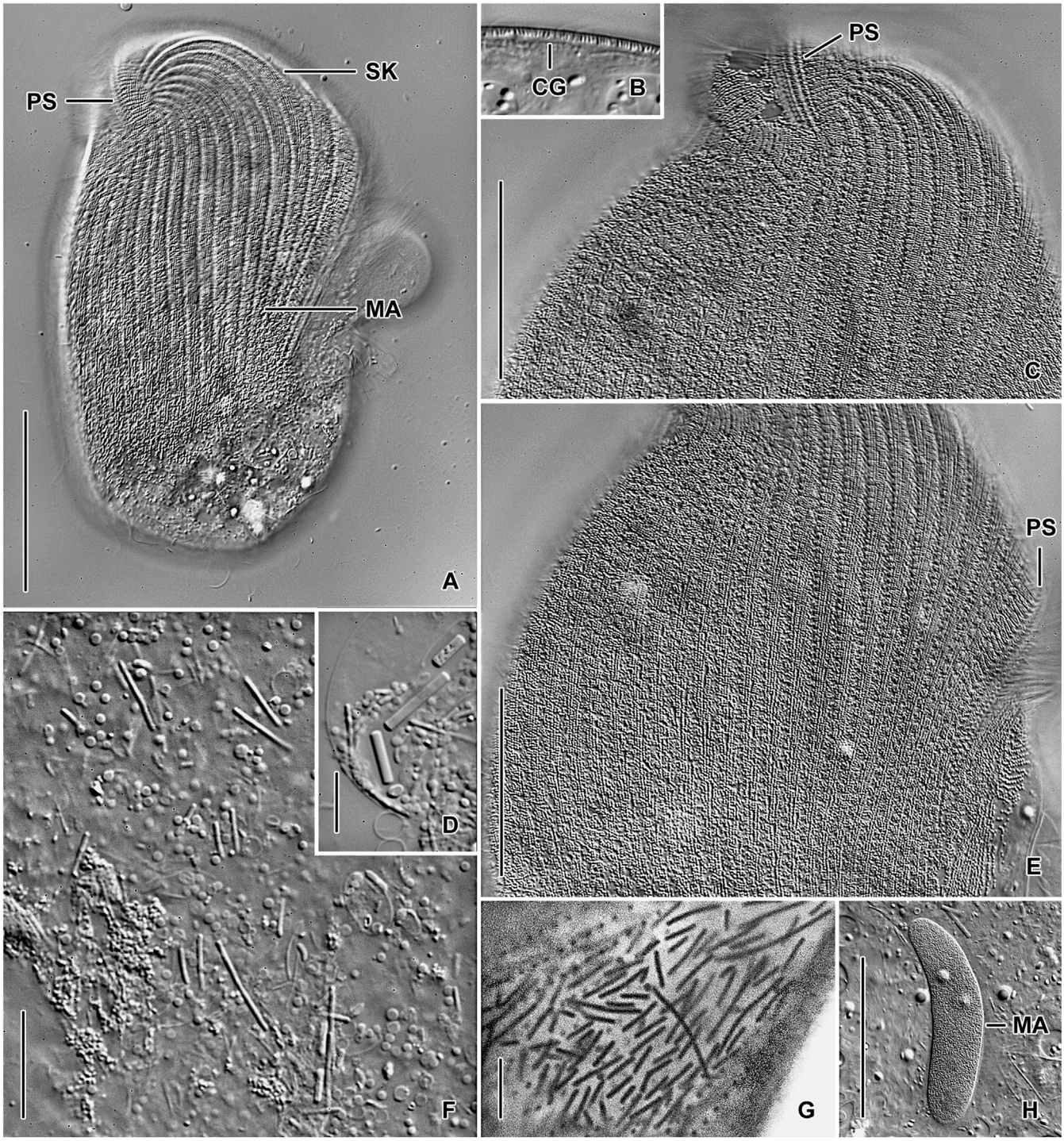

Fig. 11. A–H. *Metopus rex* nov. spec. from life (A–F, H) and after protargol impregnation (G). **A, C, E**: Dorsal views, showing ciliary pattern and cortical granulation. The dome kineties curve towards the distal end of the perizonal stripe anteriorly. The cortical granules are colourless and highly refractive. They are narrowly spaced, forming about five oblique rows between adjacent kineties. **B**: The cortical granules are oriented perpendicularly to the cell surface. They are highly refractive and about 2.0 × 0.7 μm in size. **D, F, G**: Innumerable symbiotic bacteria are scattered throughout the cytoplasm. They are oblong to filiform and 5–30 × 1–2 μm in size. They impregnate deeply with the protargol method used. **H**: The macronucleus is oblong and slightly curved. CG, Cortical granules; MA, macronucleus; PS, perizonal stripe; SK, somatic kinety. Scale bars: 10 μm (D, G), 30 μm (C, E, F) and 50 μm (A, H).

Table 3. Morphometric data on *Metopus rex* nov. spec.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	139.4	140.0	11.8	3.6	8.5	120.0	155.0	11
Body, maximum width of preoral dome	65.6	67.0	8.2	2.5	12.4	52.0	75.0	11
Body, width at cytostome	58.2	60.0	9.2	2.8	15.7	39.0	72.0	11
Body, maximum postoral width	54.5	54.0	9.6	2.9	17.6	38.0	72.0	11
Body, length:width ratio	2.6	2.5	0.4	0.1	15.6	2.2	3.7	11
Anterior body end to distal end of PS, distance	12.1	11.0	3.0	0.9	24.8	10.0	18.0	10
Anterior body end to proximal end of PS, distance	71.8	70.0	8.8	2.9	12.2	58.0	83.0	9
Perizonal stripe, percentage of body length	52.4	52.0	4.7	1.6	8.9	44.6	59.3	9
Anterior body end to distal end of AZP, distance	21.2	20.5	3.5	1.1	16.5	18.0	29.0	10
Anterior body end to proximal end of AZP, distance	81.6	81.0	6.2	1.9	7.6	70.0	92.0	11
Adoral zone of polykinetids, percentage of body length	58.8	60.0	4.9	1.5	8.4	50.3	65.0	11
Anterior body end to distal end of PM, distance	45.6	45.0	8.0	2.7	17.7	35.0	60.0	9
Anterior body end to macronucleus, distance	20.4	19.0	6.0	1.8	29.3	15.0	35.0	11
Macronucleus, length	56.4	55.0	4.3	1.3	7.7	50.0	65.0	11
Macronucleus, width	13.8	14.0	1.3	0.4	9.6	12.0	16.0	11
Macronucleus, length:width ratio	4.1	4.0	0.4	0.1	9.4	3.4	4.8	11
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	11
Micronucleus, length	4.0	4.0	1.0	0.6	25.0	3.0	5.0	3
Micronucleus, width	3.5	3.5	–	–	–	3.0	4.0	3
Micronucleus, length:width ratio	1.1	1.0	–	–	–	1.0	1.4	3
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	3
Somatic ciliary rows, total number	59.8	58.5	7.2	2.5	12.1	51.0	70.0	8
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	10
False kineties in perizonal stripe, number				>100				
Adoral polykinetids, number	61.6	61.5	3.3	1.2	5.4	57.0	68.0	8
Paroral membrane, length	39.8	38.0	4.7	1.6	11.8	32.0	48.0	9

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZP – Adoral zone of polykinetids; CV – coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; PM – paroral membrane; PS – perizonal stripe; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

ments and the morphometric data adding 15% preparation shrinkage. Body obovate to bluntly cuneate, i.e., length:width ratio 2.2–3.7:1 after protargol impregnation (Table 3), nicely spiralled anteriorly; preoral dome extends somewhat over half of body length in ventral view, slightly to distinctly projecting from body proper, overhanging portion distinctly flattened and thus comparatively narrow in dorsal view (Fig. 9B); postoral body portion bluntly obconical, sometimes wrinkled by longitudinal folds, rear end quite variable, narrowly to broadly rounded, rectangular, and sometimes irregular, depending on state of contractile vacuole (Figs 9A, E, H–M, 10A, B, 11A). Nuclear apparatus between anterior and posterior end of adoral zone. Macronucleus oblong and slightly curved with a length:width ratio of 3.4–4.8:1 after protargol impregnation, becomes ellipsoidal under moderate coverslip pressure; nucleoli small and globular. Micronucleus usually attached to mid-portion of macronucleus; globular to broadly ellipsoidal with size ranging from 3 μm to 5 μm in protargol preparations (Figs 9A, E, H–M, 10B, 11H; Table 3). A single, large contractile vacuole in posterior body end; faecal mass slimy with many bacterial spores, moves through contractile vacuole when expelled (Fig. 9E). Cortex flexible; cortical granules about 2.0 × 0.7 μm in size, colourless and highly refractive in vivo, oriented perpendicularly to cell surface and narrowly spaced forming about five oblique rows between adjacent kineties (Figs 9C, D, 11A–C, E). Cytoplasm colourless, contains some lipid droplets and many food vacuoles 3–10 μm across; studded with oblong to filiform, 5–30 × 1–2 μm-sized bacteria heavily impregnating with the protargol method used (Figs 9F, 11D, F, G).

Somatic ciliature composed of dikinetids, anterior cilium lacking in postoral kinetids as typical for metopids; somatic cilia 7–8 μm long after protargol impregnation; elongated caudal cilia about 30 μm long in vivo, not recognizable in protargol preparations. On average 60 ciliary rows; ventral and lateral kineties begin slightly posterior to adoral zone and extend to rear end following body curvature; dome kineties commence anterior to adoral zone, their anterior portion curved towards distal end of perizonal stripe (Figs 9G, 10A, B, 11A, C; Table 3). Perizonal stripe begins about 12 μm posterior to anterior body end at left margin of dorsal side, extends along dome margin over ventral side to terminate on right margin of dorsal side slightly anterior to proximal end of adoral zone, i.e., occupies about 52% of body length on average; invariably composed of five rows: rows 1–3 more narrowly spaced than rows 4 and 5, row 5 sepa-

rated from first dome kinety by a conspicuous gap; stripe rows segmented into more than 100 densely spaced false kineties; each perizonal dikinetid has two cilia 8–9 μm long in protargol preparations (Figs 9G, 10A, B; Table 3).

Type 1 oral area. Adoral zone distinctly spiralled, occupies approximately 60% of body length on average, commences slightly posterior to distal end of perizonal stripe, i.e., about 21 μm posterior to anterior body end at left margin of dorsal side, extends obliquely over ventral side to right body margin where it curves leftwards; composed of an average of 62 polykinetids. Paroral membrane dikinetal and about 40 μm long after protargol impregnation; originates at proximal end of adoral zone and follows its curvature (Figs 9G, 10A, B; Table 3). Cytopharyngeal fibres not recognizable in vivo or after protargol impregnation. Side stripe conspicuous and comparatively broad in protargol-impregnated specimens (Figs 9G, 10A, B).

Comparison with similar species: *Metopus rex* is outstanding in having an oblong macronucleus and conspicuous endosymbiotic bacteria up to 30 μm long. It resembles several large species (>100 μm long) with long caudal cilia: *M. contortus* (Quennerstedt, 1867) Kahl, 1932; *Metopus fuscus* Kahl, 1927; and *M. propagatus* Kahl, 1926. *Metopus contortus*, as redescribed by Foissner et al. (2002), differs from *M. rex* by the lower number of ciliary rows (40–55 vs. 51–70) and adoral polykinetids (33–50 vs. 59–68). *Metopus fuscus*, as redescribed by Bourland et al. (2014), is distinguished from *M. rex* by the accumulation of dark granules (bacteria) around the macronucleus and the much higher number of somatic ciliary rows (80–108 vs. 51–70) and adoral polykinetids (74–103 vs. 57–68). *Metopus propagatus*, as described by Kahl (1926, 1929, 1932), is easily separated from *M. rex* by the tail-like posterior body end (vs. rounded to bluntly cuneate) and the ellipsoidal (vs. oblong) macronucleus.

Metopus magnus nov. spec. Foissner and Vďačný (Figs 12A–G, 13A–L, 14A–G, 15A, B, 16A–E, 17A–D; Table 4)

Diagnosis: Size about 200 × 60 μm in vivo. Body oblong to narrowly oblong and twisted anteriorly. Macronucleus between proximal half of adoral zone and mid-body, lenticular; one globular to broadly ellipsoidal micronucleus in a small macronuclear concavity. Contractile vacuole terminal. On average 44 ciliary rows; many slightly elongated caudal cilia 20–25 μm long. Perizonal stripe composed of five kineties extending approximately 40% of body length and forming about 92 false kineties. Type 1 oral area. Adoral zone distinctly spiralled, composed of an average of 46 polykinetids extending about 50% of body length.

Type locality: Loamy soil and leaf litter from the floodplain of the Murray River near to the town of Albury, Australia (S36°06′ E146°54′).

Type material: The holotype slide (reg. no. 2016/16) and two paratype slides (reg. nos 2016/17, 18) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. The holotype (Fig. 12F, G) and relevant paratype specimens have been marked by black ink circles on the coverslip.

Etymology: The Latin adjective *magn·us*, *-a*, *-um* [m, f, n] (large, big) refers to the large body size. According to Aescht (2001), the genus-group name *Metopus* is neuter. It is, indeed, derived from the neuter Greek noun *métōpon* (μέτωπον, forehead). However, according to the Article 30.1.3 (International Commission on Zoological Nomenclature 1999), a genus-group name that is a latinized Greek word takes the gender normally appropriate to the changed ending. Names with the Latin masculine ending *-us*, latinized from the Greek neuter ending *-on*, are therefore masculine.

Description: Size in vivo 150–225 × 45–75 μm, usually about 200 × 60 μm, as calculated from some in vivo measurements and the morphometric data adding 15% preparation shrinkage. Body oblong to narrowly oblong, i.e., length:width ratio 2.7–4:1 after protargol impregnation (Table 4), not, or only slightly, dorsoventrally flattened, spiralled anteriorly; preoral dome rostrate, flattened, distinctly overhangs left body margin, obliquely traverses ventral side and smoothly merges into right dorsal surface; postoral body portion oblong to lenticular, rear end usually broadly rounded (Figs 12A–G, 13C–L, 14A, B, D, E, 15A, B, 17A). Nuclear apparatus between proximal half of adoral zone and mid-body, does not enter preoral dome. Macronucleus lenticular with a length:width ratio of 3.2–5.3:1; nucleoli small to medium-sized and globular, well recognizable both in vivo and after protargol impregnation. Micronucleus in small macronuclear concavity; globular to broadly ellipsoidal with a length:width ratio of 1.0–1.3:1, about 6 μm across in vivo while on average only 3.5 μm in protargol preparations; surrounded by a hyaline membrane in silver carbonate slides (Figs 12A, G, 13D–L, 14B–E; Table 4). A single, large contractile vacuole in posterior body end (Figs 12A, D, E, 13C, E–L, 14A, E). Cortex flexible, furrowed by ciliary rows in SEM micrographs (Figs 15A, B, 17A–C); cortical granules not recognizable. Cytoplasm colourless, sometimes dark at low magnification because studded with lipid droplets 1–3 μm in diameter and food vacuoles with residues of bacteria and their spores; no symbiotic bacteria recognizable in vivo and after protargol impregnation (Fig. 14A). Swims rather rapidly, appearing majestic when rotating about main body axis.

