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European Journal of Protistology 51 (2015) 210–228

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

Biogeographic specializations of two large hypotrich ciliates: *Australocirrus shii* and *A. australis* and proposed synonymy of *Australocirrus* and *Cyrtohymenides*

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Received 12 December 2014; received in revised form 27 January 2015; accepted 2 February 2015
Available online 9 February 2015

Abstract

Using standard methods, we studied the morphology and distribution of an Australian population of *Australocirrus shii* (Shi et al., 1997) nov. comb. and a Jamaican population of *A. australis* (Foissner, 1995) nov. comb. We identified three features, not or rarely used earlier, that distinguish *Australocirrus shii* from *A. australis*: the distance between the anterior pretransverse cirrus and the anteriormost transverse cirrus (5–8% vs. 1.5–1.7% of body length), the arrangement of the transverse cirri (3 + 2 vs. an oblique row), and the resting cyst macronuclear nodules (separate vs. fused). *Australocirrus shii* has been reported from Asia and Australia while *A. australis* is possibly restricted to the Neotropic. Because of problems in getting voucher materials of *A. shii*, we emphasise that permanent slides should be deposited in international repositories. Based on previous studies and new data, especially a refined interpretation of the shape of the paroral membrane, we suggest synonymy of *Cyrtohymenides* and *Australocirrus*. Thus, *Cyrtohymena* (*Cyrtohymenides*) *shii*, *C.* (*Cyrtohymenides*) *aspoecki*, and *C.* (*Cyrtohymenides*) *australis* are transferred to *Australocirrus* which is, inter alia, defined by a moderately to distinctly curved, but not recurved, paroral membrane, multiple fragmentation of dorsal kinety 3 and three or more dorsomarginal kineties.

Keywords: *Australocirrus*; Biogeography; *Cyrtohymenides*; Morphogenesis; Type slides

Introduction

The present study is an example where new morphological features and literature data from closely related species are integrated for the sake of a more accurate identification so that their distribution can be analysed (for a review, see Foissner 2006). To reach this goal, we studied a population of *Cyrtohymena* (*Cyrtohymenides*) *australis* from Central America and of *C.* (*Cyrtohymenides*) *shii* from Australia. Further, we planned to study additional features of three populations of *C.* (*Cyrtohymenides*) *shii* but only the Chinese author responded to our request, emphasising that type and voucher materials should be deposited in recognised repositories.

The subgenus *Cyrtohymenides* was created by Foissner (2004) with the Austrian *C.* (*Cyrtohymenides*) *aspoecki* as type. Presently, *Cyrtohymenides* contains three rather well established species: *Cyrtohymena* (*Cyrtohymenides*) *australis* Foissner, 1995 in Foissner, 2004; *C.* (*Cyrtohymenides*) *aspoecki* Foissner, 2004; and *C.* (*Cyrtohymenides*) *shii* (Shi et al., 1997) Shao et al., 2012. We found new features suggesting not only the validity of the species but also synonymy of *Cyrtohymenides* and *Australocirrus* Blatterer and Foissner, 1988.

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http://dx.doi.org/10.1016/j.ejop.2015.02.002
0932-4739/

Material and Methods

Australocirrus shii and *A. australis* were reactivated from resting cysts in air-dried soil samples from Australia and Jamaica using the non-flooded Petri dish method. For details on locations and samples, see the individual species descriptions. Briefly, the non-flooded Petri dish method involved placing 50–500 g litter and soil in a Petri dish and saturating, but not flooding it, with distilled water. Such a culture was analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28; for a detailed description, see Foissner et al. (2002).

Live observation, protargol impregnation, and scanning electron microscopy (SEM) were performed according to Foissner (1991, 2014). Counts and measurements on silver-impregnated specimens were conducted at a magnification of 1000×. In vivo measurements were performed at magnifications of 40–1000×. Illustrations of live specimens were based on free-hand sketches, while those of impregnated cells were made with a drawing device. Classification is according to Foissner (1989) and Berger (1999). Basal terminology is according to Wallengren (1900), Berger (1999), and Foissner and Al-Rasheid (2006).

Results

Genus *Australocirrus* Blatterer and Foissner, 1988

Improved diagnosis: Flexible oxytrichids with medium to large body size (≥150 μm long in vivo). On average ≥18 fronto-ventral-transverse cirri, frontoventral cirri in V-shaped pattern. One right and one left row of marginal cirri. Multiple fragmentation of dorsal kinety 3 and three or more dorsomarginal kineties. Caudal cirri present. Paroral membrane in *Australocirrus* pattern.

Species assignable: *Australocirrus oscitans* Blatterer and Foissner, 1988 (type species); *Australocirrus zechmeisterae* Foissner et al., 2005; *Australocirrus aspoecki* (Foissner, 2004) nov. comb. (basionym: *Cyrtohymena* (*Cyrtohymenides*) *aspoecki* Foissner, 2004); *Australocirrus shii* (Shi et al., 1997) nov. comb. (basionym: *Oxytricha shii* Shi et al., 1997); *Australocirrus australis* (Foissner, 1995) nov. comb. (basionym: *Cyrtohymena australis* Foissner, 1995).

Description of an Australian population of *Australocirrus shii* (Shi et al., 1997) nov. comb. (Figs 1A–K, 2A–H; Tables 1 and 2)

1997 *Oxytricha shii* Shi, Wei and Wang, Acta Zootaxon. Sin. 22: 225–230.
1998 *Oxytricha shii* Shi, Wei and Song, J. Ocean Univ. Qingdao 28: 269–274 (ontogenesis).
2012 *Cyrtohymena* (*Cyrtohymenides*) *shii* (Shi, Wei and Wang, 1997) comb. nov. – Shao, Song, Al-Rasheid and Berger, Acta Protozool. 50: 263–287 (combination with *Cyrtohymena* [*Cyrtohymenides*]).
2012 *Cyrtohymena australis* Foissner, 1995 – Kim, Lee, Kwon and Shin, J. Species Res. 1: 78–86 (misidentification).
2013 *Cyrtohymena* (*Cyrtohymenides*) *shii* (Shi, Wei and Wang, 1997) Shao et al., 2012 – Singh, Kamra and Sapra, Eur. J. Protistol. 49: 283–297.

Improved diagnosis: (includes data from populations of Australia, China, India, and Korea; averages are from all populations): Size in vivo 140–240 × 45–85 μm, usually about 185 × 65 μm; elongate ellipsoid or slightly obovate. Two ellipsoid macronuclear nodules and two to eight, usually five micronuclei. Cortical granules bright-citrine, in rough rows, 0.5–1.0 μm across. Anterior pretransverse cirrus 5–8% of body length away from anteriormost transverse cirrus. Five fringed transverse cirri in two groups. Left marginal row on average composed of 30–36 cirri, right of 28–31. Adoral zone occupies 37–42% of body length, on average composed of 50–57 membranelles. Paroral membrane composed of minute kineties with more than two basal bodies in anterior half. Eight to ten dorsal kineties; usually three narrowly spaced, inconspicuous caudal cirri right of body's midline. Resting cyst smooth and with separate macronuclear nodules.

Remarks: The large specimens from Korea are very likely caused by an inflation of the cells due to the preparation method used, and are thus excluded from the diagnosis. Possibly, the in vivo size was calculated from the impregnated cells. This interpretation is supported by the number of marginal cirri and adoral membranelles that is quite similar in the other populations.

Material deposited: Six voucher slides with protargol-impregnated specimens from the Australian population have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI). Reg. No. 2015,23-28. Relevant specimens have been marked by black ink circles on the coverslip.

Description: Most important features of the Australian population have an ordinary variability (CV ≤ 15%; Table 1), including the distance from anterior pretransverse cirrus to the anteriormost transverse cirrus (Table 1). Of those having higher coefficients of variation, the number of micronuclei (2–8, CV ~ 23%) and the distance from the lowermost transverse cirrus to the body end should be mentioned.

Size in vivo 170–235 × 45–70 μm, usually about 200 × 60 μm, as calculated from some in vivo measurements and the morphometric data in Table 1 adding 15% preparation shrinkage (Foissner 2014). Body elongate ellipsoidal or slightly elongate obovate; dorsoventrally flattened about 2:1 (Figs 1A–C, G, H, 2A, B, F, G; Table 1). Nuclear apparatus within central quarters of cell slightly left of midline, composed of two macronuclear nodules and two to eight, on average six micronuclei (Figs 1G, H, 2B; Table 1). Macronuclear nodules widely distant, ellipsoidal to broadly ellipsoidal, on average 17 × 12 μm in protargol preparations; contain many granular nucleoli. Micronuclei attached to and scattered around macronuclear nodules, globular to broadly ellipsoidal, on average 3.3 × 3.0 μm in protargol

Table 1. Morphometric data on an Australian population of *Australocirrus shii* (upper line) and a Jamaican population of *A. australis* (lower line).

