

Heterometopus meisterfeldi nov. gen., nov. spec. (Protozoa, Ciliophora), a new metopid from Australia

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Dedicated to Dr. Ralf Meisterfeld, Aachen University, on the occasion of his 70th birthday.

Abstract

Using standard methods, I describe *Heterometopus meisterfeldi* nov. gen., nov. spec., a new armophorean ciliate from dry soil of the bed of the Fogg Dam in northern Australia. It appeared in the non-flooded Petri dish culture three weeks after soil re-wetting, i.e., when the bottom zone of the culture became anaerobic. The new genus is characterized by a cylindroid body and a short, slightly oblique, J-shaped adoral zone extending only ventrally and about one third of body length. The species has an average size of $140 \times 30 \mu\text{m}$ and has 23 ciliary rows, 30 adoral polykinetids, and 25 false kineties in the perizonal stripe. *Metopus palaeformis*, as redescribed by Foissner et al. (2002) and in the present paper, shows very similar characteristics and is thus transferred to the new genus: *Heterometopus palaeformis* (Kahl, 1927) nov. comb. *Heterometopus meisterfeldi* and *H. palaeformis* differ by body size ($\sim 140 \times 30$ vs. 100×20 in vivo), the shape of the macronucleus (ellipsoid, $\sim 2:1$ vs. elongate ellipsoid, $\sim 3:1$), the width of the side-stripe ($12 \mu\text{m}$ vs. $4 \mu\text{m}$), the number of false kineties in the perizonal stripe (25 vs. 14), and the number of adoral polykinetids (~ 30 vs. ~ 20). Very likely, several species from Kahl's *Metopus* groups II and III belong to *Heterometopus* but a transfer should await detailed redescrptions. *Heterometopus meisterfeldi* belongs very likely to the family Metopidae. The short, J-shaped adoral zone of polykinetids highly resembles the blepharismid genera *Pseudoblepharisma* and *Blepharisma*.

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Introduction

Members of the Metopidae Kahl, 1927 live in anaerobic or microaerobic habitats (Kahl 1932; Wetzel 1928). Thus, they are important bioindicators in running and stagnant waters, wastewater treatment plants, and soil (Berger and Foissner 2003; Foissner 1999).

About 100 metopid species, subspecies, and varieties have been described, most by Kahl (1932, 1935), Vuxanovici (1961, 1962), and Jankowski (1964a,b). But less than 10 species have been redescribed with modern methods (Bourland and Wendell 2014; Bourland et al. 2014; Dragesco and Dragesco-Kernéis 1986; Foissner 1998, 2016; Foissner and Agatha 1999; Foissner et al. 1992, 2002; Vd'áčný 2007) though there is an urgent need to address whether or not many taxa are synonymous, as suggested by Wetzel (1928) and Esteban et al. (1995).

In Australia, I found many metopids some of which are similar but very likely not identical with European species

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(Foissner 2016). One of these, which represents a new genus, is described here and dedicated to Dr. Ralf Meisterfeld (Aachen University, Germany) for his long friendship and excellent studies on testate amoebae.

One of the reviewers suggested that the Australian population might be *M. palaeformis* because some cultivated monsters show a high variability of this species (Esteban et al. 1995). However, monsters are common in ciliate cultures, and those shown by Esteban et al. (1995) are dissimilar to both, the Australian population and to the neotype of *M. palaeformis* carefully described by Foissner et al. (2002). A chaos will result when we include monsters, post-conjugates etc. into the normal variability of a taxon. Thus, I present not only the description of *Heterometopus meisterfeldi* but also a second redescription of *M. palaeformis*, showing that both belong to the same genus while they are clearly different at species level.

Material and Methods

Heterometopus meisterfeldi was discovered in 1985 in a sample of grass litter and surface soil (0–5 cm) from the bottom of the dry Fogg Dam east of the town of Darwin, Australia. The soil was dark and had pH 5.0. The resting cysts were activated with the non-flooded Petri dish method. Briefly, the non-flooded Petri dish method involved placing 200 g litter and soil in a Peri dish and saturating, but not flooding it, with distilled water. This culture was analyzed for ciliates by inspecting about 1 ml of the run-off on days 2, 7, 14, 21, and 28; for a detailed description, see Foissner et al. (2002).

Metopus palaeformis (now *Heterometopus*, see below) became numerous in a non-flooded Petri dish culture from soil of the margin of brackish coastal water near to the capital of the Dominican Republic. It could be cultivated in brackish tap water (12‰ salinity) with some squashed wheat kernels so that abundant bacterial food and microaerobic conditions developed.

Live observation, Chatton-Lwoff silver nitrate impregnation, and protargol impregnation were performed according to Foissner (1991). Counts and measurements on silver-impregnated specimens were conducted at a magnification of 1000×. In vivo measurements were performed at 40–1000×. Illustrations of live specimens were based on free-hand sketches while those of impregnated cells were made with a drawing device.

Classification and terminology are according to Kahl (1932), Jankowski (1964b), and Lynn (2008). However, there is some disagreement in naming the adoral zone (of membranelles). Kahl (1932), Jankowski (1964b), Foissner et al. (2002), and Bourland et al. (2014) name it “adoral zone of membranelles” while Silva-Neto et al. (1993) introduced “paramembranelles” because of the different fine structure of the membranelles in the Hypotrichia and the Armophorea. As the fine structure needs electron microscopical investigation,

I now prefer the neutral term suggested by Lynn (2008): adoral polykinetids. Further, I resurrect the term “Nebenstreifen” (side-stripe) created by Kahl (1932) to denote the unciliated, channel-like area between the adoral zone of polykinetids and the preoral dome. “False kineties” are short, oblique kineties produced by the highly ordered kinetids of the perizonal stripe (Foissner and Agatha 1999).

Results

Heterometopus nov. gen.

Diagnosis: Cylindroid Metopidae with J-shaped adoral zone extending slightly obliquely in anterior third of ventral side. Five perizonal kineties.

Type species: *Heterometopus meisterfeldi* nov. spec.

Species assignable: As yet, there is only one other species that matches the diagnosis, viz., *Heterometopus palaeformis* (Kahl, 1927) nov. comb. (basonym: *Metopus palaeformis* Kahl, 1927). It has been redescribed by Esteban et al. (1995) and Foissner et al. (2002), and was reinvestigated in the course of the present study (Table 2).

Etymology: The genus name is a composite of the Greek adjective *héteros* (different, deviating) and the Greek genus-group name *Metopus* (forehead). Masculine gender.

