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A Reinvestigation of *Neokeronopsis* Populations, Including the Description of *N. asiatica* nov. spec. (Ciliophora, Hypotricha)

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Summary. We investigated the status of various *Neokeronopsis* populations, using protargol-impregnated type material, a new Chinese population, and literature data. This resulted not only in the recognition of a new species, *Neokeronopsis asiatica*, but also in upgrading *Afrokeronopsis* from subgenus to genus level. The genera *Neokeronopsis* and *Afrokeronopsis* differ mainly in the buccal depression (absent vs. present) and in the midventral cirri between proter and opisthe, which are either retained (*Afrokeronopsis*) or transformed into cirral anlagen (*Neokeronopsis*). *Neokeronopsis asiatica* nov. spec. differs from *N. spectabilis* (Kahl, 1932) by the following features: body size (~ 300 × 120 µm vs. 400 × 170 µm); posterior body end (acute with distinct indentation at site of caudal cirri vs. broadly rounded and without or indistinct indentation); posterior end of marginal rows (ending at different vs. same or similar level); dorsal kinety 1 (continuous vs. fragmented); and the size of the bases of the adoral membranelles (largest membranelles on average 18 µm vs. 29 µm wide). Improved diagnoses are provided for the family Neokeronopsidae and the genera contained therein, viz., *Neokeronopsis*, *Afrokeronopsis*, and *Pattersoniella*. Our study shows the importance of depositing type and voucher material in recognized repositories. Only this will allow future researchers to restudy the populations, for the sake of improved taxonomic and biogeographic knowledge.

Key words: *Afrokeronopsis* nov. stat., biodiversity, biogeography, *Pattersoniella*, type material.

INTRODUCTION

Keronopsis spectabilis, discovered by Kahl (1932) in a pond in the surroundings of the town of Hamburg (Germany), received some attention during the past decade. The investigation of a Polish population of this species from a small stream resulted in the establishment of a new genus, *Neokeronopsis* Warren *et al*., 2002, with *N. spectabilis* as the type species. In his monograph of the Urostyloidea, Berger (2006) reinvestigated *N. spectabilis*, adding valuable observations from populations of two Austrian rivers. Almost concomitantly, Wang *et al.* (2007) described ontogenesis in a population identified as *N. spectabilis* from a pond in northern China. Finally, Foissner and Stoeck (2008) added a new species, *Neokeronopsis* (*Afrokeronopsis*) *aurea*, from subtropical Africa and established the family Neokeronopsidae to include *Pattersoniella* Foissner, 1987 and the sub-

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genera *Neokeronopsis* and *Afrokeronopsis*. Establishing a new family for these conspicuous ciliates was supported by the CEUU hypothesis (convergent evolution of a midventral cirral pattern in urostylid and oxytrichid hypotrichs, Foissner *et al*. 2004) and the molecular data, which showed that *N.* (*A*.) *aurea* is an oxytrichid with midventral (urostyloid) cirral pattern.

The Neokeronopsidae Foissner and Stoeck, 2008 contain large, yellow-coloured flagship species useful for the investigation of the geographic distribution of micro-organisms in general (Foissner 2006, Foissner *et al.* 2008). Thus, they must be described very carefully to exclude misidentifications, which are so frequent in protists. Indeed, Foissner and Stoeck (2008) recognized several differences between the Asian and European *Neokeronopsis spectabilis* populations, and thus suggested that they might not be conspecific.

In the present study, we reinvestigated and compared all protargol-impregnated and described populations of *Neokeronopsis,* as well as a population from China that had previously been reported only briefly (Shi and He 1990). This resulted not only in the recognition of a new species, *Neokeronopsis asiatica*, but also in upgrading *Afrokeronopsis* to genus level. Our study shows also the importance of depositing type and voucher slides in recognized repositories, such as museums. Only this will allow future researchers to restudy the populations.

MATERIALS AND METHODS

We studied or re-studied four *Neokeronopsis* populations, all impregnated with protargol. Live observations were available only for *N.* (*Afrokeronopsis*) *aurea* Foissner and Stoeck, 2008.

Materials

Neokeronopsis spectabilis, as described by Warren *et al.* (2002): The five original slides, which are deposited in the Natural History Museum, London, with registration numbers 2005:10:10:1–5, were re-studied. About 60 specimens are distributed over the five slides; those illustrated in Warren *et al.* (2002) have not been marked. The population was from a small stream in southern Poland. The cells, which were from short-term semipure cultures, have been prepared with Wilbert's method, where specimens tend to become inflated. There were at least 30 excellently prepared cells, of which we selected the 15 most naturally looking ones for size measurements, i.e., those which were very likely not or inconspicuously inflated.

Neokeronopsis spectabilis, as described by Wang *et al.* (2007): The original slides were re-studied. They contained about 130 excellently-impregnated specimens, about half of which were dividers. This population was isolated from a freshwater pond in a suburb of the town of Harbin in northern China. Pure cultures were set up and the cells impregnated with a modification of the French protargol method, which preserves shape and size very well (Shi and Frankel 1990). Only part of the 20 slides were labeled with artificial ink and in Chinese language; those shown in the publication of Wang *et al.* (2007) have not been marked. We re-labeled the slides in red from I–XX. This population will be type of *N. asiatica* described below. The holotypes, paratypes, and the ontogenetic stages shown in the present paper have been marked with black Indian ink on the coverslip, and all slides have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI).

The third *Neokeronopsis* population is, like the second, from a pond in the surroundings of the town of Mao-Er-Shan (Morshan Town) about 100 km from the town of Harbin, northern China, 45°45´N 120°41´E. It has been briefly described in an abstract (Shi and He 1990). The cells of the 15 slides, which are labeled "*Holosticha (Keronopsis) spectabilis*," were collected and protargol-impregnated by Shi and He in 1988, using a variation of the French method (Shi and Frankel 1990). The slides contain about 180 cells, half of which are dividers, showing the complete ontogenesis. We re-studied the slides and added a label to the first slide, noting that this population is now classified in *N. asiatica*. All slides have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI).

Data for *Neokeronopsis (Afrokeronopsis) aurea* were taken from Foissner and Stoeck (2008). Additional investigations were performed on the slides kept in the Salzburg laboratory, the type slides having been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI).

Morphological methods and terminology

Counts and measurements on silvered specimens were performed at a magnification of × 1,000. The drawings were made with a drawing device. In the ontogenetic stages, parental structures were shown by contour, while newly formed structures were shaded black.

Terminology is according to Foissner and Stoeck (2008). See also Berger (2006), Foissner and Al-Rasheid (2006), and Lynn (2008).

RESULTS

Reinvestigation of Polish population of *Neokeronopsis spectabilis* (Figs 1, 5–9)

Foissner and Stoeck (2008) already investigated the protargol-prepared Polish specimens of *N. spectabilis*, but compared it mainly with *Afrokeronopsis aurea*. These investigations showed some significant differences to the data of Warren *et al.* (2002), and the present study added two more. Here, we summarize all divergent or new observations important for distinguishing *N. spectabilis* from *N. asiatica*, and also *Neokeronopsis* from *Afrokeronopsis*.

(i) The morphometrics provided by Warren *et al.* (2002, Table 1), such as body length and width as well as

the number of marginal and transverse cirri, correspond to our measurements and counts (Tables 1, 2). However, a considerable difference occurs in the size of the bases of the largest adoral membranelles, which are 20–25 µm wide according to Warren *et al.* (2002, p. 199), while 22–35 µm, on average 29 µm wide according to our measurements (Tables 1, 2). This is an unfortunate discrepancy because the width of the membranellar bases is one of the most important differences among *N. spectabilis*, *N. asiatica*, and *A. aurea* (Tables 1, 2).

(ii) The paroral membrane does not consist of dikinetids throughout, as stated by Warren *et al.* (2002), but forms short, oblique kineties in the main part, quite similar to those in *N. asiatica* (Figs 2, 4, 10, 23) and *Pattersoniella vitiphila* Foissner, 1987. The paroral membrane of *A. aurea* consists of short kineties throughout (Foissner and Stoeck 2008).

(iii) There is no indication of a buccal depression in the Polish and Austrian (Berger 2006) populations of *N. spectabilis* or in the Chinese populations of *N. asiatica*, even in underbleached, rather darkly impregnated cells with well-preserved buccal cavity. Furthermore, the anterior ends of the paroral and endoral membranes are very near together (≤ 1 µm) in most specimens, hardly leaving space for a buccal depression similar to that found in *A. aurea* (Figs 1, 2, 4, 5).

(iv) Except for a single specimen, which looks like that illustrated in Warren *et al.* (2002, Fig. 5), all well preserved cells show multiple fragmentation of dorsal kinety 1, especially in the posterior half, while only one long or no fragment is present in the anterior half of kineties 1–3 (Figs 6–8). In contrast, *N. asiatica* shows fragmentation very rarely and only in the anterior region of kineties 2 and 3 (Figs 11, 12). *Afrokeronopsis aurea* shows pronounced anterior fragmentation, especially of kinety 1 (Foissner and Stoeck 2008).

(v) The few dividers contained in the slides are not in a stage to show the whirl formation in dorsal kinety 3, so characteristic for *A. aurea* (Foissner and Stoeck 2008).

(vi) Warren *et al.* (2002) illustrate parental midventral cirri between the cirral anlagen fields (Fig. 9). Later, these are resorbed. This matches the findings of Foissner and Stoeck (2008) for *A. aurea*, but disagrees with the observations of Wang *et al.* (2007), who did not comment on this matter, and the present study which shows that these cirri contribute to cirral anlagen formation in *N. asiatica* (Figs 16–27). We re-examined the two specimens illustrated by Warren *et al.* (2002) in their Figures 6 and 8. Unfortunately, the impregnation was so poor in the area concerned that we could not decide whether cirri are present or absent. As there is no indication that the specimens have bleached, one must assume that these cirri have been added, possibly because they are maintained in some urostylids (Berger 2006).

Description of *Neokeronopsis asiatica* nov. spec. (Figs 2–4, 10–38; Tables 1, 2)

Diagnosis: Size about 290 × 120 µm in protargol preparations. Oblanceolate with more or less distinct indentation at site of caudal cirri. Two ellipsoidal macronucleus nodules. Cortical granules present. Midventral rows composed of an average of 14 pairs of cirri. Frontal cirral corona and pseudobuccal row composed of 18 cirri on average; pseudobuccal cirral row curves away from right margin of buccal cavity. On average 18 transverse cirri, forming a J-shaped row in rear body half. Marginal cirral rows terminating posteriorly at different levels, i.e., right row ends above left row. Dorsal kinety 1 continuous (not fragmented). Adoral zone impressive because occupying about 38% of body length and composed of an average of 81 membranelles with bases of longest membranelles about 18 µm wide. Anterior ends of undulating membranes almost abutting.

