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# Morphogenesis in the Marine Spirotrichous Ciliate *Apokeronopsis crassa* (Claparède & Lachmann, 1858) n. comb. (Ciliophora: Stichotrichia), with the Establishment of a New Genus, *Apokeronopsis* n. g., and Redefinition of the Genus *Thigmokeronopsis*

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ABSTRACT. Morphogenetic events during the division of the marine spirotrichous ciliate, *Apokeronopsis crassa* (Claparède & Lachmann 1858) n. comb. were investigated. Compared with members of the well-known genera *Thigmokeronopsis*, *Uroleptopsis*, and *Pseudokeronopsis*, *A. crassa* has one row of buccal cirri, high number of transverse cirri, clearly separated midventral rows, lacks thigmotactic cirri and a gap in adoral zone, its undulating membranes (UMs) anlage forms one cirrus and marginal rows and dorsal kineties form apokinetally during division. All these characteristics indicate that this organism represents a new taxon at the generic level, and hence a new genus is suggested, *Apokeronopsis* n. g. It is defined as thus: Pseudokeronopsidae with *Pseudokeronopsis*-like bicorona of frontal cirri and one marginal row on each side; one row of two or more buccal cirri in ordinary position; two midventral rows distinctly separated, hence of cirri that are not in a typical zig-zag pattern; high number of transverse cirri, caudal cirri absent, and frontoterminal cirri present; thigmotactic cirri absent, many macronuclear nodules fuse into many masses as well as marginal and dorsal kineties form apokinetally during morphogenesis. At the same time, the genus *Thigmokeronopsis* Wicklow, 1981 is redefined, and one new combination, *Apokeronopsis antarctica* (Petz, 1995) n. comb. is proposed. The morphogenetic events of *A. crassa* are characterized as follows: (1) In the proter, the adoral zone of membranelles and UMs are completely renewed by the oral primordium. The UM anlage is formed apokinetally on the dorsal wall of the buccal cavity and is hence clearly separated from the frontoventral-transverse (FVT) cirral anlagen in the proter. (2) Frontoventral-transverse cirral anlagen are generated de novo in the outermost region of the cortex to the right of the old UMs. (3) A row of buccal cirri arises from FVT cirral streak I. (4) The marginal rows and dorsal kineties originate de novo in both dividers; no caudal cirri are formed. (5) The last FVT-streak contributes two frontoterminal cirri. (6) The many macronuclear nodules fuse into many masses (about 50 segments) during division, unlike a singular or branched mass as described in other urostylids.

**Key Words.** Cell regeneration, Pseudokeronopsidae, Spirotrichea, systematics, Urostylida.

THE large and unique marine pseudokeronopsid ciliate, *Thigmokeronopsis crassa* (Claparède & Lachmann, 1858) Berger, 2006 was originally described as *Oxytricha crassa* by Claparède and Lachmann (1858) (Berger, 2006; Song, Wilbert, and Hu, 2004b). However, its detailed morphology, especially its infraciliature, was not redescribed until Hu and Song (2000) and Song et al. 2004b reinvestigated it under the name of *Pseudokeronopsis qingdaoensis* Hu & Song, 2000. Berger (2006) recognized the synonymy of *O. crassa* and *P. qingdaoensis* and simultaneously recombined it into the genus *Thigmokeronopsis* based mainly on its morphological features, especially its similarities to *Thigmokeronopsis antarctica*. According to our understanding, *T. crassa* is characterized by a row of buccal cirri, separated midventral rows, high number of transverse cirri, the brown-reddish ''blood cell-shaped'' cortical granules, the strongly bent distal end of the adoral zone of membranelles (AZM), and macronuclear nodules fuse into many masses (Berger 2006; Hu and Song 2000; Song et al. 2004b).

Developmental and infraciliature patterns in most other well-known pseudokeronopsids exhibit great similarities, which implies that they are closely related species (Berger 2004; Hu, Warren, and Suzuki 2004b). Details of the morphogenetic events in *T. crassa*, however, have yet to be reported, hence its phylogenetic relationship with other pseudokeronopsids remains unknown and its status as a *Thigmokeronopsis* species remains to be confirmed.

In the summer of 2005, a further population of *T. crassa* was collected and a uniprotistan culture was established, giving us the opportunity to investigate in detail the morphogenetic processes during binary fission and cell regeneration. As a result of the present investigations a new genus (*Apokeronopsis*) was erected for this organism and its phylogenetic position within the pseudokeronopsids was confirmed.

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## MATERIALS AND METHODS

The population of *Apokeronopsis crassa* used for morphogenetic studies was isolated in May 2005 from offshore fish-farming water off Qingdao (Tsingtao, 120°18′E; 36°04′N), China. Glass slides were used as artificial substrates to collect ciliates (Gong, Song, and Warren 2005). Briefly, the slides were carefully taken out after being exposed in the tanks for about 7–10 days and transferred to Petri dishes with seawater from the sampling site. Isolated specimens were maintained in the laboratory for about 1 wk as uniprotistan cultures (water temperature about 20 °C, salinity ca 30‰) in Petri dishes for observation and further studies.

Protargol staining (Wilbert 1975) was used in order to reveal the infraciliature. Drawings were made with the help of a camera lucida at a magnification of 1,250X. To illustrate the changes during morphogenesis, parental cirri are depicted by contour whereas new ones are shaded black.

Terminology and systematics are according to Song et al. (2004b) and Lynn and Small (2002).

## RESULTS

***Apokeronopsis crassa* (Claparède & Lachmann, 1858) n. comb.**

*Short list of synonyms.* 1858 *Oxytricha crassa* sp. n.—Claparède & Lachmann, Mém. Inst. natn. Genèv., **5**:1–260 (original description; no type material available).

1932 *Trichotaxis* (*Oxytricha crassa*) (Claparède & Lachmann, 1858)—Kahl, Tierwelt Dtl., **25**:399–650 (revision; *Trichotaxis* is an incorrect spelling of *Trochototaxis*).