Somatic ciliature composed of dikinetids, anterior cilium lacking in postoral kinetids as typical for metopids (Fig. 17C); somatic cilia 12–15 μm long in vivo; caudal cilia slightly elongated and about 20–25 μm long in vivo (Fig. 12A), recognizable also in silver carbonate preparations (Fig. 14D). On average 44 ciliary rows; ventral and lateral rows commence slightly posterior to adoral polykinetids and extend meridionally towards rear end; dome kineties gradually shortened

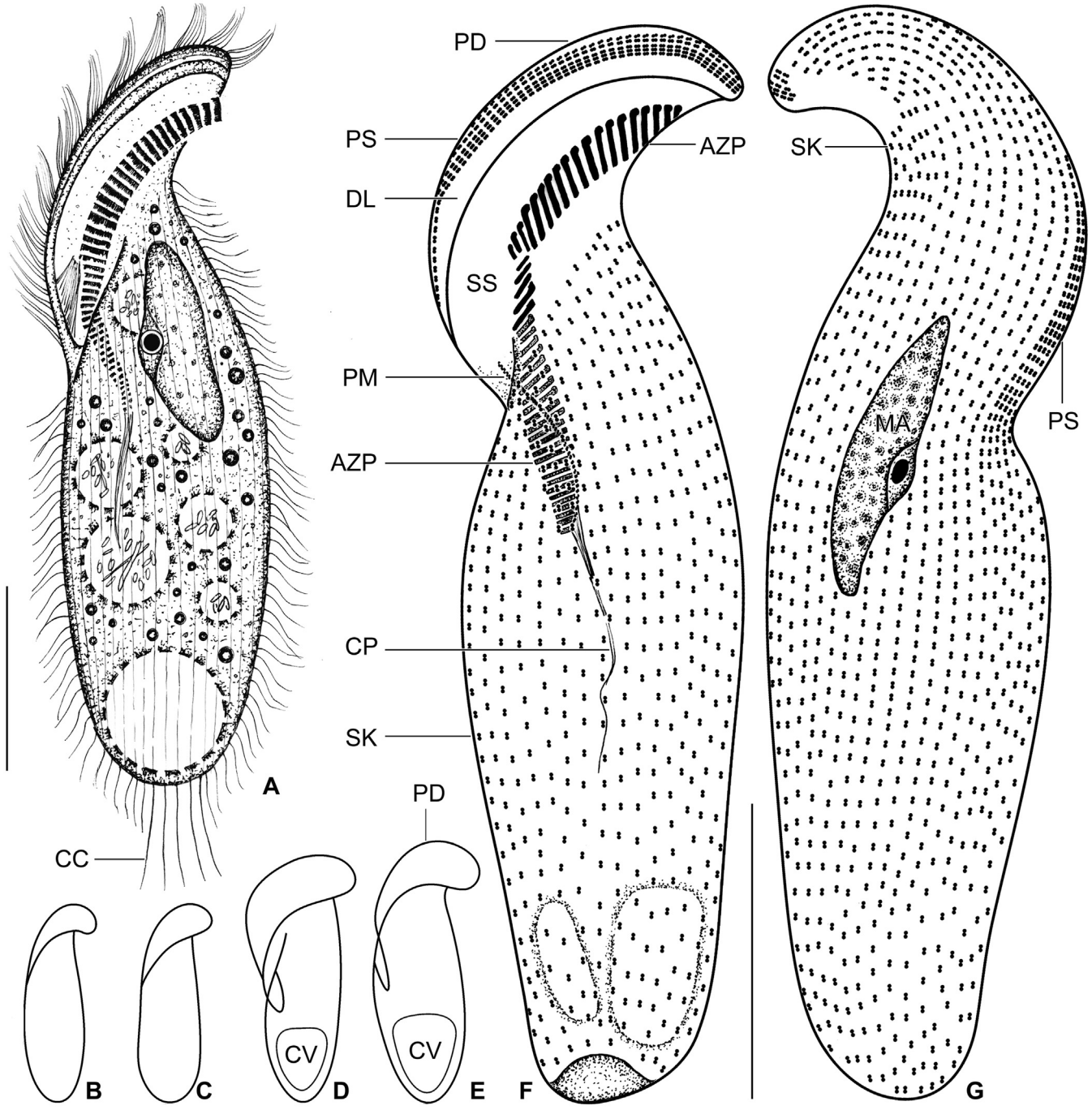

Fig. 12. A–G. *Metopus magnus* nov. spec. from life (A–E) and after protargol impregnation (F, G). **A**: Ventral view of a representative specimen, length 200 μm. Note the inconspicuous caudal cilia. **B–E**: Shape variants. The body is oblong to narrowly oblong and not or only slightly dorsoventrally flattened. The oral portion is spiralled. The preoral dome is rostrate and massive but flattened at base; it extends about third of body length in ventral view, overhangs the left body margin, obliquely traverses the ventral side, and smoothly merges into the dorsolateral surface. The postoral body portion is oblong to lenticular and the rear body end is usually broadly rounded. **F, G**: Ventral and dorsal view of ciliary pattern and nuclear apparatus of holotype specimen, length 185 μm. The ventral somatic kineties commence slightly posterior to the adoral polykinetids and extend towards the rear end. The dorsal kineties extend onto the preoral dome and curve leftwards and shorten gradually, i.e., towards the distal end of the perizonal stripe. Note the single micronucleus in a small concavity of the lenticular macronucleus. AZP, Adoral zone of polykinetids; CC, caudal cilia; CP, cytopharynx; CV, contractile vacuole; DL, dome lip; MA, macronucleus; PD, preoral dome; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 50 μm.

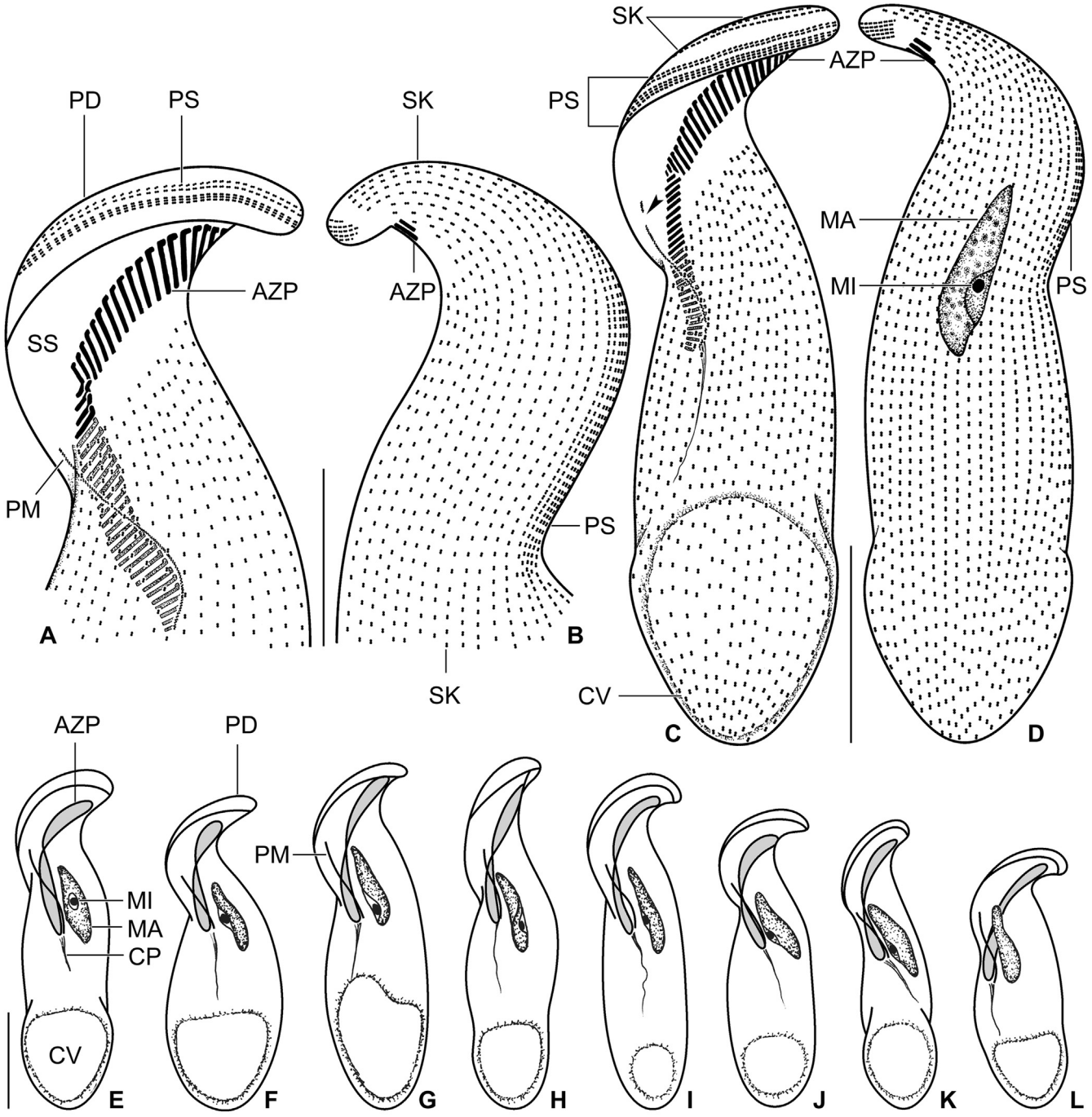

Fig. 13. **A–L**. *Metopus magnus* nov. spec. after protargol impregnation. **A, B**: Ventral and dorsal view of anterior body portion of a paratype specimen. **C, D**: Ventral and dorsal view, showing the ciliary pattern, the nuclear apparatus, and the contractile vacuole of another paratype specimen, length 189 μm. Arrowhead marks the anteriorly fragmented paroral membrane. **E–L**: Variability of body shape and size and of nuclear apparatus. The body is oblong; the rostrate preoral dome extends only about third of body length in ventral view. The postoral body portion is oblong or lenticular, and the rear end is narrowly to broadly rounded, depending on state of the contractile vacuole. Drawn to scale. AZP, Adoral zone of polykinetids; CP, cytopharynx; CV, contractile vacuole; MA, macronucleus; MI, micronucleus; PD, preoral dome; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 30 μm (A, B) and 50 (C–L).

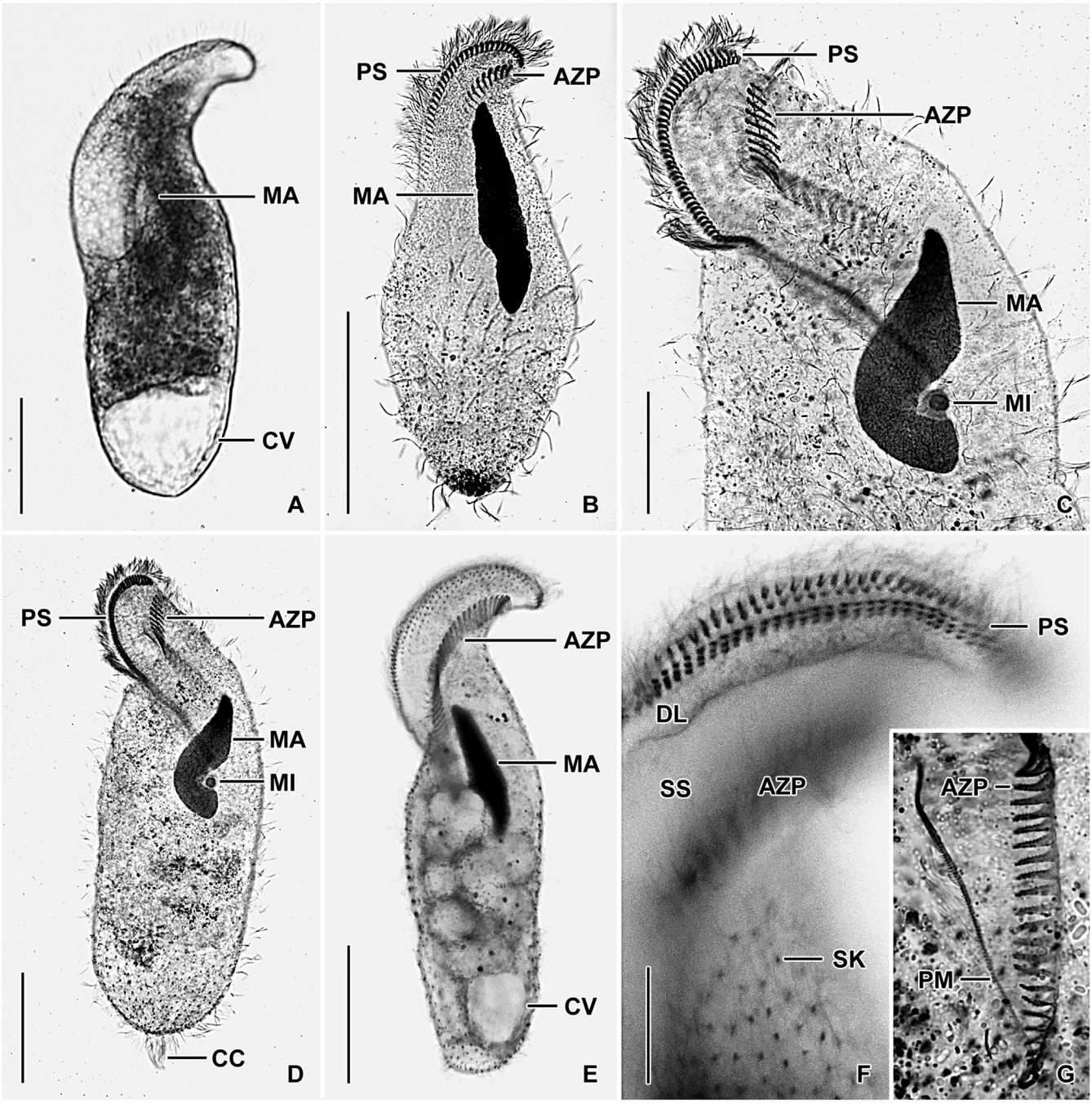

Fig. 14. **A–G**. *Metopus magnus* nov. spec. from life (A) and after silver carbonate (B–D, G) and protargol (E, F) impregnation. **A**: Ventral view of a representative specimen. **B, D, E**: Overviews showing the adoral zone of polykinetids, the perizonal stripe, and the nuclear apparatus. The caudal cilia are only 20–25 μm long in vivo and are thus inconspicuous. **C**: Ventral view of anterior body portion, showing the twisted adoral zone, the perizonal stripe regularly segmented into many false kineties, and the lenticular, slightly curved macronucleus. The globular micronucleus is surrounded by a hyaline membrane and is in a small concavity of the macronucleus. **F**: Detail of anterior body portion, showing the perizonal stripe which follows the curvature of the preoral dome. Stripe rows 1–3 are arranged more closely and are separated from the more widely spaced rows 4 and 5 by a more or less wide gap. **G**: Detail of oral ciliature. The paroral membrane is dikinetal; usually it starts slightly posterior to mid-portion of the adoral zone of polykinetids, and extends along the margin of the preoral dome to sink into the deep buccal cavity where it optically intersects the adoral zone. AZP, Adoral zone of polykinetids; CC, caudal cilia; CV, contractile vacuole; DL, dome lip; MA, macronucleus; MI, micronucleus; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 10 μm (F), 30 μm (C), and 50 μm (A, B, D, E).