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Body, length	168.8	168.0	15.2	2.8	9.0	146.0	203.0	29
	144.5	142.0	13.1	2.9	9.0	121.0	169.0	21
Body, width	49.9	51.0	6.1	1.1	12.3	41.0	60.0	29
	41.1	40.0	5.7	1.2	13.8	29.0	51.0	21
Body length:width, ratio	3.4	3.4	0.4	0.1	11.4	2.8	4.2	21
	3.6	3.5	0.5	0.1	12.7	3.0	4.8	21
Body width:length, ratio (%)	29.8	29.7	3.2	0.7	10.8	24.0	35.1	21
	28.5	28.7	3.2	0.7	11.3	20.7	33.8	21
Anterior body end to proximal end of adoral zone, distance	69.1	69.0	4.6	1.0	6.6	60.0	77.0	21
	46.6	46.0	4.8	1.1	10.3	40.0	59.0	21
Body length:AZM length, ratio	2.4	2.4	0.1	0.0	5.4	2.2	2.6	21
	3.1	3.1	0.2	0.1	7.9	2.7	3.6	21
Anterior body end to proximal end of adoral zone, percentage of body length	41.6	41.6	2.3	0.5	5.5	37.8	45.6	21
	32.3	32.5	2.6	0.6	8.1	28.0	36.8	21
Anterior body end to distal end of adoral zone, distance	25.8	24.0	4.4	1.1	17.0	20.0	35.0	15
	15.3	15.0	2.4	0.6	15.9	11.0	21.0	15
Anterior body end to distal end of adoral zone, percentage of body length	15.5	15.7	2.1	0.5	13.7	12.4	19.0	15
	10.5	10.4	1.3	0.3	12.6	8.2	13.3	15
DE-value	0.4	0.4	0.1	0.0	15.0	0.3	0.5	15
	0.3	0.3	0.0	0.0	11.1	0.2	0.4	15
Adoral membranelles, longest membranellar base	12.4	12.0	1.0	0.2	8.3	11.0	14.0	21
	10.1	10.0	1.0	0.3	9.8	9.0	12.0	15
Adoral membranelles, number	49.6	49.0	3.6	0.8	7.3	44.0	56.0	21
	47.8	48.0	4.4	1.0	9.2	40.0	55.0	21
Anterior body end to paroral membrane, distance	15.5	15.0	2.0	0.4	12.7	10.0	21.0	21
	9.6	9.5	1.2	0.3	12.9	7.0	11.5	21
Paroral membrane, length of cilia	8.1	8.0	1.1	0.3	14.0	7.0	11.0	19
	9.7	10.0	0.9	0.2	9.1	8.0	11.0	15
Anterior body end to endoral membrane, distance	16.5	16.0	1.5	0.3	9.1	14.0	21.0	21
	11.0	11.0	1.3	0.3	11.8	8.5	14.0	21
Anterior body end to anterior macronuclear nodule, distance	54.2	54.0	3.0	0.7	5.5	49.0	59.0	21
	39.4	38.5	4.5	1.0	11.4	33.0	49.0	21
Macronuclear nodules, distance in between	25.5	26.0	3.7	0.8	14.5	17.0	32.0	21
	19.1	19.0	6.4	1.4	33.2	9.0	33.0	21
Macronuclear figure, length	61.9	62.0	5.9	1.3	9.5	50.0	72.0	21
	59.3	58.0	6.1	1.3	10.4	50.5	72.0	21
Anterior macronuclear nodule, length	16.7	16.0	1.8	0.4	10.9	13.0	21.0	21
	20.1	20.0	2.4	0.5	12.0	16.0	27.5	21
Anterior macronuclear nodule, width	12.2	12.0	1.8	0.4	14.8	9.0	18.0	21
	10.4	10.0	0.9	0.2	8.9	8.0	12.5	21
Macronuclear nodules, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
Anteriormost micronucleus, length	3.3	3.0	–	–	–	3.0	4.0	21
	2.9	3.0	–	–	–	2.5	3.5	21
Anteriormost micronucleus, width	3.0	3.0	–	–	–	2.5	3.5	21
	2.6	2.5	0.4	0.1	13.4	2.0	3.5	21
Micronuclei, number	6.0	6.0	1.4	0.3	22.8	2.0	8.0	21
	5.3	5.0	1.6	0.4	29.8	3.0	9.0	21
Anterior body end to right marginal row, distance	37.1	36.0	4.7	1.0	12.7	31.0	48.0	21
	24.0	23.0	4.1	0.9	16.9	18.0	32.5	21
Posterior body end to right marginal row, distance	7.0	7.0	1.8	0.4	25.6	4.0	11.0	21
	6.1	6.0	1.3	0.3	20.7	4.0	9.0	21
Right marginal row, number of cirri	31.0	31.0	2.1	0.5	6.7	27.0	34.0	21
	33.1	33.0	2.7	0.6	8.0	29.0	38.0	21

Table 1. (*Continued*)

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Posterior body end to left marginal row, distance	0.9	1.0	0.4	0.1	41.8	0.5	2.0	21
	1.0	1.0	–	–	–	0.5	1.5	21
Left marginal row, number of cirri	35.6	36.0	2.7	0.6	7.6	30.0	39.0	21
	36.9	37.0	2.7	0.6	7.4	32.0	41.0	21
Frontal cirri, number	2.9	3.0	–	–	–	2.0	3.0	21
	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Anterior body end to buccal cirrus, distance	25.5	26.0	2.7	0.6	10.4	20.0	30.0	21
	17.8	17.0	2.7	0.6	15.2	13.5	22.5	21
Buccal cirrus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Anterior body end to posteriormost frontoventral cirrus, distance	47.8	47.0	5.1	1.1	10.8	40.0	58.0	21
	31.4	31.0	4.1	0.9	12.9	26.0	40.0	21
Frontoventral cirri, number	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
	4.0	4.0	0.0	0.0	0.0	4.0	4.0	21
Anterior body end to posteriormost postoral cirrus, distance	87.4	86.0	7.2	1.6	8.3	72.0	104.0	21
	61.6	62.0	4.2	1.2	6.8	56.0	69.0	13
Postoral cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Posterior body end to posteriormost transverse cirrus, distance	11.9	12.0	3.5	0.8	29.1	7.0	20.0	21
	8.5	8.0	1.9	0.4	23.0	5.5	12.5	21
Transverse cirri, number	4.9	5.0	–	–	–	4.0	5.0	21
	5.1	5.0	–	–	–	5.0	6.0	21
Anterior transverse cirrus to anterior pretransverse cirrus, distance	13.5	14.0	1.8	0.4	13.4	10.0	17.0	21
	2.6	3.0	1.1	0.3	44.7	0.5	4.0	19
Anterior transverse cirrus to anterior pretransverse cirrus, percentage of body length	8.1	8.4	1.0	0.2	11.9	6.4	9.6	21
	1.7	1.9	0.8	0.2	43.5	0.4	3.1	19
Posterior body end to anterior pretransverse cirrus, distance	46.4	46.0	5.0	1.1	11.0	37.0	58.0	21
	22.7	23.0	3.2	0.8	13.9	17.0	27.0	15
Posterior body end to rear pretransverse cirrus, distance	25.7	26.0	3.3	0.7	13.0	18.0	30.0	21
	13.7	14.0	2.6	0.7	19.2	10.0	18.0	15
Pretransverse cirri, distance in between	17.2	17.0	2.4	0.5	13.8	13.0	21.0	21
	6.5	7.0	1.4	0.4	21.0	4.0	9.0	15
Pretransverse cirri, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
	2.1	2.0	–	–	–	2.0	3.0	21
Dorsal kineties, number	10.0	10.0	0.7	0.2	7.4	9.0	11.0	21
	10.0	10.0	0.9	0.2	8.7	9.0	11.0	21
Dorsal kinety 1, number of bristles	36.8	38.0	4.2	0.9	11.3	27.0	42.0	21
	34.9	34.0	5.2	1.1	14.8	24.0	44.0	21
Dorsal kinety 2, number of bristles	29.8	30.0	3.1	0.7	10.3	24.0	35.0	21
	31.9	31.0	3.8	0.8	12.1	27.0	41.0	21
Dorsal kinety 3, number of bristles	16.0	16.0	3.2	0.7	19.8	10.0	24.0	21
	20.4	20.0	2.7	0.6	13.3	16.0	26.0	21
Dorsal kinety 4, number of bristles	5.5	5.0	2.3	0.5	42.3	3.0	12.0	21
	4.3	4.0	1.9	0.4	43.4	2.0	9.0	20
Dorsal kinety 5, number of bristles	12.1	13.0	4.9	1.1	40.7	3.0	21.0	21
	17.8	19.0	3.6	0.8	20.3	6.0	22.0	21
Dorsal kinety 6, number of bristles	18.8	21.0	5.6	1.2	29.8	4.0	27.0	21
	16.5	16.5	3.3	0.7	20.1	11.0	24.0	20
Dorsal kinety 7, number of bristles	19.4	20.0	4.1	0.9	21.1	9.0	26.0	21
	13.8	13.0	2.8	0.7	20.6	8.0	19.0	19
Dorsal kinety 8, number of bristles	15.0	14.0	4.1	0.9	27.5	8.0	21.0	21
	10.6	10.0	3.4	0.8	31.6	6.0	21.0	20

Table 1. (*Continued*)

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	n
Dorsal kinety 9, number of bristles	8.8	9.0	3.0	0.7	34.4	4.0	15.0	21
	5.3	5.0	2.7	0.6	51.7	1.0	10.0	20
Dorsal kinety 10, number of bristles	6.5	5.0	4.4	1.1	67.7	1.0	14.0	15
	2.8	1.5	2.5	0.7	89.2	1.0	7.0	12
Dorsal kinety 11, number of bristles	3.6	3.0	0.9	0.4	24.8	3.0	5.0	5
	1.7	1.5	0.8	0.3	49.0	1.0	3.0	6
Caudal cirri, number	3.0	3.0	–	–	–	2.0	3.0	21
	3.1	3.0	–	–	–	3.0	4.0	21
Resting cyst, diameter in vivo	not investigated							
	66.4	65.0	5.2	1.2	7.9	60.0	80.0	19

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

preparations. Contractile vacuole slightly anterior of mid-body at left cell margin, with lacunar collecting canals (Fig. 2H). Cortex very flexible, contains bright citrine granules 1.0–1.2 μm across, some dispersed, most around bases of cirri and dorsal bristles, about four rows between marginal cirral rows and about 10 intrakinetal rows on dorsal surface; rarely impregnate with the protargol method used (Figs 1D, E, 2F). Posterior half of body studded with crystals of usual shape and some lipid droplets appearing dark at low magnification (Fig. 1A, F). Food vacuoles scattered throughout body, in vivo up to 50 μm across, contain naked and testate amoebae (*Trinema lineare*), flagellates, brown fungal spores, and rotifers (Figs 1A, 2H). Swims and glides rather rapidly.

Cirral pattern oxytrichid (Berger 1999), number of frontoventral-transverse cirri comparatively variable, on average 18 as typical for oxytrichids s. str. (Figs 1A, G, I, J, 2A, B, E; Table 1). Usually three thickened, in vivo about 22 μm long frontal cirri, right cirrus posterior of distal end of adoral zone, middle cirrus anterior of buccal cirrus, left cirrus anterior of distal end of undulating membranes; only two frontal cirri in three out of 21 specimens. Invariably one slightly thickened buccal cirrus about 15 μm long in protargol preparations. On average four hook-like arranged frontoventral cirri, anteriormost cirrus usually slightly posterior to level of buccal cirrus. On average three postoral cirri, last cirrus near mid-body. Usually two slightly obliquely arranged pretransverse cirri, anterior cirrus on average 14 μm distant (8% of body length) from anteriormost transverse cirrus in protargol preparations (Figs 1A, G, 2A, B, E; Table 1). On average five transverse cirri in two groups of three and two, usually slightly narrowed and fringed distally, about 40 μm long in vivo and 30 μm in protargol preparations, distal half projects from body proper. Marginal cirri in two non-confluent rows about 22 μm long in vivo, gradually decrease in size posteriorly; left row extends around posterior margin, composed of an average of 36 cirri, right row extends to body margin posteriorly, composed of an average of 31 cirri (Figs 1A, G, J, 2A, B, E; Table 1).

On average ten dorsal kineties with bristles 3–4 μm long in vivo and in protargol preparations: kineties 1, 2, 6 bipolar; kinety 3 slightly shortened anteriorly, occasionally posteriorly; kinety 5 slightly shortened anteriorly; kineties 4, 7–10 slightly or distinctly shortened anteriorly and posteriorly (Figs 1H, 2G; Table 1). Usually three caudal cirri at right posterior margin of cell, about 22 μm long in protargol preparations, conspicuously thin and thus easily confused with last cirri of left marginal row; only two caudal cirri in one out of 21 specimens investigated (Figs 1H, J, 2E, G; Table 1).