Description of *Heterometopus meisterfeldi* nov. spec. (Figs 1A–F, 2A–C, 3A–E, 4B; Table 1)

Diagnosis: In vivo about $140 \times 30 \mu\text{m}$; cylindroid. Macronucleus posterior to proximal end of adoral zone, ellipsoid. On average 23 ciliary rows and five perizonal kineties extending 24% of body length and forming 25 false kineties on average. Oral apparatus extends about 33% of body length, composed of a short paroral membrane and an average of 30 adoral polykinetids; side-stripe about $12 \mu\text{m}$ width.

Type locality: Australia, east of the town of Darwin, grass litter and soil from the dry bottom of the Fogg Dam, $\sim 12^\circ 34' \text{ S } 131^\circ 18' \text{ E}$.

Type material: The holotype slide (reg. no. 2015/848) with silver nitrate-impregnated cells and three paratype slides (reg. no. 2015/849–851) with protargol-impregnated specimens have been deposited in the Museum of Natural History (Biologiezentrum) in Linz (LI). The holotype (Figs 1B, C, 3A) and other important specimens have been marked by black ink circles on the coverslip.

Etymology: Dedicated to Dr. Ralf Meisterfeld, an eminent researcher on testate amoebae.

Description: *Heterometopus meisterfeldi* is ordinarily variable, i.e., most important features have variation coefficients <15%, for instance, body length and the number of ciliary rows and adoral polykinetids. Specimens fixed in Bouin's solution for protargol impregnation are about 15% smaller than those fixed for silver nitrate impregnation (only about 5% due to the osmium tetroxide contained in Champy's fixative) and live cells (Table 1).

Size in vivo $110\text{--}170 \times 20\text{--}35 \mu\text{m}$, usually about $140 \times 30 \mu\text{m}$, as calculated from some in vivo measurements

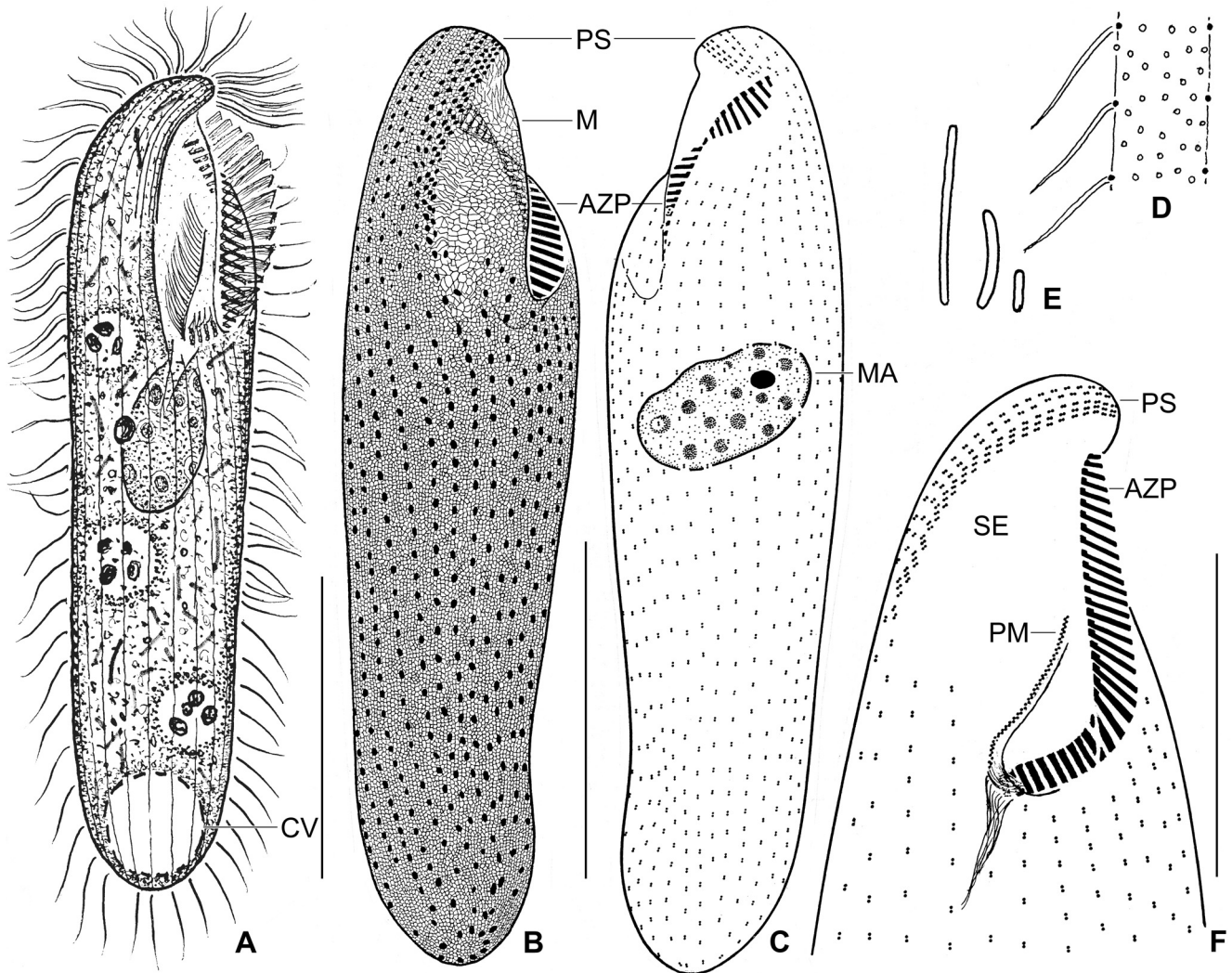


Fig. 1. (A–F) *Heterometopus meisterfeldi* from life (A, D, E), after silver nitrate impregnation (B, C), and protargol-impregnated (F). (A) Right side view of a representative specimen, length 140 μm . The cytoplasm contains many anaerobic bacteria. (B, C) Right and left side view of holotype specimen, length 144 μm . The silverline pattern is shown only on the right side and in the side-stripe, where the meshes are larger. (D) Surface view, showing the cortical granulation. (E) Cytoplasmic bacteria are 3–14 μm long. (F) Ventrolateral view, showing the J-shaped adoral zone of polykinetids. AZP, adoral zone of polykinetids; CV, contractile vacuole; M, margin of side-stripe; MA, macronucleus; PM, paroral membrane; PS, perizonal stripe; SE, side-stripe. Scale bars 30 μm (F) and 50 μm (A–C).