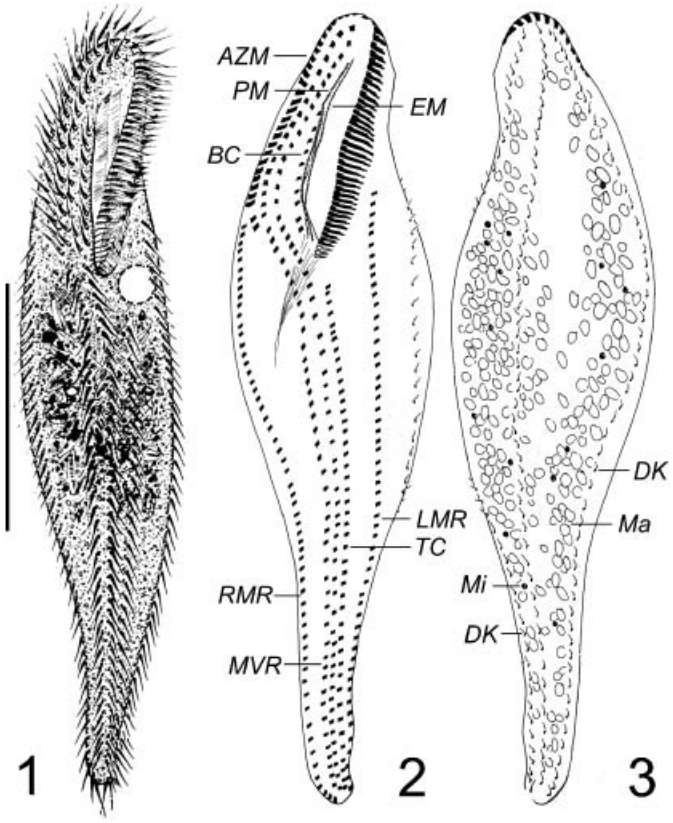



Fig. **1–3.** *Apokeronopsis crassa* n. comb. from life (Fig. 1) and after protargol impregnation (Fig. 2, 3) (after Song et al. 2004b, under the name *Pseudokeronopsis qingdaoensis* Hu & Song, 2000). **1.** Ventral view of a typical individual. **2, 3.** Ventral (Fig. 2) and dorsal (Fig. 3) views of the same specimen, to demonstrate the general infraciliature. AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kineties; EM, endoral membrane; FTC, frontal terminal cirri; LMR, left marginal row; Ma, macronuclei; Mi, micronuclei; MVR, midventral row; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri. Scale bar = 100 μm.

2000 *Pseudokeronopsis qingdaoensis* sp. n.—Hu & Song, Acta Zootaxo. Sin., **25**:361–364 (original description of junior synonym and deposition of type material in China).

2004 *Pseudokeronopsis qingdaoensis* Hu & Song, 2000—Song, Wilbert & Hu, Cah. Biol. Mar., **45**:335–342 (revision).

2006 *Thigmokeronopsis* (*Oxytricha*) *crassa* (Claparède & Lachmann, 1858)—Berger, Monographiae Biol., **85**:i–xvi, 1–1304. (synonymy of *O. crassa* and *P. qingdaoensis* and detailed review).

For details on synonyms see Berger (2006).

**Morphology and infraciliature of the Qingdao population of *Apokeronopsis crassa* (Fig. 1–3).** The in vivo morphology and infraciliature of the present population of *A. crassa* closely resembles previous descriptions (Claparède and Lachmann 1858; Hu and Song 2000; Song et al. 2004b).

Cell in vivo ca 250 × 50 μm in size; body flexible and highly contractile, basically elongate with its widest part at midbody. The posterior portion more or less narrowed while the anterior one is considerably narrowed. Buccal field is moderately narrow, and occupies about one-third of body length. There are two types of cortical granules: type I colourless and tiny, about 0.2 μm across in vivo; type 2 large, about 2 μm across, brownish to brown-reddish in colour, and red blood-cell shaped, which is sparsely distributed. Contractile vacuole positions in anterior third of body. More than 100 macronuclear nodules, each ovoid to ellipsoid and about 2 μm long, are distributed throughout the body. Locomotion by slow, crawling without pause on debris or on bottom of Petri dish. Distal end of AZM curves strongly posteriad along right margin and extends to about level of cytostome. Bases of membranelles about 6–10 μm long. Paroral and endoral membranes about equal in length, lying parallel. Most somatic cirri are relatively fine, 10–15 μm long. Bicorona comprises of about 30 slightly enlarged frontal cirri, which are sometimes separated from the midventral complex by an inconspicuous gap. Usually two frontoterminal cirri are near the distal end of adoral zone and hence often difficult to recognize. One long row of buccal cirri locates close to the paroral membrane. Midventral rows terminate at posterior end of cell, with cirri of each pair conspicuously separated from each other. Transverse cirri are arranged in an unusually long row which is parallel and to the left of the midventral rows and extends anteriorly to the proximal portion of the adoral zone. Consistently three complete dorsal kineties exist, with cilia about 2–3 μm long.

**Morphogenesis of *Apokeronopsis crassa* (Fig. 4–21, 26–56).**

*Stomatogenesis and cirral streaks.* Cortical morphogenesis in *A. crassa* mainly occurs in two zones: an anterior field in the proter and a posterior field in the opisthe. The first morphogenetic event within these zones is the formation of the oral primordia by a proliferation of basal bodies. In the proter, the oral primordium develops on the dorsal wall of the buccal cavity while the paroral and endoral membranes are apparently still intact (Fig. 4, POP). In the opisthe, the oral primordium is formed as an anarchic field posterior to the AZM, between the transverse cirral row and the left midventral row (Fig. 4, 26, OP). During this process, all midventral cirri remain unchanged (ciliature and fibres present), indicating that parental basal bodies are not incorporated into the primordium (Fig. 4, 26).