Adoral zone occupies about 42% of body length, commences far posteriorly on right margin of cell (about 16% of body length, DE-value 0.42), on average composed of 49 ordinary membranelles with up to 20 μm long cilia, bases of largest membranelles on average 12 μm long in vivo and in protargol preparations (Figs 1A, G, I, 2A, B; Table 1). Buccal cavity conspicuous, wide and deep with distinct buccal horn. Buccal lip narrow and hyaline, covers proximal membranelles. Undulating membranes right of body's midline, indistinctly cyrtohymenid because only moderately curved, but not re-curved anteriorly, and cilia only about 8 μm long in protargol preparations (Fig. 2C), intersect optically near rear end of paroral. Paroral membrane commences 15 μm posterior from anterior body end, endoral membrane commences left of anterior end of paroral and extends to buccal vertex; pharyngeal fibres of ordinary length (Figs 1I, 2C, D; Table 1).

Occurrence and ecology: As yet found in China (Shi et al. 1997), India (Singh et al. 2013), Korea (Kim et al. 2012), and Australia, i.e., in freshwater puddles of Maoershan town, Shangzhi Count, Heilongjiang Province, China, 45°15′ N 127°30′ E; in a water catchment area close to the entry of the Barsey Rhododendron Sanctuary, Sikkim, India, 27°15′ to 27°27′ N and 88°01′ E to 88°23′ E; in freshwater of Beonam-myeon, Jangsu-gun, Jeollabuk-do, Korea, 35°30′ 58″ N 127°32′20″ E; and in surface soil, pH 5.8, of the Holmes jungle, Botanical Gardens of the town of Darwin, Australia, 12°26′40.70″ S 130°50′14.82″ E. Omnivorous, i.e., feeds on naked and testate amoebae (*Trinema*

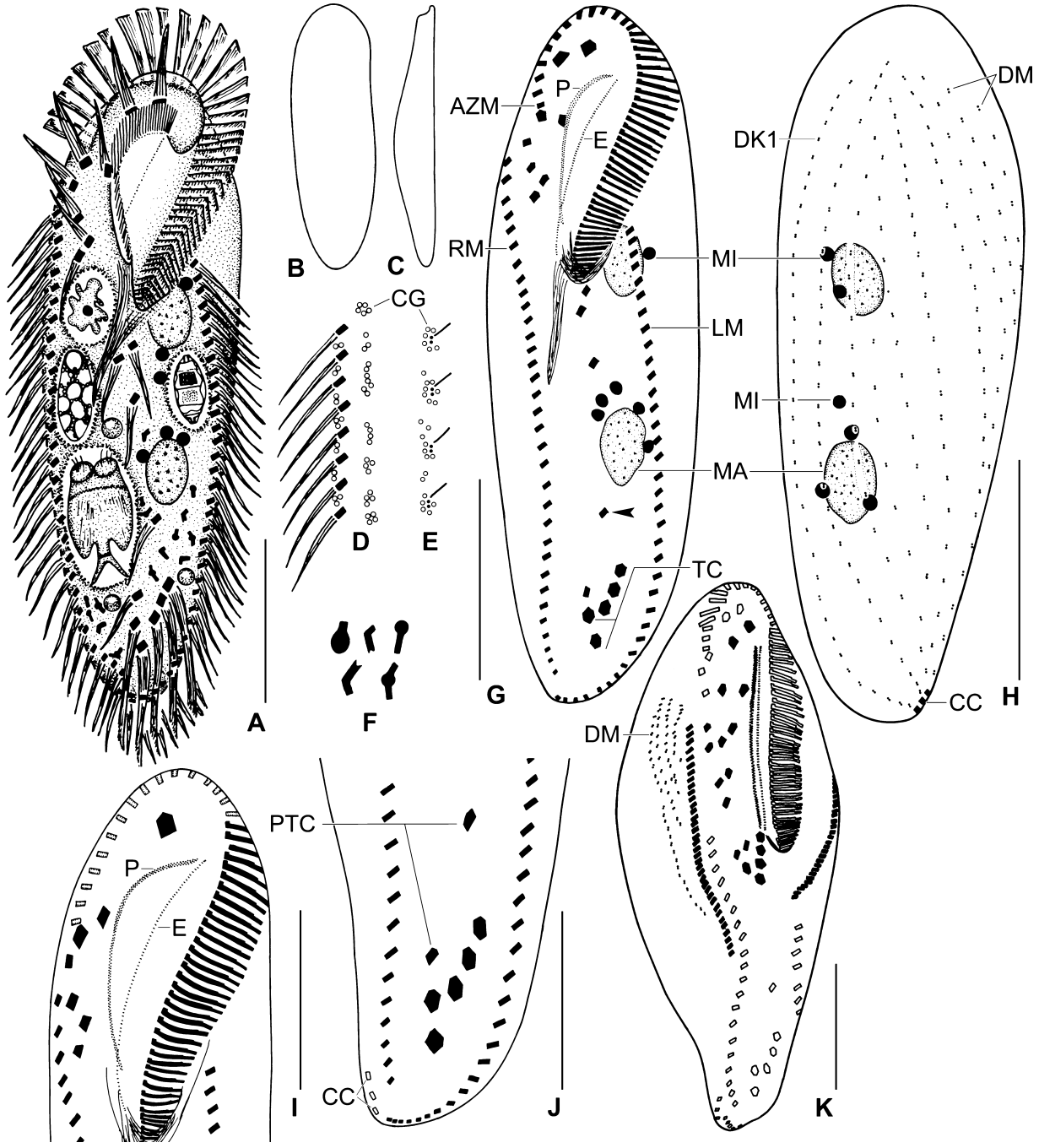

Fig. 1. A–K. *Australocirrus shii*, Australian specimens from life (A–F) and after protargol impregnation (G–K). **A:** Ventral view of a representative specimen, length 190 μm. **B, C:** Ventral and lateral view of same specimen. **D, E:** Cortical granules cluster around bases of cirri (D) and dorsal bristles (E). **F:** Cytoplasmic crystals. **G, H:** Ventral and dorsal view of main voucher specimens, showing infraciliature and nuclear apparatus. Arrowhead in (G) points to the far anteriorly placed cirrus V/2. **I:** A specimen with only two frontal cirri. The paroral membrane is composed of minute ciliary rows in anterior half. **J:** Posterior region, showing arrangement of caudal, marginal and transverse cirri (3 + 2). **K:** A reorganiser, showing formation of dorsomarginal kineties close to right marginal row. AZM, adoral zone of membranelles; CC, caudal cirri; CG, cortical granules; DK1, dorsal kinety 1; DM, dorsomarginal kineties; E, endoral membrane; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; P, paroral membrane; PTC, pretransverse cirri; RM, right marginal row; TC, transverse cirri. Scale bars 40 μm (I–K) and 50 μm (A, G, H).

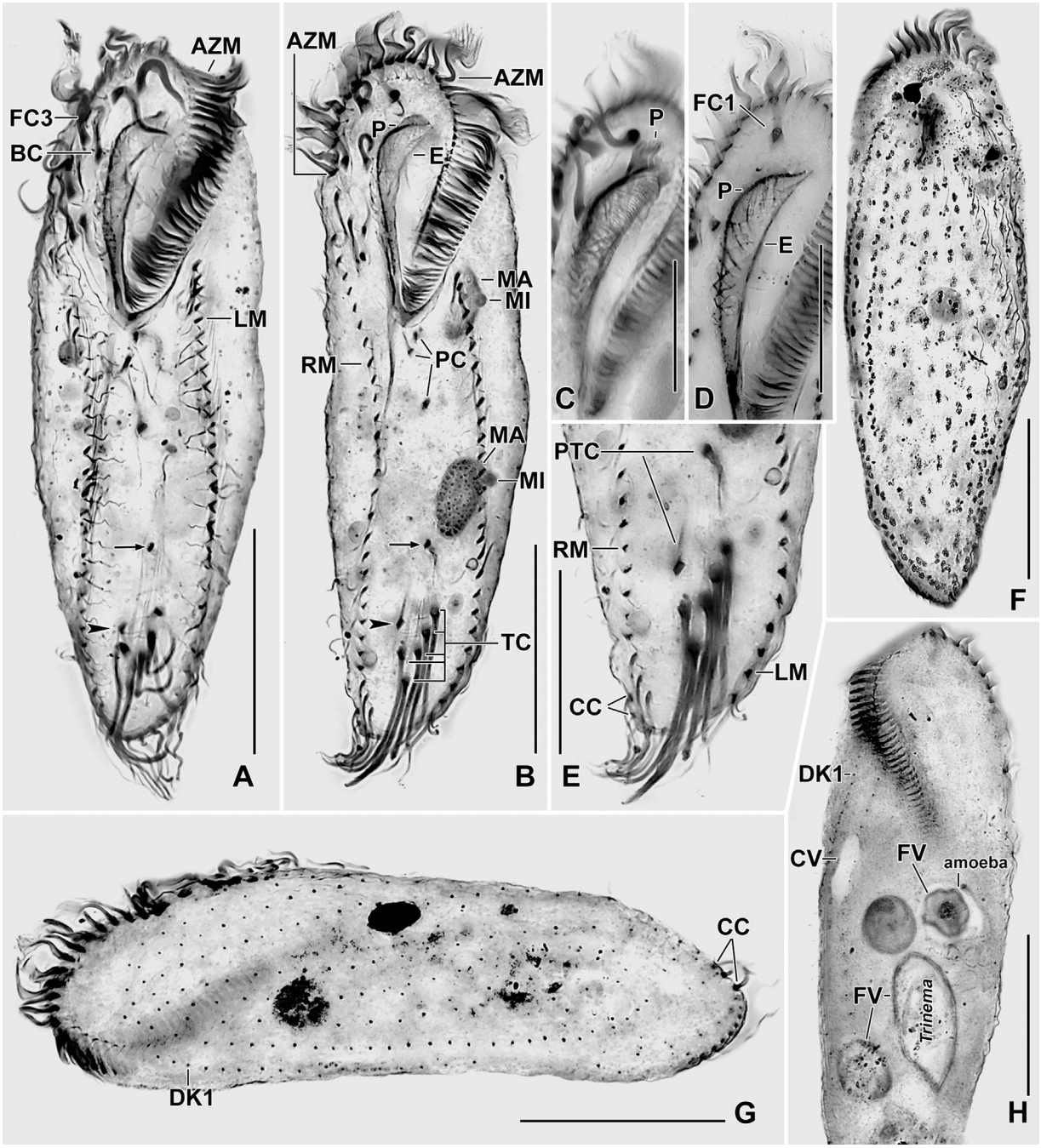

Fig. 2. A–H. *Australocirrus shii*, Australian specimens after protargol impregnation. **A, B:** Ventral views of an ordinary and of a rather slender specimen with adoral zone commencing far posteriorly of anterior body end. Arrows point to cirrus V/2 placed far anteriorly; arrowheads mark posterior pretransverse cirrus. **C, D:** Oral area, showing the paroral cilia only about 8 μm long in protargol preparations (C) and the *Australocirrus* pattern of the paroral membrane. **E:** Posterior region, showing the arrangement of the transverse cirri (3 + 2). Note the caudal cirri at right margin of cell making them difficult to separate from the left marginal cirri. **F:** Dorsal surface, showing the protargol-impregnated cortical granules accumulated around the dorsal bristles. **G:** Dorsal infraciliature of a slightly obovate specimen with broad posterior end. **H:** A specimen studded with food vacuoles. AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; CV, contractile vacuole; DK1, dorsal kinety 1; E, endoral membrane; FC1,3, frontal cirri; FV, food vacuoles; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; P, paroral (cilia) membrane; PC, postoral cirri; PTC, pretransverse cirri; RM, right marginal row; TC, transverse cirri. Scale bars 30 μm (C)–(E) and 50 μm (A, B, F–H).