Fig. 15. A, B. *Metopus magnus* nov. spec. in the scanning electron microscope. Overviews showing general body organization. Arrows mark the paroral membrane which extends along the margin of the preoral dome; together with the adoral zone of polykinetids, it sinks into the deep buccal cavity. The side stripe is smooth or finely ribbed. Note the furrowed cortex. AZP, Adoral zone of polykinetids; DL, dome lip; PS, perizonal stripe; SS, side stripe. Scale bars: 50 μm.

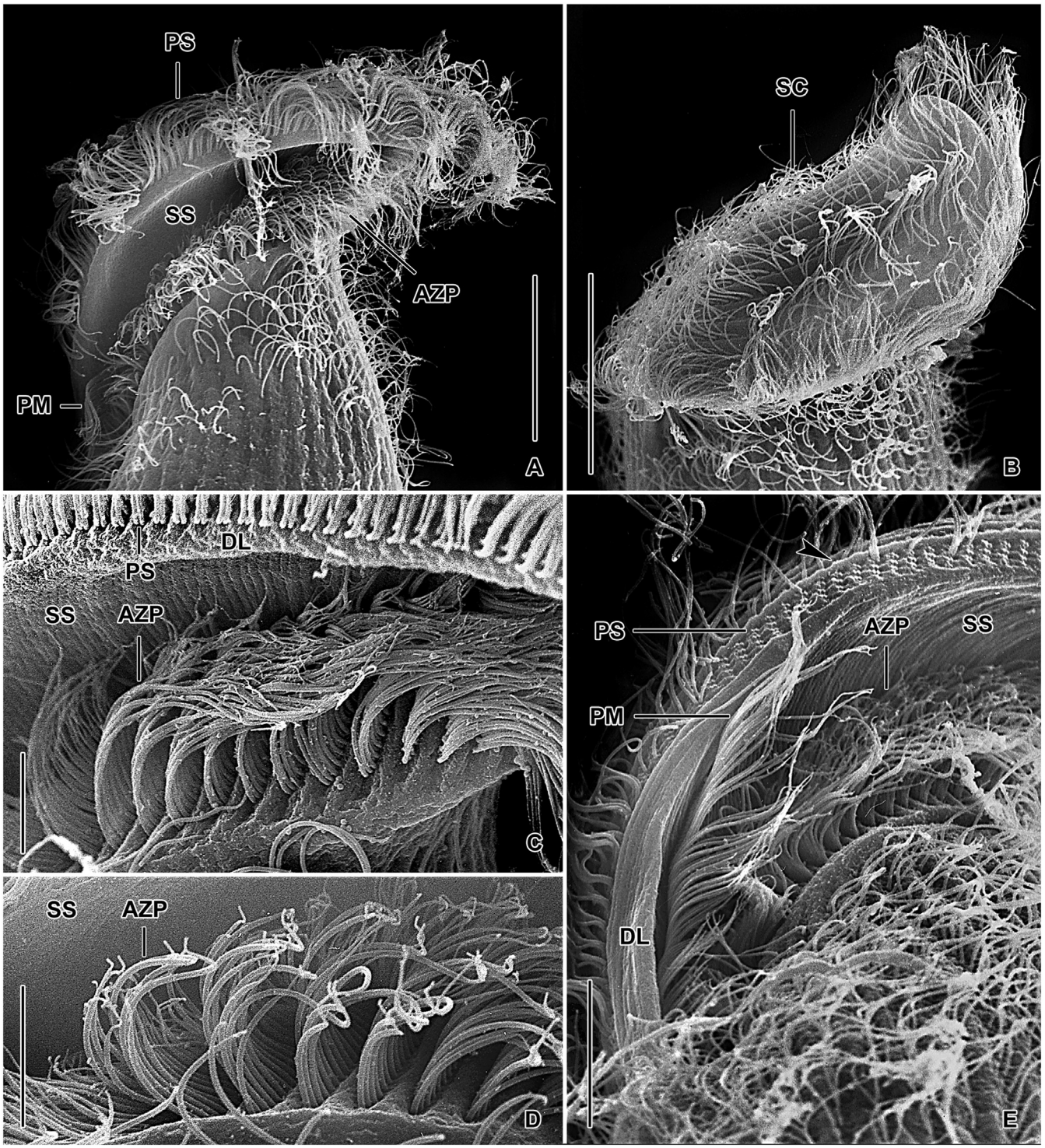

Fig. 16. A–E. *Metopus magnus* nov. spec. in the scanning electron microscope. **A**: Ventral view of anterior body portion, showing the beautiful metachronal waves produced by the perizonal cilia, the smooth side stripe, and the obliquely extending adoral zone of polykinetids. **B**: Dorsal view of anterior body portion, showing the preoral dome and its ciliature. **C, D**: Details of adoral zone of polykinetids. The individual polykinetids consist of two long rows of very narrowly spaced and zigzagging cilia; they are separated by distinct cortical ridges. **E**: Detail showing the oral apparatus and the perizonal stripe. The paroral membrane extends along the margin of the preoral dome and sinks, together with the adoral zone of polykinetids, into the deep buccal cavity. Stripe rows 1–3 are deciliated to show their separation from rows 4 and 5 by a cortical ridge (arrowhead). AZP, Adoral zone of polykinetids; DL, dome lip; PM, paroral membrane; PS, perizonal stripe; SC, somatic cilia; SS, side stripe. Scale bars: 5 μm (C, D), 10 μm (E), and 20 μm (A, B).

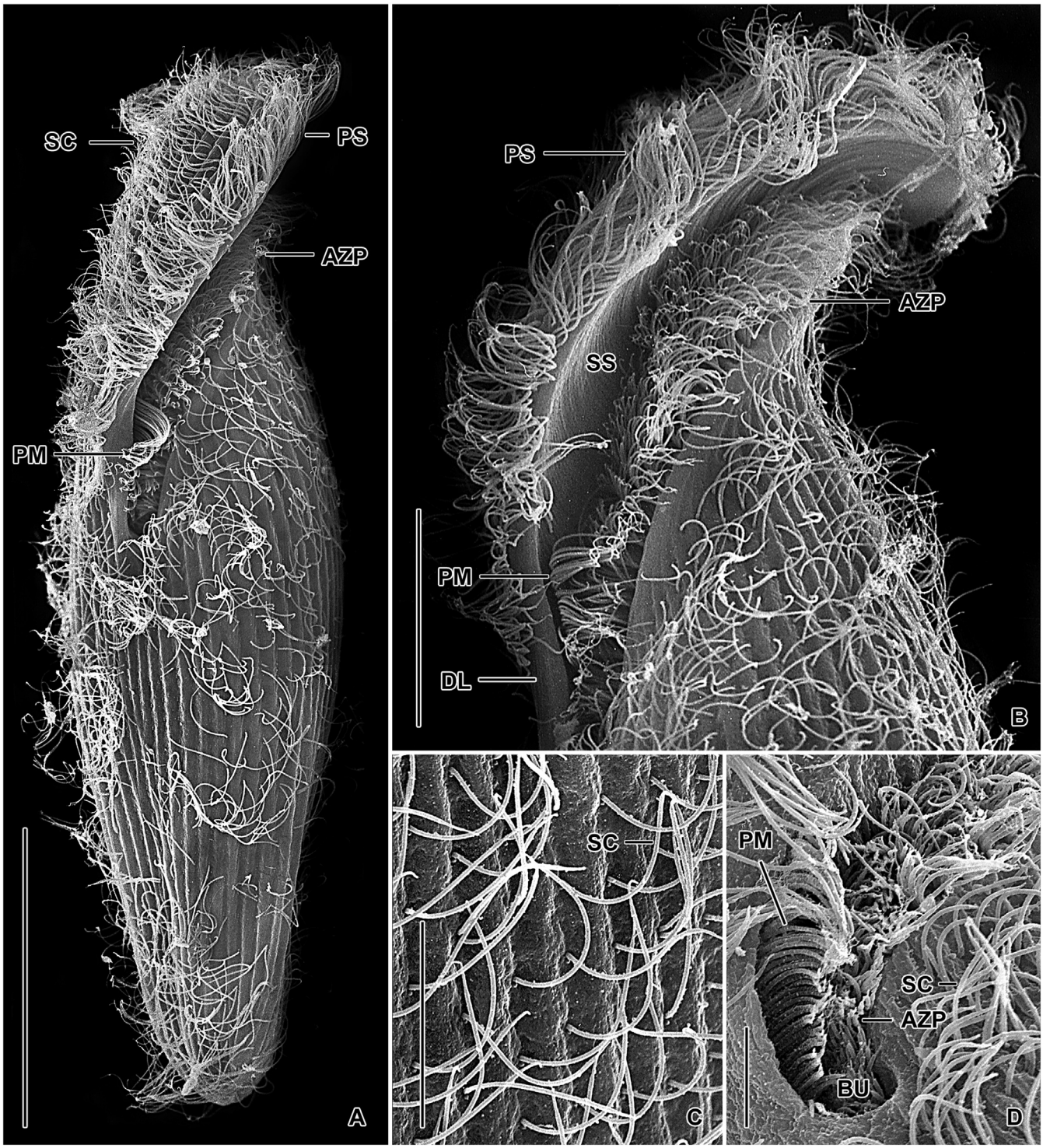

Fig. 17. A–D. *Metopus magnus* nov. spec. in the scanning electron microscope. **A**: Ventrolateral overview, showing the comparatively broad preoral dome. **B**: Ventral view of anterior body portion, showing the densely ciliated perizonal stripe and the smooth or finely ribbed side stripe overhanging the obliquely extending adoral zone of polykinetids. The paroral membrane extends along the posterior margin of the preoral dome and sinks into the buccal cavity. **C**: Surface view in postoral body region, showing that only one basal body of the somatic dikinetids is ciliated. This is a usual feature in metopids. The cortex is furrowed by the ciliary rows. **D**: Detail of proximal portion of oral apparatus, showing that the paroral membrane and the adoral zone of polykinetids sink into the buccal cavity. AZP, Adoral zone of polykinetids; BU, buccal cavity; DL, dome lip; PM, paroral membrane; PS, perizonal stripe; SC, somatic cilia; SS, side stripe. Scale bars: 5 μm (D), 10 μm (C), 20 μm (B), and 50 μm (A).

Table 4. Morphometric data on *Metopus magnus* nov. spec.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	175.4	180.0	15.9	3.5	9.1	140.0	197.0	21
Body, maximum width of preoral dome	53.2	53.0	7.6	1.7	14.3	35.0	68.0	21
Body, width at cytostome	51.2	52.0	6.2	1.4	12.1	36.0	62.0	21
Body, maximum postoral width	55.1	57.0	7.8	1.7	14.1	39.0	66.0	21
Body, length:width ratio	3.2	3.1	0.4	0.1	12.3	2.7	4.0	21
Anterior body end to distal end of PS, distance	8.4	8.5	3.4	0.8	40.9	2.0	15.0	20
Anterior body end to proximal end of PS, distance	72.3	72.0	5.1	1.1	7.1	58.0	80.0	21
Perizonal stripe, percentage of body length	41.5	41.1	4.8	1.0	11.6	35.0	55.0	21
Anterior body end to distal end of AZP, distance	10.8	11.0	2.3	0.5	21.6	7.0	15.0	21
Anterior body end to proximal end of AZP, distance	85.0	86.0	6.3	1.4	7.4	71.0	95.0	21
Adoral zone of polykinetids, percentage of body length	48.6	48.9	3.3	0.7	6.8	43.0	56.9	21
Anterior body end to distal end of PM, distance	50.9	51.0	5.9	1.3	11.7	40.0	61.0	21
Anterior body end to macronucleus, distance	46.6	48.0	6.6	1.4	14.1	33.0	61.0	21
Macronucleus, length	47.2	48.0	3.9	0.9	8.3	38.0	53.0	21
Macronucleus, width	11.2	11.0	1.1	0.2	9.6	10.0	13.0	21
Macronucleus, length:width ratio	4.3	4.2	0.5	0.1	12.8	3.2	5.3	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length	3.6	4.0	–	–	–	3.0	4.0	19
Micronucleus, width	3.4	3.0	–	–	–	3.0	4.0	19
Micronucleus, length:width ratio	1.1	1.0	–	–	–	1.0	1.3	19
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	19
Somatic ciliary rows, total number	44.1	45.0	3.0	0.7	6.8	38.0	48.0	21
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	21
False kineties in perizonal stripe, number	91.9	93.0	6.5	1.4	7.0	75.0	106.0	21
Adoral polykinetids, number	46.3	46.0	2.4	0.5	5.2	42.0	51.0	20
Paroral membrane, length	35.7	36.0	3.3	0.7	9.3	29.0	41.0	21

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. CV – Coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

and curved leftwards following body curvature (Figs 12F, G, 13A–D, 15A, B, 16B, 17A, B; Table 4). Perizonal stripe begins about 8 μm posterior to anterior body end at left margin of dorsal side, with similar course as in congeners; extends 42% of body length on average; invariably composed of five rows: rows 1–3 narrowly spaced and separated from slightly more widely arranged rows 4 and 5 by a cortical ridge recognizable in SEM (Fig. 16E, arrowhead); a conspicuous gap between row 5 and first dome kinety; stripe rows divided into 92 false kineties on average; dikinetids of rows 1–4 more or less parallel to kinety axis while dikinetids of row 5 slightly inclined to kinety axis (Figs 12F, G, 13A–D, 14C, F; Table 4).

Type 1 oral area. Adoral zone distinctly spiralled, occupies about 50% of body length, commences slightly posterior to distal end of perizonal stripe at left margin of dorsal side; extends obliquely over ventral side, twisted in mid-portion where it sinks into a deep buccal cavity; composed of an average of 46 polykinetids: proximal- and distalmost polykinetids rectangular each consisting of two rows of basal bodies, other polykinetids made of two long rows of basal bodies and a third short row with cilia 5 μm long in vivo; bases of polykinetids about 12 μm wide in vivo, separated by distinct ridges in SEM (Figs 12F, 13A–D, 14C, D, E, G, 16C–E; Table 4). Paroral membrane dikinetal and usually 36 μm long in protargol preparations; begins on average 51 μm posterior to anterior body end, i.e., near entrance to buccal cavity and then continues to proximal end of zone; cilia 7–10 μm long in SEM; rarely fragmented anteriorly (Figs 12F, 13A, C, 14G, 15A, B, 16E, 17A, B, D; Table 4). Cytopharyngeal fibres originate from proximal end of adoral zone and paroral membrane, extend backwards forming a slender funnel in protargol preparations (Figs 12F, 13C, E–L). Dome lip smooth and 1.3–2.9 μm wide (mean = 1.9 μm, $n = 15$) in SEM. Side stripe a deep, smooth or finely ribbed channel, 5.0–9.1 μm wide (mean = 6.2 μm, $n = 16$) in SEM (Figs 15A, B, 16A, C–E, 17B).

Comparison with similar species: *Metopus magnus* has a combination of features rare or as yet not described in large metopids with caudal cilia: (i) the macronucleus is lenticular and the micronucleus is in a small macronuclear concavity, (ii) the caudal cilia are only slightly elongated to 20–25 μm, and (iii) cortical granules are absent or not recognisable. On the other hand, most large metopids display an ellipsoidal or oblong macronucleus without concavity, their caudal cilia are about 40 μm long, and the cortical granules are distinct and rod-shaped. With respect to the lack of cortical granules, *M. magnus* needs to be compared with two species, *M. ovalis* Kahl, 1927 and *M. ventrosus* Vuxanovici, 1962; having a

similar size and shape. The former differs from *M. magnus* in having an ellipsoidal macronucleus and lacks elongated caudal cilia (Kahl 1927, 1932; Foissner 1998). The insufficiently known *M. ventrosus* is only 100 μm long and was synonymised with *M. ovalis* by Esteban et al. (1995) and Foissner (1998).