and the morphometric data in Table 1 adding 5% and 15% preparation shrinkage for silver nitrate and protargol-impregnated specimens, respectively (Foissner 2016). Body cylindroid, often slightly widened in posterior half of oral area; anterior end slightly rostrate, posterior rounded (Figs 1A–C, 2A, B, 3A, B, E). Nuclear apparatus usually between proximal end of adoral zone and mid-body (Figs 1A, C, 3A, B; Table 1). Macronucleus usually ellipsoid, rarely broadly ellipsoid or indistinctly reniform, $23 \times 12 \mu\text{m}$ in silver nitrate-impregnated specimens; nucleoli of ordinary shape and size. Micronucleus attached to macronucleus, globular to broadly ellipsoid, compact and thus easily recognizable in vivo. Contractile vacuole in posterior end (Fig. 1A). Cortex flexible, contains loosely arranged, bright granules

about 0.5 μm across in vivo; do not impregnate with the protargol method used (Fig. 1D). Cytoplasm colorless, studded with two size classes of bacteria: 2–5 μm ($\times 3.4 \mu\text{m}$) and 7–14 μm ($\times 10.9 \mu\text{m}$), impregnate with protargol and thus very likely anaerobes (Figs 1A, E, 2C, 3C, E). Food vacuoles about 10 μm across, contain granular material, possibly bacterial spores. Swims slowly rotating about main axis.

Somatic ciliature composed of dikinetids, cilia 12 μm long in vivo, both basal bodies ciliated in oral portion of cell, anterior basal body usually barren in postoral region; arranged in an average of 23 meridional rows commencing rather close to proximal margin of adoral zone and extending to posterior body end, slightly more narrowly spaced on ventral side; elongated caudal cilia absent in vivo and in protargol

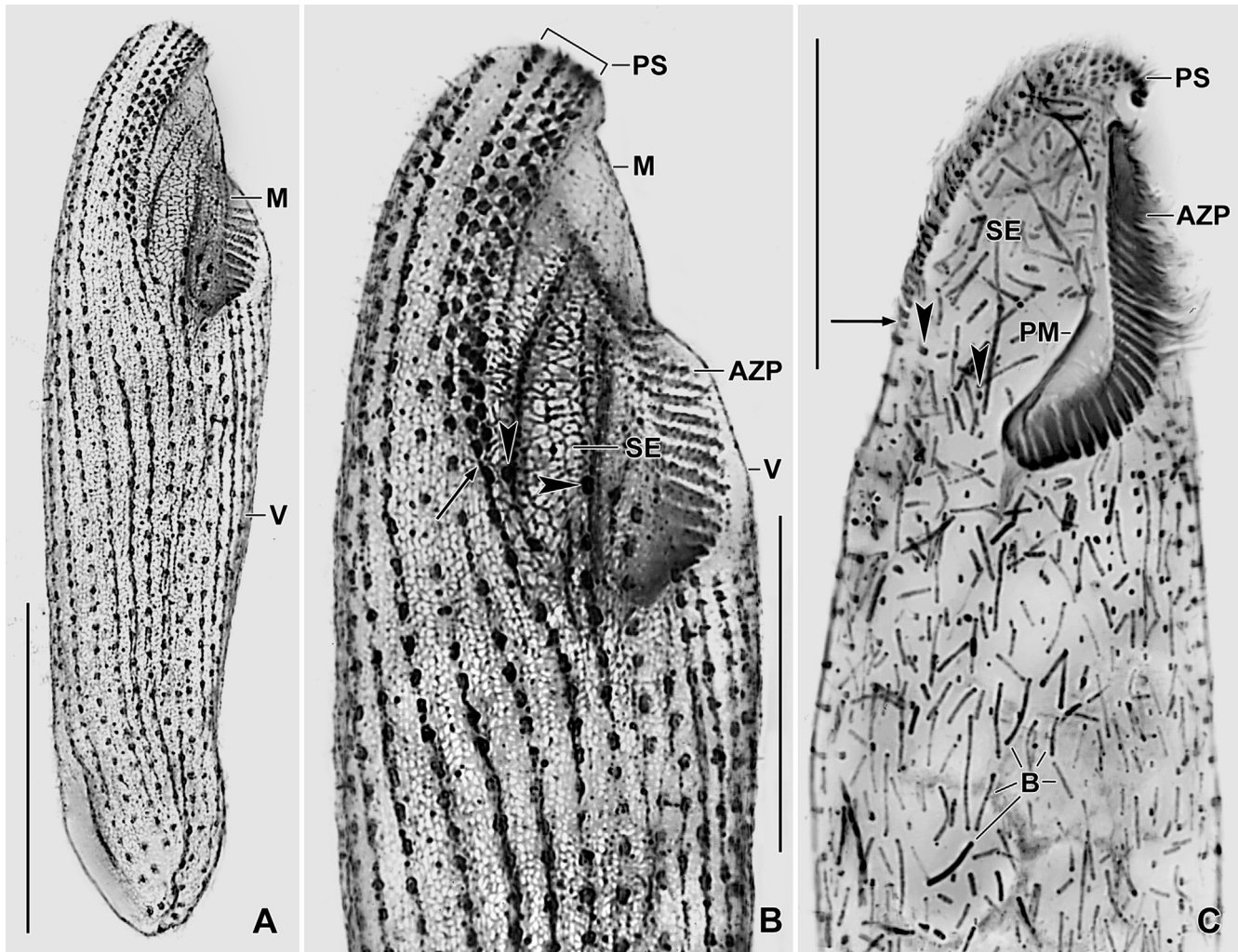


Fig. 2. (A–C) *Heterometopus meisterfeldi* after silver nitrate impregnation (A, B) and protargol-impregnated (C). (A, B) Ventrolateral overview and oral detail. The adoral zone of polykinetids extends vertically on the ventral side, i.e., does not traverse the right side of the body. The arrow marks the site where a single somatic kinety spreads and becomes the three-rowed part of the perizonal stripe. The arrowheads mark the beginning of the right side ciliary rows. The silverline pattern is very fine-meshed in the somatic cortex and coarser-meshed in the side-stripe. (C) Ventrolateral view, showing the J-shaped adoral zone of polykinetids, the paroral membrane, and the perizonal stripe the proximal end of which is marked by an arrow. The arrowheads denote the begin of the right side ciliary rows. The cytoplasm is studded with up to 14 μm long anaerobic bacteria. AZP, adoral zone of polykinetids; B, bacteria; M, margin of side-stripe; PM, paroral membrane; PS, perizonal stripe; SE, side-stripe; V, ventral side. Scale bars 25 μm (B, C) and 50 μm (A).

preparations (Figs 1A–C, F, 2A–C, 3A–C, E; Table 1). Perizonal stripe short, i.e., extends only 24% of body length on average; composed of five longitudinal rows forming an average of 25 oblique false kineties (Fig. 3D) with cilia about 15 μm long in vivo; anterior end abuts to distal end of adoral zone; perizonal rows 1 and 2 each connected to an ordinary somatic kinety, rows 3 to 5 connected to only one somatic kinety (Figs 1A, E, F, 2A–C, 3A–E; Table 1).