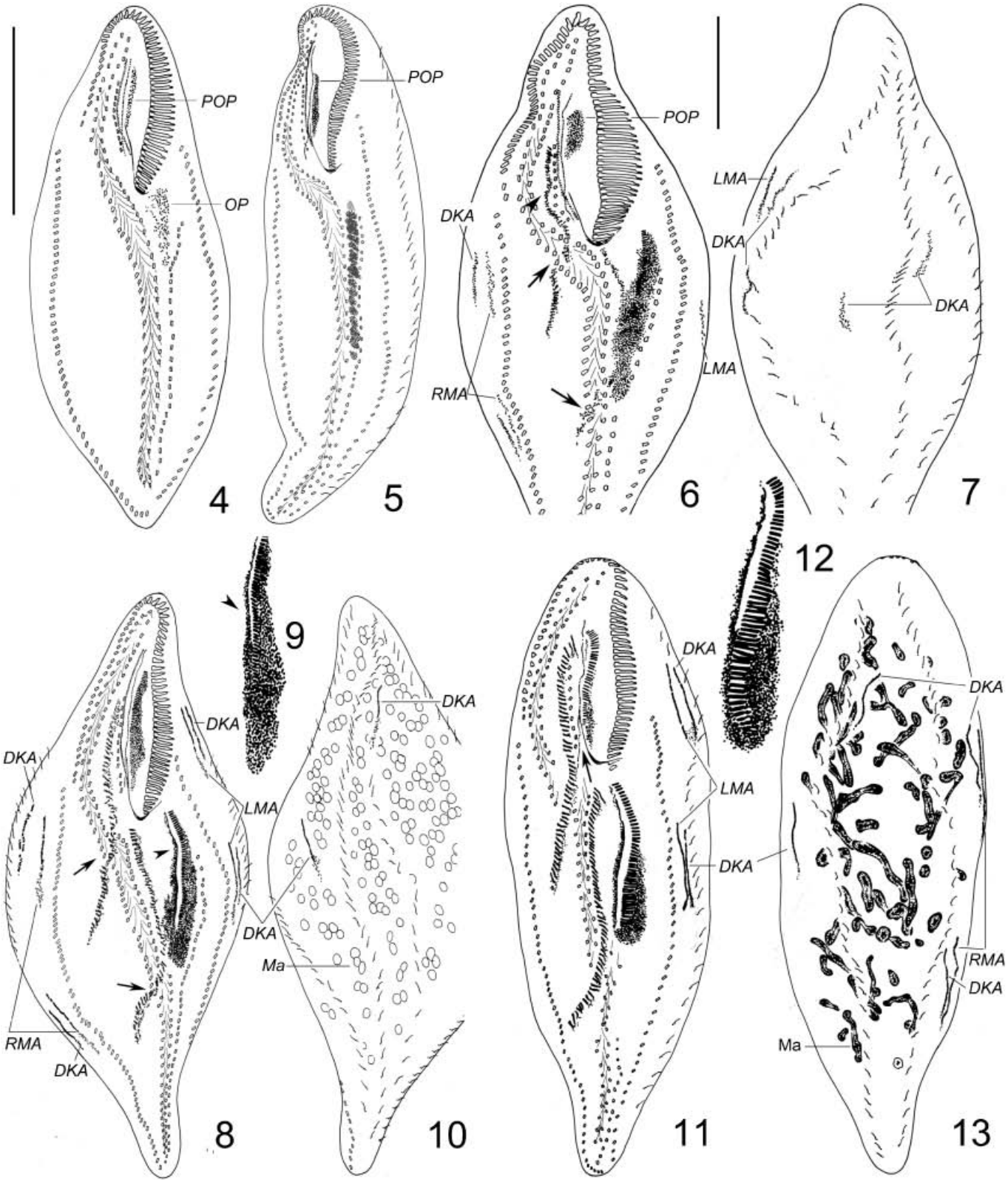
In the intermediate stage, the oral primordia continue to grow by further proliferation of basal bodies. The oral primordium in the opisthe forms groups of closely spaced basal bodies near the ventral cirri (Fig. 5, 27).

Eventually, the opisthe's primordium separates into anlagen for the adoral membranelles and frontoventral transverse cirri (FVT cirri, Fig. 6). In the proter, one group of basal bodies appears to the right of the old buccal cirri; this is the FVT anlagen of the proter (Fig. 6, arrowhead). The midventral and the buccal cirri are still

Fig. **4–13.** Early and middle stages of morphogenesis in *Apokeronopsis crassa* n. comb. **4.** Ventral view of an early divider, to show the formation of oral primordia in the proter (POP) and the opisthe (OP). **5.** Ventral view of a slightly later divider, to show the proliferation of basal bodies of two oral primordia. **6, 7.** Ventral view (Fig. 6) and dorsal (Fig. 7) views of the same specimen, to demonstrate the FVT anlagen in the opisthe are differentiated from the opisthe's oral primordium; FVT anlagen in the proter are formed in the outermost region of the cortex de novo to the right of the old undulating membranes (arrowhead); old midventral rows do not contribute to the construction of the FVT anlagen in both dividers (arrows). Note the anlagen of the marginal rows (LMA, RMA) and dorsal kineties (DKA) are generated de novo. **8–10.** Ventral (Fig. 8, 9) and dorsal (Fig. 10) views of the same specimen, to demonstrate the undulating membranes anlagen in both dividers are differentiated from the oral primordia (arrowheads in Fig. 8, 9). Note the FVT anlagen cross the parental midventral rows (arrows in Fig. 8). **9.** Proter's oral primordium as shown in Fig. 8 at high magnification. **11–13.** Ventral (Fig. 11, 12) and dorsal (Fig. 13) views of a middle-stage divider. **11.** Arrow marks the undulating membranes and buccal cirri in the proter beginning to dedifferentiate. **12.** Proter's oral primordium at high magnification. **13.** Showing the partial fusion of the macronuclear nodules (Ma). DKA, dorsal kineties anlagen; FVT, frontoventral-transverse; LMA, left marginal anlagen; RMA, right marginal anlagen. Scale bars in Fig. 4, 5, 8, 10, 11, 13 = 100 μm; in Fig. 6, 7 = 50 μm.

present, indicating that they are not involved in the formation of the new anlagen (Fig. 29, arrow). This is strong evidence for a de novo origin of the anlagen for the AZM, undulating membranes (UMs) and FVT cirri.