Metopus murrayensis nov. spec. Foissner and Vďačný (Figs 18A–N, 19A–H, 20A–F, 21A–C, 22A, B; Table 5)

Diagnosis: Size about 90 × 40 μm in vivo. Body obovate to obconical and twisted anteriorly. Macronucleus in or slightly anterior to mid-body, globular and surrounded by numerous highly refractive granules; one globular to broadly ellipsoidal micronucleus. Contractile vacuole terminal. Cortical granules about 2.0 × 0.7 μm in size, colourless, narrowly spaced forming about five rows between adjacent kineties. On average 35 ciliary rows; ventral kineties shortened posteriorly leaving a glabrous excretion area; elongated caudal cilia about 40 μm long. Perizonal stripe composed of five kineties extending approximately 53% of body length and forming about 100 false kineties. Type 3 oral area. Adoral zone distinctly spiralled, composed of an average of 40 polykinetids extending about 60% of body length.

Type locality: Loamy soil and leaf litter from the floodplain of the Murray River near to the town of Albury, Australia (S36°06′ E146°54′).

Type material: The holotype slide (reg. no. 2016/19) and six paratype slides (reg. nos 2016/20–25) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. The holotype (Fig. 18M, N) and relevant paratype specimens have been marked by black ink circles on the coverslip.

Etymology: The Latin adjective *murrayens·is*, *-is*, *-e* [m, f, n] refers to the Murray River floodplain where this species was discovered.

Description: Size in vivo 70–120 × 35–50 μm, usually about 90 × 40 μm, as calculated from some in vivo measurements and the morphometric data adding 15% preparation shrinkage. Body massive due to the thick preoral dome; obovate to obconical and twisted anteriorly, length:width ratio fairly stable, i.e., ranging from 2.0:1 to 2.7:1 after protargol impregnation and in SEM preparations (Table 5), dorsoventrally flattened about 0.5:1; preoral dome conspicuous because extending almost half of body length in ventral view, only slightly broader than mid-body and thus indistinctly overhanging left body margin; postoral body portion obconical, with rear body end narrowly to broadly rounded (Figs 18A, E–N, 19A, F–H, 20A–F, 21A, C, 22A, B). Nuclear apparatus in or slightly above mid-body, remarkable because surrounded by innumerable highly refractive granules of globular and irregular shape; individual granules 1–3 μm, usually 2 μm long in vivo, deeply impregnate with the protargol method used and thus appearing as a dense cloud around nuclear apparatus (Figs 18A, B, G–L, N, 19A–C, E, H, 20C, F). Macronucleus globular, becomes ellipsoidal under coverslip pressure; about 35 μm across in vivo, shrinks strongly in protargol preparations to about 10 μm; impregnates deeply with protargol. Micronucleus attached to macronucleus at various positions but usually at or near anterior pole; globular to broadly ellipsoidal with a size of 3–4 μm in protargol preparations (Figs 18A, G–L, N, 19A, B, E, H; Table 5). A single, large contractile vacuole in posterior body end (Figs 18A, G–L, 19A). Cytopyge well recognizable in SEM micrographs, slit-like and subterminal on ventral side, likely serves also as discharge device for the contractile vacuole because an excretory pore was not found (Fig. 21B, C). Cortex flexible and slightly furrowed by ciliary rows in SEM (Figs 21A, C, 22A, B). Cortical granules about 2.0 × 0.7 μm in size; colourless; oriented perpendicularly to cell surface; narrowly spaced forming about five rows between adjacent kineties and about two rows between adoral polykinetids; impregnate deeply with silver carbonate leaving kineties as bright lines; absent in excretion area (Figs 18C, D, 19D, F, G, 20A, B). Cytoplasm colourless, contains some lipid droplets and food vacuoles with residues of bacteria and their spores; symbiotic bacteria not recognizable in vivo and in protargol preparations. Swims rather rapidly, rotating about main body axis.

Somatic ciliature composed of dikinetids, both basal bodies ciliated in oral portion of cell while anterior basal body barren in postoral region as usual for metopids; somatic cilia about 15 μm long in vivo; elongated caudal cilia about 40 μm long in vivo (Figs 18, 19A), fragile and thus often missing in prepared specimens. On average 35 ciliary rows; ventral kineties begin slightly posterior to adoral polykinetids, most gradually shortened posteriorly leaving a glabrous area around the cytopyge; dorsolateral kineties commence also slightly posterior to adoral polykinetids while ventrolateral kineties commence approximately at same level as ventral kineties; both dorso- and ventrolateral kineties extend towards rear end; dorsal kineties gradually shortened and curved leftwards on preoral dome (Figs 18E, F, M, N, 19F, G, 20A, B, D, 21A–C, 22A, B; Table 5). Perizonal stripe begins about 8 μm away from anterior body end at left margin of dorsal side, follows curvature of ventral dome margin and terminates on right margin of dorsal side slightly above level of proximal end of adoral zone, i.e., extends about 53% of body length on average; invariably composed of five rows: rows 1–3 usually more narrowly spaced than rows 4 and 5, rarely only first two rows being closer together; segmented into an average of 100 narrowly spaced false kineties; perizonal dikinetids with two about 10 μm long cilia in vivo; rarely some irregularities at beginning of perizonal stripe (Figs 18E, F, M, N, 20A, B, E; Table 5).

Type 3 oral area. Adoral zone distinctly spiralled; occupies about 60% of body length; commences slightly posterior to perizonal stripe at left margin of dorsal side; extends obliquely over ventral side to right body margin where it curves leftwards to sink into a deep buccal cavity; composed

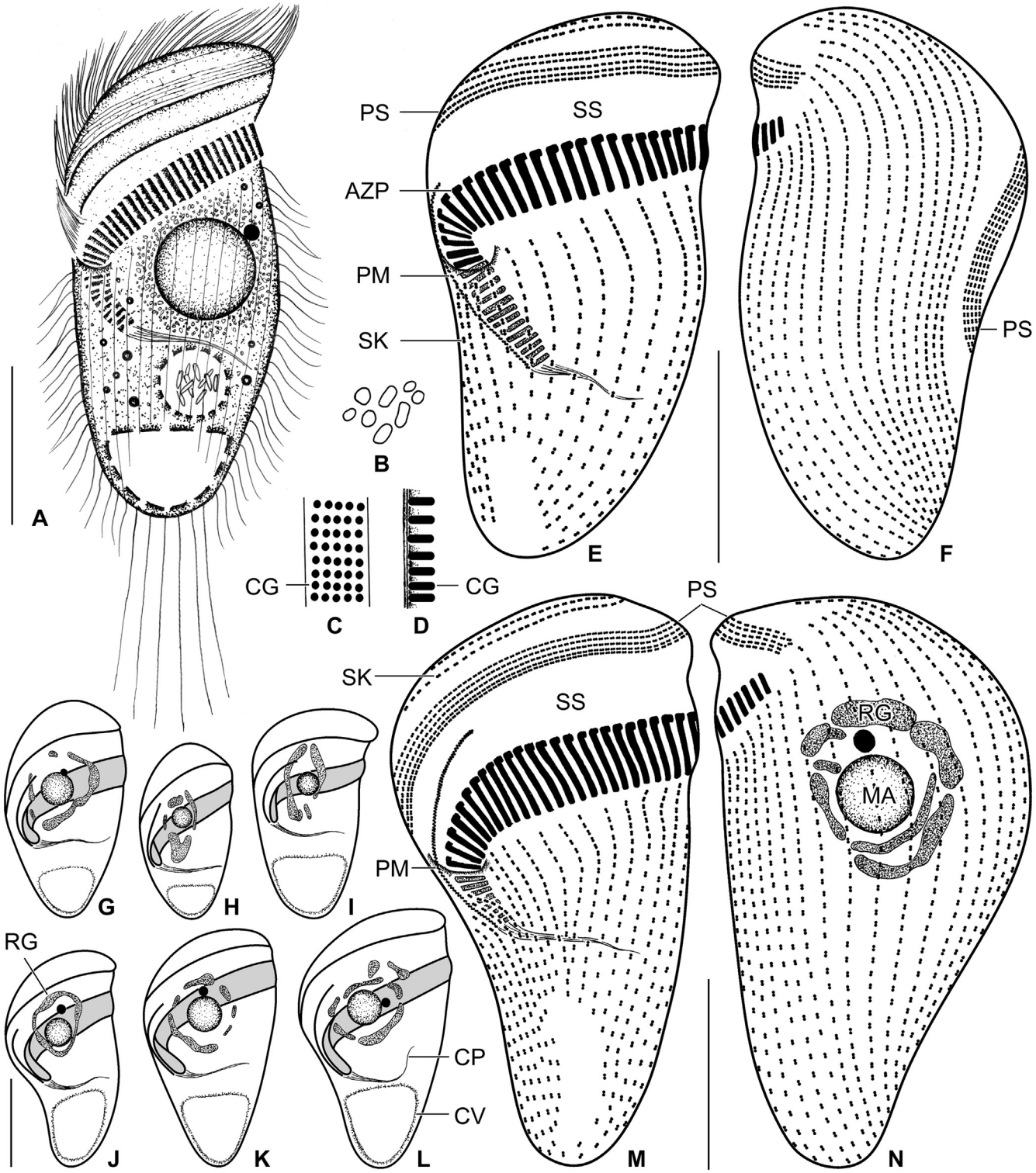

Fig. 18. A–N. *Metopus murrayensis* nov. spec. from life (A–D) and after protargol impregnation (E–N). **A**: Ventral view of a representative specimen, length 90 μm. **B**: Refractive granules 1–3 μm long surround the globular macronucleus. **C, D**: Surface view and optical section showing cortical granulation. **E, F, M, N**: Ventral and dorsal views of ciliary pattern and nuclear apparatus of the holotype (M, N) and of a paratype (E, F) specimen. **G–L**: Variability of body shape and size as well as of nuclear apparatus. Drawn to scale. AZP, Adoral zone of polykinetids; CG, cortical granules; CP, cytopharynx; CV, contractile vacuole; MA, macronucleus; PM, paroral membrane; PS, perizonal stripe; RG, refractive granules; SK, somatic kineties; SS, side stripe. Scale bars: 30 μm.

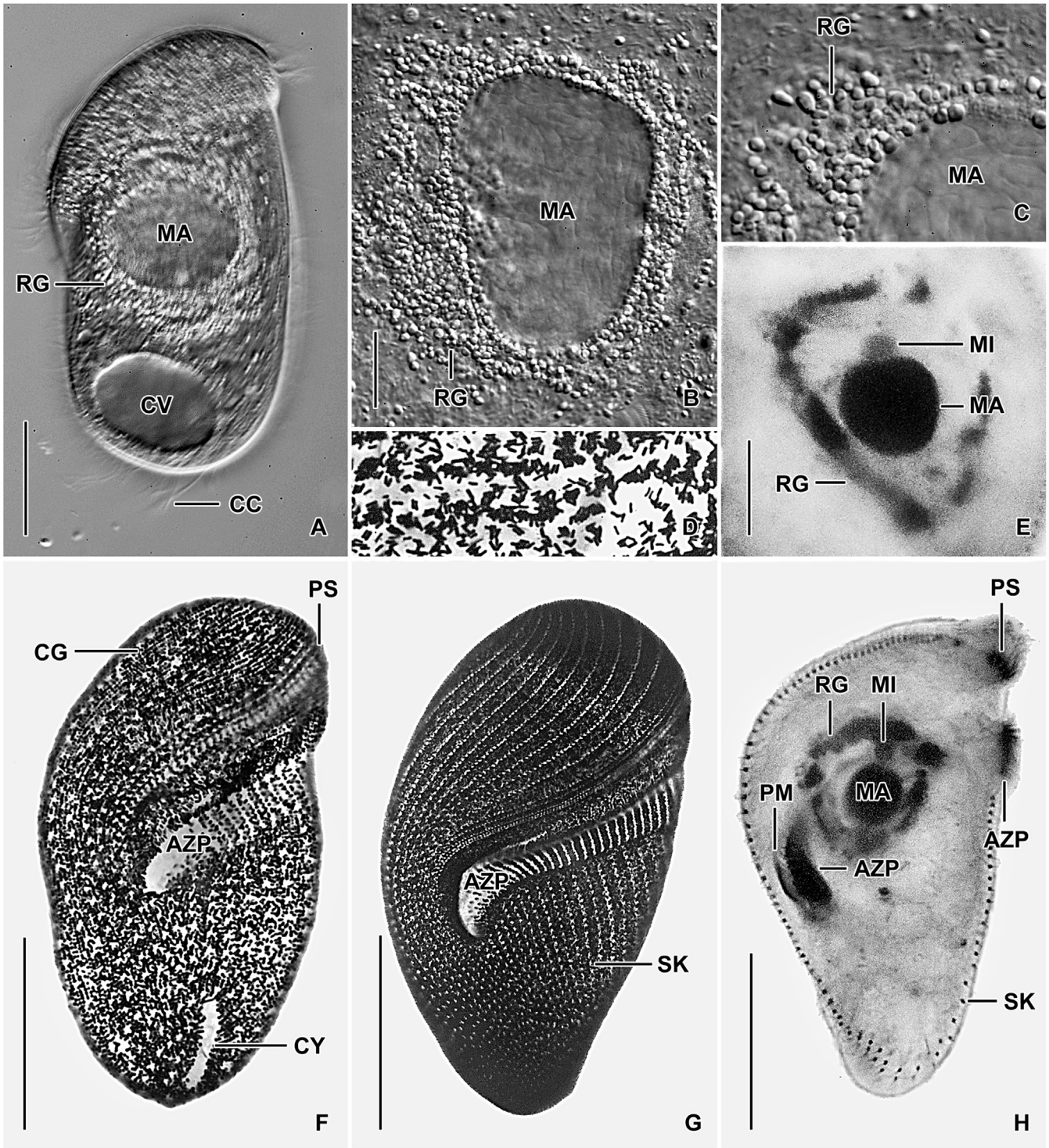

Fig. 19. A–H. *Metopus murrayensis* nov. spec. from life (A–C) and after silver carbonate (D, F, G) and protargol (E, H) impregnation. **A**: Overview showing the globular macronucleus surrounded by many refractive granules, the large contractile vacuole, and the elongated caudal cilia. **B, C, E**: The macronucleus is surrounded by innumerable, highly refractive granules. The individual granules are 1–3 μm long in vivo and are heavily impregnated with the protargol method used, appearing as a dense cloud around the macronucleus. **D**: The cortical granules are about 2.0 × 0.7 μm in size. **F, G**: Overviews showing cortical granulation of ventral side. The cortical granules impregnate deeply with silver carbonate leaving kineties as bright lines. **H**: Overview showing general body organization. AZP, Adoral zone of polykinetids; CC, caudal cilia; CV, contractile vacuole; CY, cytopyge; MA, macronucleus; MI, micronucleus; PM, paroral membrane; PS, perizonal stripe; RG, refractive granules; SK, somatic kineties. Scale bars: 10 μm (B, E) and 30 μm (A, F–H).