Adoral zone extends slightly obliquely on ventral side for about one third of body length, rather distinctly spiraled about main axis, J-shaped: long anterior portion straight to slightly convex, short posterior portion curves by about 90° when entering cell; composed of an average of 30 obliquely

arranged polykinetids up to 8 μm wide and with cilia up to 10 μm long in vivo; composed of two long rows of basal bodies and a very short proximal row recognizable when the cell is observed in a certain angle (Fig. 1F). Paroral membrane about 20 μm long, extends obliquely in proximal region of side stripe, dikinetal, cilia 10 μm long in vivo. Side stripe only slightly concave but comparatively wide, i.e., 12 μm on average, extends onto right side of body (Figs 1A–C, 2A–C, 3A–C, E; Table 1).

Silverline pattern very narrowly meshed; meshes about 0.5 μm in size, form five to eight rows between two ciliary rows each, become irregular and up to 2 μm in size in wall of side-stripe (Figs 1B, 2B).

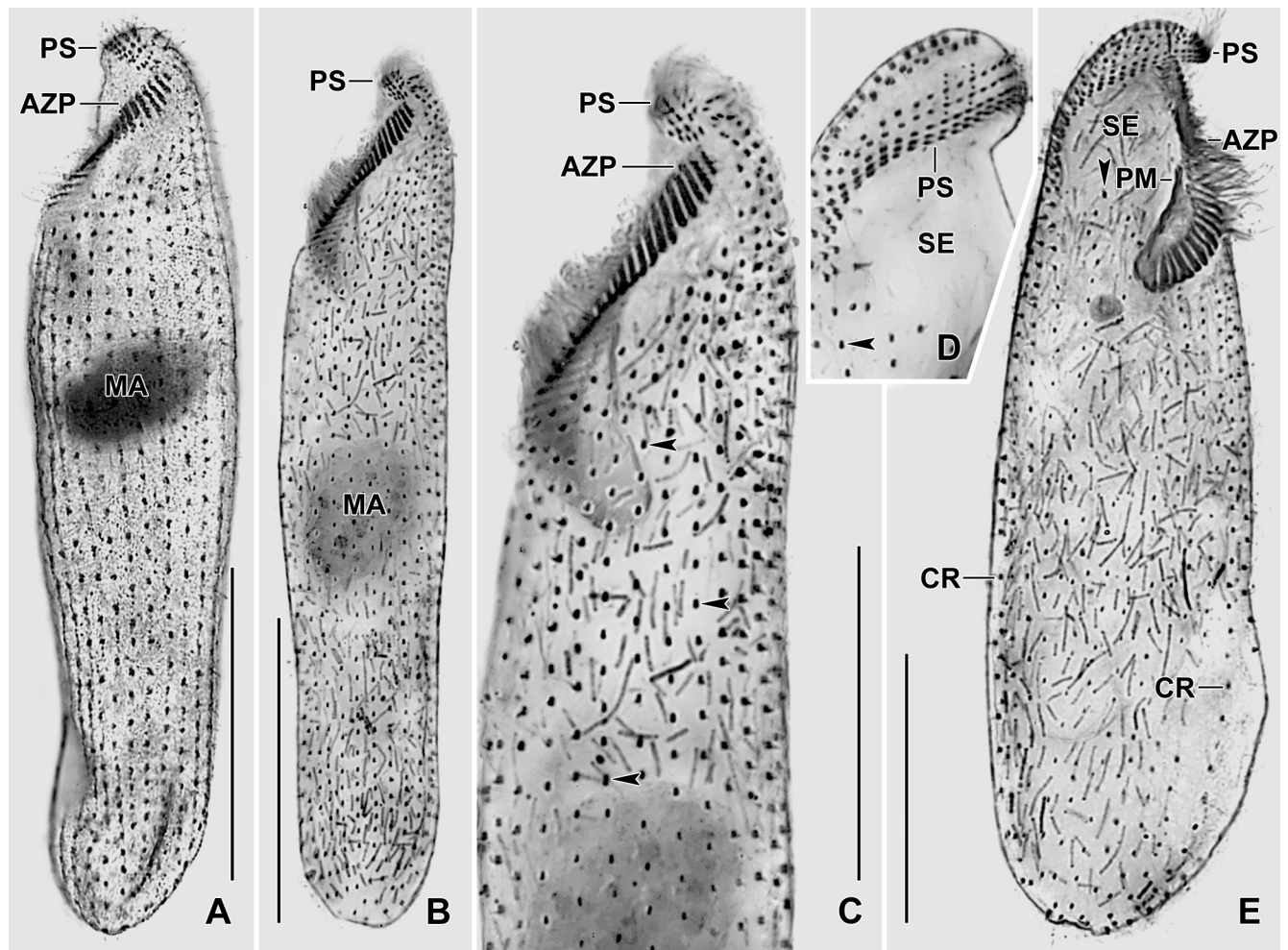


Fig. 3. (A–E) *Heterometopus meisterfeldi* after silver nitrate impregnation (A) and protargol-impregnated (B–E). (A) Left side view of holotype specimen (cp. Fig. 1C). (B, C) Left side overview and detail, showing the dorsally directed adoral zone and the meridional ciliary rows composed of dikinetids (arrowheads). (D) The perizonal stripe consists of five rows of dikinetids which produce more or less oblique false kineties (dotted). The three proximal rows are associated with a single somatic kinety (arrowhead). (E) Ventrolateral overview, showing the J-shaped adoral zone of polykinetids and the large side-stripe. The arrowhead marks the begin of a somatic ciliary row. The cytoplasm is studded with anaerobic bacterial rods. AZP, adoral zone of polykinetids; CR, ciliary rows; MA, macronucleus; PM, paroral membrane; PS, perizonal stripe; SE, side-stripe. Scale bars 30 μm (C, E) and 50 μm (A, B).