In the next stage, the FVT anlagen grow by increasing the number of basal bodies and organize into many oblique streaks posteriad (Fig. 8, 30). Both FVT anlagen cross the parental mid-ventral rows but resorption of cirri was not observed in these



regions (Fig. 8, arrows; 34, 38, arrow). The anlagen for the UMs (UM anlage) in both proter and opisthe are differentiated (Fig. 8, 9, 31, 32, arrowhead). New adoral membranelles begin to differentiate in a posteriad direction (Fig. 8, 9, 31, 32).

Differentiation of the adoral membranelles proceeds simultaneously in both proter and opisthe (Fig. 11, 12, 35). In the proter, the old UMs and buccal cirrus begin to dedifferentiate (Fig. 11, arrow). Development of the FVT streaks and the UM anlage in both proter and opisthe is very similar and takes place at about the same pace (Fig. 11, 12, 37).

By the next stage the differentiation of membranelles is almost complete, forming the new oral structures for both proter and opisthe (Fig. 14). The anterior ends of the two new AZMs arch strongly to the right. The old UMs and buccal cirri are already resorbed. The FVT anlagen begin to differentiate. A single cirrus develops from the anterior end of the UMs anlage and later becomes the leftmost frontal cirrus (Fig. 14, arrowheads; 41, arrowhead). Frontoventral-transverse streak I segregates into a row of ca 10 cirri (Fig. 14, arrows; 41, arrow).

Later, the UM anlagen of both proter and opisthe split giving rise to the paroral and endoral membranes (Fig. 16, arrowheads; 43, arrowhead). The posterior end of the new AZM in the proter is still in deep within the cortex (Fig. 44). The segregation of cirri from the FVT anlagen is almost complete. Each FVT streak, apart from the rightmost two, develops into two to three cirri that form three oblique rows (Fig. 16, 45, 48). All the cirri developed from FVT streak I, apart from the anterior one, migrate posteriad alongside the UMs to become the buccal cirri (Fig. 16, arrows; 43, arrow).

The frontoterminal cirri form, as is usual, as the two rightmost ( = anteriormost) cirri in the last FVT streak and subsequently move anteriad (Fig. 18, arrowheads; 49 arrowhead); the leftmost streak ''file'' (posterior cirrus in each streak) becomes the transverse cirri (Fig. 18). It was observed that in all dividers, several buccal cirri migrate to the bottom surface of the buccal cavity (Fig. 18, arrows; 51, arrow) and intersect the FVT anlagen at different depths within the cell (Fig. 18, 51, 52, arrow).

In later dividers the anterior portions of the new AZMs become distinctly curved and the parental AZM is almost completely resorbed (Fig. 20, 53). Disaggregation of the old midventral, marginal, and dorsal rows is not yet evident (Fig. 20, 53).

*Marginal and dorsal anlagen.* Two anlagen originate de novo dorsally to each old marginal row (Fig. 6, 7, LMA, RMA; 29, arrowhead). Likewise, the dorsal kineties anlagen form de novo: two anlagen are located near each old dorsal kinety (Fig. 6, 7, DKA).

The anlagen of the marginal rows and dorsal kineties develop with further proliferation of basal bodies and gradually lengthen (Fig. 8, 10, 11, 13, LMA, RMA, DKA; 15, LMA, RMA, arrows; 16, 17, LMA, RMA, double-arrowheads; 19, 40, 47, DKA, RMA; 56, LMA, RMA, arrows), until they stretch continuously in both directions across almost the whole dorsal surface (Fig. 20, 21, LMR, RMR, arrows; 53, 54). The parental marginal rows are evidently fully intact at these stages. Dorsal kinety fragmentation and dorsomarginal kineties are lacking.

*Division of the nuclear apparatus.* The most striking feature of the macronuclear division process was that the more than 100 macronuclear segments fuse into many masses before division (Fig. 10, 13, 17, 19, 39, Ma; 17, 21, 42, 50).

*Physiological regeneration (Fig. 22–25, 57–62).* Several stages of physiological regeneration were observed and these indicated that the main process of the cortical development in reorganizers is very similar to that in dividers. These include: (a) oral primordium and anlage of the UMs are formed de novo on the dorsal wall of the buccal cavity; (b) the FVT anlagen develop de novo in the outmost region of the cortex to the right of the old apparatus; (c) the buccal cirri are generated from FVT streak I; (d) the anlagen of the marginal rows and dorsal kineties are formed de novo, each dorsal kinety anlage being in the same position as those in the dividers; (e) and the most posterior FVT streak contributes the two frontoterminal cirri.

## DISCUSSION

For discussion of history and synonymy see detailed review by Berger (2006).

**Comparison with similar genera and rationale for a new combination.** *Apokeronopsis* resembles the well-known genera *Pseudokeronopsis*, *Uroleptopsis*, and *Thigmokeronopsis* in the possession of the following characters: the bicorona of frontal cirri; the strongly curved AZM, the distal end of which extends posteriorly down the right side of the cell; the macronuclear nodules that do not fuse into a single round to elliptical mass as in other urostylids (except for *Pseudokeronopsis similis*) (Gruber 1884; Hu and Song 2001; Hu, Warren, and Song 2004a; Hu et al. 2004b; Petz 1995; Shi and Xu 2003; Shi et al. 2007; Sun and Song 2005; Wicklow 1981; Wirnsberger 1987).