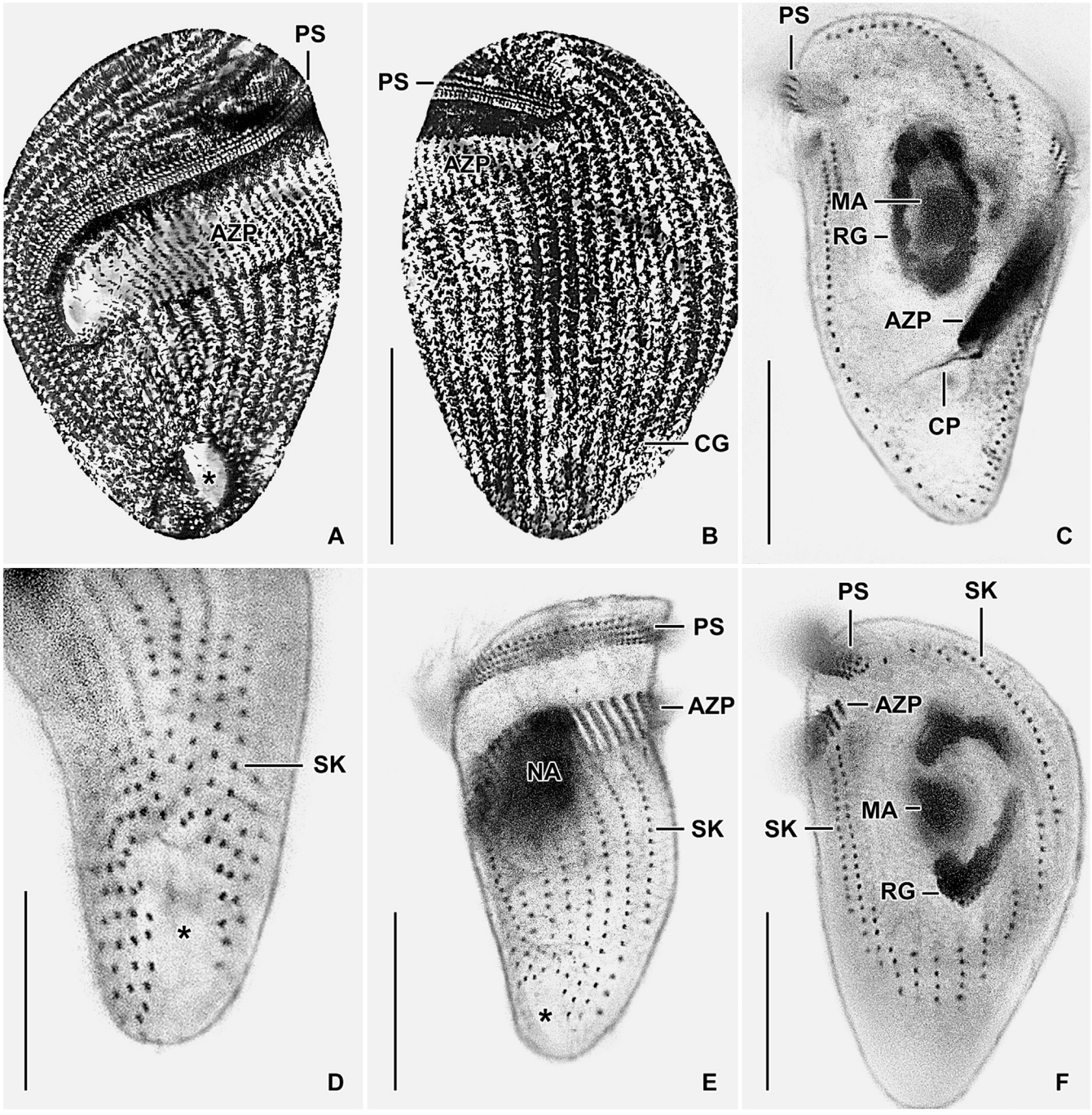

Fig. 20. A–F. *Metopus murrayensis* nov. spec. after silver carbonate (A, B) and protargol (C–F) impregnation. **A, B**: Overviews showing cortical granulation of ventral and dorsal side. The cortical granules are narrowly spaced and form four to six rows between adjacent kineties to and about two rows between the adoral polykinetids. They are lacking in the excretion area marked by an asterisk. **C, E, F**: Overviews showing oral and somatic ciliary pattern as well as the nuclear apparatus. The adoral zone of polykinetids and the perizonal stripe commence subapically on the left margin of dorsal side, extend obliquely over the ventral side to end at right body margin. The refractive granules around the macronucleus impregnate deeply with the protargol method used. The cytopharyngeal fibres originate from the proximal end of the adoral polykinetids and the paroral membrane; they curve transversely towards the left body margin, forming a slender funnel. The asterisk marks the unciliated excretion area. **D**: Ventral view of ciliary pattern in posterior body region. The middle somatic kineties are gradually shortened posteriorly, leaving a glabrous area around the cytopyge (asterisk). AZP, Adoral zone of polykinetids; CG, cortical granules; CP, cytopharynx; MA, macronucleus; NA, nuclear apparatus; PS, perizonal stripe; RG, refractive granules; SK, somatic kineties. Scale bars: 20 μm (D) and 30 μm (B, C, E, F).

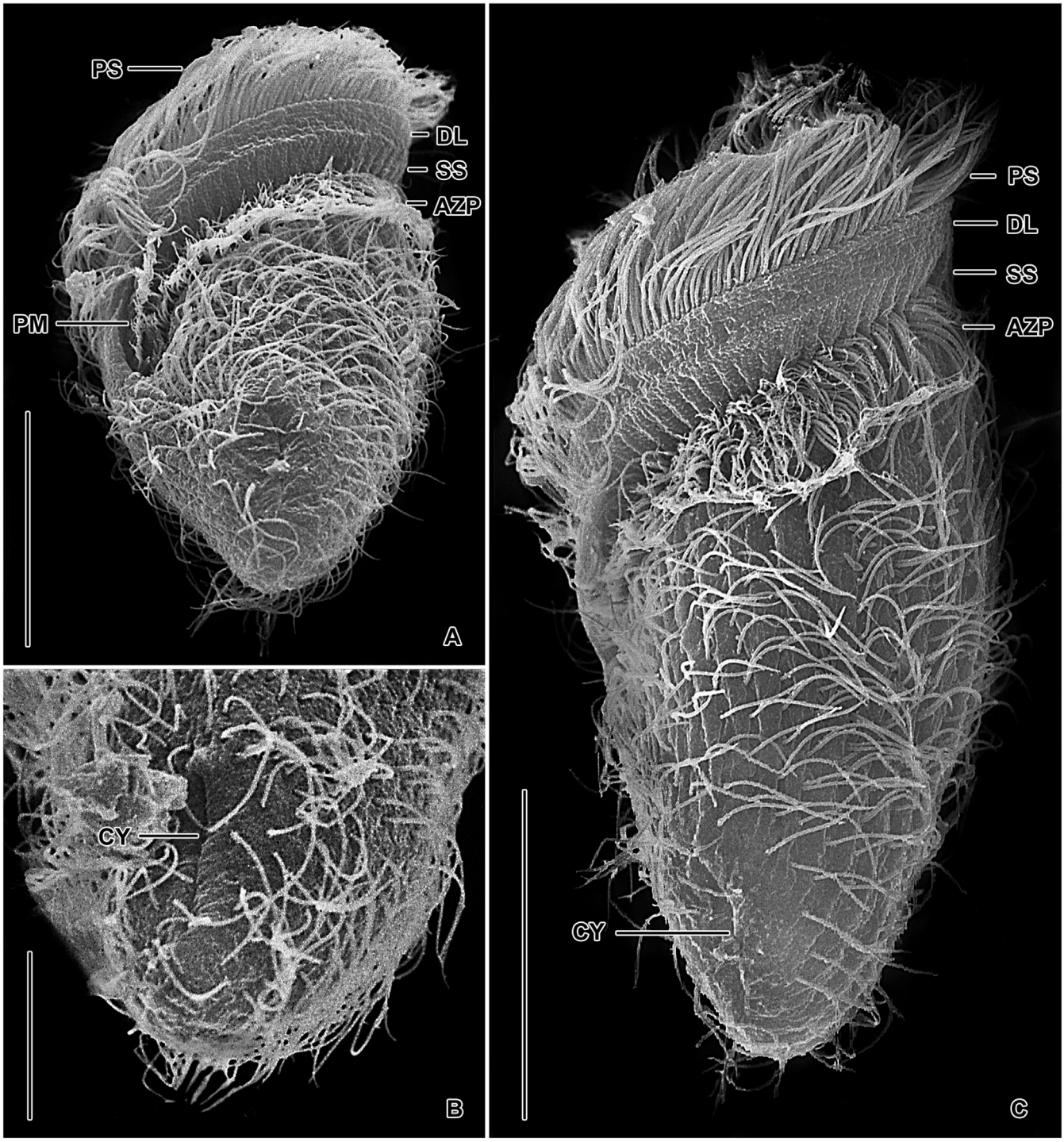

Fig. 21. A–C. *Metopus murrayensis* nov. spec. in the scanning electron microscope. **A, C**: Overviews showing the massive, anteriorly distinctly twisted obovate body. The densely ciliated perizonal stripe extends anteriorly to the the broad dome lip and the ribbed side stripe. The adoral zone of polykinetids begins subapically at the left margin of the dorsal side, runs obliquely over the ventral side where it curves leftwards to sink into the buccal cavity. The paroral membrane extends along the posterior margin of the preoral dome and, together with the adoral zone, sinks into the buccal cavity. The ventral somatic kineties commence slightly posterior to the adoral polykinetids, extend slightly helically and are gradually shortened posteriorly to leave a glabrous area around the slit-like cytopyge. The elongated caudal cilia are fragile and thus often missing in prepared specimens. **B**: Detail of posterior body portion, showing the slit-like cytopyge. The excretion area is unciliated. AZP, Adoral zone of polykinetids; CY, cytopyge; DL, dome lip; PM, paroral membrane; PS, perizonal stripe; SS, side stripe. Scale bars: 10 μm (B) and 20 μm (A, C).

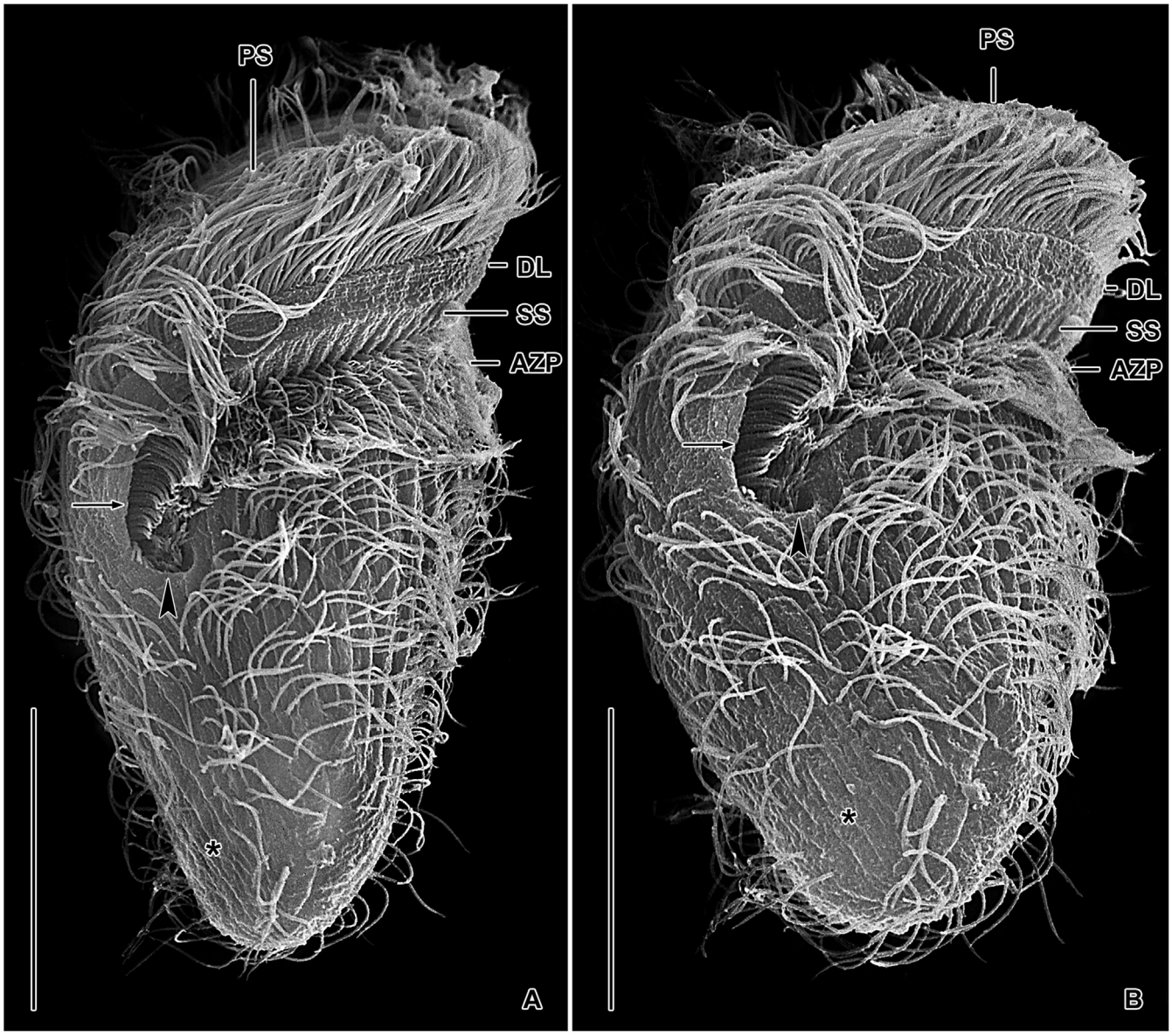

Fig. 22. A, B. *Metopus murrayensis* nov. spec. in the scanning electron microscope. Overviews showing the massive, obovate body carrying a densely ciliated perizonal stripe and an obliquely extending adoral zone of polykinetids. Arrows mark the paroral membrane, arrowheads point to the entrance to buccal cavity, and asterisks denote the unciliated excretion area. AZP, Adoral zone of polykinetids; DL, dome lip; PS, perizonal stripe; SS, side stripe. Scale bars: 20 μm.

of 40 polykinetids: proximal- and distalmost polykinetids rectangular each consisting of two rows of basal bodies, other polykinetids made of two long rows of basal bodies and a third short row (Figs 18E, F, M, N, 19F, G, 20A–C, E, F, 21A, C, 22A, B; Table 5). Paroral membrane dikinetal and usually 25 μm long after protargol impregnation; begins on average 22 μm posterior to anterior body end and extends along adoral zone to sink into buccal cavity; cilia about 10 μm long in vivo and forming beautiful, tongue-like structures in SEM (Figs 18E, M, 19H, 21A, 22A, B; Table 5). Cytopharyngeal fibres originate from proximal end of adoral zone and paroral membrane, curve transversely towards left body margin, forming a slender funnel 15–20 μm long in protargol preparations (Figs 18E, G–M, 20C). Dome lip conspicuously broad and striated by rows of cortical granules, 2.9–3.7 μm wide (mean = 3.2 μm, $n = 5$) in SEM. Side stripe a flat channel with prominent ribs, 2.9–4.2 μm wide (mean = 3.6 μm, $n = 5$) in SEM (Figs 21A, C, 22A, B).