Ecology and occurrence: The sample was collected in 1985 and investigated in 1988. *Heterometopus meisterfeldi* and seven other metopids appeared three weeks after re-wetting the air-dried sample, i.e., when the bottom zone of the non-flooded Petri dish culture became anaerobic. Occurs also in the floodplain of the Amazon River, Brazil, but not in about 1000 other soil samples, indicating that it is very rare and possibly restricted to Gondwana.

***Heterometopus palaeformis* (Kahl, 1927) nov. comb.** (Fig. 4C, D, F–H; Table 2)

As a redescription of *H. palaeformis* is available (Foissner et al. 2002), I do not provide a detailed description of the Dominican population but refer the readers to the figures and the morphometric analysis cited above.

The Dominican specimens are shorter, but not broader, than those from England and Madagascar (Table 2) and match

well the size (length 70–80 μm) given by Kahl (1927, 1932) when preparation shrinkage is added. The body shape is more stout and highly variable (the three “forms” described by Kahl were present in my cultures), as shown by the length: width ratio (Table 2). The shape of the macronucleus is also highly variable, i.e., varies from broadly ellipsoid to $30 \times 6 \mu\text{m}$, often it is curved to a globular mass between proximal end of adoral zone and mid-body. The somatic and oral infraciliature is highly similar to the Madagascan specimens (Fig. 4C, D, F–H). Scanning electron microscopy shows that the paroral membrane consists of a single row of cilia about 7 μm long. Likewise, the adoral polykinetids consist of three rows of cilia of which one is very short (Fig. 4C, D, F–H).

Material deposited: Three voucher slides (reg. no. 2015/852–854) with protargol-impregnated specimens have

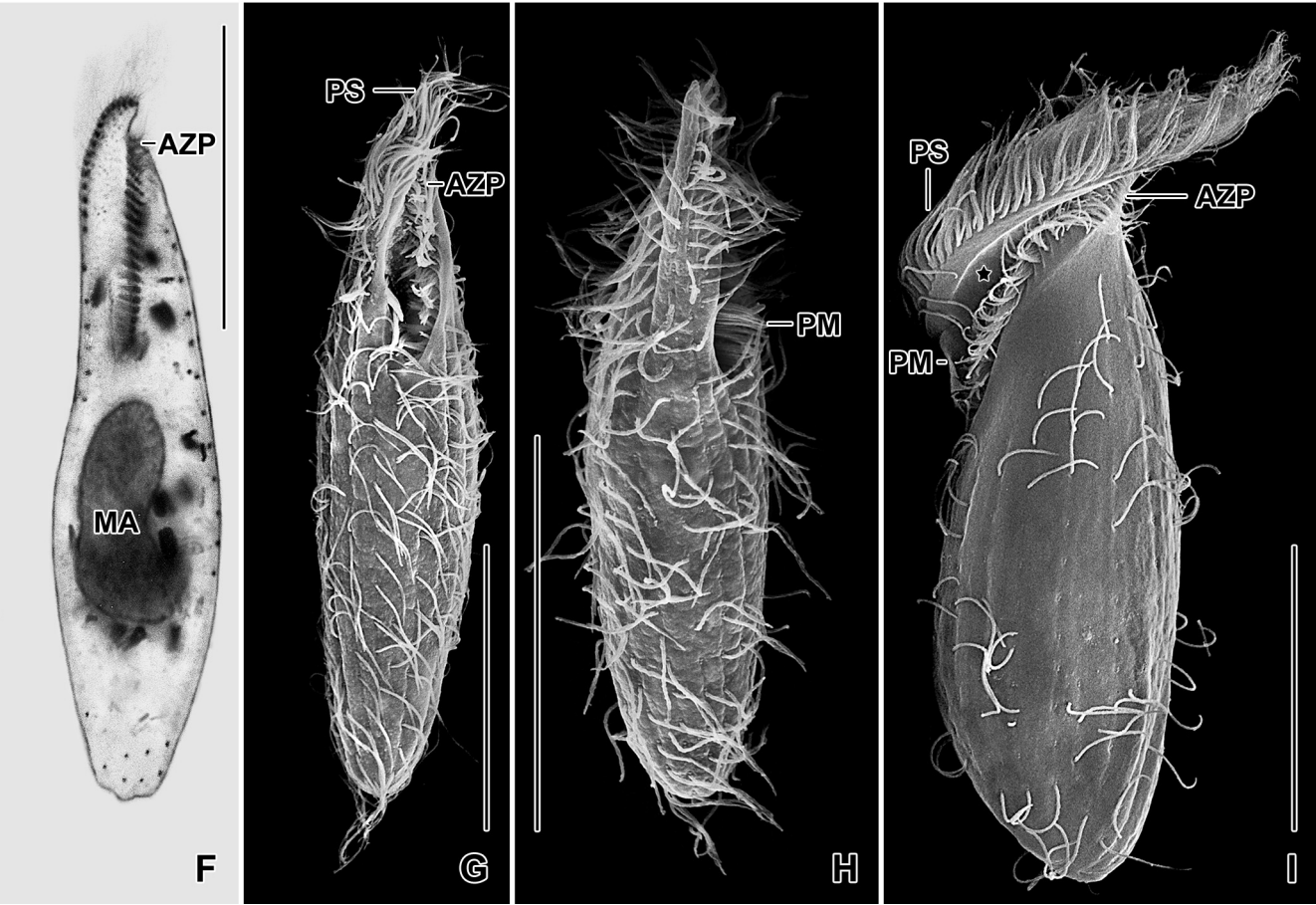
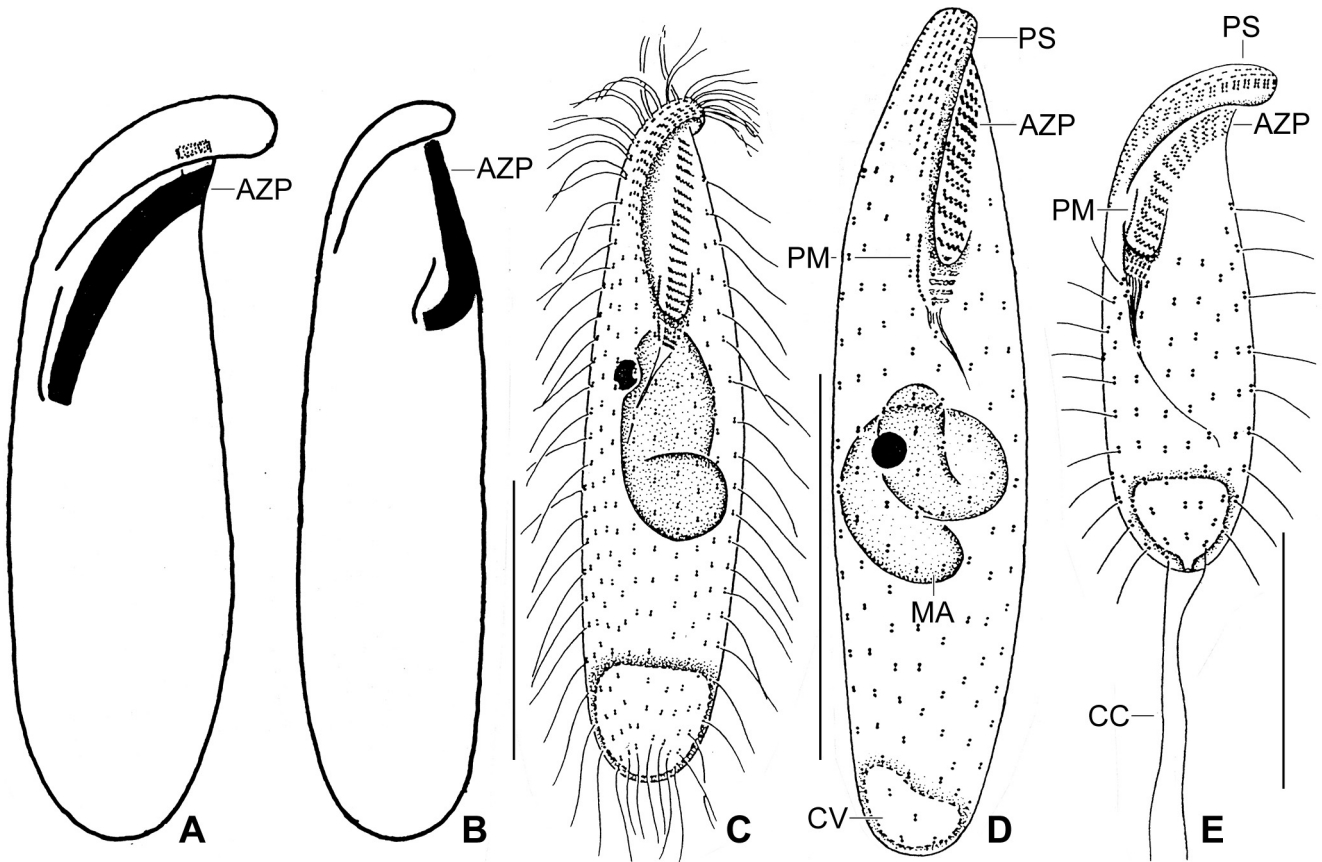