Table 2. Comparison of main morphometrics of several populations of *Australocirrus shii* and *A.australis*. Distinguishing features bold.

Species	*Australocirrus shii*[a]				*Australocirrus australis*[a]		
Characteristics	Australia Present study	China Shi et al. (1997)	India Singh et al. (2013)	Korea Kim et al. (2012)	Jamaica Present study	Peru Foissner (1995)	Costa Rica Foissner (1995)
Material	Environmental	Culture	Culture	Culture	Environmental	Environmental	Environmental
Protargol method	Foissner (1991)	Shi et al. (1997)	Foissner (1991)	Wilbert (1975)	Foissner (1991)	Wilbert (1975)	Foissner (1991)
Body, length (μm)	169 (29)	175 (20)	138 (20)	252 (10)	145 (21)	285 (15)	179 (15)
Body, width (μm)	50 (29)	63 (20)	51 (20)	103 (10)	41 (21)	97 (15)	68 (15)
Body length: width, ratio	3.4 (29)	2.8[b] (20)	2.7[b] (20)	2.5[b] (10)	3.6 (21)	2.9[b] (15)	2.6[b] (15)
Adoral membranelles, number	50 (21)	52 (20)	50 (20)	57 (10)	48 (21)	57 (15)	48 (15)
Adoral zone, percentage of body length	42 (21)	38 (20)	37 (20)	41[b] (10)	32 (21)	32[b] (15)	35[b] (15)
Anterior transverse cirrus to anterior pretransverse cirrus, percentage of body length	**8.1** (21)	**5.3**[e] (10)	**5.0**[c] (1)	**6.8**[c] (1)	**1.7** (21)	**1.5**[d] (15)	**1.5**[d] (19)
Arrangement of transverse cirri	**3 + 2** (21)	**3 + 2** (2)	**3 + 2** (2)	**3 + 2** (2)	**row** (21)	**row** (15)	**row** (19)
Left marginal row, number of cirri	36 (21)	33 (20)	30 (20)	35 (10)	37 (21)	39 (15)	36 (15)
Right marginal row, number of cirri	31 (21)	31 (20)	29 (20)	28 (10)	33 (21)	37 (15)	34 (15)
Indent at posterior body end	no (21)	no (2)	yes (2)	no (2)	no (21)	no (15)	no (19)
Dorsal kineties, number	10 (21)	8 (20)	9 (20)	8 (10)	10 (21)	8 (?)	7–10 (?)
Macronuclear nodules in resting cyst	**?**	**?**	**separate**[c] **(protargol)**	**?**	**fused (in vivo)**	**?**	**?**

[a]Data based on protargol-impregnated, randomly selected specimens. Number of specimens analysed in parentheses.
[b]Calculated from arithmetic means.
[c]According to the related illustrations.
[d]Data from present study or reinvestigation.
[e]Data from personal communication.

lineare), flagellates, brown fungal spores, and rotifers. In cultures, also on algae (*Chlorogonium elongatum*, Singh et al. 2013), and wheat and rice starch (Kim et al. 2012).

Observations on a Jamaican population of *Australocirrus australis* (Foissner, 1995) nov. comb. (Figs 3A–D, 4A–C, 5A–C, 6A, B, 7A–D, 8A–D; 9A–D; Tables 1 and 2)

1995 *Cyrtohymena australis* Foissner, Arch. Protistenkd. 145, 37–79.
1999 *Cyrtohymena australis* Foissner, 1995 – Berger, Monogr. Biol. 78, 1–1080.
2004 *Cyrtohymena* (*Cyrtohymenides*) *australis* Foissner, 1995 – Foissner, Denisia 13, 369–382.

Improved diagnosis: (includes data from populations of Costa Rica, Peru, and Jamaica; averages are from all populations): Size in vivo 140–265 × 35–95 μm, usually about 185 × 65 μm; elongate ellipsoid or slightly obovate. Two ellipsoid macronuclear nodules and two to nine, usually five micronuclei. Cortical granules bright-citrine, in rough rows, 0.4–1.2 μm across. Anterior pretransverse cirrus 1.5–1.7% of body length away from anteriormost transverse cirrus. Five transverse cirri arranged in an oblique, slightly concave row. Left marginal row on average composed of 36–39 cirri, right of 33–37. Adoral zone occupies 32–35% of body length, composed of an average of 48–57 membranelles. Paroral membrane composed of minute kineties with more than two basal bodies in anterior half. Eight to ten dorsal kineties; usually three narrowly spaced, inconspicuous caudal cirri right of body's midline. Resting cyst smooth, many granules between ectocyst and mesocyst, macronuclear nodules fused.

Remarks: Sizes of Peruvian population studied by Foissner (1995) excluded for the same reason as the Korean population of *A. shii*.

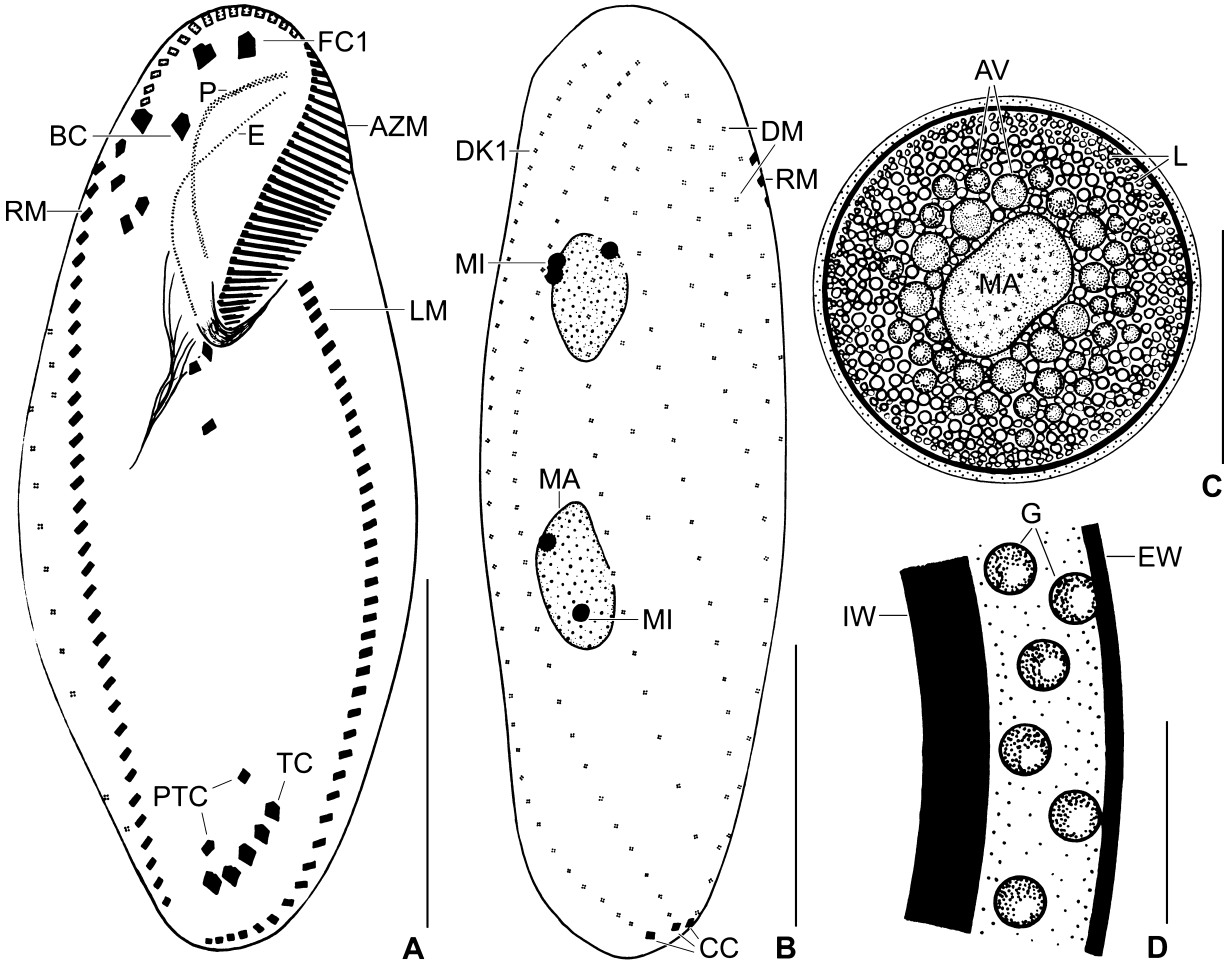

Fig. 3. A–D. A*ustralocirrus australis*, Jamaican specimens after protargol impregnation (A, B) and from life (C, D). **A, B:** Ventral and dorsal view of ordinary specimens, showing the infraciliature and the nuclear apparatus. **C, D:** Overview and detail of resting cyst in optical section. Note the presence of granules between the thin external and thick internal cyst wall. AV, autophagous vacuoles; AZM, adoral zone of membranelles; BC, buccal cirrus; CC, caudal cirri; DK1, dorsal kinety 1; DM, dorsomarginal kineties; E, endoral membrane; EW, external cyst wall; FC1, frontal cirrus 1; G, granules; IW, internal cyst wall; L, lipid droplets; LM, left marginal row; MA, macronuclear nodules or mass; MI, micronuclei; P, paroral membrane; PTC, pretransverse cirri; RM, right marginal row; TC, transverse cirri. Scale bars 2 μm (D), 40 μm (C), and 50 μm (A and B).

Material deposited: Four voucher slides with protargol-impregnated specimens from the Jamaican population have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI). Reg. No. 2015,29-32. Relevant specimens have been marked by black ink circles on the coverslip.

Observations: Most important features of the Jamaican population have an ordinary variability (CV ≤ 15%; Table 1). Of those having higher coefficients, the distance between the macronuclear nodules, the number of micronuclei (3–9, CV ~ 30%), the distance between the anterior pretransverse cirrus and the anteriormost transverse cirrus (CV ~ 45%), and the distance from the lowermost transverse cirrus to body end should be mentioned.