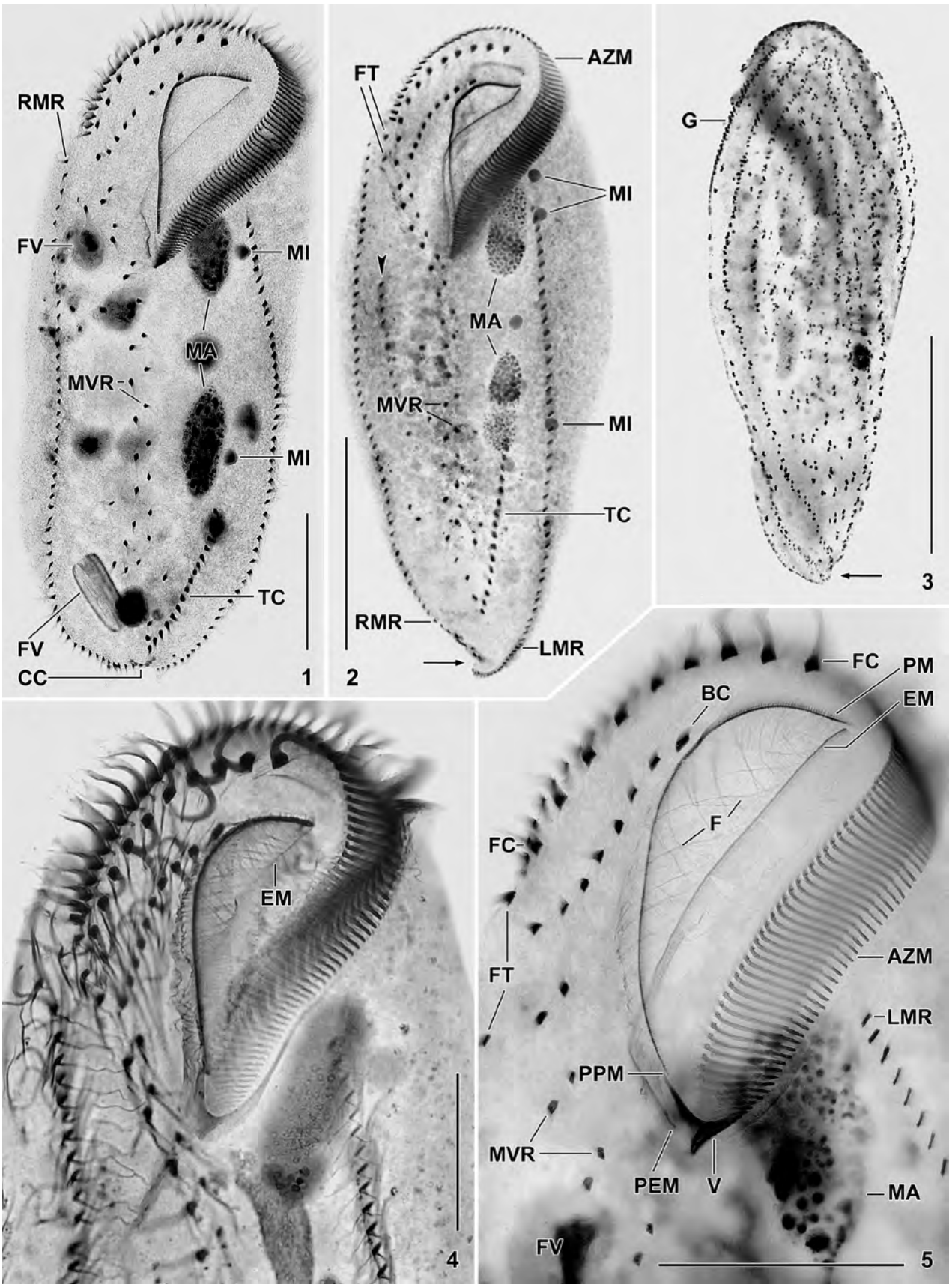
Type locality: Freshwater pond in a suburb of the town of Harbin, northern China, E 127° N 45°10′.

Type material: See Materials section.

Etymology: Named after the continent (Asia) it was discovered.

Description: We studied two populations from northern China, as described in the Materials section, viz., those from Shi and He (1990) and Wang *et al.* (2007). They are very similar morphologically (cp. Figs 1–5, 10, 28), morphometrically (Table 1), and ontogenetically (cp. Figs 18, 29; 19, 30; 20, 21; 22, 25–27; 20, 30, 33). Thus, conspecificity is beyond doubt. Nonethe-less, the description of *N. asiatica* is based, if not stated otherwise, on the population studied by Wang *et al.* (2007). We re-analysed some important morphometrics of the Wang *et al.* (2007) population, and obtained only slightly different values (cp. Tables 1 in the present and the Wang *et al.* 2007 study). Therefore, we refer to this study for more detailed morphometrics. Neither Shi and He (1990) nor Wang *et al.* (2007) studied live specimens. Thus, all data were based on the excellent protargol slides.

Size 220–330 × 85–150 µm, on average 290 × 120 µm in protargol preparations, of ordinary variability (~8%, Table 1). Length: width ratio 1.9–2.8:1, on average 2.4:1. Shape slightly to distinctly oblanceolate, i.e., posteriorly becoming more or less acute and rather dis-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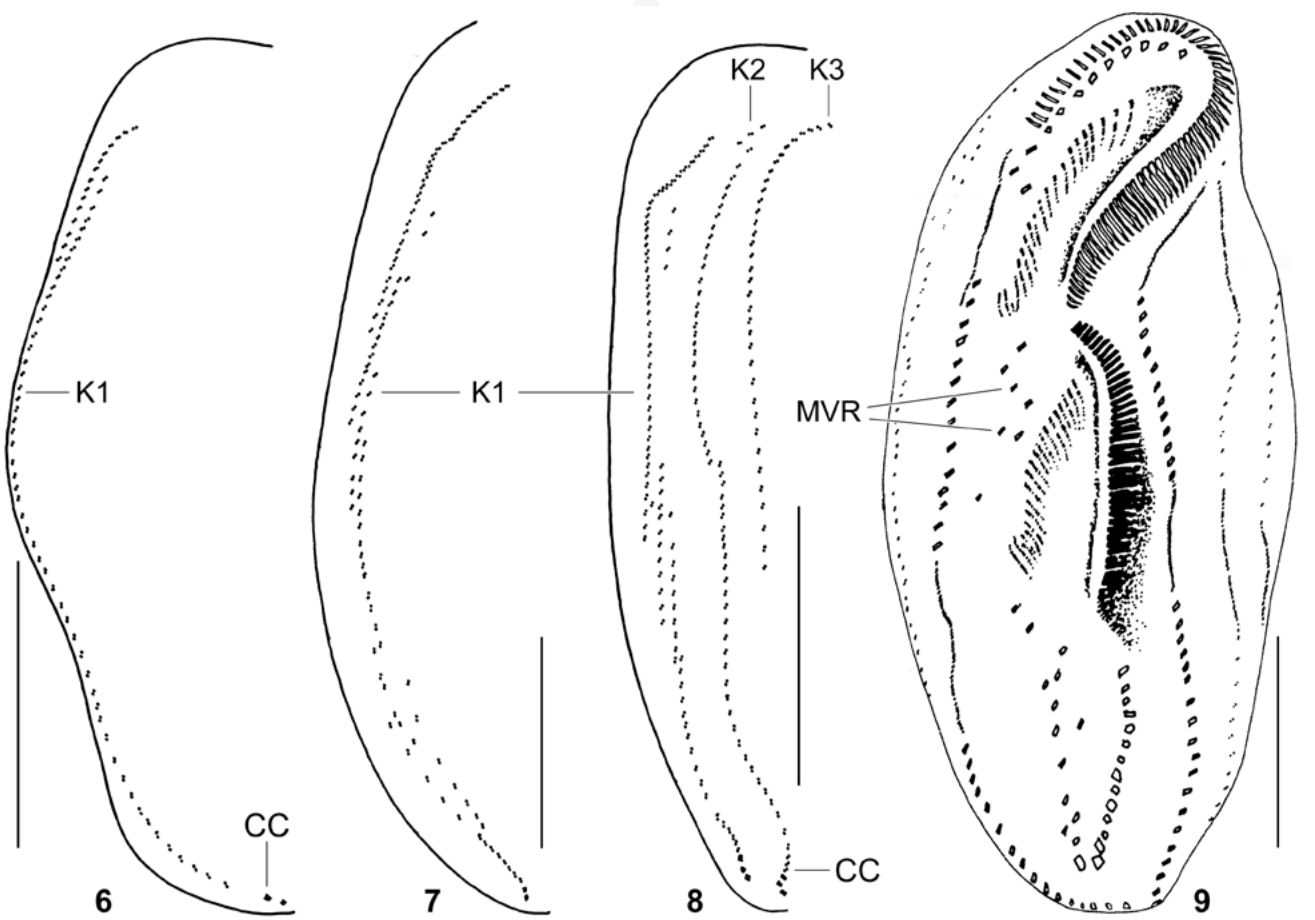
tinctly indented at site of caudal cirri (Figs 2, 3, 10–12, 28, 31, 32). Two, very rarely three or four ellipsoidal macronucleus nodules in middle third of cell slightly left of midline. Two to nine micronuclei near or attached to macronucleus nodules. Cortical granules around and between cirral and bristle bases, forming about 12 rows on dorsal side (Fig. 3).

Cirral pattern urostyloid (Berger 2006), frequently with small irregularities, such as breaks and/or some supernumerary cirri; frontal cirri and adoral membranelles



Figs 6–9. *Neokeronopsis spectabilis*, protargol-impregnated Polish specimens, originals (6–8) and from Warren *et al.* 2002 (9). **6–8** – rare (6) and frequent (7, 8) fragmentation patterns of dorsal kinety 1; **9** – ventral view of a mid-divider, still having parental midventral cirri (MVR) between the cirral anlagen of proter and opisthe. However, this might be an unfortunate misobservation. CC – caudal cirri, K1–3– dorsal kineties. Scale bars: 100 μm.