Table 1. Morphometric data on *Heterometopus meisterfeldi* after protargol impregnation (upper line) and Chatton-Lwoff silver nitrate impregnation (lower line), if not stated otherwise.

Characteristics ^a	Mean	<i>M</i>	SD	SE	CV	Min	Max	<i>n</i>
Body, length in vivo	143.0	–	–	–	–	120.0	170.0	3
Body, width in vivo	30.0	–	–	–	–	25.0	35.0	3
Body, length	115.9	115.0	13.5	3.0	11.7	96.0	145.0	21
	132.0	129.5	11.8	3.7	8.9	116.0	148.0	10
Body, width	26.1	27.0	3.9	0.8	14.8	18.0	31.0	21
	31.9	31.0	5.3	1.7	16.7	23.0	43.0	10
Body length:width, ratio	4.6	4.2	1.1	0.2	23.1	3.5	7.2	21
	4.2	4.2	0.6	0.2	13.9	3.4	5.3	10
Anterior body end to proximal end of adoral zone, distance	31.7	32.0	3.7	0.8	11.8	25.0	40.0	21
	43.6	45.0	2.3	0.7	5.3	40.0	46.0	10
Anterior body end to proximal end of adoral zone, percentage of body length	27.7	28.0	4.5	1.0	16.2	17.0	35.0	21
	33.2	33.0	2.2	0.7	6.5	30.0	37.0	10
Anterior body end to macronucleus, distance	41.0	40.0	8.4	1.9	20.4	26.0	56.0	21
	48.4	50.0	12.3	4.1	25.3	26.0	65.0	9
Anterior body end to proximal end of perizonal stripe, distance	23.0	23.0	2.2	0.5	9.5	20.0	26.0	21
	31.8	31.0	1.6	0.5	4.9	30.0	35.0	10
Anterior body end to proximal end of perizonal stripe, percentage of body length	20.0	19.0	3.2	0.7	16.2	15.0	26.0	20
	24.2	24.5	2.5	0.8	10.3	20.0	28.0	10
Macronucleus, length	20.3	20.0	3.2	0.7	15.7	14.0	26.0	21
	23.5	23.0	4.3	1.4	18.2	18.0	30.0	10
Macronucleus, width	13.5	14.0	2.1	0.5	15.3	9.0	17.0	21
	12.0	12.5	2.4	0.8	19.6	9.0	15.0	10
Micronucleus, length	3.9	4.0	0.5	0.1	13.8	3.0	5.0	21
	3.9	4.0	–	–	–	3.0	4.0	10
Micronucleus, width	3.5	4.0	0.7	0.2	19.3	2.0	5.0	21
	3.0	3.0	0.5	0.2	15.7	2.0	4.0	10
Somatic ciliary rows in mid-body, number	23.2	23.0	1.3	0.3	5.6	21.0	26.0	21
	24.3	24.0	1.0	0.3	3.9	23.0	26.0	10
Somatic cilia, length in anterior region	9.9	10.0	1.6	0.3	15.6	7.0	13.0	21
	Not recognizable							
Perizonal stripe, number of ciliary rows	5.0	5.0	0.0	0.0	0.0	5.0	5.0	21
	5.0	5.0	0.0	0.0	0.0	5.0	5.0	10
False kinetics in perizonal stripe, number	25.1	25.0	1.3	0.3	5.1	23.0	27.0	14
	23.3	24.0	1.1	0.5	4.6	23.0	25.0	5
Adoral zone, number of polykinetids	31.6	30.0	2.8	0.6	8.7	27.0	37.0	21
	28.1	28.0	1.5	0.5	5.4	26.0	31.0	10
Largest polykinety, width	4.6	5.0	0.9	0.2	18.7	3.0	7.0	21
	5.9	5.5	1.1	0.4	18.7	5.0	8.0	10
Paroral membrane, length	14.6	14.0	1.4	0.3	9.8	12.0	17.0	21
	19.6	20.6	1.7	0.6	8.5	16.0	22.0	9
Side-stripe, width	11.0	12.0	1.9	0.6	17.1	9.0	14.0	10
	12.5	12.0	1.6	0.7	13.1	11.0	15.0	6

^aData based on mounted, silver-impregnated, and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm . CV, coefficient of variation in %; *M*, median; Mean, arithmetic mean; Max, maximum; Min, minimum; *n*, number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean.