Comparison with similar species: *Metopus murrayensis* is almost unique in having a globular macronucleus surrounded by highly refractive granules. Only *M. fuscus* displays a similar pattern (Kahl 1927). Bourland et al. (2014) assume that these are perinuclear endosymbionts. *Metopus murrayensis* differs from *M. fuscus* in body length

Table 5. Morphometric data on *Metopus murrayensis* nov. spec.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	77.3	78.0	8.1	2.5	10.5	60.0	90.0	11
Body, maximum width of preoral dome	41.5	40.0	5.4	1.6	13.0	34.0	53.0	11
Body, width at cytostome	35.2	36.0	3.9	1.2	11.1	30.0	40.0	11
Body, maximum postoral width	33.8	35.0	4.0	1.2	11.7	28.0	39.0	11
Body, length:width ratio	2.3	2.3	–	–	–	2.0	2.7	11
Anterior body end to distal end of PS, distance	7.2	8.0	2.2	0.7	30.4	3.0	10.0	11
Anterior body end to proximal end of PS, distance	40.6	42.0	6.0	1.8	14.9	30.0	49.0	11
Perizonal stripe, percentage of body length	52.7	53.8	6.9	2.1	13.1	41.7	62.8	11
Anterior body end to distal end of AZP, distance	15.5	16.0	3.1	0.9	19.9	10.0	20.0	11
Anterior body end to proximal end of AZP, distance	46.5	46.0	5.3	1.6	11.3	40.0	56.0	11
Adoral zone of polykinetids, percentage of body length	60.2	61.3	3.9	1.2	6.6	53.3	66.7	11
Anterior body end to distal end of PM, distance	22.2	22.0	3.0	0.9	13.6	18.0	27.0	11
Anterior body end to macronucleus, distance	23.4	23.0	2.8	0.8	12.0	18.0	27.0	11
Macronucleus, length	10.7	10.0	2.4	0.7	22.5	7.0	14.0	11
Macronucleus, width	9.8	10.0	2.2	0.7	22.2	6.0	13.0	11
Macronucleus, length:width ratio	1.1	1.1	–	–	–	1.0	1.2	11
Macronucleus, number	1.0	1.0	–	–	–	1.0	1.0	11
Micronucleus, length	4.1	4.0	–	–	–	4.0	5.0	9
Micronucleus, width	3.3	3.0	–	–	–	3.0	4.0	9
Micronucleus, length:width ratio	1.3	1.3	–	–	–	1.0	1.7	9
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	9
Somatic ciliary rows, total number	35.9	35.0	3.4	1.0	9.5	30.0	42.0	11
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	11
False kineties in perizonal stripe, number	99.5	100.0	16.4	5.0	16.5	78.0	122.0	11
Adoral polykinetids, number	39.6	41.0	7.0	2.1	17.6	28.0	52.0	11
Paroral membrane, length	25.0	25.0	3.8	1.2	15.4	18.0	32.0	11

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZP – Adoral zone of polykinetids; CV – coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; PM – paroral membrane; PS – perizonal stripe; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

(70–120 μm vs. 180–300 μm), shape of the macronucleus (globular vs. dumbbell-shaped to elongate ellipsoidal), and the type of oral area. *Metopus pulcher* Kahl, 1927 also has a globular macronucleus but lacks the surrounding refractive granules typical for *M. murrayensis*. Further, both species are clearly distinguished by the adoral zone which extends about 60% of body length in *M. murrayensis* while about 90% in *M. pulcher*. *Metopus murrayensis* resembles *M. setosus* in body shape, the conspicuous cortical granulation and the long caudal cilia (see description below). However, *M. setosus* has an ellipsoidal macronucleus not surrounded by refractive granules (vs. globular and surrounded by refractive granules) and a lower number of ciliary rows (17–28 vs. 30–42) and adoral polykinetids (18–27 vs. 28–52).

Remarks: The affiliation of *M. murrayensis* is questionable because it has an outstanding oral area, i.e, a thick preoral dome hardly projecting from body proper, a very broad dome lip, and a very flat side stripe. Possibly, it represents a distinct genus.

Metopus setosus Kahl, 1927 (Figs 23A–S, 24A–G, 25A–D, 26A–F; Table 6)

Voucher material: Five voucher slides (reg. nos 2016/26–30) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI), Austria. Relevant specimens have been marked by black ink circles on the coverslip.

Description: Size in vivo 45–85 × 20–40 μm, usually about 65 × 30 μm, as calculated from some in vivo measurements and the morphometric data adding 15% preparation shrinkage. Body obovate to slightly obconical, length:width ratio fairly stable, i.e., 2.0:1 to 3.0:1 after protargol impregnation and in SEM preparations (Table 6), twisted anteriorly; preoral dome extends almost half of body length in ventral view, only slightly overhangs left body margin; rear body end narrowly to broadly rounded (Figs 23A–C, F, G, I–S, 24A–E, 25A, B, 26A, B). Nuclear apparatus in anterior body half but usually does not extend into preoral dome. Macronucleus broadly to narrowly ellipsoidal, usually slightly curved, with a length:width ratio of 1.4–3.7:1 after protargol impregnation; nucleoli small and globular, evenly distributed. Micronucleus attached to mid-portion of macronucleus; globular to ellipsoidal, 2–5 μm long after protargol impregnation (Figs 23A, G, I–Q, S, 24C, D; Table 6). A single, large contractile vacuole in posterior body end; faecal mass moves through contractile vacuole when expelled via cytopyge (Figs 23A, F, I–Q, 24A). Cytopyge well recognizable in some SEM micrographs, subterminal on ventral side, slit-like, likely serves also as discharge device for

Table 6. Morphometric data on Australian population of *Metopus setosus* Kahl, 1927.

Characteristic[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	55.2	55.0	8.2	1.8	14.9	41.0	72.0	21
Body, maximum width of preoral dome	27.6	28.0	4.1	0.9	15.0	21.0	35.0	21
Body, width at cytostome	25.2	25.0	3.6	0.8	14.3	19.0	31.0	21
Body, maximum postoral width	24.3	24.0	3.7	0.8	15.4	18.0	32.0	21
Body, length:width ratio	2.3	2.3	–	–	–	2.0	3.0	21
Anterior body end to distal end of PS, distance	3.5	3.0	2.4	0.5	68.1	1.0	8.0	21
Anterior body end to proximal end of PS, distance	30.8	30.0	3.5	0.8	11.4	25.0	42.0	21
Perizonal stripe, percentage of body length	56.4	55.6	7.3	1.6	13.0	45.3	72.9	21
Anterior body end to distal end of AZP, distance	10.0	10.0	2.3	0.5	23.0	4.0	16.0	21
Anterior body end to proximal end of AZP, distance	33.3	33.0	3.3	0.7	9.9	27.0	40.0	21
Adoral zone of polykinetids, percentage of body length	61.0	60.0	5.6	1.2	9.3	50.0	72.9	21
Anterior body end to distal end of PM, distance	16.9	18.0	3.2	0.7	19.1	10.0	21.0	21
Anterior body end to macronucleus, distance	13.9	13.0	3.4	0.7	24.3	10.0	26.0	21
Macronucleus, length	16.5	17.0	2.3	0.5	14.1	13.0	22.0	21
Macronucleus, width	7.5	7.0	1.3	0.3	17.8	6.0	10.0	21
Macronucleus, length:width ratio	2.3	2.2	0.6	0.1	24.5	1.4	3.7	21
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length	3.6	3.5	0.6	0.1	17.0	3.0	5.0	20
Micronucleus, width	2.6	2.5	0.6	0.1	23.7	2.0	4.0	20
Micronucleus, length:width ratio	1.5	1.5	–	–	–	1.0	2.0	20
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	20
Somatic ciliary rows, total number	24.1	25.0	3.4	0.7	13.9	17.0	28.0	21
Perizonal ciliary rows, number	5.0	5.0	0.0	0.0	0.0	5.0	5.0	21
False kineties in perizonal stripe, number	52.0	52.0	8.4	1.8	16.1	40.0	65.0	21
Adoral polykinetids, number	23.2	23.0	2.5	0.5	10.7	18.0	27.0	21
Paroral membrane, length	16.7	17.0	2.3	0.5	13.8	11.0	21.0	21

[a]Data based on mounted, protargol-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. AZP – Adoral zone of polykinetids; CV – coefficient of variation (%); M – median; Max – maximum; Mean – arithmetic mean; Min – minimum; PM – paroral membrane; PS – perizonal stripe; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean.

the contractile vacuole because an excretory pore is absent (Figs 25, 26B). Cortex flexible; not, or only slightly, furrowed by ciliary rows and densely dotted by tips of cortical granules in SEM; sometimes with scattered epibiotic bacteria 1–2 μm long in SEM (Figs 24G, 25A, B, 26A–C, E, F). Cortical granules about 1.0 × 0.5 μm in size; colourless; oriented perpendicularly to cell surface; narrowly spaced forming about five rows between adjacent kineties, one or two rows between adoral polykinetids; impregnate deeply with silver carbonate, neither stained and nor extruded with methyl green-pyronin; absent in excretion area (Figs 23D, E, 24B). Cytoplasm colourless, contains few to many lipid droplets about 3 μm across and some food vacuoles with residues of bacteria and their spores; defecation vacuole about 10 μm across and near contractile vacuole; no accumulation of refractive granules in preoral dome; no symbiotic bacteria recognizable in vivo or after protargol impregnation. Swims moderately fast, rotating about main body axis.

Somatic ciliature composed of dikinetids, both basal bodies ciliated in oral portion of cell while anterior basal body barren in postoral region; somatic cilia about 12 μm long in vivo; caudal cilia very conspicuous because 40–60 μm long in vivo, of variable length even in the same cell, form a flexible bundle curving rightwards when cell is moving leftwards, under coverslip easily lost and often absent or not impregnated in prepared specimens (Figs 23A–C, 24A, C, 25A, 26A). On average 24 ciliary rows; ventral kineties begin slightly posterior to adoral polykinetids, some gradually shortened posteriorly leaving a glabrous area around cytopyge, remaining kineties run towards rear end following body curvature; dorsal kineties extend from anterior to posterior body end in slightly sigmoid pattern (Figs 23F, G, R, S, 24B, 25A, B, 26A, B; Table 6). Perizonal stripe begins about 4 μm posterior to anterior body end at left margin of dorsal side, extends along ventral dome margin and terminates on right margin of dorsal side at or slightly anterior to level of proximal end of adoral zone, i.e., occupies about 56% of body length on average; invariably composed of five rows: rows 1–3 arranged more closely than rows 4 and 5; a conspicuous gap between stripe row 5 and first dome kinety; stripe rows segmented into an average of 52 false kineties; each perizonal dikinetid has two cilia 9–13 μm long in SEM (Figs 23F, G, R, S, 24B, E, 25A, B, D, 26A, B, E, F; Table 6).

Type 2 oral area. Adoral zone distinctly spiralled; extends about 60% of body length; commences posterior to perizonal stripe at left margin of dorsal side; runs obliquely over ventral side to right body margin where it curves leftwards to sink into buccal cavity; composed of an average of

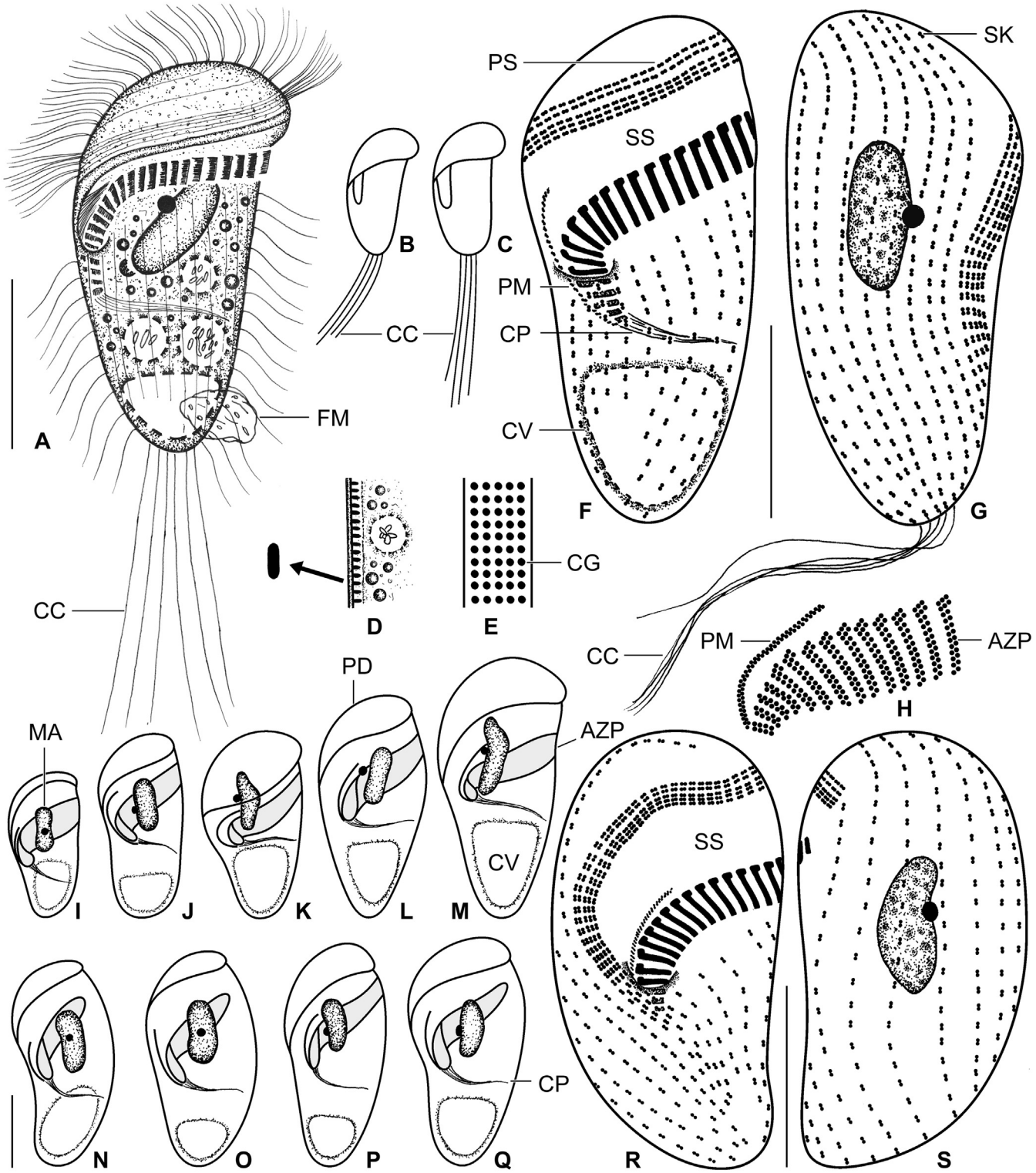

Fig. 23. A–S. *Metopus setosus*, Australian population from life (A–E) and after protargol impregnation (F–S). **A**: Ventral view of a representative specimen, length 70 μm. **B, C**: Shape variants. **D, E**: Optical section and surface view, showing cortical granulation. The cortical granules are about 1.0 × 0.5 μm in size. **F, G, R, S**: Ventral (F, R) and dorsal (G, S) views of ciliary pattern as well as nuclear apparatus and contractile vacuole. **H**: Semi-schematic diagram of proximal region of oral ciliature. **I–Q**: Variability of body shape and size as well as of the nuclear apparatus. Drawn to scale. AZP, Adoral zone of polykinetids; CC, caudal cilia; CG, cortical granules; CP, cytopharynx; CV, contractile vacuole; FM, faecal mass; MA, macronucleus; PD, preoral dome; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 20 μm (F, G, I–S) and 30 μm (A).