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Figs 1–5. Comparison of protargol-impregnated Polish *Neokeronopsis spectabilis* (1, 5) and Chinese (Wang *et al.* 2007) *N. asiatica* (2–4). **1**, **2**, **4**, **5 –** note the high similarity in the ventral and oral ciliary pattern as well as the nuclear apparatus, while body size and shape are distinctly different (Tables 1, 2): *N. spectabilis* has an average size of 440 μm and a rounded posterior end, while *N. asiatica* has an average size of 287 μm and an acute body end with a distinct indentation at the site of the caudal cirri (Figs 2, 3, arrows). The arrowhead in Fig. 2 marks a short extra cirral row; **3** – dorsal view showing cortical granulation within the dorsal kineties. Very likely, *N. spectabilis* has a similar granulation. AZM – adoral zone of membranelles, BC – pseudobuccal cirral row, CC – caudal cirri, EM – endoral membrane, F – fibres originating from paroral membrane, FC – frontal cirral corona, FT – frontoterminal cirri, FV – food vacuoles, G – cortical granule rows, LMR – left marginal row, MA – macronucleus nodules, MI – micronuclei, MVR – midventral rows, PM – paroral membrane, PEM – posterior end of endoral membrane, PPM – posterior end of paroral membrane, RMR – right marginal row, TC – transverse cirri, V – buccal vertex. Scale bars: 100 μm (Figs 1–3) and 50 μm (Figs 4, 5).

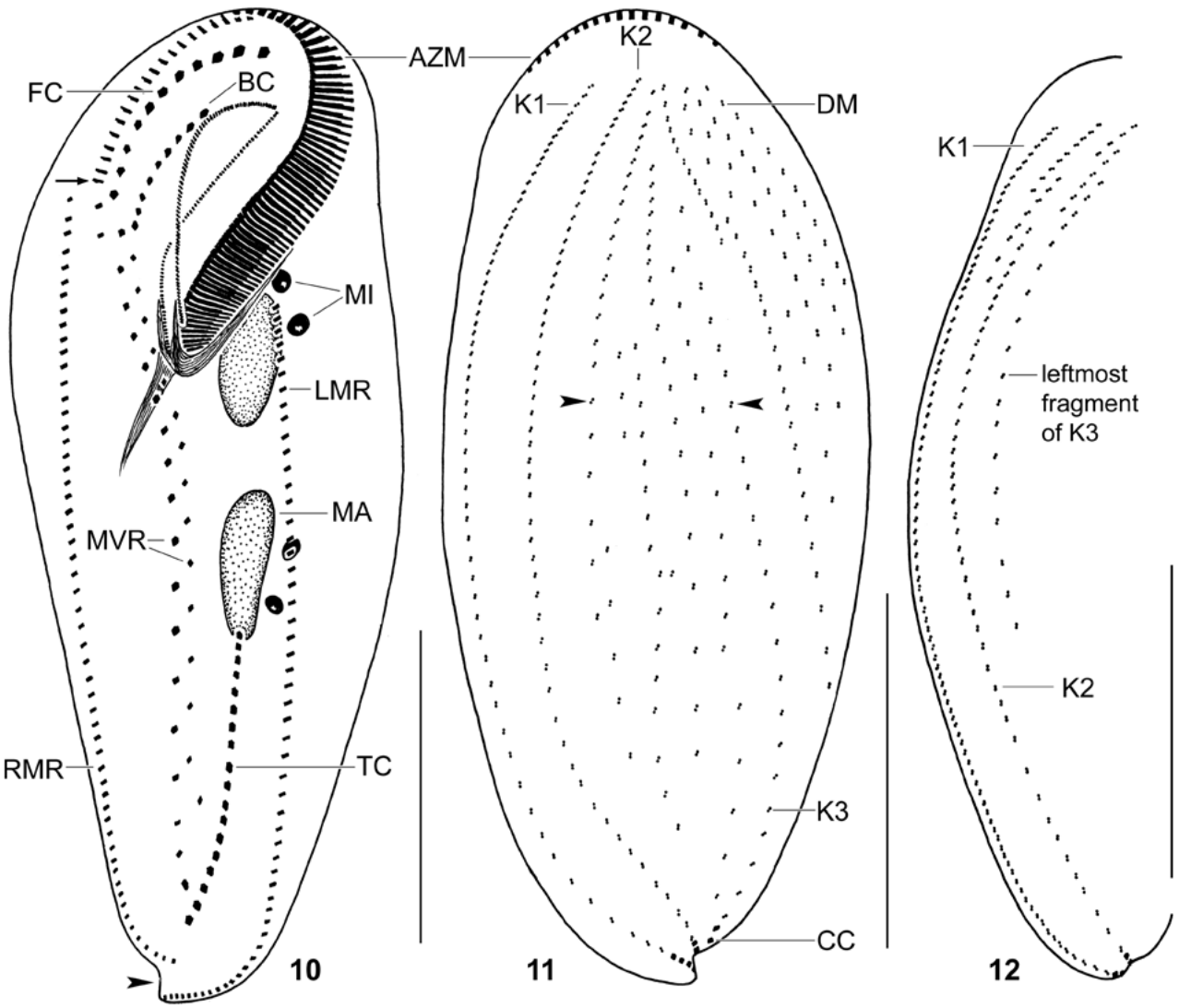
Table 1. Morphometric data on various *Neokeronopsis* and *Afrokeronopsis* populations.

Characteristics[a]	Pop.[e]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	PO	440.0	440.0	36.7	9.5	8.3	360.0	500.0	15
	WA	287.6	290.0	23.4	4.7	8.1	220.0	330.0	25
	SH	291.7	290.0	24.0	5.5	8.2	253.0	342.0	19
	AF[d]	306.4	303.0	25.9	5.7	8.5	260.0	350.0	21
Body, width	PO	176.0	180.0	13.5	3.5	7.7	150.0	200.0	15
	WA	119.2	120.0	12.7	2.5	10.7	85.0	150.0	25
	SH	111.1	110.0	12.9	3.0	11.6	90.0	130.0	19
	AF[d]	111.8	110.0	12.9	2.8	11.5	86.0	135.0	21
Proximal end of adoral zone of membranelles to first proximal transverse cirrus, distance	PO	143.3	150.0	36.4	9.4	25.4	70.0	200.0	15
	WA	82.0	84.0	17.0	3.9	20.7	48.0	100.0	19
	SH	86.8	88.0	20.7	4.7	23.8	43.0	135.0	19
	AF[d]	9.2 (calculated from Foissner and Stoeck 2008)							
Base of largest adoral membranelles, width	PO	29.0	29.0	2.8	0.5	9.7	22.0	35.0	33
	WA	18.2	18.0	1.5	0.3	8.3	15.0	20.0	19
	SH	18.9	19.0	0.9	0.2	4.6	17.0	20.0	19
	AF[d]	17.4	17.0	1.2	0.3	6.7	16.0	20.0	21
Adoral membranelles, number	PO[b]	81.5	–	4.3	–	5.0	72.0	90.0	20
	WA[c]	80.9	–	7.0	1.1	8.6	66.0	93.0	40
	SH	78.6	79.0	2.7	0.6	3.4	73.0	82.0	19
	AF[d]	100.3	100.0	2.6	0.6	2.6	95.0	104.0	21
Right marginal cirri, number	PO[b]	47.5	–	3.0	–	6.0	42.0	54.0	21
	WA[c]	47.8	–	4.1	0.6	8.5	41.0	57.0	40
	SH	46.0	47.0	2.5	0.6	5.5	40.0	49.0	19
	AF[d]	50.4	52.0	4.7	1.0	9.3	43.0	57.0	21
Left marginal cirri, number	SH	52.1	51.0	1.9	0.4	3.6	49.0	55.0	19
Transverse cirri, number	SH	18.3	19.0	1.1	0.2	9.1	15.0	21.0	19
Caudal cirri, number	SH	8.0	8.0	1.1	0.2	13.2	6.0	10.0	19

[a] Data based on protargol-impregnated, morphostatic specimens. All measurements in µm. For details, see footnote (e). CV – coefficient of variation in %, M – median; Max – maximum; Min – minimum; n – number of specimens investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.

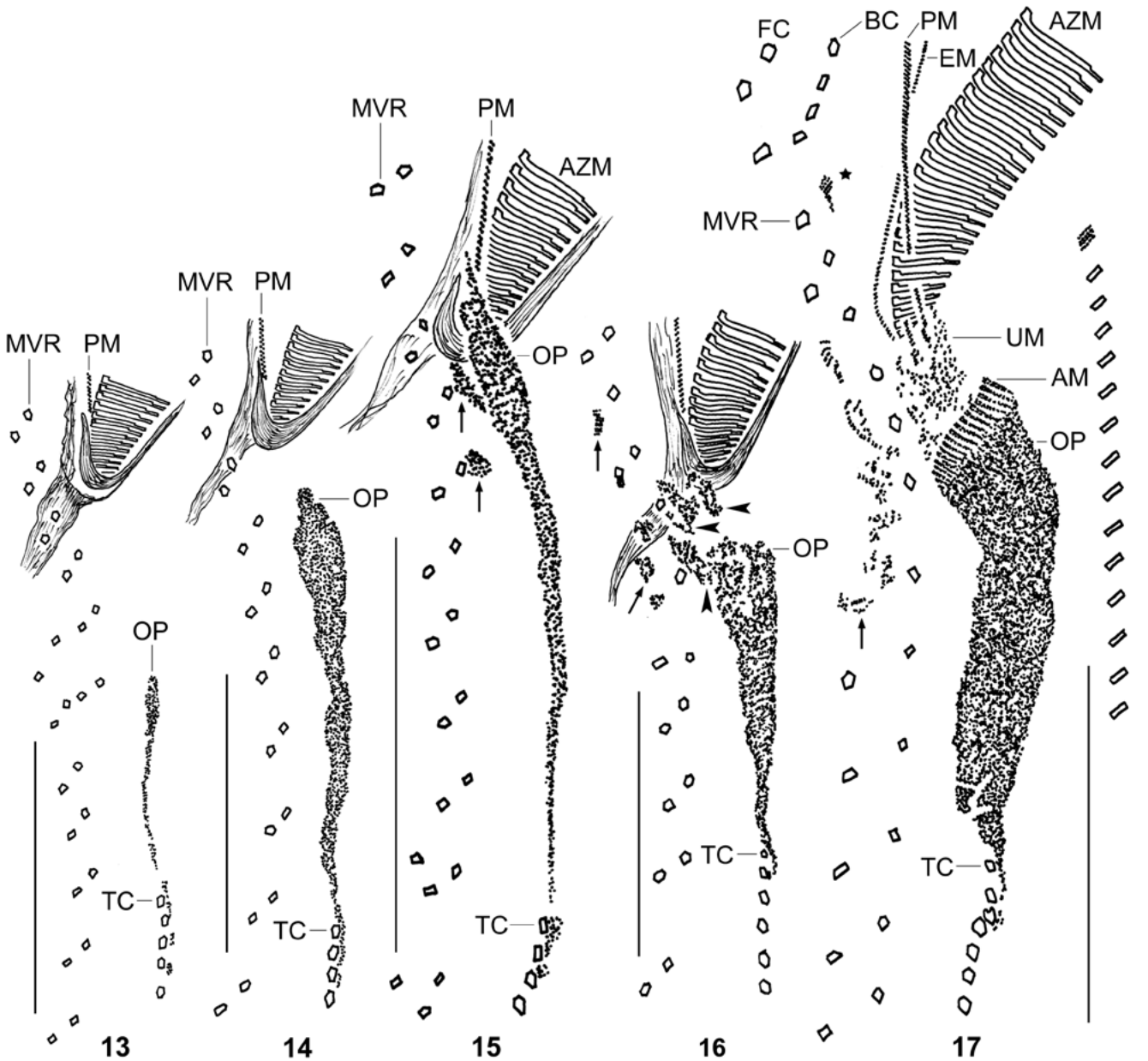
[b–d] From Warren *et al.* (2002), Wang *et al.* (2007), and Foissner and Stoeck (2008).