However, *Apokeronopsis* differs from *Pseudokeronopsis* in having a row of buccal cirri (vs. only one buccal cirrus in *Pseudokeronopsis*), distinctly separated midventral rows (vs. midventral rows close together, the cirri forming a conspicuous urostylid zig-zag pattern in *Pseudokeronopsis*), anlagen for the marginal rows and dorsal kineties formed de novo (vs. within parental structures in the latter), and developed transverse cirri in a long row (vs. fewer than 10 weakly developed transverse cirri in a short row in *Pseudokeronopsis*) (Borrow and Wicklow 1983; Hu et al. 2004b; Shi and Xu 2003; Shi et al. 2007; Song, Sun, and Ji 2004a; Song, Wilbert, and Warren 2002; Song et al. 2004b, 2006; Sun and Song 2005; Wirnsberger 1987).

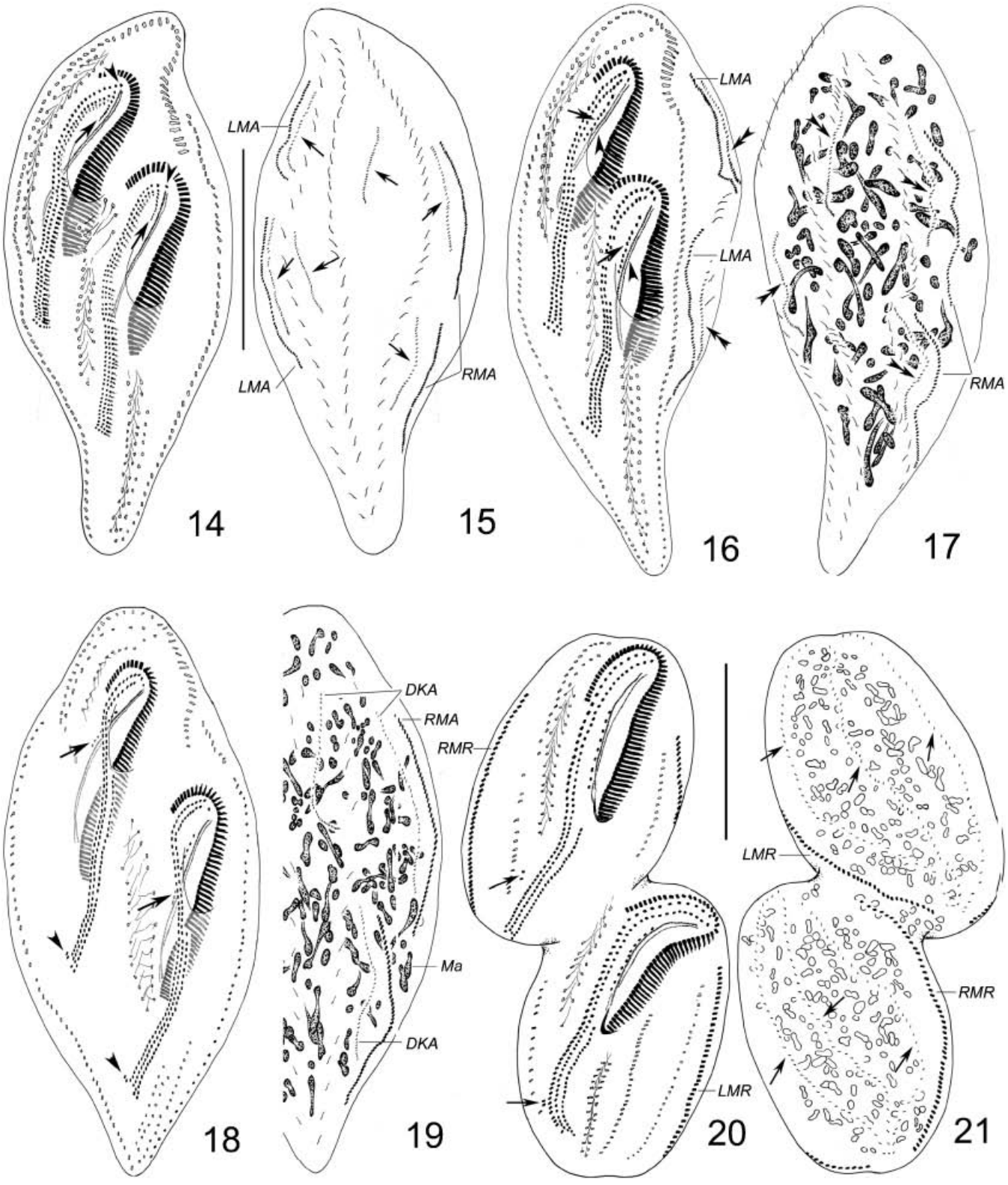
Different from *Uroleptopsis*, *Apokeronopsis* has two or more buccal cirri (vs. only one or no buccal cirrus in *Uroleptopsis*), high number of transverse cirri (vs. transverse cirri absent), distinctly separated midventral rows (vs. typical midventral rows as in *Pseudokeronopsis*), the anlage for the UMs generating one cirrus (vs. two cirri) and no gap in adoral zone (vs. present) (Berger 2004; Mihailowitsch and Wilbert 1990).

Compared with *Thigmokeronopsis* (except for *T. antarctica* Petz, 1995), *Apokeronopsis* is distinguished by possessing transverse cirri (developed and aligned in long row vs. fewer, fine cirri in a short row in *Thigmokeronopsis*), the macronuclear segments