Fig. 4. (A–I) Comparison of *Metopus* (A, E, I) and *Heterometopus* (B–D, F–H) in protargol preparations (A–F) and scanning electron micrographs (G–I). (A, E) *Metopus hasei*, right side views, schematic (A, adoral zone of polykinetids shaded black) and in a protargol preparation (E, from Foissner and Agatha 1999). The adoral zone extends obliquely on the right side. (B) *Heterometopus meisterfeldi*, scheme showing that the adoral zone (shaded black) has another location (ventral vs. mainly right lateral) and shape (J-shaped vs. convex and slightly sigmoid) than in *Metopus* (A, E, I). (C, D, F–H) *Heterometopus palaeformis* (formerly *Metopus palaeformis*, see Discussion) from Foissner et al. (2002; C, D) and originals from slightly saline soil of the Dominican Republic (F–H). The shape and location of the adoral zone of polykinetids is quite similar to that of *H. meisterfeldi* (3E, 4B), i.e., it is on the ventral side (B, C, F, G) and J-shaped (G). (I) Ventrolateral view of *Metopus rostratus* from Botswana (Africa). The asterisk denotes the narrow side-stripe. This species, which has a similar size as *H. meisterfeldi* and *H. palaeformis*, looks quite different, showing that both very likely belong to different genera. AZP, adoral zone of polykinetids; CC, caudal cilia; CV, contractile vacuole; MA, macronucleus; PM, paroral membrane; PS, perizonal stripe. Scale bars 30 μm (C, D, F–I) and 40 μm (E).

Table 2. Morphometric data on *Heterometopus palaeformis* from England (En, Esteban et al. 1995), Madagascar (Ma, neotype, Foissner et al. 2002), and the Dominican Republic (DR, original).

Characteristics ^a	Site	Method	Mean	<i>M</i>	SD	SE	CV	Min	Max	<i>n</i>
Body, length	En	IV	101 ± 1 ^c	–	–	–	–	70.0	132.0	403
	Ma ^b	P	81.7	81.0	9.1	2.7	11.1	70.0	100.0	11
	DR ^b	P	62.4	63.0	4.1	1.0	6.5	57.0	73.0	17
Body, width	En	IV	18 ± 1	–	–	–	–	8.0	31.0	403
	Ma ^b	P	19.3	20.0	2.8	0.9	14.7	13.0	23.0	11
	DR ^b	P	18.5	18.0	2.7	0.7	14.9	15.0	25.0	17
Body length:width, ratio	En	IV	5.6	–	–	–	–	–	–	403
	Ma	P	4.3	4.4	0.6	0.2	12.9	3.7	5.6	11
	DR	P	3.5	3.3	0.5	0.1	15.7	2.6	4.9	17
	DR	SEM	3.8	3.8	0.5	0.1	13.2	2.6	5.5	56
Anterior body end to proximal end of adoral zone of polykinetids, distance	Ma	P	27.8	28.0	3.0	0.9	10.9	23.0	35.0	11
Paroral membrane, length	DR	P	24.1	24.0	1.4	0.3	5.7	22.0	26.0	17
Side-stripe, width	DR	P	12.4	12.0	2.0	0.5	16.1	10.0	15.0	17
Macronucleus, length	DR	P	4.2	4.0	0.8	0.2	19.4	3.0	6.0	17
Macronucleus, width	Ma	P	32.6	35.0	–	–	–	17.0	50.0	11
	DR	P	23.1	23.5	4.6	1.0	19.9	13.0	30.0	20
Micronucleus, length	Ma	P	10.3	10.0	2.3	0.7	22.7	6.0	15.0	11
	DR	P	6.0	5.5	1.2	0.3	19.5	5.0	8.0	20
Micronucleus, width	Ma	P	3.6	3.5	–	–	–	3.0	4.0	11
	DR	P	2.7	3.0	–	–	–	2.0	3.0	17
Somatic kineties, number	Ma	P	3.1	3.0	–	–	–	3.0	3.5	11
	DR	P	2.7	3.0	–	–	–	2.0	3.0	17
False kineties in perizonal stripe, number	En ^e	?	?	?	?	?	?	8.0	14.0	?
	Ma	P	17.6	18.0	1.4	0.4	7.8	15.0	20.0	11
	DR	P	13.4	13.0	1.2	0.3	9.2	10.0	15.0	17
Adoral polykinetids, number	Ma	P ^d	16.0	–	–	–	–	14.0	18.0	2
	DR	P	13.6	13.0	1.7	0.4	12.2	10.0	17.0	17
	En ^e	?	?	?	?	?	?	10.0	20.0	?
	Ma	P	19.9	20.0	2.2	0.7	10.9	17.0	24.0	11
	DR	P	19.1	19.0	0.9	0.2	4.6	18.0	21.0	17

^a Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a non-flooded Petri dish culture (Ma) or from a pure culture (DK). Measurements in μm . CV, coefficient of variation in %; *M*, median; Mean, arithmetic mean; Max, maximum; Min, minimum; *n*, number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean, SEM, unselected scanning electron micrographs.

^b When 15% preparation shrinkage are added (Foissner 2014, 2016), the following means are obtained: Ma $94 \times 22.2 \mu\text{m}$, DR $72 \times 21 \mu\text{m}$.

^c "Typical trophs". Arithmetic mean with 95% confidence interval for the unknown population mean. In the text, Esteban et al. (1995) provide different values: $70\text{--}200 \times 8\text{--}31 \mu\text{m}$.

^d From two figures provided by Foissner et al. (2002). Three kineties were added for the unseen frontal rows.