[e] PO – Polish population, as described by Warren *et al.* (2002). Prepared with Wilbert's method. If not stated otherwise, original data from selected specimens very likely not or inconspicuously inflated by the preparation procedures. WA – Harbin (northern China) population, as described by Wang *et al.* (2007). Otherwise as population (SH). SH – A Chinese (Harbin) population ("Shi population") of *N. asiatica*, briefly described by Shi and He (1990), prepared with a variation of Truffau's method. AF – *Afrokeronopsis aurea*, as described by Foissner and Stoeck (2008). Based on mercury chloride-osmium fixed, well-preserved specimens from three different, exponentially growing cultures.



Figs 10–12. *Neokeronopsis asiatica* nov. spec., Wang *et al.* (2007) population after protargol impregnation. **10** – ventral holotype, length 315 µm. The arrow marks the distal end of the adoral zone of membranelles. The arrowhead denotes the indentation at the site of the caudal cirri. Note also the marginal cirral rows, which end at different levels, i. e., do not touch each other though slightly overlapping; **11** – dorsal holotype, length 270 µm. Note that kineties 1 and 2 are not fragmented and the caudal cirri are in an indentation of the posterior body end. Arrowheads delimit kineties originating from kinety 3; **12** – very rarely, the anterior portion of kineties 1, 2 and/or 3 is fragmented, similar as in *Afrokeronopsis aurea*. AZM – adoral zone of membranelles, BC – pseudobuccal cirral row, CC – caudal cirri, FC – frontal cirral corona, K1–3 – dorsal kineties, LMR – left marginal row, MA – macronucleus nodules, MI – micronuclei, MVR – midventral rows, RMR – right marginal row, TC – transverse cirri. Scale bars: 100 µm.

form an impressive, apical corona (Figs 2, 4, 10, 28); cirri associated with a complex fibre system highly similar to that of *Afrokeronopsis aurea* described by Foissner and Stoeck (2008). Most cirri of ordinary thickness and length (~15 µm), except for enlarged frontal cirri; thickness gradually decreasing from anterior to posterior, except of transverse cirral row; distances between cirri within rows rather constant, except for narrowly spaced cirri in rear region of marginal rows. Both marginal rows commence slightly above level of buccal vertex; right row almost straight ending subterminally in body midline; left row J-shaped, curved over body midline and more extended posteriorly than right row, forming an angular indentation containing the caudal



Figs 13–17. *Neokeronopsis asiatica* nov. spec., very early and early ontogenetic stages of Wang *et al.* (2007) population after protargol impregnation. **13** – very early stage, corresponding to that shown in Figs 3 and 17 of Wang *et al.* (2007). The oral primordium (OP) develops left of the uppermost transverse cirri; **14** – very early stage, corresponding to that shown in Figs 5 and 19 of Wang *et al.* (2007). The oral primordium has grown and extends to the buccal vertex; **15** – early stage not shown in Wang *et al.* (2007). The oral primordium extends over the buccal vertex and two small primordia (arrows) develop between the oral primordium and the left midventral row; **16** – early stage not shown in Wang *et al.* (2007). The oral primordium broadens and has three extensions anteriorly (arrowheads). The cirri of the right midventral row dissolve in the region of the buccal vertex (arrows); **17** – early stage not shown in Wang *et al.* (2007). The oral primordium has grown considerably and adoral membranelles develop in its anterior region, where remaining basal bodies form the anlage for the undulating membranes. Cirral anlagen develop within the right (arrow) and left (asterisk) midventral row. The anterior cirri of the transverse row (TC) become disordered and smaller (see also Fig. 16). The first cirrus of the left marginal row transforms to the anlage of the new row of the proter. AM – forming adoral membranelles, AZM – parental adoral zone of membranelles, BC – parental pseudobuccal cirral row, EM – parental endoral membrane, FC – parental frontal cirri, MVR – parental midventral rows, OP – opisthe oral primordium, PM – parental paroral membrane, TC – parental transverse cirri, UM – anlage for the undulating membranes of the opisthe. Scale bars: 60 μm.

cirri on dorsal side of cell. Midventral rows about 3 µm apart in mid-body, cirri of right row visibly thicker than those of left, extend slightly obliquely and sigmoidally from right anterior end of body to near posterior end; spread at level of right (distal) end of adoral zone of membranelles to become the frontal cirral corona (right row) and the pseudobuccal row (left row), which curves away from right margin of buccal cavity; first frontal cirrus produced by the undulating membranes, frontal bow thus contains one more cirrus than buccal bow. Two inconspicuous frontoterminal cirri posterior of right end of adoral zone and close to right midventral row. Transverse cirri unusual because slightly thickened and arranged in a long, J-shaped row commencing underneath mid-body and extending subterminally, ending right of cell's midline; possibly, there are one or two pretransverse cirri (Figs 2, 4, 10, 28, 31, 32; Table 1).

Dorsal bristles densely spaced in rows 1 and 2 and in leftmost dorsomarginal kinety, while loosely arranged in row 3, in the fragments originating from row 3, and in the right dorsomarginal kineties (Fig. 11); encaged by long fibres in fusiform pattern (Fig. 37). Bristle pattern complex and thus appearing fairly disordered at first glance, composed of an average of 11 rows originating by different processes (Figs 11, 37): (i) rows 1–3 ontogenetically active and almost as long as body, i.e., commence subapically and end posteriorly right of midline with several caudal cirri each; (ii) row 1 and 2 usually continuous, very rarely with one or two fragments anteriorly (Fig. 12); (iii) row 3 with multiple posterior fragmentation, producing about five loosely ciliated kineties extending in middle body third and shortened anteriorly and posteriorly; (iv) in right anterior body third about five dorsomarginal kineties decreasing in length from left to right.

Oral apparatus conspicuous due to the huge adoral zone of membranelles occupying 38% of body length and extending to and along right body margin, forming a tail-like elongation (Figs 2, 4, 10, 28; Tables 1, 2). Adoral zone thus inverted U-shaped, respectively, narrowly spoon-shaped in plane projection; right third of zone (spoon-handle) composed of minute, 3–4 µm wide membranelles gradually increasing to 15–20 µm in left half, that is, at level of buccal cavity, and then gradually decreasing again to about 4 µm in proximal region of zone covered by the buccal vertex. Individual membranelles of usual structure, possibly very similar to that described by Foissner and Stoeck (2008) in *Afrokeronopsis aurea*.

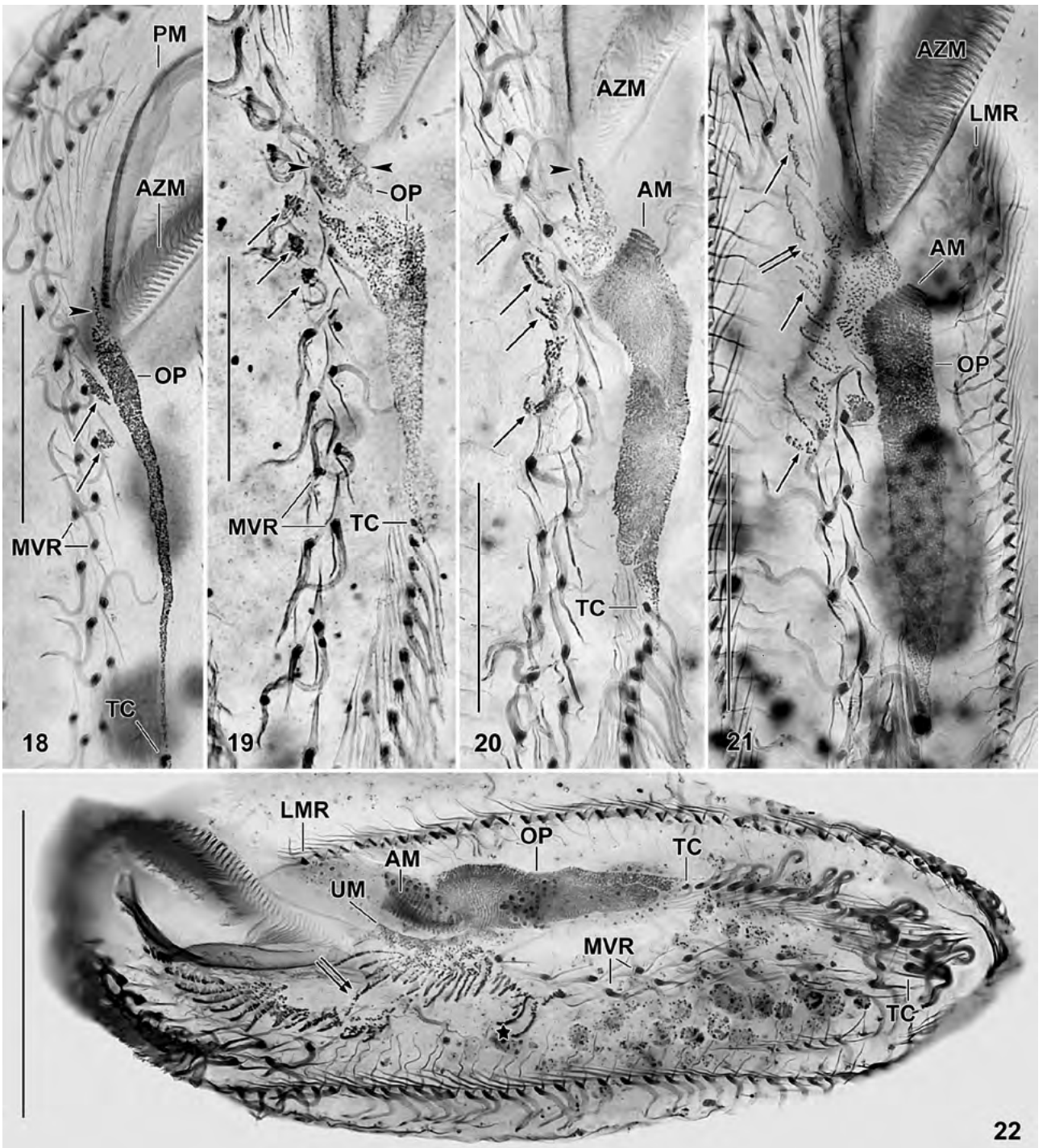
Buccal cavity and undulating membranes basically of ordinary size and structure, very similar to the *Cyrtohymena* pattern described by Berger (1999): cavity large and deep; paroral membrane distinctly curved anteriorly, polystichad, i.e., massive because consisting of very narrowly spaced, short, oblique kineties, except in proximal dikinetal region; endoral membrane extends obliquely across buccal area, optically traversing paroral slightly below middle of cavity, composed of very narrowly spaced mono-or dikinetids (Figs 2, 4, 10, 28). Any indication of a buccal depression lacking in both Chinese populations and in Polish specimens. Pharyngeal fibres inconspicuous, extend obliquely backwards (Figs 2, 4, 10, 28).