Fig. **14–21.** Middle and late stages of morphogenesis in *Apokeronopsis crassa* n. comb. **14, 15.** Ventral and dorsal views of a same divider to show the FVT anlagen beginning to differentiate; In Fig. 14 arrows mark the FVT streak I generating a row of cirri, arrowheads mark the leftmost frontal cirrus which is separated from the UMA. Arrows in Fig. 15 indicate the dorsal kineties anlagen (DKA). **16, 17.** Ventral and dorsal views of a slightly later divider. Arrows depict the row of cirri coming from FVT streak I which migrate along the undulating membranes to form the buccal cirri; arrowheads mark the undulating membranes anlage splitting to form the endoral and paroral membranes, while double-arrowheads depict DKA. Note posterior portion of the proter's new membranelles are still beneath the cortex (Fig. 16) and the macronuclear nodules are fusing to many masses (Fig. 17). **18, 19.** Ventral and dorsal views of the same specimen. Arrows demonstrate the buccal cirri migrating into the buccal cavity. Arrowheads indicate the frontoterminal cirri. Note the macronuclear nodules (Ma) are in the process of division. **20, 21.** Ventral and dorsal views of a late divider, arrows in Fig. 20 demonstrate the frontoterminal cirri and arrows in Fig. 21 show the dorsal kineties. FVT, frontoventral-transverse; LMA, left marginal anlagen; LMR, left marginal row; RMA, right marginal anlagen; RMR, right marginal row; UMA, undulating membranes anlage. Scale bar = 100 μm.

fused to many masses (vs. fused completely forming a single branched mass) before division, and the absence of thigmotactic cirri (vs. conspicuously present in *Thigmokeronopsis*, which render the body very sticky as described in the type species *Thigmokeronopsis jahodai* and the recently reported species *Thigmokeronopsis magna* and *Thigmokeronopsis rubra* (Hu et al. 2004a; Petz 1995; Wicklow 1981).

Based on the above understanding, the following improved diagnosis for the genus *Thigmokeronopsis* is suggested: Pseudokeronopsidae with *Pseudokeronopsis*-like bicorona of frontal cirri



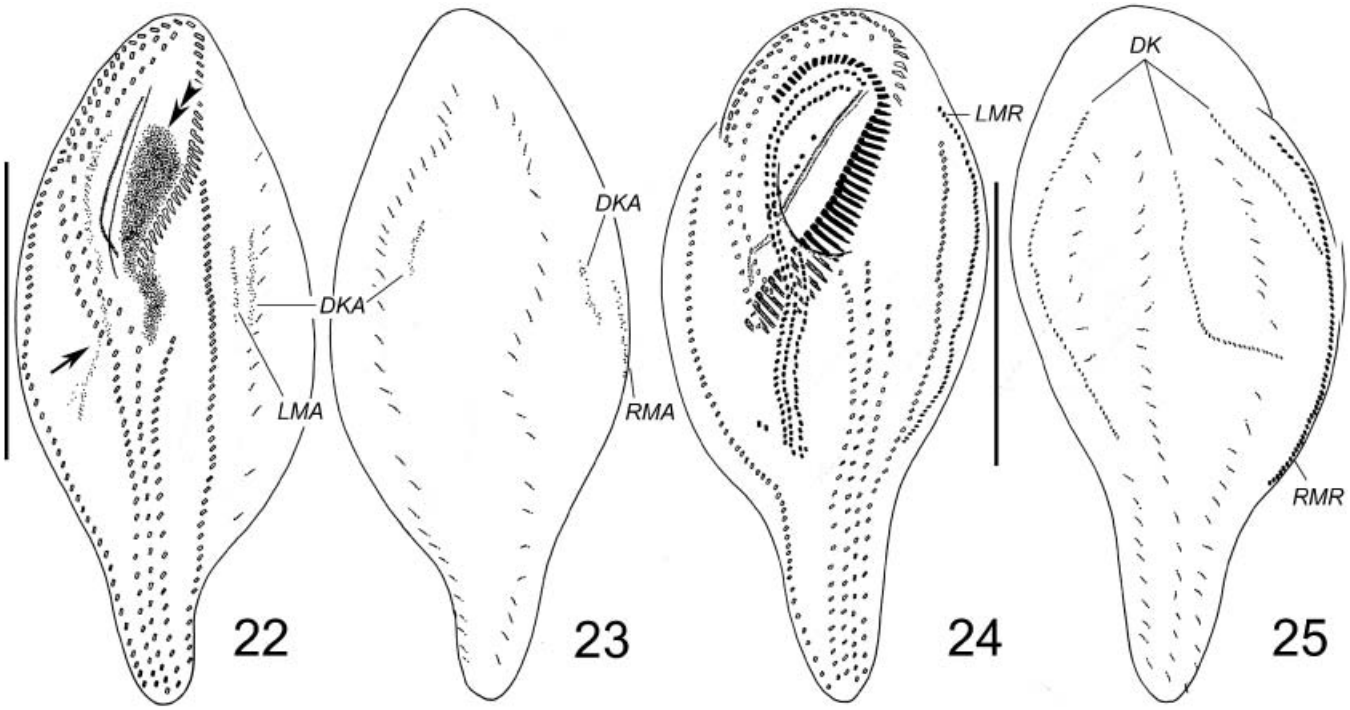



Fig. **22–25.** Physiological regeneration in *Apokeronopsis crassa* n. comb. **22, 23.** Ventral and dorsal views of the same specimen at an early stage of physiological regeneration, arrow marks the FVT anlagen, double arrowhead marks the oral primordium. Note the marginal anlagen and dorsal kineties anlagen (DKA) are formed de novo. **24, 25.** Ventral and dorsal views of the same specimen at a late stage of physiological regeneration, demonstrating that the general developmental pattern is similar to that in the proter during morphogenesis. DK, dorsal kineties; FVT, frontoventral-transverse; LMA, left marginal anlagen; LMR, left marginal row; RMA, right marginal anlagen; RMR, right marginal row. Scale bar = 100 μm.

and one marginal row on each side; two midventral rows distinctly separated, hence cirri that are not in a typical urostylid zig-zag pattern; frontoterminal cirri present; few, weak transverse cirri in a short row; thigmotactic cirri present as well as marginal and dorsal kineties form apokinetally during morphogenesis. According to the present definition, *T. antarctica* Petz, 1995 should be assigned to the genus *Apokeronopsis*: *Apokeronopsis antarctica* Petz, 1995 n. comb.