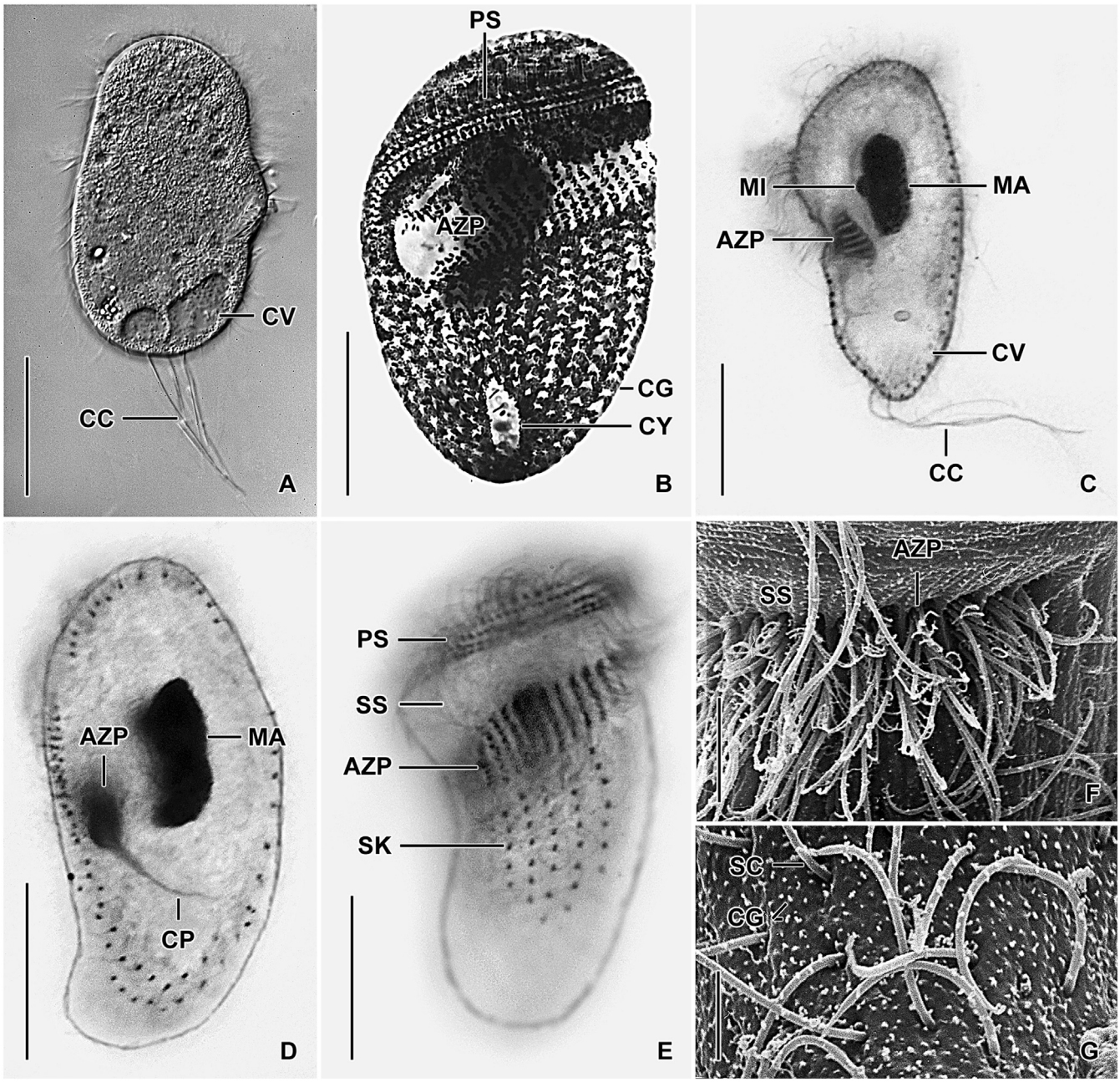

Fig. 24. A–G. *Metopus setosus*, Australian population from life (A), after silver carbonate (B) and protargol (C–E) impregnation, and in the scanning electron microscope (F, G). **A**: Overview showing the long caudal cilia. **B**: Overview showing cortical granulation of ventral side. The cortical granules are narrowly spaced, forming about five rows between adjacent kineties and one or two rows between the adoral polykinetids; they are lacking in the surroundings of the cytopyge. **C–E**: Overviews showing general body organisation. The perizonal stripe and the adoral zone of polykinetids extend obliquely over the ventral side. They are separated by the side stripe. The broadly to narrowly ellipsoidal macronucleus is in the anterior body half but does not extend into the preoral dome. The cytopharynx originates at the proximal end of the adoral zone of polykinetids and paroral membrane; then, it curves transversely towards the left body margin forming a slender funnel. **F**: Detail of the adoral zone of polykinetids. The cortex of the side stripe is finely ribbed and densely dotted by the tips of the cortical granules. **G**: Surface view in postoral body region, showing that only one basal body of the somatic dikinetids is ciliated, as usual for metopids. The cortex is densely dotted by the tips of the cortical granules. AZP, Adoral zone of polykinetids; CC, caudal cilia; CG, cortical granules; CV, contractile vacuole; CY, cytopyge; MA, macronucleus; MI, micronucleus; PS, perizonal stripe; SC, somatic cilia; SK, somatic kineties; SS, side stripe. Scale bars: 2 μm (G), 5 μm (F), 20 μm (B–E), and 30 μm (A).

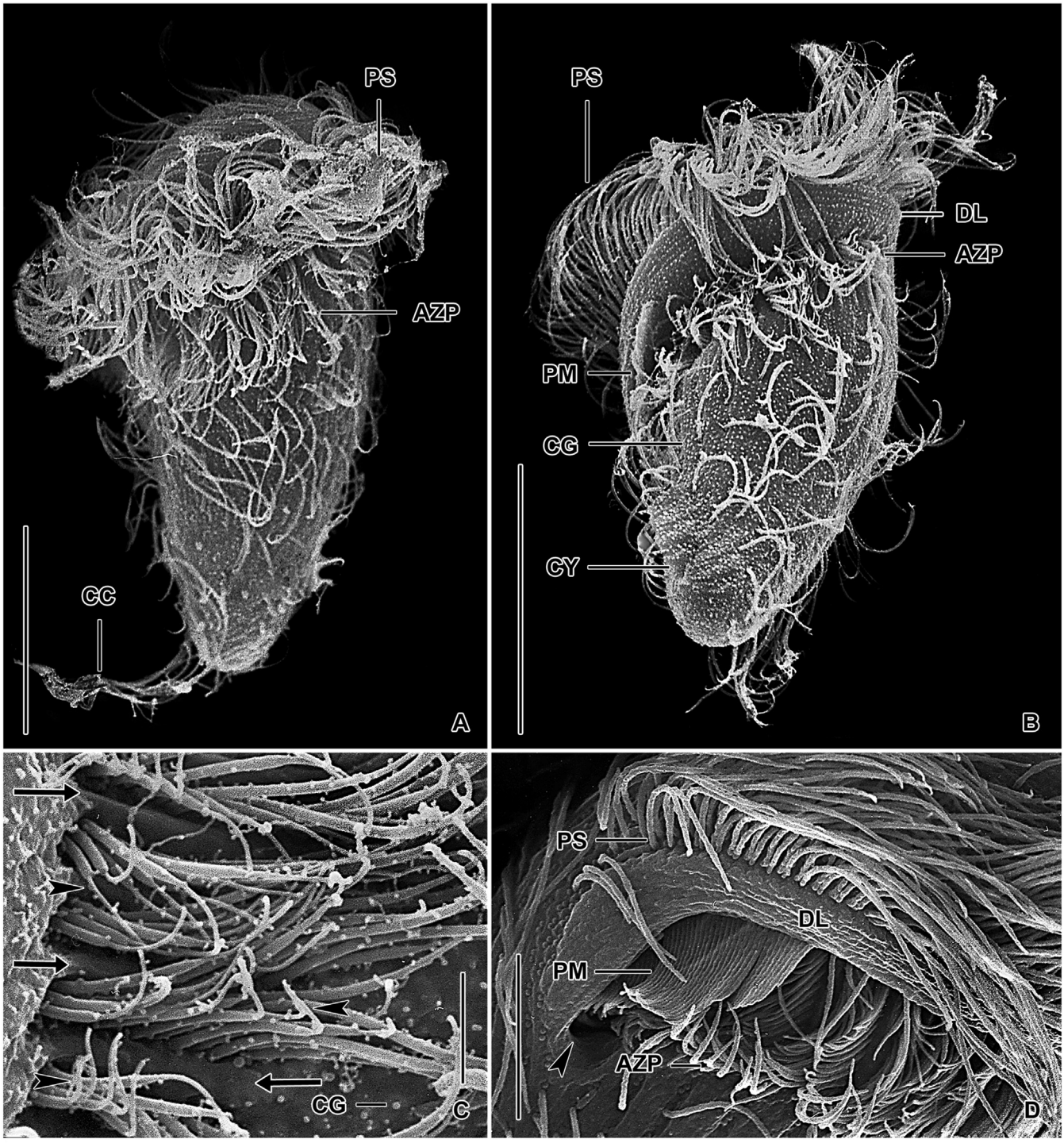

Fig. 25. A–D. *Metopus setosus* in the scanning electron microscope. **A, B**: Ventral overviews, showing the obovate to narrowly obovate body carrying a densely ciliated perizonal stripe and an obliquely extending adoral zone of polykinetids. The long caudal cilia are sometimes preserved also in prepared specimens (A). The subterminal cytopyge is slit-like. The cortex of the specimen shown in (B) is densely dotted by the tips of the cortical granules. **C**: Detail of adoral zone of polykinetids. The individual polykinetids are made of two long rows of basal bodies and a third short row. The polykinetids are separated by distinct cortical ridges marked by arrows. Some distalmost adoral cilia are anteriorly shortened and narrowed to a filiform structure marked by arrowheads. **D**: Detail showing the dense perizonal stripe, the adoral zone, and the beautiful tongue-like paroral membrane which runs along the margin of the preoral dome and, together with the adoral zone, sinks into the buccal cavity. Arrowhead points the entrance to buccal cavity. AZP, Adoral zone of polykinetids; CC, caudal cilia; CG, cortical granules; CY, cytopyge; DL, dome lip; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties. Scale bars: 2 μm (C), 5 μm (D), and 20 μm (A, B).

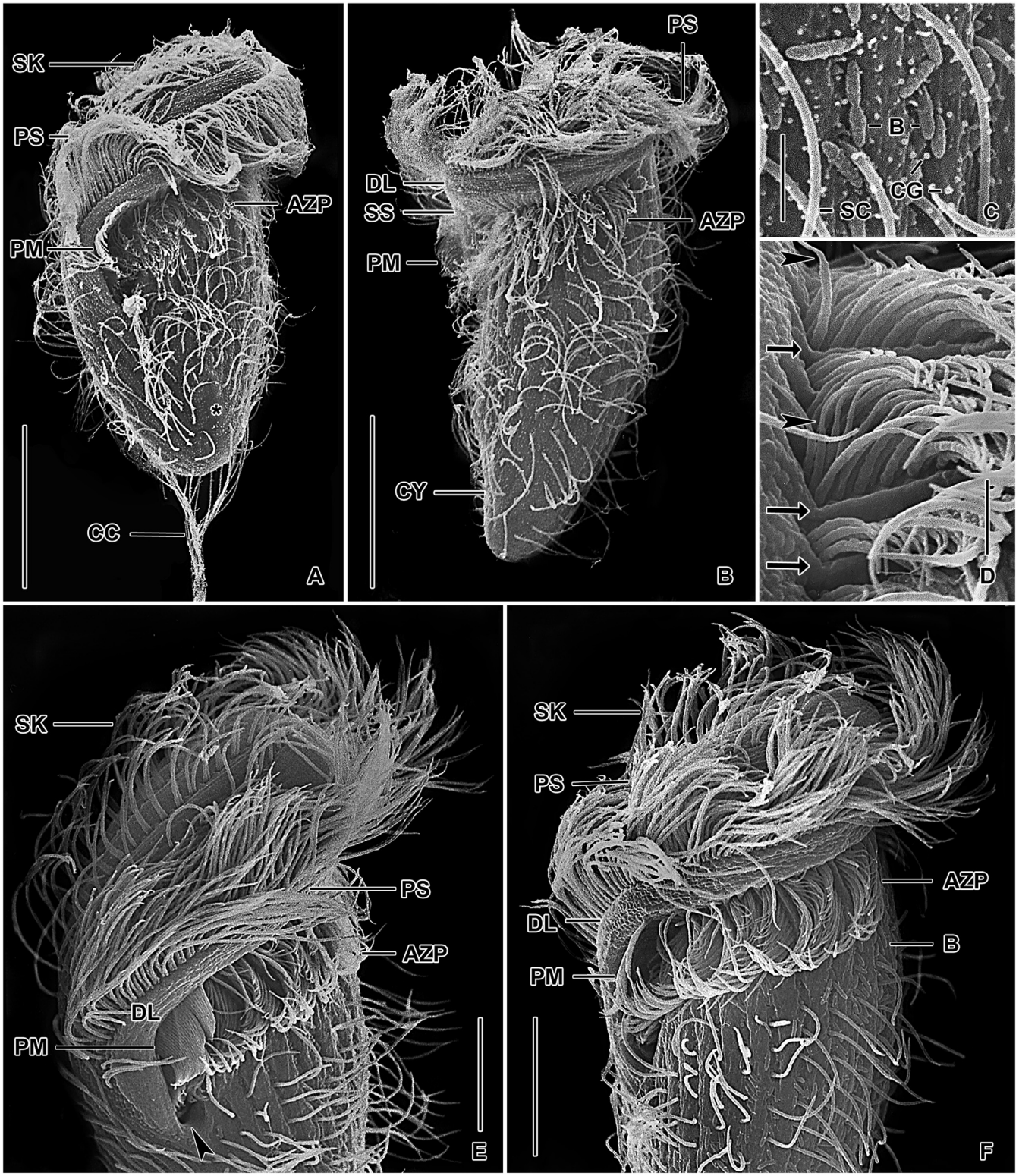

Fig. 26. A–F. *Metopus setosus* in the scanning electron microscope. **A, B**: Ventral overviews, showing the ellipsoidal to obconical body. The asterisk denotes the unciliated excretion area. **C**: The cortex is dotted by the tips of the cortical granules and sometimes covered with epibiotic bacteria. **D**: Detail of adoral zone of polykinetids which are separated by distinct ridges (arrows). The arrowheads denote the anteriorly narrowed part of the adoral cilia. **E, F**: Details in anterior body portion. Arrowhead in (E) marks the entrance to the buccal cavity. AZP, Adoral zone of polykinetids; B, bacteria; CC, caudal cilia; CG, cortical granules; CY, cytopyge; DL, dome lip; PM, paroral membrane; PS, perizonal stripe; SK, somatic kineties; SS, side stripe. Scale bars: 2 μm (C, D), 10 μm (E, F), and 20 μm (A, B).