Detailed morphometrics on *Australocirrus australis* from Jamaica are presented in Table 1, and the main characteristics are compared with those from other populations in Table 2. The three populations are similar in most morphological and morphometrical features. Thus, we do not provide a further live drawing but present the first SEM-micrographs of this genus.

Size in vivo 140–195 × 35–70 μm, usually about 165 × 50 μm, as calculated from the morphometric data in Table 1 adding 15% preparation shrinkage (Foissner 2014).

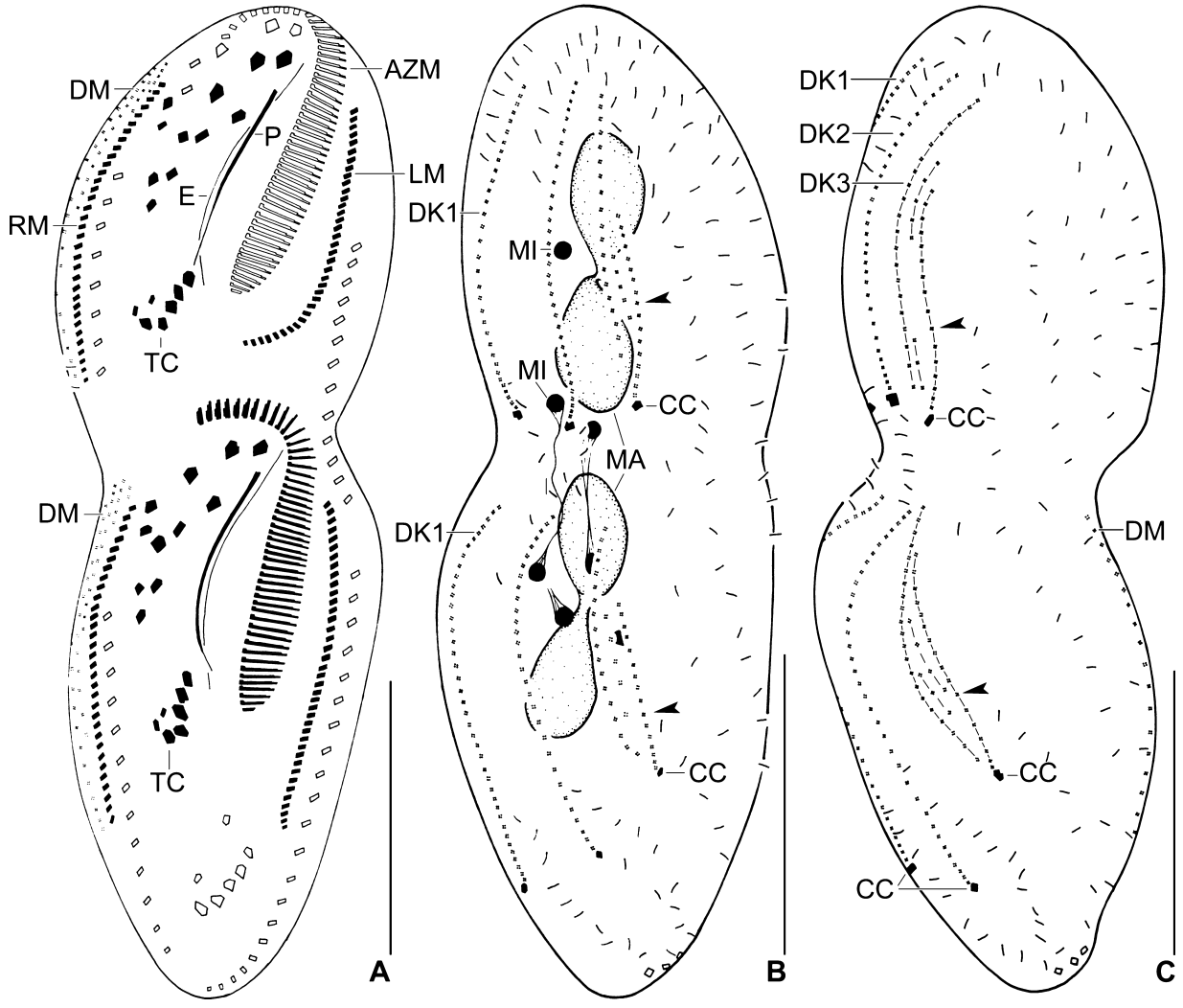

Fig. 4. A–C. *Australocirrus australis*, ventral (A) and dorsal (B, C) view of late dividers from Jamaican population after protargol impregnation; parental structures shown by contour, newly formed structures shadded black. The fronto-ventral-transverse cirri migrate to their specific sites and the dorsomarginal kineties originate close to the right marginal row (A). Dorsal kinety 3 splits into three (B) or four fragments (connected by broken lines in C), the rightmost (arrowheads) producing a caudal cirrus each in proter and opisthe. The macronucleus completes the second division (B). AZM, adoral zone of membranelles; CC, caudal cirri; DK1,3, dorsal kineties, DM, dorsomarginal kineties; E, endoral membrane; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; P, paroral membrane; RM, right marginal row; TC, transverse cirri. Scale bars 50 μm.

Shape as in specimens from type locality, i.e. elongate ellipsoidal or slightly elongate obovate. Macronuclear nodules usually ellipsoid in protargol preparations. Three to nine, on average five globular micronuclei (Figs 3A, B, 5A–C, 6A, B; Table 1).

Cirral pattern as in type population, i.e., usually 18 fronto-ventral-transverse cirri (Figs 3A, 5A–C, 6A; Table 1). Cirri rather thick, except of last marginal cirri; transverse cirri arranged in an oblique, slightly concave row, fringed distally, about 21 μm long in protargol preparations and in SEM, and thus projecting from body proper. Marginal cirri in two non-confluent rows, gradually decrease in size posteriorly; left row extends around posterior margin, composed of an average of 37 cirri, right row extends to body margin posteriorly, composed of an average of 33 cirri (Figs 3A, 5A–C, 6A, 8A, B; Table 1).

On average 10 dorsal kineties with bristles about 3 μm long in protargol preparations and in SEM: kineties 1, 2, 3 bipolar; 5, 6 slightly shortened anteriorly; 4, 7–10 slightly shortened anteriorly and posteriorly (Figs 3B, 6B). Three caudal cirri at right posterior margin of cell and indistinctly separate from left marginal cirri, each composed of about 10 cilia, circa 20 μm long in protargol preparations and in SEM (Figs 3B, 6B, 8C, D; Table 1).

Adoral zone extends about 32% of body length, commences far posteriorly on right margin of cell (about 11% of body length, DE-value 0.33), on average composed of 48 ordinary membranelles. Buccal cavity conspicuous, wide

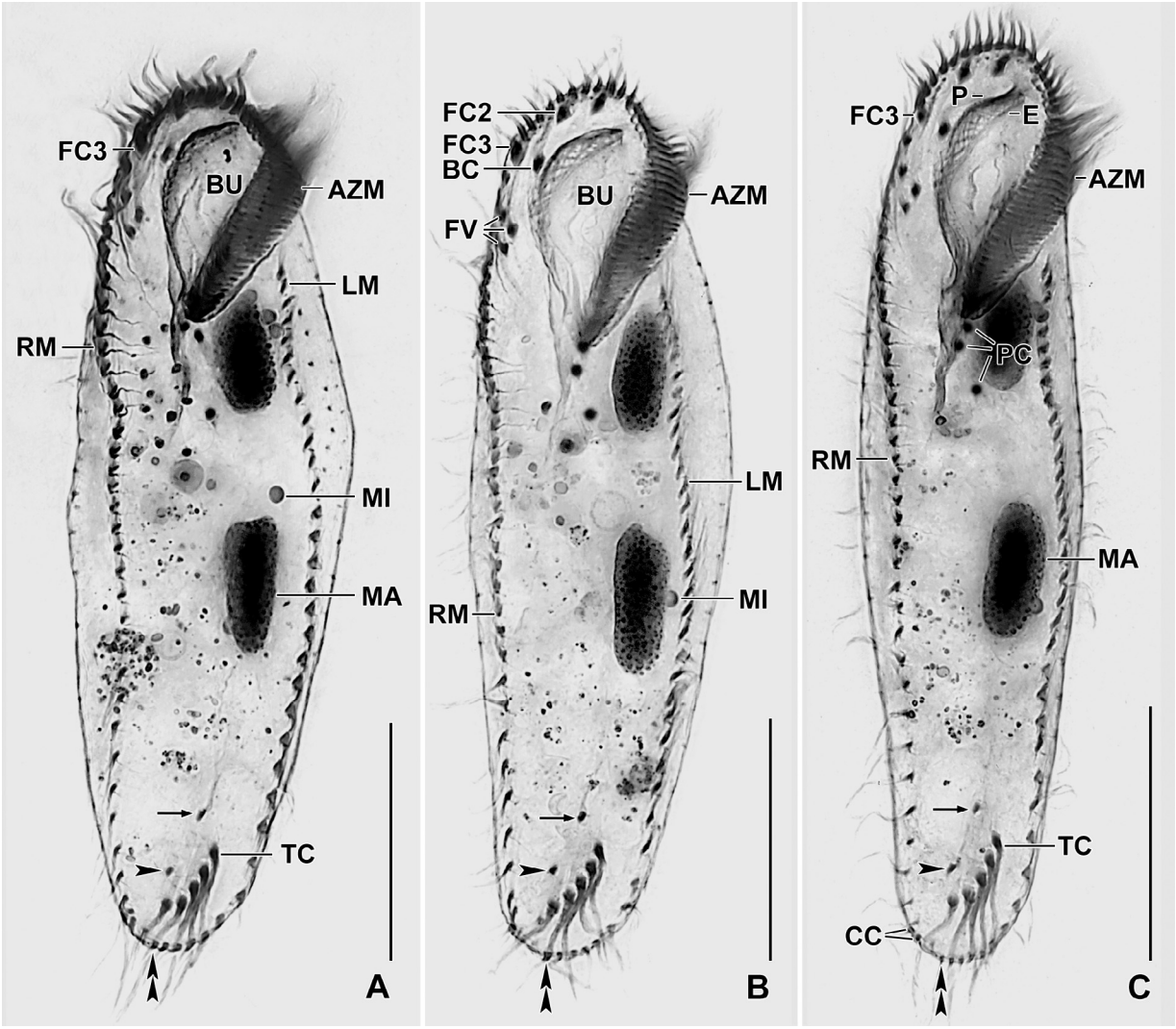

Fig. 5. A–C. *Australocirrus australis*, ventral view of elongate to very elongate (4:1, C) elliptical Jamaican specimens after protargol impregnation. Note the typical 18 fronto-ventral-transverse cirral pattern; the wide and deep buccal cavity; the *Australocirrus* pattern of the paroral membrane; the nuclear apparatus; the anterior pretransverse cirrus (arrows) close to the anteriormost transverse cirrus; the transverse cirri forming a single, oblique group; and the posterior end of the left marginal row (double arrowheads). Arrowheads mark the posterior pretransverse cirrus. AZM, adoral zone of membranelles; BC, buccal cirrus; BU, buccal cavity; CC, caudal cirri; E, endoral membrane; FC2,3, frontal cirri; FV, frontoventral cirri; LM, left marginal row; MA, macronuclear nodules; MI, micronuclei; P, paroral membrane; PC, postoral cirri; RM, right marginal row; TC, transverse cirri. Scale bars 40 μm.