### Family Pseudokeronopsidae

### *Apokeronopsis* n. g.

**Diagnosis.** Pseudokeronopsidae with *Pseudokeronopsis*-like bicorona of frontal cirri and one marginal row on each side; one row of two or more buccal cirri in ordinary position; two midventral rows distinctly separated, hence cirri that are not in a typical urostylid zig-zag pattern; high number of transverse cirri, caudal cirri absent and frontoterminal cirri present; thigmotactic cirri absent, many macronuclear nodules fuse into many masses as well as marginal and dorsal kineties form apokinetally during morphogenesis.

**Type species.** *Apokeronopsis crassa* (Claparède & Lachmann, 1858) n. comb.

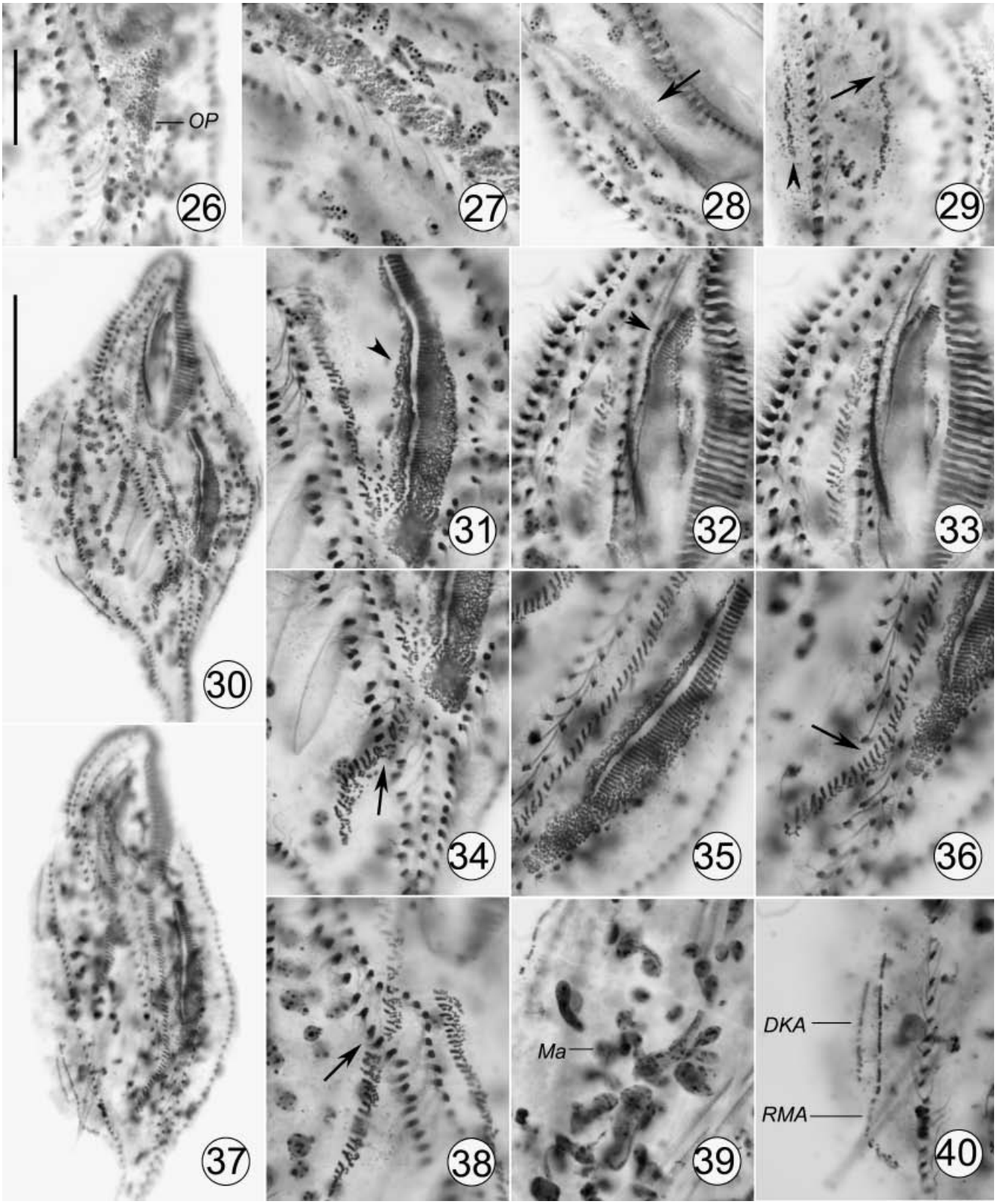
**Etymology.** Composite of Greek *apo* (derived from) and the generic name *Keronopsis*. Feminine gender.

**Neotype.** One slide with protargol-impregnated specimens has been deposited in the Natural History Museum, London, UK, with Registration number 2007: 3: 15: 1.

**Species assignable.** *Apokeronopsis crassa* (Claparède & Lachmann, 1858) n. comb. (basionym: *Oxytricha crassa* Claparède & Lachmann, 1858) and *A. antarctica* (Petz, 1995) n. comb. (basionym: *T. antarctica* Petz, 1995).

**Comparative morphogenesis.** Morphogenesis has been investigated in four species of *Pseudokeronopsis*: *Pseudokeronopsis carnea*, *Pseudokeronopsis rubra*, *Pseudokeronopsis flava*, and *Pseudokeronopsis flavicans* (Hu and Song 2001; Hu et al. 2004b; Shi and Xu 2003; Sun and Song 2005; Wirnsberger 1987) and the general pattern of morphogenesis in the genus *Pseudokeronopsis* has recently been documented (Hu and Song 2001; Hu et al. 2004b). Division morphogenesis of *Thigmokeronopsis*, which contains at least four species (*T. jahodai*, *Thigmokeronopsis crystallis*, *T. rubra*, *T. magna*), has been described for three species