23 polykinetids: proximal- and distalmost polykinetids rectangular and consisting of two rows of basal bodies, other polykinetids made of two long rows of basal bodies and a third short row (Fig. 23H); polykinetids separated from each other by distinct cortical ridges; adoral cilia 5 μm long in vivo and some distinctly narrowed distally in SEM (Figs 25C, 26D, arrowheads). Paroral membrane dikinetal, on average 17 μm long after protargol impregnation; begins about 17 μm away from anterior body end and extends along adoral polykinetids to sink into buccal cavity; cilia about 8 μm long in vivo and forming a tongue-like structure emerging from buccal cavity in SEM (Figs 23F, H, R, 25D, 26A, B, E, F; Table 6). Cytopharyngeal fibres originate from proximal end of adoral zone and paroral membrane, curve transversely leftwards forming a slender funnel about 15 μm long in protargol preparations (Figs 23F, I–Q, 24D). Dome lip dotted by tips of cortical granules, 2.0–3.2 μm wide (mean 2.7 μm, $n = 7$) in SEM. Side stripe a moderately deep channel, 2.2–3.3 μm wide (mean = 2.7, $n = 4$) in SEM, usually regularly dotted by tips of cortical granules and/or finely striated by ridges (Figs 24F, 25B, D, 26A, B, E, F).

Comparison with original description: The Australian population of *M. setosus* strongly resembles the European specimens described by Kahl (1927). All important features match: (i) body shape and size are similar (60–90 μm vs. 45–85 μm), (ii) the macronucleus is broadly to narrowly ellipsoidal and in anterior body half but usually posterior to the preoral dome, (iii) the adoral zone extends slightly posterior to mid-body, and (iv) conspicuous caudal cilia are present. According to Kahl's (1927, 1932) figures, the number of ciliary rows and adoral polykinetids is also similar. Thus, conspecificity is beyond reasonable doubts. However, neotypification should await the investigation of a European population.

Comparison with similar species: There are several small to medium-sized metopids with long caudal cilia and thus resembling *M. setosus*, viz., *M. setosus* var. *minor* Kahl, 1927; *M. setifer* Kahl, 1932; *M. recurvatus* Vuxanovici, 1962 and *M. recurvatus* var. *pusillus* Vuxanovici, 1962. Foissner (1980) and Foissner et al. (2002) elevated *M. setosus* var. *minor* to species level. *Metopus minor*, as described by Kahl (1927, 1932) and re-described by Foissner (1980) and Foissner et al. (2002), is distinguished from *M. setosus* by the much smaller body length (30–40 μm vs. 45–90 μm) and the much lower number of the ciliary rows (8–10 vs. 17–28) and adoral polykinetids (6–7 vs. 18–27). Foissner (1980) suggested the superficially described *M. recurvatus* and *M. recurvatus* var. *pusillus* as junior synonyms of *M. minor*. *Metopus setifer* has a similar size as *M. setosus* (60–90 μm vs. 45–90 μm) and was, therefore, considered as its junior synonym by Esteban et al. (1995). However, according to Kahl (1932), *M. setifer* clearly differs from *M. setosus* by body shape which looks similar to *M. es* var. *rectus*. Moreover, *M. setifer* has many fewer ciliary rows and adoral polykinetids than *M. setosus* according to Kahl's drawings.

Amongst the Australian metopids, *M. setosus* has some similarity with *M. murrayensis* in having an obovate, anteriorly twisted body with the adoral zone and perizonal stripe usually extending posterior to mid-body. Both species have conspicuous caudal cilia. However, they are clearly separated by the oral area pattern (Fig. 2) and the presence/absence of highly refractive granules surrounding the macronucleus. Moreover, the macronucleus is broadly to narrowly ellipsoid in *M. setosus* while globular in *M. murrayensis*. Both differ also in the number of the ciliary rows (17–28 in *M. setosus* vs. 30–42 in *M. murrayensis*) and adoral polykinetids (18–27 vs. 28–52).

Multivariate analyses

Cluster analyses (Fig. 27A): Altogether, we conducted eight cluster analyses, using four different grouping algorithms and two coefficients of distance. Since single linkage, centroid and UPGMA methods brought similar dendrograms that were consistent with results from the principal component analyses, we present here only one dendrogram that was produced by the centroid method in a combination with the Manhattan city block distance (Fig. 27A).

Six analyses (single linkage, centroid and UPGMA clustering in a combination with the Euclidean distance and the Manhattan city block distance) consistently depicted *M. filum*, *M. palaeformides*, *M. rex* and *M. magnus* as a distinct group each. However, complete linkage showed only *M. filum* as a separate cluster and individuals from the three remaining species were not classified into homogeneous groups, which might be an artefact of this algorithm. In most analyses, *M. murrayensis* and *M. setosus* formed a well-defined cluster consisting of two sub-clusters: the first contained all *M. murrayensis* specimens and a single *M. setosus* cell; the second sub-cluster comprised all remaining *M. setosus* specimens. Again, only complete linkage did not generate two distinct sub-clusters but mixed *M. murrayensis* and *M. setosus* specimens.

Principal component analyses (Fig. 27B, C): We performed two principal component analyses. The first PCA was conducted with 106 individuals from six species and 24 morphometric features. Five mutually well-isolated groups of specimens were generated and four were homogenous. The first group was placed in the upper left quadrant of the ordination diagram and belongs to *M. filum*. The second group, which is attributed to *M. palaeformides*, was plotted in the middle part of the upper half of the diagram and was subdivided by the second ordination axis. The third group, representing *M. magnus*, was depicted in the upper right quadrant slightly above the first ordination axis. On the other hand, the fourth group, which belongs to *M. rex*, was localized in the lower right quadrant slightly below the first axis. The fifth group was placed in the middle of the lower ordination quadrants and contained specimens of possibly two closely related species, *M. murrayensis* and *M. setosus*.

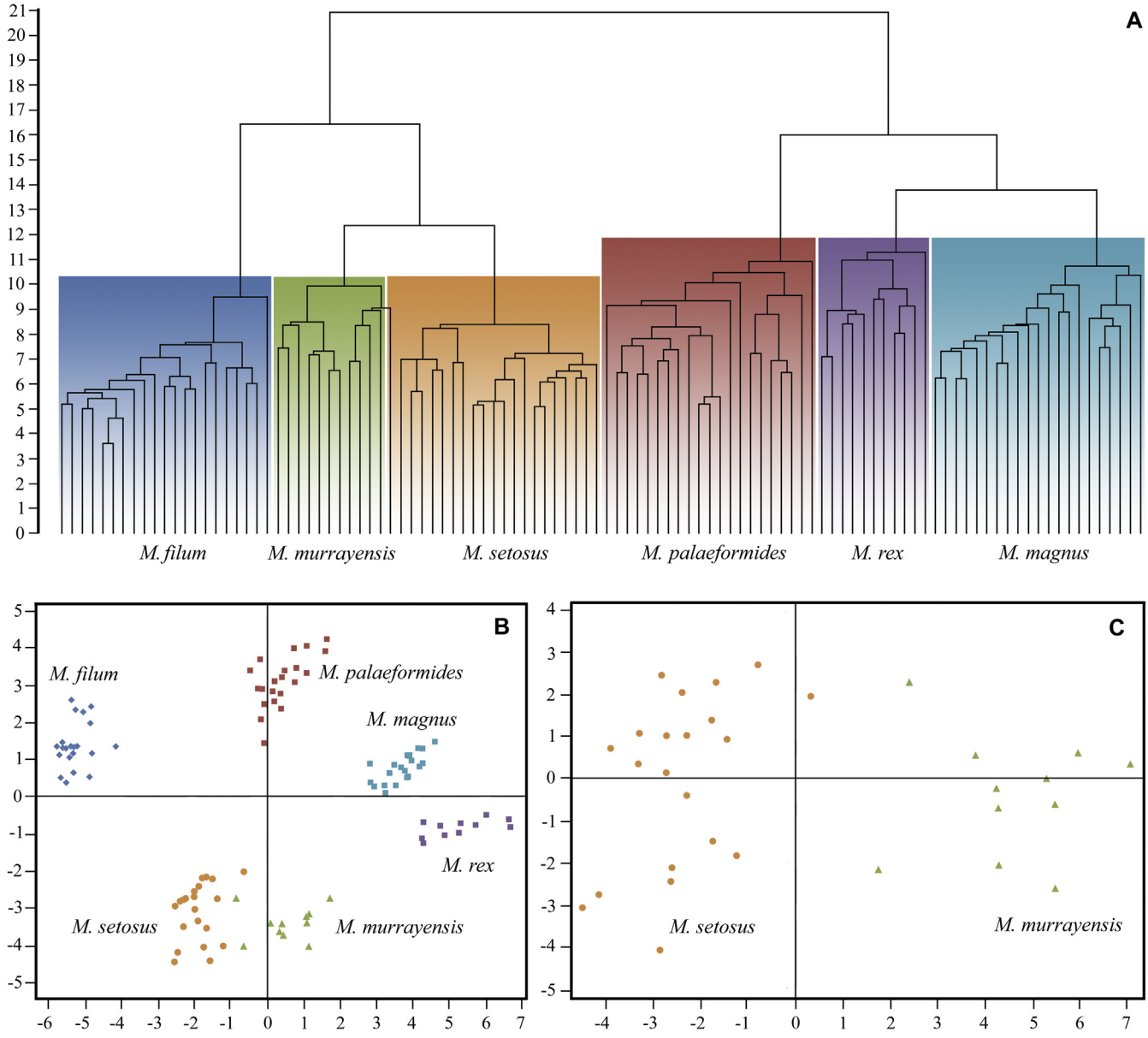

Fig. 27. A–C. Multivariate analyses of Australian metopids. **A**: Cluster analysis (centroid method in a combination with the Manhattan city block distance) of 106 specimens from six species based on 24 morphometric features. **B**: Principal component analysis of 106 individuals from six species based on 24 features. Eigenvalues of the first two components are $\lambda_1 = 12.229$ and $\lambda_2 = 5.951$, explaining 75.8% of the total variation. **C**: Principal component analysis of 32 individuals from two closely related species (*M. murrayensis* and *M. setosus*) and 23 features (shape of adoral zone of polykinetids excluded because uniform across the dataset). Eigenvalues of the first two components are $\lambda_1 = 12.812$ and $\lambda_2 = 3.062$, explaining 69.0% of the total variation.

Nonetheless, specimens of these two species were not mixed together but showed a trend to separate along the first ordination axis: specifically, all *M. setosus* specimens were left of the second axis while nine *M. murrayensis* individuals were right of the axis; only two *M. murrayensis* specimens transcended the second axis and were thus localized near to the *M. setosus* group (Fig. 27B). Separation of metopids along the first ordination axis was governed, especially, by the following morphometric features (Table 7): (i) maximum width of preoral dome, (ii) body width at level of cytostome; (iii) maximum postoral body width, (iv) length of perizonal stripe, adoral zone of polykinetids and paroral membrane as well as (v) the number of ciliary rows and adoral polykinetids. The following characteristics contributed most to the distinction of groups along the second ordination axis: (i) body length, (ii) body length:width ratio, (iii) percentage of perizonal stripe and adoral zone of body length, (iv) length and length:width ratio of macronucleus, (v) width and length:width ratio of micronucleus, and (vi) course of adoral zone of polykinetids.

Table 7. Component weights of 24 features used in the principal component analyses. Eigenvector values show correlations of characteristics with principal component axes 1 and 2. Numbers in boldface (>0.25) mark features strongly correlated with the ordination axes.

Characteristic	PCA1[a]		PCA2[b]	
	Axis 1	Axis 2	Axis 1	Axis 2
Body, length (μm)	0.195800	**0.280793**	**0.259903**	0.164996
Body, maximum width of preoral dome (μm)	**0.272343**	−0.063999	**0.262111**	0.015756
Body, width at cytostome (μm)	**0.277754**	−0.007822	**0.257178**	0.028083
Body, maximum postoral width (μm)	**0.273385**	0.032976	**0.255715**	0.039260
Body, length:width ratio	−0.175122	**0.262582**	0.004357	**0.301081**
Anterior body end to distal end of perizonal stripe, distance (μm)	0.212437	−0.164954	0.171204	**−0.291058**
Anterior body end to proximal end of perizonal stripe, distance (μm)	**0.274876**	0.059014	0.233381	−0.059383
Perizonal stripe, percentage of body length	0.141391	**−0.310472**	−0.093696	−0.397437
Anterior body end to distal end of adoral zone of polykinetids, distance (μm)	0.216106	−0.109657	0.227743	0.094079
Anterior body end to proximal end of adoral zone of polykinetids, distance (μm)	**0.269497**	0.114995	**0.268017**	0.000418
Adoral zone of polykinetids, percentage of body length	0.131954	**−0.334104**	−0.048099	−0.466036
Anterior body end to distal end of paroral membrane, distance (μm)	0.241132	0.191962	0.208070	0.148465
Anterior body end to macronucleus, distance (μm)	0.144051	0.248593	**0.259278**	0.019525
Macronucleus, length (μm)	0.185797	**0.271423**	−0.157233	0.129679
Macronucleus, width (μm)	0.240355	0.069288	0.191685	0.006648
Macronucleus, length:width ratio	0.017002	**0.315729**	−0.194345	0.069915
Micronucleus, length (μm)	0.070548	0.135242	0.127330	**−0.319558**
Micronucleus, width (μm)	0.079806	**0.270060**	0.171391	0.172486
Micronucleus, length:width ratio	−0.055056	**−0.252663**	−0.097078	**−0.416160**
Somatic ciliary rows, total number	**0.257146**	−0.124645	0.248783	−0.140465
Adoral polykinetids, number	**0.271094**	−0.045857	**0.250056**	−0.132941
Paroral membrane, length (μm)	**0.275349**	−0.004476	0.238177	−0.128402
Granules surrounding macronucleus (absence/presence)	0.015094	−0.195337	**0.260219**	−0.064726
Course of adoral zone of polykinetids (oblique/spiralled)[c]	0.161256	**−0.308742**	–	–

[a] PCA1–principal component analysis performed on 106 individuals from six metopid species and 24 features.
[b] PCA2–principal component analysis performed on 32 individuals from two species (*M. murrayensis* and *M. setosus*) and 23 features.
[c] This feature was excluded from PCA2 because the adoral zone is spiralled in both species.

The second PCA was carried out with a reduced dataset, containing 23 features from 32 individuals of *M. murrayensis* and *M. setosus*. This approach caused better differentiation amongst these taxa than the first PCA did. Specimens of both species spread along the first ordination axis, forming two independent groups separated by the second ordination axis (Fig. 27C). The strongest influence on the separation along the first axis can be attributed to (Table 7): (i) body length, (ii) maximum width of preoral dome, (iii) body width at level of cytostome, (iv) maximum postoral body width, (v) length of adoral zone and number of adoral polykinetids, (vi) distance of macronucleus from anterior body end, and (vii) presence/absence of granules around macronucleus. The second ordination axis reached highest correlations with (i) body length:width ratio, (ii) distance of distal end of perizonal stripe from anterior body end, and (iii) length and length:width ratio of micronucleus.

To summarise, multivariate analyses support the distinctness of the six metopids described in the present study. This not only corroborates the validity of the morphospecies concept but also documents that the traditionally used morphometrics can be used for the characterization of metopid taxa. Although metopid species are considered to be comparatively variable, they form distinct and homogenous groups in the phenotypic space. Therefore, most of the synonymisations proposed by Esteban et al. (1995) and Wetzel (1928) need to be reconsidered.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejop.2016.12.001.

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