Ontogenesis of *Neokeronopsis asiatica*

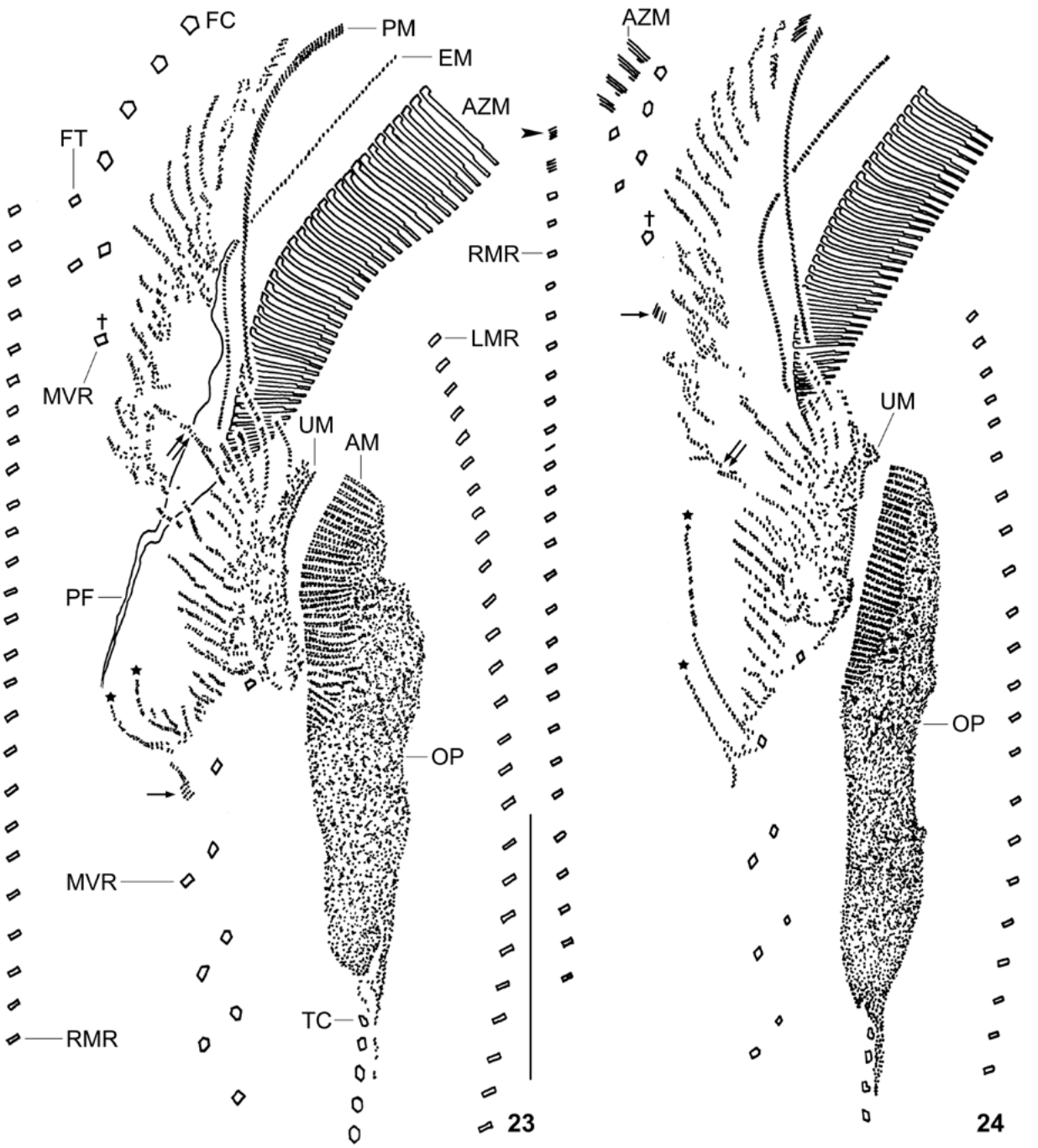
The ontogenesis of *N. asiatica* has been described by Wang *et al.* (2007). The re-analysis of their slides showed slightly different results and details not mentioned, for instance, the fate of the parental midventral cirri between the cirral anlagen fields and primary primordium-like streaks between the proter and opisthe cirral anlagen. As concerns the two Chinese *Neokeronopsis* populations, their ontogenesis is highly similar, for instance, primary primordia-like patterns occur in both (Figs 21–23).

We concentrated our analysis on very early, early, and early mid-dividers because they show the greatest diversity of events and differences between *Neokeronopsis* and *Afrokeronopsis*. No significant differences to *Afrokeronopsis aurea* and other oxytrichids were found in middle and late dividers nor in the fission of the cell and nuclear apparatus.

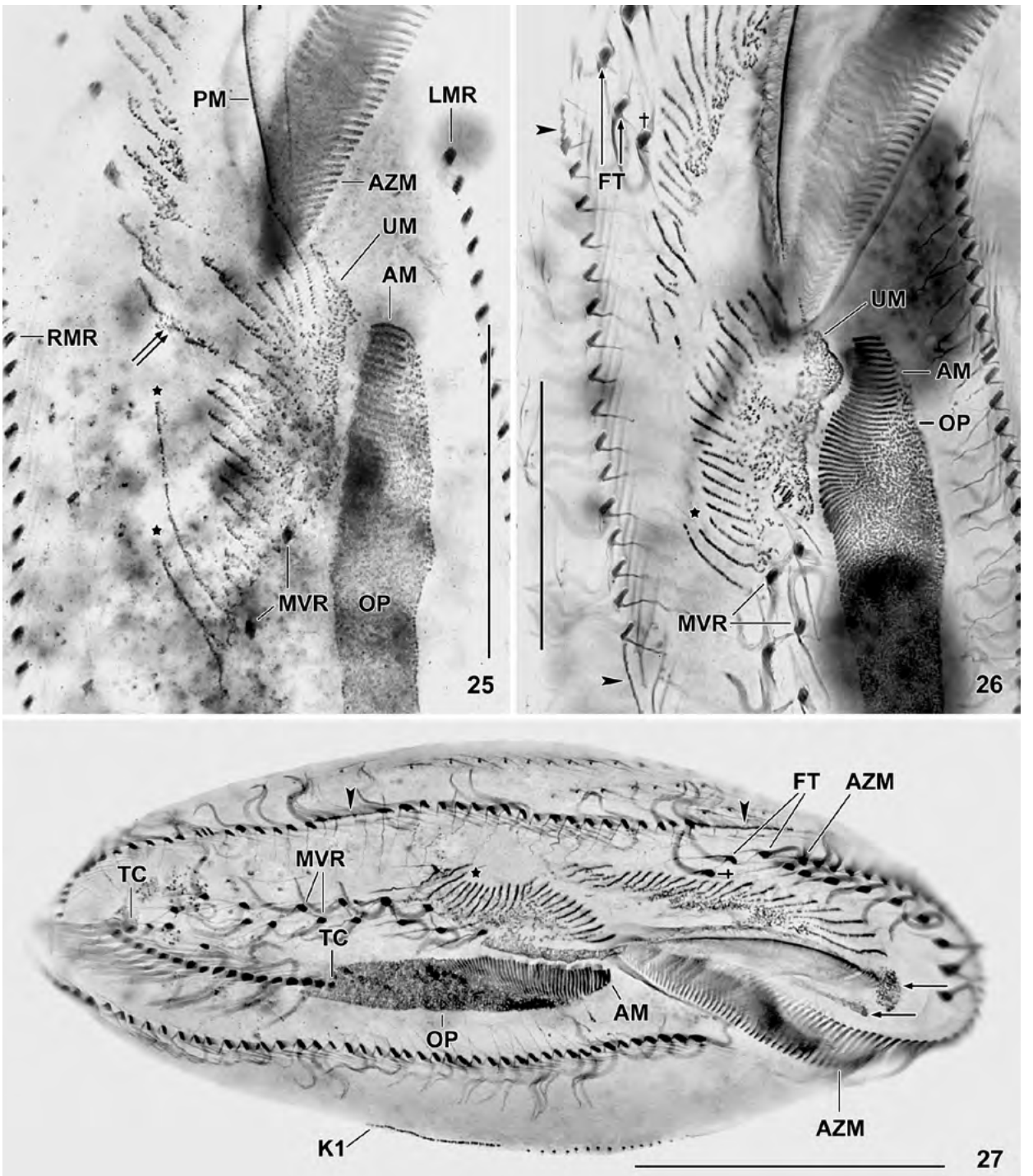
Oral apparatus and ventral cirral pattern: The oral primordium develops close to the left of the uppermost three or four transverse cirri, which are not or only partially included into the anlage (Figs 13, 27). Soon, the oral primordium extends underneath the buccal vertex, showing a bluntly conical anterior end (Fig. 14), corresponding to Fig. 5 in Wang *et al.* (2007). We could not find a stage as depicted by Wang *et al.* (2007) in Fig. 4, i.e., where three primordia are recognizable: one at the transverse cirri and two underneath the buccal vertex. In the next stage, the oral primordium extends over the buccal vertex, showing a finger-like extension anteriorly (Figs 15, 18, 29). Concomitantly, two primordia appear left of the first two postoral cirri of the left midventral row. These primordia unite with the oral primordium, leaving unchanged the cirri of the left midventral