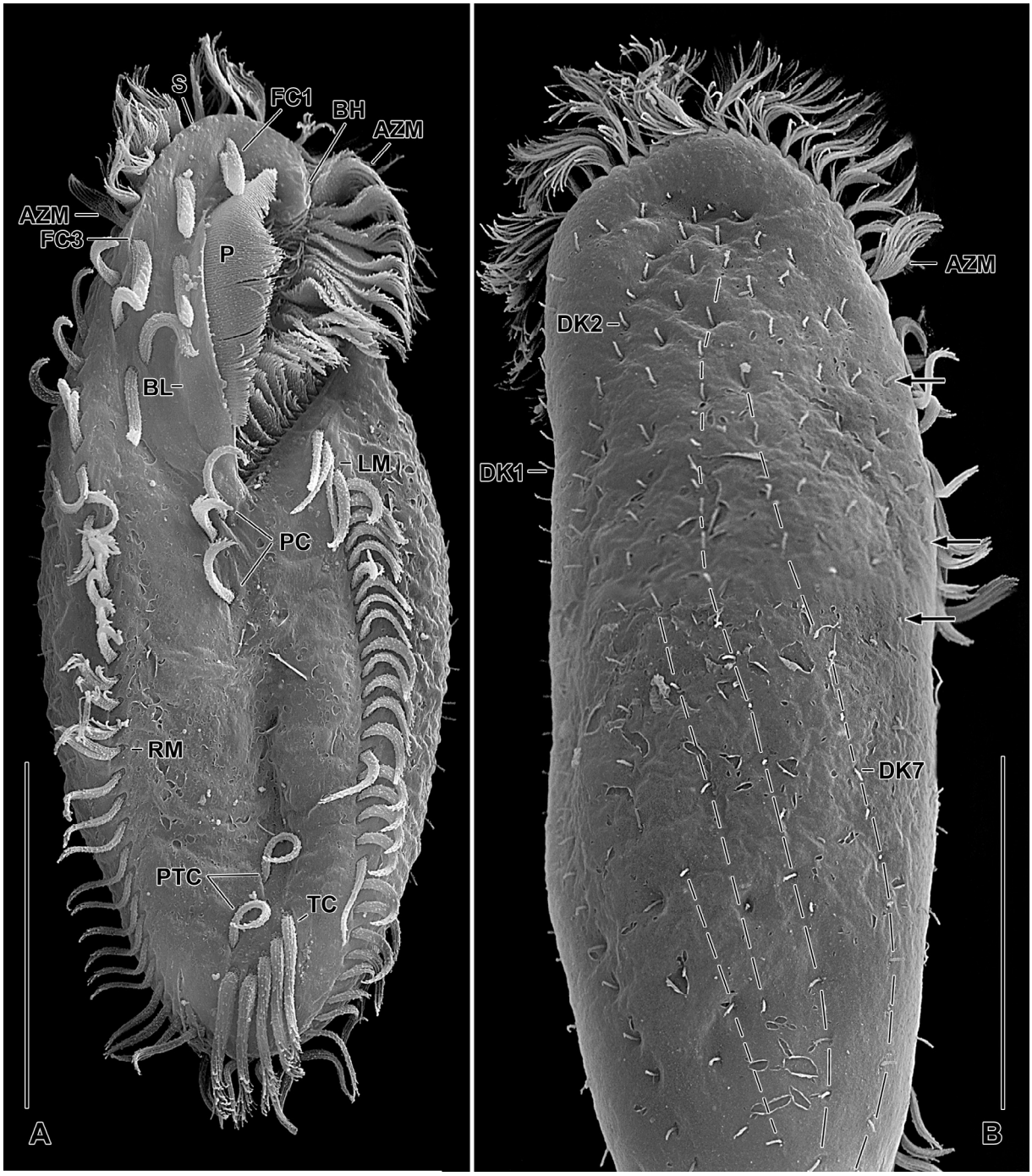

Fig. 6. A B. *Australocirrus australis*, Jamaican specimens in the scanning electron microscope. **A:** Ventral overview, showing the somatic and oral infraciliature as well as the distally fringed transverse cirri forming an oblique slightly concave row. **B:** Dorsal view, showing the kinety fragments (broken lines) resulting from the multiple split of dorsal kinety 3, and the posteriorly shortened dorsomarginal kineties (arrows) which originate close to the right marginal cirral row (see Fig. 4B, C). The adoral zone commences far posterior of the anterior body end. AZM, adoral zone of membranelles; BH, buccal horn; BL, buccal lip; DK1,2,7, dorsal kineties; FC1,3, frontal cirri; LM, left marginal row; P, paroral membrane; PC, postoral cirri; PTC, pretransverse cirri; RM, right marginal row; S, scutum; TC, transverse cirri. Scale bars 50 μm.

and deep with distinct buccal horn and two buccal seals (Fig. 7A–C): upper seal covers buccal cavity and buccal horn; lower seal covers endoral membrane. Buccal lip comparatively narrow and hyaline, covers some proximal membranelles, with a distal cleft from which the paroral cilia emerge. Paroral membrane commences 15 μm posterior of anterior body end, distinctly curved, but not re-curved anteriorly, and composed of minute kineties in anterior half, cilia about 8 μm long in protargol preparations and about 11 μm in SEM; endoral membrane commences slightly

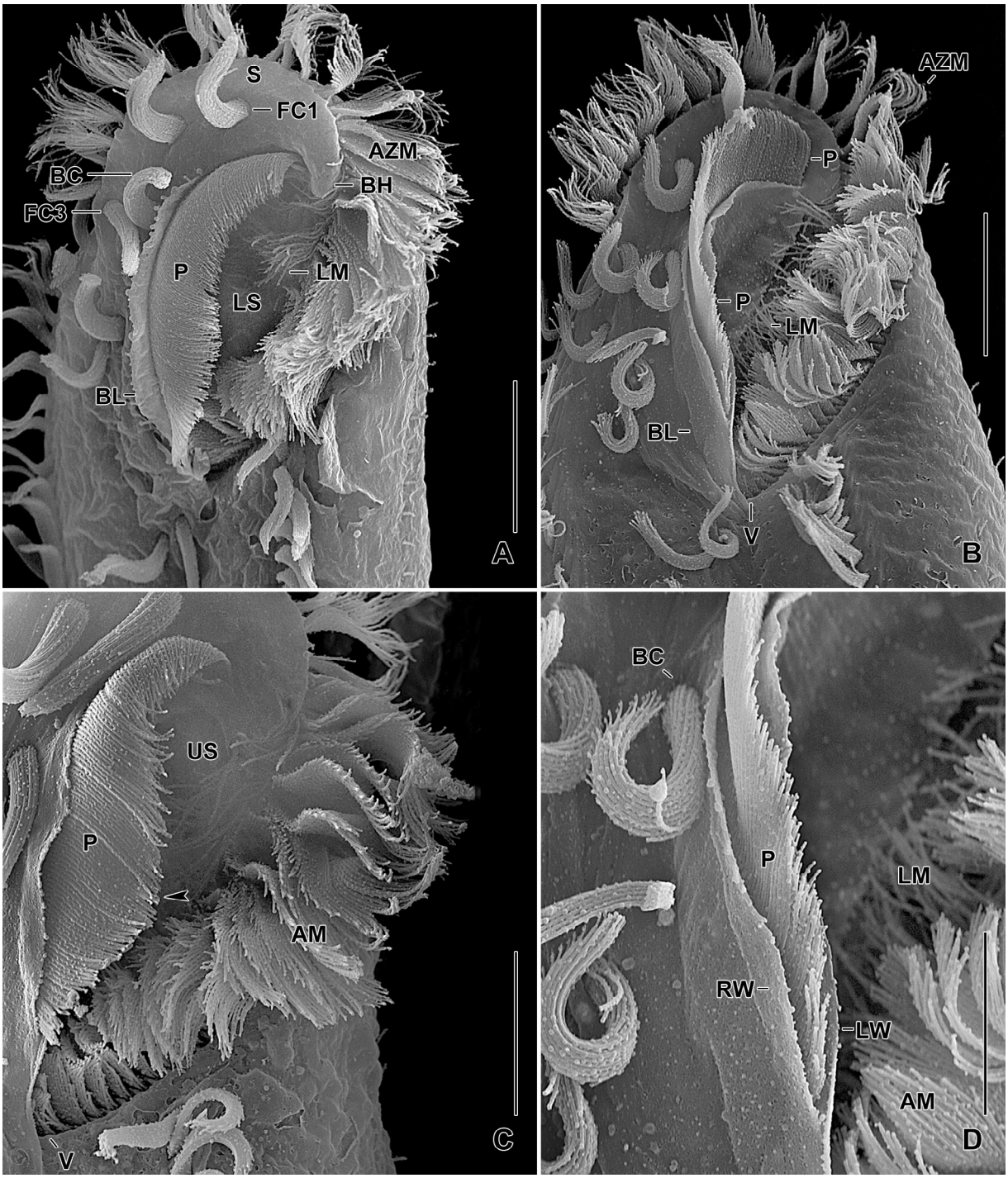

Fig. 7. A–D. *Australocirrus australis*, oral structures of Jamaican specimens in the scanning electron microscope. **A:** Overview, showing the wide buccal cavity, the conspicuous buccal horn, the almost intact lower buccal seal which covers the endoral membrane and the posterior part of the lateral membranellar cilia. **B, D:** This specimen shows the rather narrow buccal lip and the partially destroyed buccal seal exposing the lateral membranellar cilia. The buccal lip has a cleft from which the paroral cilia emerge. **C:** A specimen with intact upper buccal seal covering the buccal horn and the lateral membranellar cilia. The arrowhead points to the longest paroral cilia. AM, adoral membranelles; AZM, adoral zone of membranelles; BC, buccal cirrus; BH, buccal horn; BL, buccal lip; FC1,3, frontal cirri; LM, lateral membranellar cilia; LS, lower buccal seal; LW, left wall of buccal lip; P, paroral membrane; RW, right wall of buccal lip; S, scutum; US, upper buccal seal; V, buccal vertex. Scale bars 10 μm (D), 15 μm (C), and 20 μm (A and B).