^e Method and number of specimens investigated not provided!

been deposited in the Museum of Natural History (Biologisches Zentrum) in Linz (LI).

Discussion

Justification and comparison of the genus *Heterometopus*

Heterometopus differs from *Metopus* s. str. mainly by the shape and location of the adoral zone of polykinetids, a feature used to split *Metopus* s. l. into several subgenera, especially by Jankowski (1964a,b, 2007). This has been widely acknowledged (Bourland and Wendell 2014; Foissner 2016; Lynn 2008) and the subgenera raised to genera. In

Heterometopus, the J-shaped adoral zone extends slightly obliquely on the ventral side and makes a sharp proximal turn when it enters the cell (Fig. 2A–C). Indeed, this pattern is highly similar to those found in various heterotrichs, for instance, *Blepharisma* (Fig. 5C) and *Pseudoblepharisma* (Fig. 5A, B). In *Metopus* s. str., the adoral zone extends ventrally and right laterally becoming more or less sigmoid and slightly to distinctly convex on the right body side. This causes a very different appearance when *Metopus* and *Heterometopus* are seen side by side (Fig. 4A, B, F–I).

The most conspicuous and diagnostically important features of *Heterometopus* are the J-shaped zone of adoral polykinetids and the slender, cylindroid body (Figs 1A, 2A). These features are present also in various heterotrich genera, for instance, *Pseudoblepharisma* (Fig. 5A–C). In vivo, they can be separated only by the presence (*Heterometopus*) vs.

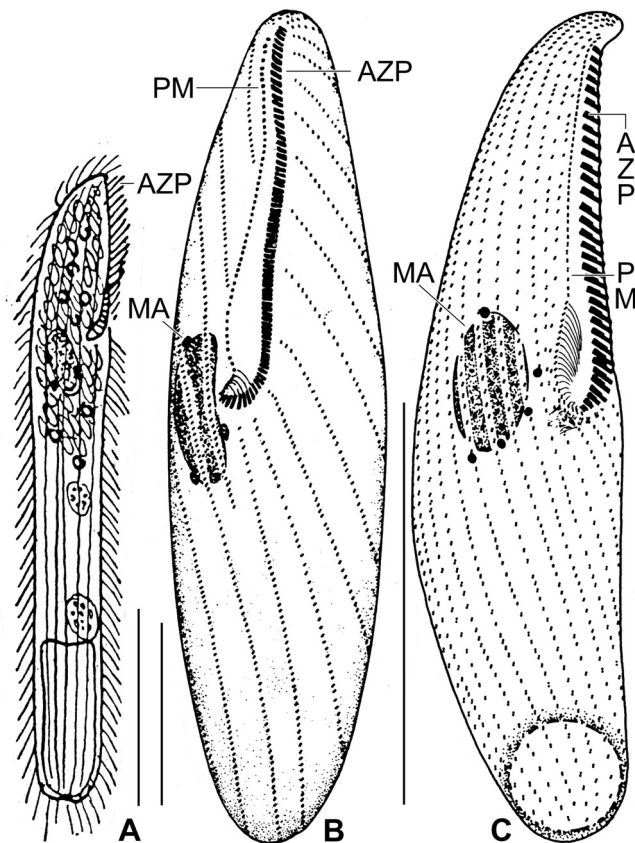


Fig. 5. (A–C) Generic comparison. (A, B) *Pseudoblepharisma tenuis*, length 150 µm (A, from Kahl 1932) and *P. crassum*, length 210 µm (B, from Grolière 1977) are in vivo highly similar to *Heterometopus meisterfeldi* (Figs 1A, 3E), i.e., have a slender body and a short, ventrally extending adoral zone. (C) *Blepharisma steinii* (from Foissner 1989) and *Blepharisma* spp. also resemble *Heterometopus* because they have the same location and shape of the adoral zone. AZP, adoral zone of polykinetids; MA, macronucleus; PM, paroral membrane. Scale bars 50 µm.

absence (*Pseudoblepharisma*, *Blepharisma*) of a perizonal stripe (cp. Figs 2A–C, 4C–E with Fig. 5C).

Another rather similar genus is *Tesnospira* which Jankowski (1964b) diagnosed as follows: “Small bag-like ciliates with reduced anterior body part, narrow, split-like small subanterior buccal cavity with reduced number of membranelles, with no visible cilia-free zone between the adoral zone of membranelles and a highly shortened perizonal stripe.” Unfortunately, the description of the type species, *T. alba*, is rather incomplete, casting doubts on the generic diagnosis. Esteban et al. (1995) synonymized *T. alba* with *Metopus palaeformis*. I disagree because *T. alba* has only 6–7 adoral polykinetids while *M. palaeformis* has 20 (Table 2).

Species comparison

Using the reviews by Kahl (1932, 1935), Jankowski (1964b), and Esteban et al. (1995) as well as several

isolated publications, e.g., Vuxanovici (1961, 1962), it becomes clear that the Australian population is an undescribed species characterized by the features detailed in the diagnosis.

In the literature, I found only one species with features similar to those of *H. meisterfeldi*, viz., *Metopus palaeformis* Kahl, 1927 (Fig. 4C, D, F–H), as redescribed by Esteban et al. (1995) and Foissner et al. (2002) who neotypified it with a Madagascan population. It differs from *H. meisterfeldi* by body size ($\sim 100 \times 25$ µm, Foissner et al. 2002 or 100×18 µm, Esteban et al. 1995 vs. 140×30 µm), by the number of adoral polykinetids (~ 20 vs. 29), the width of the side-stripe (4 µm vs. 12 µm), the number of false kineties in the perizonal stripe (16 vs. 25), and by the shape of the macronucleus (elongate ellipsoid, $\sim 3:1$ vs. ellipsoid, $2:1$). This is confirmed by a new population from the Dominican Republic (Table 2). Although body length and number of somatic ciliary rows are rather different (Table 2), the general morphology (Fig. 4G, H), the shape of the macronucleus, the narrow side-stripe, and the number of adoral polykinetids and false kineties in the perizonal stripe match well with the Madagascan neotype specimens. Thus, I disagree with one of the reviewers that the Australian population is *Metopus palaeformis*. Very likely, several metopids from Kahl’s groups II and III belong to *Heterometopus* but a transfer should wait for detailed redescrptions.

Classification

Heterometopus is very likely most closely related to the “group II” and “group III” metopids of Kahl (1932), i.e., to species like *Metopus hasei* (Fig. 4E) and *M. setosus* (Kahl 1932).

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