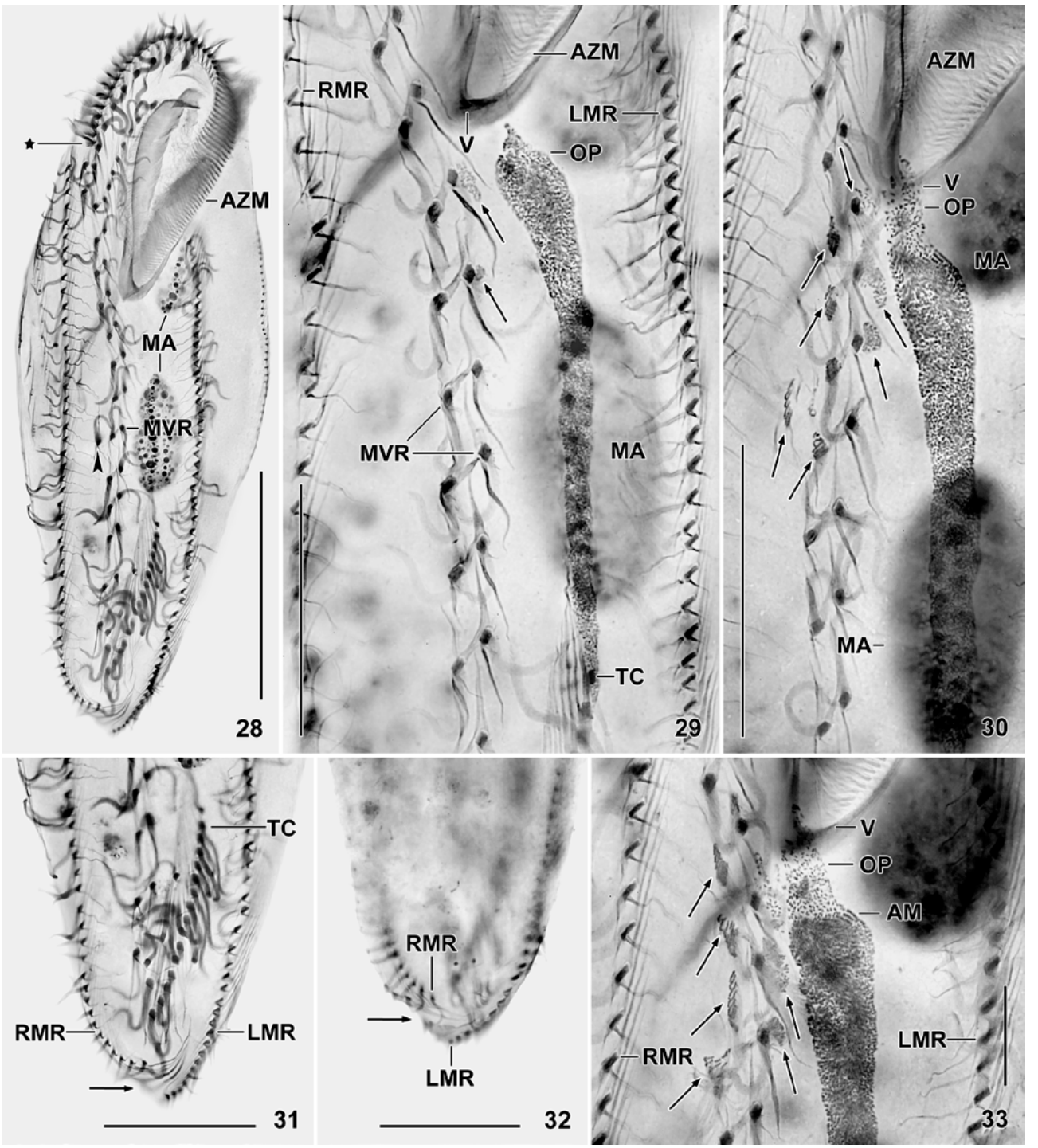
Figs 18–22. *Neokeronopsis asiatica* nov. spec., early (18–21) and early mid-dividers (22) of two Chinese populations (18–20, 22, Wang *et al.* 2007; 21, Shi and He 1990) after protargol impregnation. **18 –** an anlage (arrowhead) develops at the anterior end of the oral primordium and extends above the buccal vertex. Arrows mark two anlagen between left midventral row and oral primordium; **19**, **20** – anlagen (arrowheads) develop at the anterior end of the oral primordium and some postoral midventral cirri (arrows) transform to cirral anlagen; **21** – most midventral cirri of the oral area have transformed to cirral anlagen (arrows). The doubled arrow marks an abutting proter and opisthe cirral anlage; **22** – the midventral cirri between the proter and opisthe anlagen transformed into cirral anlagen which may abut (doubled arrow). The asterisk marks the elongated posteriormost cirral anlagen. AM – developing adoral membranelles, AZM – adoral zone of membranelles, LMR – left marginal row, MVR – midventral rows, OP – oral primordium, PM – paroral membrane, TC – transverse cirri, UM – anlage for the undulating membranes. Scale bars: 50 µm (Figs 18–21) and 100 µm (Fig. 22).



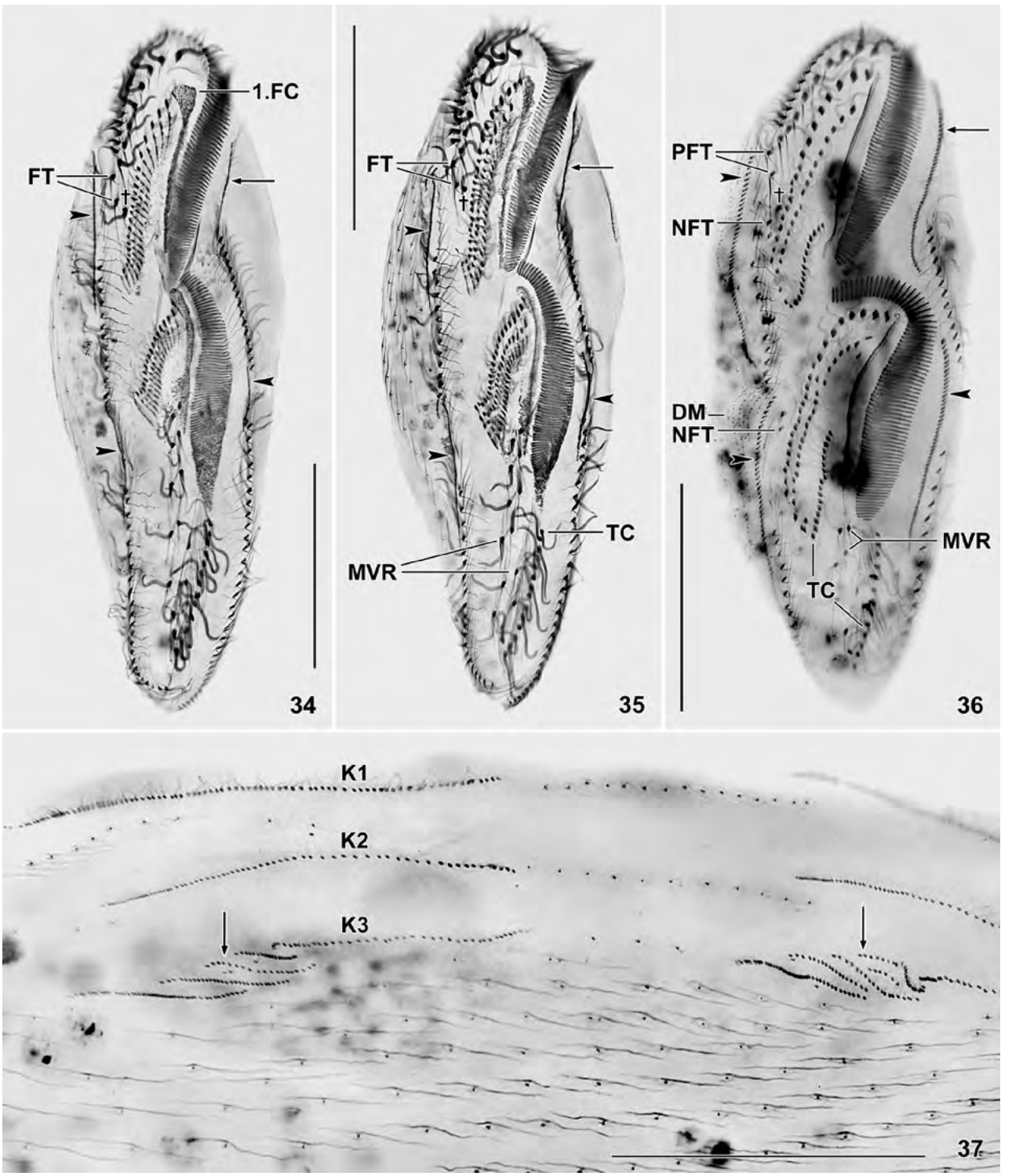
Figs 23, 24. *Neokeronopsis asiatica* nov. spec., early mid-dividers of the Wang *et al.* (2007) population after protargol impregnation. Both stages are very similar, differing only in minor features, for instance, in the right marginal row, where the anterior cirri transform into anlagen in the right specimen (arrowhead). On the other hand, anlagen formation in the buccal cirral row is more advanced in the left specimen. Although dissolution of the midventral cirri is still in progress (arrows), both specimens show that the midventral cirri transform into cirral anlagen between proter and opisthe, a main difference with *N.* (*Afrokeronopsis*) *aurea* and, possibly, *N. spectabilis* (Fig. 9). In the proter, a single cirrus (cross) of the right midventral row does not form anlagen and remains to the end of division. The doubled arrows mark sites where the cirral primordia of proter and opisthe meet, seemingly forming a primary primordium. The asterisks mark the posteriormost cirral anlagen, which are frequently distinctly elongated. Note that some opisthe cirral anlagen extend over the buccal vertex. AM – developing adoral membranelles, AZM – adoral zone of membranelles, EM – parental endoral membrane, FC – parental frontal cirri, FT – parental frontoterminal cirri, LMR – parental left marginal row, MVR – parental midventral rows, OP – oral primordium, PF – parental pharyngeal fibres, pM – parental paroral membrane, RMR – parental right marginal row, TC – parental transverse cirri, UM – anlage for the undulating membranes. Scale bar: 50 μm for both specimens.