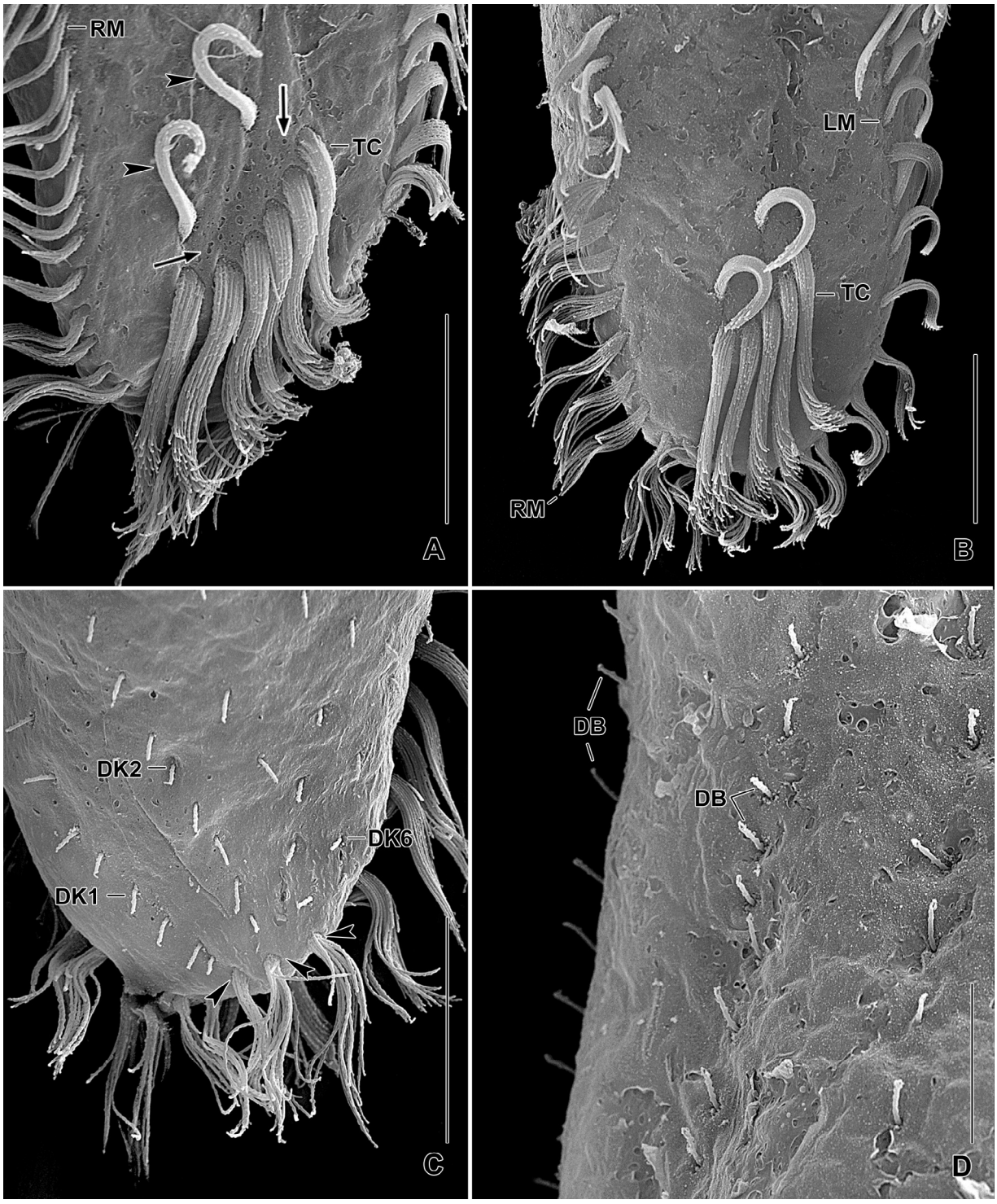

Fig. 8. A–D. *Australocirrus australis*, Jamaican specimens in the scanning electron microscope. **A:** A specimen with six transverse cirri arranged in an oblique slightly concave row and fringed distally; arrowheads mark the pretransverse cirri, arrows denote the cortex holes after the release of cortical granules. **B:** As (A) but a specimen with the usual five transverse cirri. **C:** Posterior dorsal region with arrowheads denoting the caudal cirri at the end of the first, the second, and the sixth dorsal kinety. **D:** Dorsal bristles. DB, dorsal bristles; DK1, 2,6, dorsal kineties; LM, left marginal row; RM, right marginal row; TC, transverse cirri. Scale bars 10 μm (D) and 20 μm (A–C).

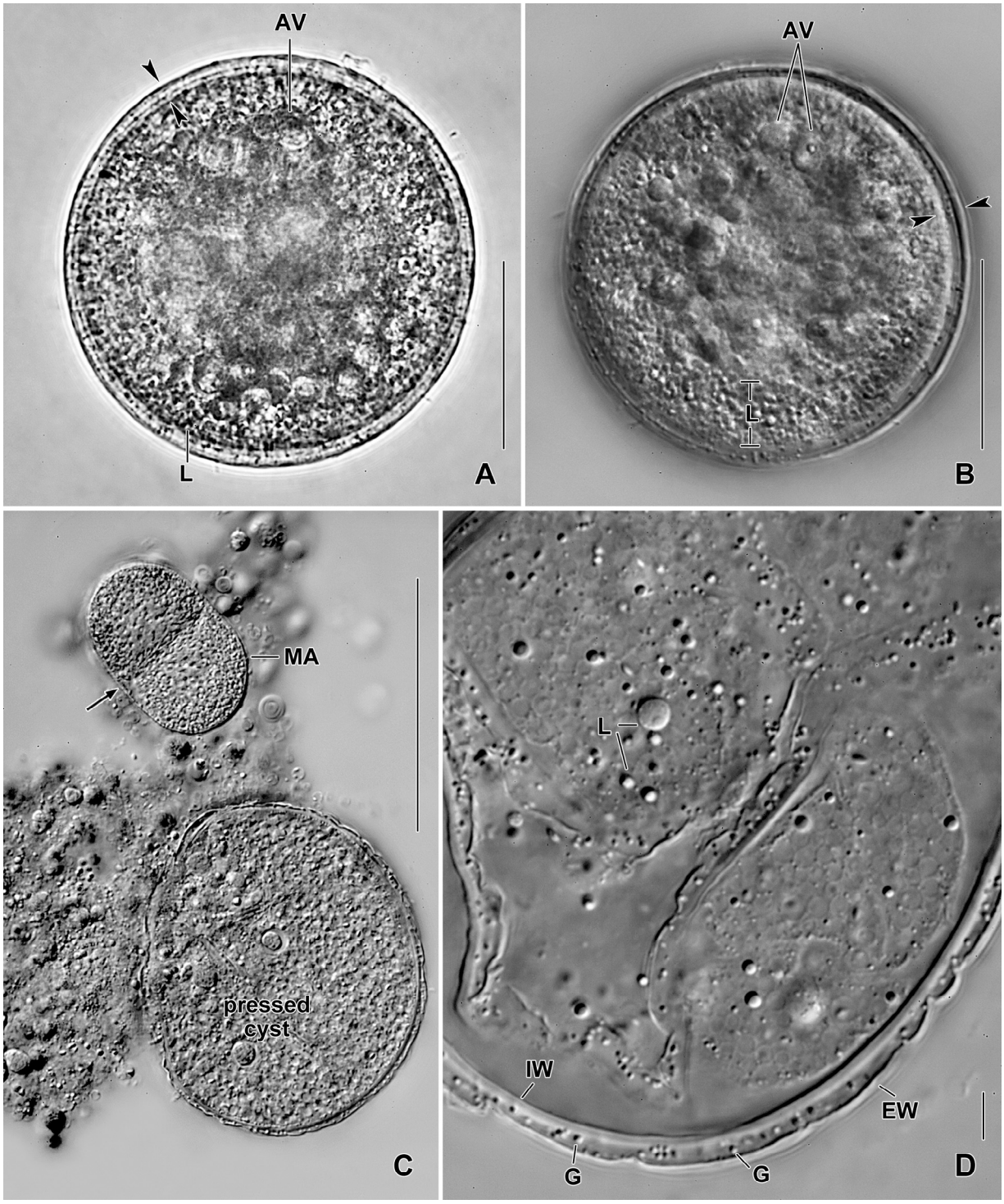

Fig. 9. A–D. *Australocirrus australis*, resting cysts of Jamaican specimens from life. **A, B:** Optical section of cysts in the bright field and interference contrast microscope. The cyst wall is marked by opposed arrowheads. The globular inclusions are very likely autophagous vacuoles covered by a thick zone of small lipid droplets. **C:** When slightly squeezed with the coverslip the fused (arrow) macronuclear nodules and some cytoplasm leave the cyst. **D:** Squashed cyst, showing minute granules between the thin external (ectocyst?) and the thick (meso- and endocyst?), moderately refractive internal wall. AV, autophagous vacuoles; EW, external cyst wall; G, granules; IW, internal cyst wall; L, lipid droplets; MA, macronucleus. Scale bars 5 μm (D), 30 μm (A and B), and 50 μm (C).