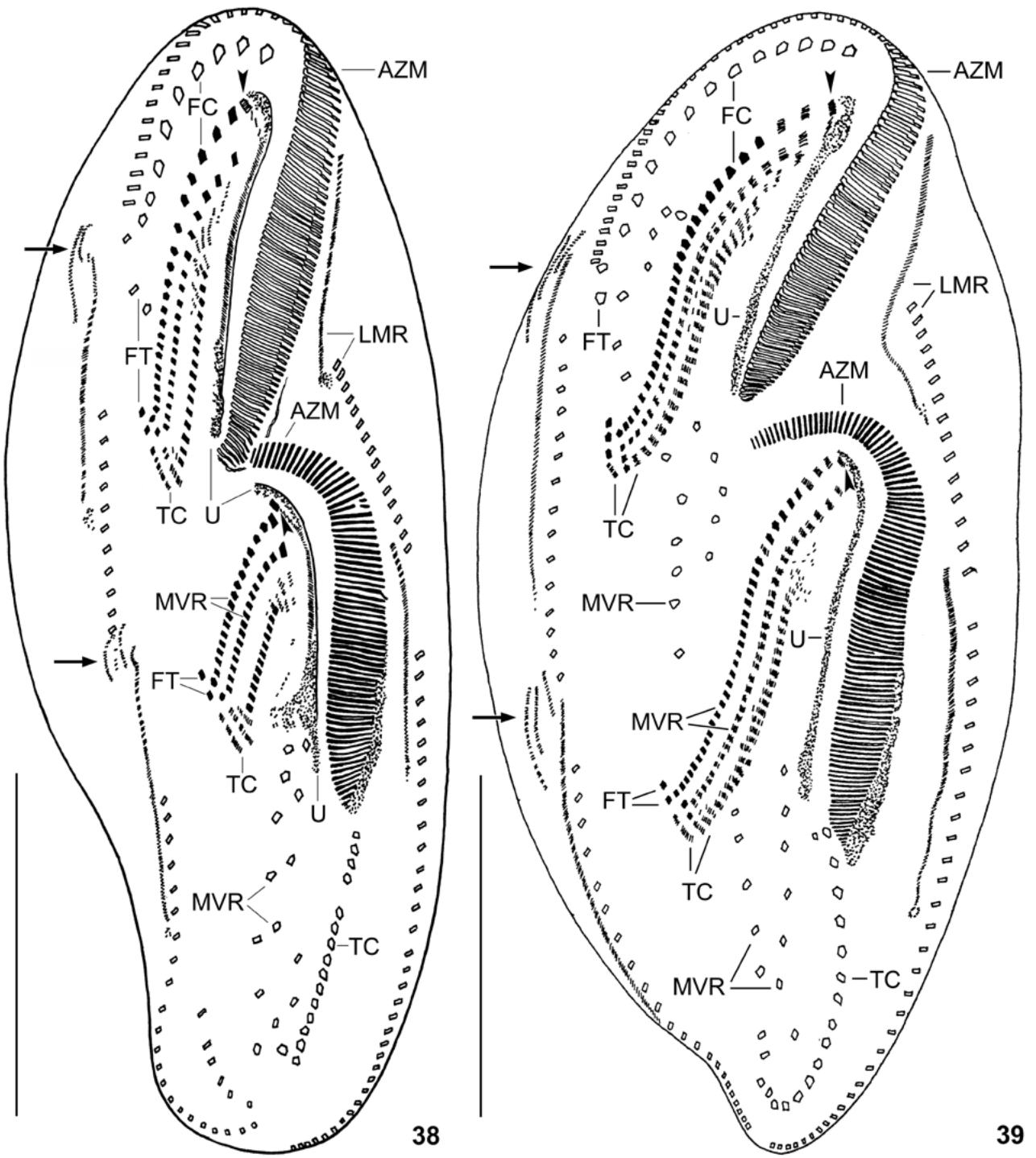
Figs 25–27. *Neokeronopsis asiatica* nov. spec., early mid-dividers of populations fromWang *et al.* 2007 (25) and Shi and He 1990 (26, 27) after protargol impregnation. Crosses mark a single cirrus which does not form an anlage. **25**, **26 –** these specimens are at a similar stage of division, showing that the midventral cirri are resorbed where cirral anlagen develop. The double arrows mark a site where cirral primordia of proter and opisthe meet. The asterisks denote the posteriormost cirral anlagen, which are frequently enlarged. For further information, see Figures 23 and 24; **27** – a slightly later stage than that shown in Fig. 26. The parental undulating membranes begin to dissolve (arrows), the right marginal row forms anlagen (arrowheads), and the posteriormost opisthe anlagen are slightly elongated (asterisk). AM – new adoral membranelles, AZM – parental adoral zone of membranelles, FT – parental frontoterminal cirri, K1 – anlage of dorsal kinety 1, LMR – left marginal row, MVR – midventral rows, OP – oral primordium, PM – parental paroral membrane, RMR – right marginal row, TC – parental transverse cirri, UM – undulating membranes in process of formation. Scale bars: 25 μm (Fig. 26), 50 μm (Fig. 25) and 100 μm (Fig. 27).



Figs 28–33. *Neokeronopsis asiatica* nov. spec., Shi and He (1990) population after protargol impregnation. **28**, **31** – ventral overview showing, inter alia, the typical indentation (arrow) at right posterior end; the marginal cirral rows, which end at different levels; the distal end of the adoral zone of membranelles (asterisk); and a surplus cirrus in the midventral rows (arrowhead); **29 –** very early ontogenetic stage, showing the oral primordium extending between buccal vertex and the uppermost transverse cirri. Arrows mark two anlagen close to the left midventral row (cp. Fig. 18); **30**, **33** – two very similar ontogenetic stages, showing the oral primordium extending over the buccal vertex, and several cirral anlagen (arrows) in the postoral region of both midventral rows; **32** – as Fig. 31, but from another specimen, showing the species-specific posterior body indentation (arrow) and the divergent marginal cirral rows. AM – adoral membranelles, AZM – adoral zone of membranelles, LMR – left marginal row, MA – macronucleus nodules, MVR – midventral rows, OP – oral primordium, RMR – right marginal row, TC – transverse cirri, V – buccal vertex. Scale bars: 20 µm (Figs 31, 32), 50 µm (Figs 29, 30, 33), and 100 µm (Fig. 28).



Figs 34–37. *Neokeronopsis asiatica* nov. spec., middle and late dividers of Shi and He (1990) population after protargol impregnation. Crosses mark a single midventral cirrus which did not form an anlage and is resorbed only in very late dividers. **34**, **35 –** middle dividers showing cirral segregation and the anlagen of the marginal cirral rows (arrowheads), of which the left proter anlage extends far anteriorly (arrows); **36** – a late divider with fully developed proter and opisthe cirral pattern. Arrow and arrowheads denote the same structures as in Figs 34, 35. **37** – dorsal view of an early mid-divider during maximum fragmentation of kinety 3 (arrows). 1. FC – anlage for the first frontal cirrus, FT – parental frontoterminal cirri, MVR – parental midventral rows, NFT – new frontoterminal cirri, PFT – parental frontoterminal cirri, TC – parental and newly formed transverse cirri. Scale bars: 50 µm (Fig. 37) and 100 µm (Figs 34–36).



Figs 38, 39. Comparison of middle dividers of *Neokeronopsis asiatica* nov. spec. (Shi and He 1990 population) and *Afrokeronopsis aurea* (from Foissner and Stoeck 2008) after protargol impregnation. In this stage, the macronucleus nodules have fused and the new cirri have segregated forming three conspicuous, sigmoidal rows each in proter and opisthe: the upper two rows will produce the new midventral rows, while the lower row will generate the transverse cirral row. The undulating membranes (U) are forming and have produced the first frontal cirrus (arrowheads). Arrows mark developing dorsomarginal kineties. Further, these figures show the origin of the frontoterminal cirri (FT) from the rearmost cirral anlage and a most important difference between the two genera: in *Neokeronopsis*, the parental midventral cirri disappeared between the proter and opisthe anlagen field because they were transformed into cirral anlagen; in *Afrokeronopsis*, the middle portion of the parental midventral rows is still recognizable because it did not generate cirral anlagen. AZM – adoral zone of membranelles, FC – frontal cirral coronas, FT – frontoterminal cirri, LMR – left marginal rows, MVR – midventral rows, TC – transverse cirral rows, U – undulating membranes. Scale bar: 100 μm.

row, while about five cirri of the right row transform to anlagen at level the buccal vertex (Figs 16, 19, 30, 33). Next, the three anarchic fields in the anterior region of the oral primordium fuse and commence to form dikinetidal anlagen streaks on the buccal vertex. Further, adoral membranelles assemble at the anterior end of the oral primordium, which is now a large anarchic field of basal bodies. Concomitantly, further postoral cirri of the right midventral row disaggregate and transform into cirral anlagen, while in the left midventral row only the anteriormost cirrus disappears (Figs 17, 20). However, slightly later, the cirri of the left midventral row also transform to anlagen (Figs 21, 30, 33). This complex pattern, which is only partially recognizable in the illustrations of Wang *et al*. (2007), occurs in both Chinese populations (Figs 15–17, 18–21, 29, 30, 33).

The next stages, which are not clearly recognizable in Wang *et al.* (2007), show two main processes (Figs 22–27): (i) adoral membranelles and an undulating membrane develop at the right anterior half of the oral primordium and the proximal end of the opisthe cirral anlagen, respectively; (ii) most cirri of the anterior half of the midventral rows have transformed into anlagen, except of those of the frontal corona, the two frontoterminal cirri and, curiously, the first cirrus of the right midventral row, which is resorbed only in late dividers (marked by † in Figs 23, 24, 26, 27, 34–36).

When the cirral anlagen have grown into long streaks, those of the proter and opisthe may touch each other in the mid-region, forming primary primordia-like streaks (Figs 22–25, double arrows). In about 10% of the dividers, the two last anlagen are distinctly elongated, especially in the opisthe (Figs 22–27, asterisks). Thus, we checked the possibility that the proter frontoterminal cirri are produced via these elongated streaks, i.e., via primary primordia. However, this is not the case, which matches the observation that such elongated streaks occur in few specimens. The frontoterminal cirri are very likely produced by the last anlage (Fig. 38). However, we cannot entirely exclude that the penultimate anlage is also involved, especially in that few cases were three frontoterminal cirri are produced. This uncertainty is caused by the rather high number of cirri generated at the end of the anlagen field, i.e., the J-shaped portion of the transverse cirral row, the pretransverse and/or last cirri of the midventral rows, and the frontoterminal cirri.