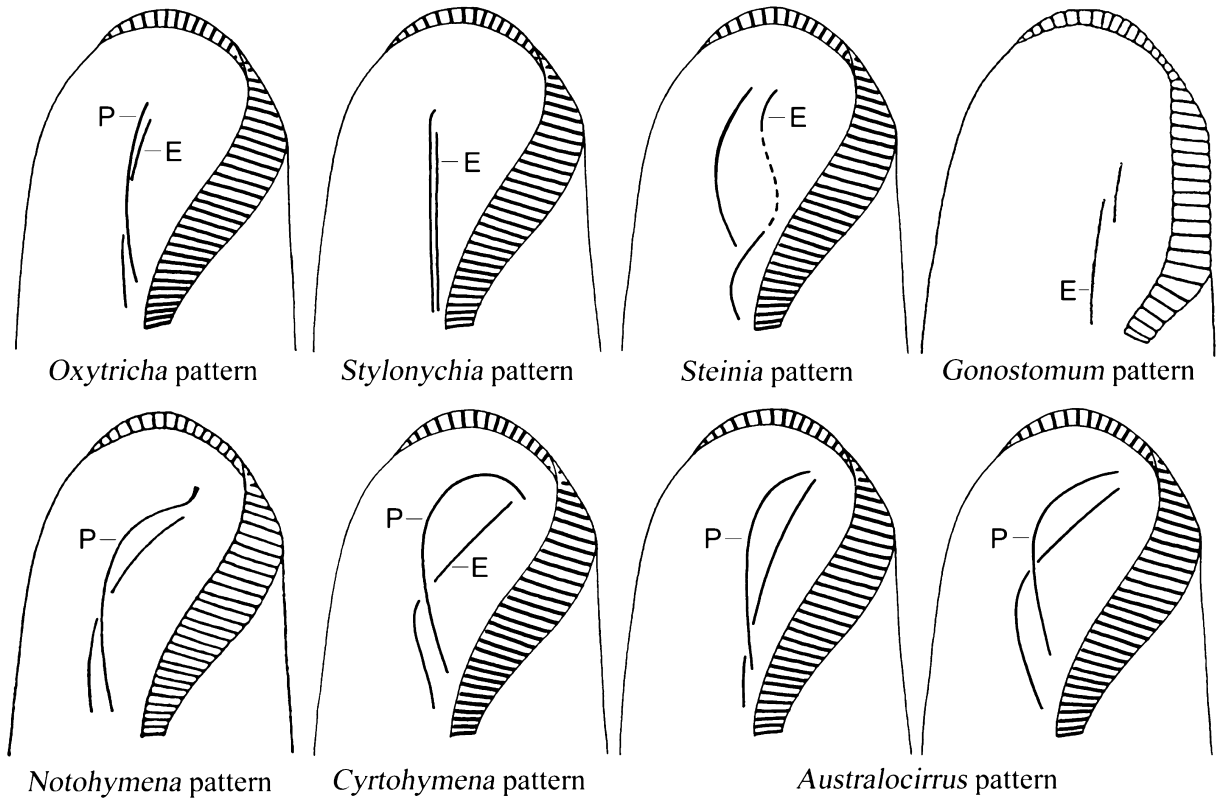

Fig. 10. Scheme of the undulating membranes in various oxytrichids, originals and modified from Foissner (1989) and Berger and Foissner (1997). *Oxytricha* pattern: paroral and endoral slightly curved and intersecting optically; *Stylonychia* pattern: paroral and endoral straight or slightly curved and side by side; *Steinia* pattern: paroral curved and middle portion of endoral fragmented; *Gonostomum* pattern: paroral consists of few, widely spaced cilia and extends far anteriorly of endoral; *Notohymena* pattern: paroral with hooked distal end; *Cyrtohymena* pattern: paroral and endoral intersecting optically, paroral semicircular because recurved distally; *Australocirrus* pattern: paroral moderately to distinctly curved but not recurved distally. E, endoral membrane; P, paroral membrane.

posterior and left of anterior end of paroral membrane and extends to buccal vertex, optically intersects with paroral slightly posterior of buccal cirrus. Pharyngeal fibres comparatively short and thin (Figs 3A, 5A–C, 6A, 7A–D; Table 1).

Resting cyst: Cysts 60–80 μm across in vivo, on average 66 μm (Table 1); slightly to distinctly yellowish due to a yellowish fluid in cytoplasm. Cyst wall smooth and bipartite: internal wall (meso- and endocyst?) moderately refractive, about 2 μm thick (3 μm in pressed cells), external wall (ectocyst?) fragile, about 0.5 μm thick. Refractive granules 0.5–1.0 μm in size between internal and external wall. Cyst contents yellowish bipartite, central area with fused macronuclear nodules and many autophagous vacuoles 3–7 μm across; marginal area studded with lipid droplets 0.5–2.0 μm across (Figs 3C, D, 9A–D; Table 1).

Notes on morphogenesis: The oral primordium originates close to the anteriormost transverse cirrus (data not shown). The developing oral primordium incorporates cirrus V/3. The dorsal morphogenesis is similar to that of *Australocirrus shii*, i.e., shows multiple fragmentation of dorsal kinety 3 and more than three dorsomarginal kineties (Fig. 4A–C).

Occurrence and ecology: As yet found in the Amazonian rain forest near to the town of Iquitos, Peru, 4° S 74° W; in a tropical dry forest of the Santa Rosa National Park about 5 km east of the ranch "La Casona", near a path to the Pacific Ocean, Costa Rica; in a saline sample collected in a garden in the town of Salina, Curaçao (Foissner 1995); and in mud from tree holes and mosses of Jamaica (exact site not known because label disappeared; present study). Feeds on testate amoebae (*Trinema lineare*, *Euglypha* spp.), fungal spores, ciliates (*Leptopharynx costatus*, *Drepanomonas pauciciliata*), and cysts of *Polytoma* sp. (Foissner 1995).

Discussion

The new *Australocirrus* paroral pattern

Six undulating membrane patterns have been defined in oxytrichid ciliates (Fig. 10): *Oxytricha* pattern, *Stylonychia* pattern, *Steinia* pattern, *Gonostomum* pattern, *Notohymena* pattern, and *Cyrtohymena* pattern (Berger and Foissner 1997; Berger, 1999; Shao et al. 2015). Our observations and

literature data show a seventh pattern which has been confused with the *Cyrtohymena* pattern several times because the paroral is more or less curved and the large and deep buccal cavity in vivo usually rather distinctly curved anteriorly. The new *Australocirrus* pattern differs from the *Cyrtohymena* pattern in that the distal region of the paroral is not recurved and thus not semicircular. In a broader view, the *Australocirrus* pattern is an enlarged *Oxytricha* pattern found, e.g., also in *Sterkiella cavicola* (Foissner et al. 2002), *Pattersoniella* Foissner, 1987, and in *Apoterritricha* Kim et al., 2014. Recently, Shao et al. 2015 provided a very helpful review on the cirral and oral patterns of 18 typical oxytrichid genera; however, they did not separate the *Oxytricha* pattern from the *Australocirrus* pattern.

Proposed synonymy of the genus *Australocirrus* Blatterer and Foissner, 1988 and the subgenus *Cyrtohymenides* Foissner, 2004

Foissner (2004) mentioned that the sole difference between *Australocirrus* and *Cyrtohymenides* is the undulating membrane in *Oxytricha* vs. *Cyrtohymena* pattern; later, the diagnosis was improved by Singh et al. (2013). The overall appearance of the oral apparatus of *Cyrtohymenides* resembles a mixture of the *Oxytricha* and the *Cyrtohymena* pattern: the buccal cavity is large and deep and the paroral membrane is distinctly curved but not recurved anteriorly, as typical for *Cyrtohymena* (Fig. 10) and *Rigidohymena candens* (Kahl, 1932) Berger, 2011 (formerly *Cyrtohymena candens*, see Berger 1999, 2011). Shao et al. (2012) transferred *Oxytricha shii* to *Cyrtohymena* (*Cyrtohymenides*) and Singh et al. (2013) provided a description of an Indian population; none discussed the shape similarity of the undulating membranes in *Australocirrus* and *Cyrtohymenides*. Our data show that both share a very similar shape and are thus synonymous. The molecular analysis provided by Singh et al. (2013) shows a close relationship of *Cyrtohymena* and *Australocirrus*, while that of Jung et al. (in press) relates *Australocirrus* more closely to *Neokeronopsis* which has the paroral in *Australocirrus* pattern. This suggests that the shape of the paroral membrane has some phylogenetic significance although it evolved convergently several times, for instance, the *Cyrtohymena* pattern in *Cyrtohymena* Foissner, 1989 and in *Rigidohymena* Berger, 2011 (Jung et al. in prees).

Foissner (2004) suggested raising *Cyrtohymenides* to genus level if ontogenesis shows fragmentation of dorsal kineties 1 and 2, and parental dorsal kinetids are preserved. However, he mentioned that multiple fragmentation of dorsal kineties 1 and 2 could be a preparation artefact. This is supported by the data on morphogenesis in *Australocirrus shii* (Shi et al. 1998; Singh et al. 2013) and *A. australis* (present study), which show fragmentation only in dorsal kinety 3 and no retention of parental dorsal kinetids. This corroborates the conclusion above that *Cyrtohymenides* falls into synonymy with *Australocirrus* at the present state of knowledge.

Comparison of populations and species

Most of the morphometric characteristics of *Australocirrus shii* and *A. australis* overlap suggesting that they could belong to a single species (Table 2). However, on thorough observation we found three features that separate these populations unequivocally (Table 2): the distance between the anterior pretransverse cirrus and the anteriormost transverse cirrus (5–8% vs. 1.5–1.7% of body length); the arrangement of the transverse cirri (3 + 2 vs. an oblique row); and the resting cyst macronuclear nodules (separate vs. fused). The first two features can also be observed in the micrographs of the Korean population, indicating that it belongs to *A. shii* (Kim et al. 2012).

The DE-value (for *D*istal *E*nd of adoral zone), a feature first used by Wicklow (1981) to define the Keronopsidae, was later quantified by Wiackowski (1988) in dividing the distance between the anterior body end and the distal end of the adoral zone by the distance between the anterior body end and the proximal end of the adoral zone. He distinguished two states: up to 0.10 (designated as plesiomorphy) and 0.11–0.28 and more (increasingly derived). Low DE-values have been reported for some taxa (e.g. *Gonostomum*) while they are usually very high in, e.g., the Retroextendia (Berger 2006). The DE-value is 0.4 for *Australocirrus shii* and 0.3 for *A. australis* which corroborates the high DE-values and the close molecular relationship of *Neokeronopsis* and *Cyrtohymena*.

The Costa Rican and the Jamaican population of *Australocirrus australis* differ from the Peruvian type population by the smaller body size and the number of adoral membranelles (48 vs. 57) and cirri in the right marginal row (33 vs. 39) as well as in the length of the macronuclear nodules (20 vs. 43 μm). The higher number of adoral membranelles and marginal cirri indicate that the Peruvian specimens are indeed slightly larger than those from Costa Rica and Jamaica. In contrast, the differences in body and macronuclear size are very likely mainly caused by the different protargol methods used: while the Wilbert (1975) method tends to inflate the specimens they shrink with the Foissner (1991, 2014) method (Table 2).

Australocirrus aspoecki is easily separated from *A. shii* and *A. australis* by the colour of the cortical granules (brilliant red vs. bright citrine). *Australocirrus oscitans* and *A. zechmeisterae* differ mainly by the number of macronuclear nodules (2 vs. 4).

Notes on biogeography

Reliable data on the distribution of heterotrophic protists are very rare and have been controversially discussed (for a review, see Foissner 2006). *Australocirrus australis* is very likely widely distributed in wet soils and mosses of Central and South America while the records of *Australocirrus shii* indicate a Paleotropic and Australic distribution; however, we would not be surprised to find it also in Europe, where

only the red *A. aspoecki* Foissner, 2004 has been recorded, an eye-catching species with large body size (in vivo usually 300 × 140 μm) and red cortical granules.

Type slides

Our efforts to obtain additional data were successful only for the Chinese population of *Australocirrus shii* while the describers of the Korean and the Indian population of *A. shii* did not provide the lacking data or slides so that we could do the analysis. Thus, type and voucher materials should be deposited in an international repository from which researchers can loan it, as recommended by the International Code of Zoological Nomenclature. In addition, the accession numbers should be mentioned.

Acknowledgements

Financial support was provided by the Austrian Science Fund (FWF Project to W.F., P26325_B16). The technical assistance of Johannes Rattey M.Sc., Michael Gruber M.Sc., Robert Schörghofer, and Andreas Zankl is greatly acknowledged. We thank Dr. habil. Klaus W. Wolf (Electron Microscopy Unit at the University of the West Indies, Kingston, Jamaica) for help with sampling in Jamaica. We also thank Prof. Weibo Song (Laboratory of Protozoology, Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, China) for providing data on the Chinese population of *Australocirrus shii*.

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