The marginal and dorsomarginal kineties develop in the usual fashion, for instance, as described by Wang *et al.* (2007) in *N. asiatica* and by Foissner and Stoeck (2008) in *A. aurea*. The sigmoidal anlage of the left marginal row of the proter is very long and thus prominent (Figs 24, 27, 34–36).

Dorsal ciliature (Figs 11, 12, 37): Generally, the dorsal ciliature develops as described by Wang *et al.* (2008) and by Foissner and Stoeck (2008) in *Afrokeronopsis aurea*. Specifically, kinety 3 splits into several fragments, of which the leftmost fragment produces the anlage with the kinety whirl, while the rightmost fragment produces caudal cirri. With respect to *A. aurea*, two main differences occur: (i) more than three caudal cirri are formed by kineties 1–3 (Tables 1, 2); and (ii) the kinety whirls, which generate the posterior fragments of kinety 3 in proter and opisthe, are much more pronounced in *A. aurea* than in *N. asiatica*. The whirls shown in Fig. 37 are the most distinct ones found in both Chinese populations (see also Wang *et al.* 2007 and compare with Foissner and Stoeck 2008).

DISCUSSION

Comparison of *Neokeronopsis asiatica* with *N. spectabilis*

Using the data available, these species differ in the following features (Table 2): body size (~300 × 120 µm vs. 400 × 170 µm); posterior body end (acute with distinct indentation at site of caudal cirri vs. broadly rounded without indentation); posterior end of marginal rows (at different vs. same level); dorsal kinety 1 (continuous vs. fragmented); and the size of the bases of the adoral membranelles (largest membranelles on average 18 µm vs. 29 µm wide). No significant differences were found in the ontogenesis, if the interpretation suggested in the Results section is accepted.

However, all features, except of dorsal kinety fragmentation, are somewhat problematic because we cannot fully exclude that the Polish specimens are heavily inflated and slightly distorted due to the preparation method used (see Materials and Methods section). This is indicated by the numbers of transverse and marginal cirri and adoral membranelles, which are highly similar in both species (Table 2), in spite of the very different body size (288 × 119 µm and 442 × 176 µm).

The most simple and distinct difference between *N. spectabilis* and *N. asiatica* is the width of the membranellar bases, which was, unfortunately, miscalculated by Warren *et al.* 2002 (see Results section). Al-

Table 2. Comparison of main and/or distinctive features in *Neokeronopsis spectabilis* (from Warren *et al.* 2002), *Neokeronopsis asiatica* n.sp. (from Wang *et al*. 2007), and *Afrokeronopsis aurea* (from Foissner and Stoeck 2008).

Characteristics[a]	*N. spectabilis*	*N. asiatica*	*A. aurea*
Body, size (µm)	442 × 176	288 × 119[b]	306 × 112
Base of largest adoral membranelles, width (µm)	29	18	17
Proximal end of adoral zone to first (uppermost) transverse cirrus, distance (µm)	143	82	9
Adoral membranelles, number	81	81	100
Transverse cirri, number	18	18	24
Caudal cirri, number	7	8	3
Right marginal cirri, number	48	48	50
Cortical granules	present according to Berger (2008)	present	present
Buccal depression	absent	absent	present
Anterior end of paroral and endoral	almost abutting	almost abutting	distinctly separate
Posterior body end, shape	broadly rounded	acute with distinct indentation at site of caudal cirri	narrowly rounded to acute
Fragmentation of dorsal kinety 1	posterior fragmentation	usually absent	anterior fragmentation
Dorsal whirl in mid-dividers	indistinct (?)	indistinct (also in re-analysis)	distinct
Midventral cirri between anlagen in early dividers	maintained (?)	resorbed	maintained
Distribution	Europe	Far East	Africa

[a] Averages from ≥ 15 protargol-impregnated specimens; for exact numbers, see Table 1.
[b] Corrected values from Table 1.

though the adoral membranelles and the adoral zone formed by them are comparatively massive structures, one cannot entirely exclude inflation by the preparation procedures. Fortunately, a notion by Berger (2006), who made live observations on a Salzburg (Austrian) population of *N. spectabilis*, solves the problem: "Largest adoral membranelles up to 28 µm wide," which closely matches our measurements on the Polish specimens (average width 29 µm). Berger (2006) investigated also a Carithian population, from which he obtained a single, protargol-impregnated specimen that was, however, very small (236 µm; usual length 300–400 µm). The figure shows that the longest membranellar bases are about 18 µm wide, like those of *N. asiatica*. However, if this value is increased in proportion to the size of a typical cell (360 µm; Fig. 242b in Berger 2006), 27 µm is obtained, which is close to that of the Polish specimens (29 µm).

These considerations and those outlined in the following section, show the need for further investigations, especially of *N. spectabilis*, in order to obtain more reliable information about the following features: body size (larger than in *N. asiatica*?), body shape (broadly rounded and not indented at rear end?), marginal cirral rows (terminate at same or similar level?), width of adoral membranellar bases (over 25 µm on average?), cortical granules (colour and arrangement), buccal depression (absent?), and the midventral cirri (early resorption between cirral anlagen?).

The subgenus *Afrokeronopsis*

The status of *Afrokeronopsis* has been carefully discussed by Foissner and Stoeck (2008). They proposed raise to genus level, if further investigations show the absence of a buccal depression in *Neokeronopsis*. The excellent protargol impregnations of *N. asiatica* strongly support this assumption. Thus, *Afrokeronopsis* is raised to genus level (see diagnostic section).

Improved diagnoses of the family Neokeronopsidae and the genera *Neokeronopsis*, *Afrokeronopsis*, and *Pattersoniella*

Family Neokeronopsidae Foissner and Stoeck, 2008

Diagnosis: Rigid or flexible, oxytrichid Hypotricha (Dorsomarginalia) with midventral (urostyloid) cirral pattern, including a more or less distinct corona of frontal and pseudobuccal cirri both originating from the midventral rows. Midventral cirri maintained or transformed into cirral anlagen between proter and opisthe. Oral apparatus in *Oxytricha* or mixed *Oxytricha*/*Cyrtohymena* pattern, with or without buccal depression. Dorsal ciliature composed of dorsomarginal kineties and three ordinary rows, of which row 3 produces further kineties by multiple fragmentation, showing a distinct or indistinct kinety whirl; three or more caudal cirri; parental dorsal ciliature resorbed or partially retained after ontogenesis.

Type genus (by original designation)**:** *Neokeronopsis* Warren, Fyda and Song, 2002.

Taxa assignable: *Neokeronopsis* Warren *et al*., 2002; *Afrokeronopsis* Foissner and Stoeck, 2008; *Pattersoniella* Foissner, 1987. See Foissner and Stoeck (2008, p. 29, 30) for a detailed discussion of the classification of *Pattersoniella*.

Remarks: For nomenclature, see Foissner and Stoeck (2008). *Afrokeronopsis* is raised from subgenus to genus level (see below). See Figures 38 and 39 for a conspicuous ontogenetic difference in *Neokeronopsis* and *Afrokeronopsis*.

Genus *Neokeronopsis* Warren, Fyda and Song, 2002

Improved diagnosis: Very flexible Neokeronopsidae with conspicuous midventral rows and frontal cirral corona, each composed of many cirri, producing a midventral cirral pattern. Midventral cirri transformed to cirral anlagen between proter and opisthe. Oral apparatus in *Oxytricha*/*Cyrtohymena* pattern and without buccal depression. Oral primordium developing postorally and above transverse cirral row. Parental dorsal ciliature resorbed during ontogenesis; left fragment of kinety 3 with indistinct whirl; more than three caudal cirri.

Type species (by original designation)**:** *Neokeronopsis spectabilis* (Kahl, 1932) Warren *et al*., 2002. Basionym: *Holosticha* (*Keronopsis*) *spectabilis* Kahl, 1932.

Remarks: This genus now consists of the type species and *N. asiatica* described above. See Warren *et al.* (2002) and Berger (2006) for redescriptions of the type species, neotypification problems, and etymology.

Genus *Afrokeronopsis* Foissner and Stoeck, 2008 nov. stat.

Improved diagnosis: Semirigid Neokeronopsidae with conspicuous midventral rows and frontal cirral corona, each composed of many cirri, producing a midventral cirral pattern. Midventral cirri maintained between proter and opisthe. Oral apparatus in *Oxytricha*/*Cyrtohymena* pattern and with buccal depression. Oral primordium developing along transverse cirral row. Parental dorsal ciliature resorbed during ontogenesis; left fragment of kinety 3 with distinct whirl; three caudal cirri.

Type species (by original designation)**:** *Neokeronopsis* (*Afrokeronopsis*) *aurea* Foissner and Stoeck, 2008.

Remarks: Foissner and Stoeck (2008) established *Afrokeronopsis* as a subgenus of *Neokeronopsis*. The present investigations suggest raising *Afrokeronopsis* to genus level (see above). Thus, the following new combination is necessary: *Afrokeronopsis aurea* (Foissner and Stoeck, 2008) nov. comb. As yet, the genus is monotypic. Feminine gender. See Foissner and Stoeck (2008) for a detailed discussion of the characteristics contained in the diagnosis.

Genus *Pattersoniella* Foissner, 1987

Improved diagnosis: Rigid Neokeronopsidae with inconspicuous midventral rows and frontal cirral corona, each consisting of comparatively few cirri, producing a mixed oxytrichid/urostylid cirral pattern. Oral apparatus oxytrichid, without buccal depression. Midventral cirri maintained between proter and opisthe. Oral primordium developing very near to two postoral midventral cirri and mainly above transverse cirral row. Parental dorsal ciliature partially maintained; left fragment of kinety 3 with indistinct whirl; three caudal cirri.

Type species: *Pattersoniella vitiphila* Foissner, 1987.

Remarks: See Foissner and Stoeck (2008) for a detailed discussion of this genus.

Type material and biogeographic aspects

The deposited type and voucher material was indispensable for the present investigations. Unfortunately, systematic slide deposition started late in ciliates, and thus type material is lacking for most species (Foissner 2002, Aescht 2008). Although several recognized repositories for protists exist (Aescht 2008), some colleagues

still prefer to deposit their type specimens in laboratory collections. History shows that such "depositions" are frequently lost when key personnel die suddenly or become an emeritus, or if institutions or departments close or reorganize.

Type and voucher material is also indispensable for biogeographic research. The present study is an impressive example, showing distinct species each in different biogeographic regions. The widespread view that protists are cosmopolitans is partially based on misidentifications and the lack of type and voucher material (Foissner 2006). Indeed, if not investigated carefully, *Neokeronopsis spectabilis*, *N. asiatica*, and even *Afrokeronopsis aurea* could have been identified as a single, cosmopolitan species!

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