Fig. **26–40.** Photomicrographs of *Apokeronopsis crassa* n. comb. during morphogenesis. **26.** Ventral view of an early divider, to show the opisthe's oral primordium (OP). **27, 28.** Ventral views of a slightly divider showing the OP in the opisthe (Fig. 27) and proter (Fig. 28), arrow in Fig. 28 demonstrates the OP in the proter. **29.** Ventral view of an early-stage divider. Arrowhead marks the right marginal anlagen and arrow demonstrating the old structures do not participate in the formation of the new FVT anlagen. **30–34, 38.** Ventral views of the same divider. **30.** Whole cell. **31.** Opisthe's OP. Arrowhead showing that the undulating membranes anlage is differentiated. **32.** Proter's OP. Arrowhead marks the undulating membranes anlage. **33.** Showing that the old buccal cirri are not involved in the formation of FVT anlagen in the proter. **34, 38.** FVT anlagen in the opisthe (arrow in Fig. 34) and proter (arrow in Fig. 38) cross the old midventral rows. **35–37, 39, 40.** Ventral (Fig. 35–37, 39) and dorsal (Fig. 40) views of the same specimen. **35.** Opisthe's OP. **36.** FVT anlagen crossing the old midventral rows (arrow). **37.** Whole cell. **39.** Macronuclear nodules (Ma). Fig. **40** Right marginal anlagen (RMA) and dorsal kineties anlagen (DKA). FVT, frontoventral-transverse. Scale bars in Fig. 26–29, 31–36, 38–40 = 25 μm; in 30, 37 = 100 μm.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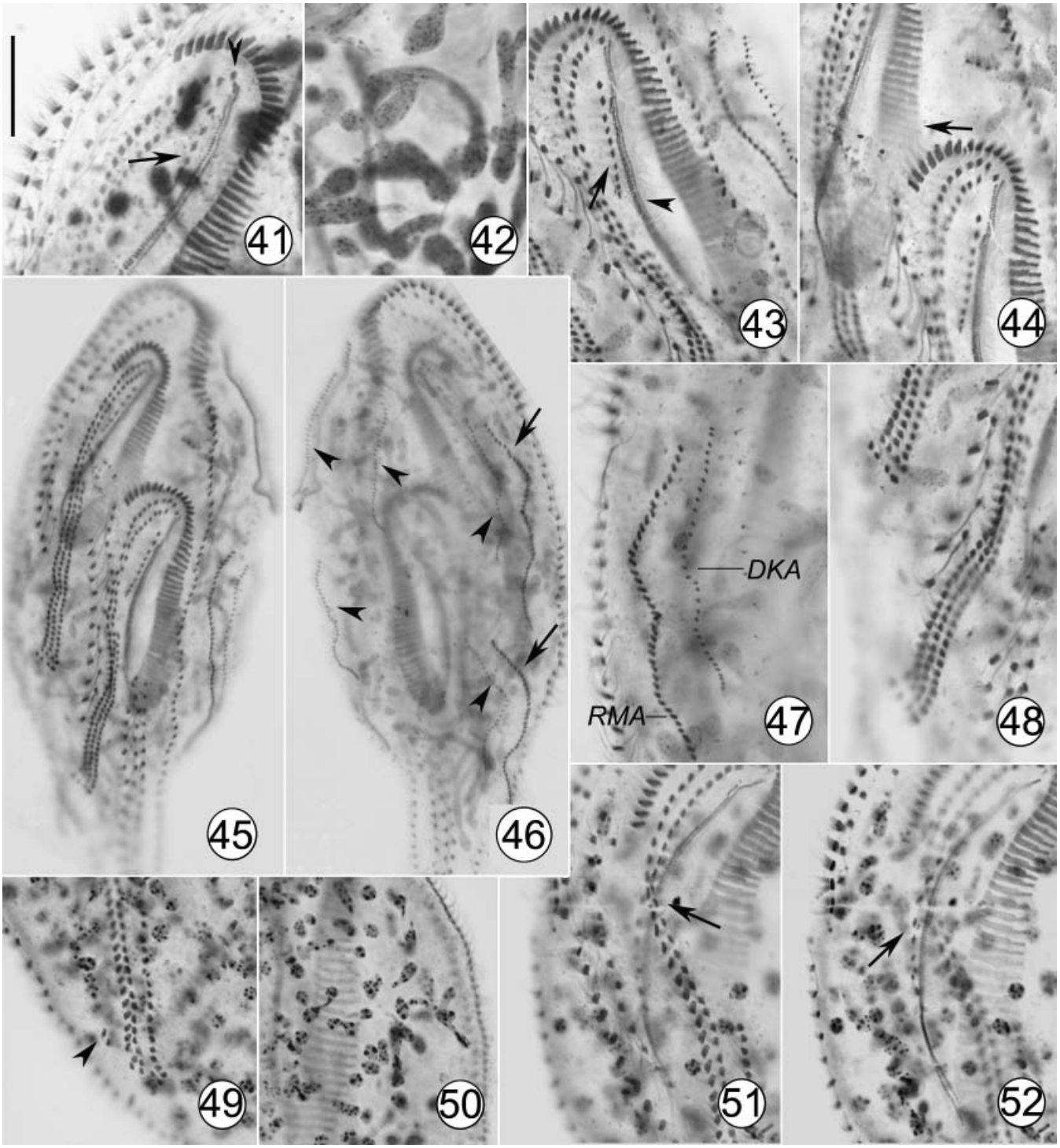


Fig. **41–52.** Photomicrographs of *Apokeronopsis crassa* n. comb. during morphogenesis. **41.** Ventral view, to show that the leftmost frontal cirrus is separated from the undulating membranes anlage (arrowhead). Arrow marks the buccal cirri. **42.** Partial fusion of the macronuclear nodules. **43–48.** Ventral (Fig. 43–45, 48) and dorsal (Fig. 46, 47) views of the same specimen. **43.** Opisthe's oral primordium. Arrow showing the buccal cirri and arrowhead marking the undulating membranes anlage splitting into the endoral and paroral membranes. **44.** Showing that the posterior portion of the new membranelles in the proter is still beneath the cortex (arrow). **45, 46.** Showing the whole cell. Arrows in Fig. 46 show the right marginal anlagen while arrowheads point to the dorsal kineties anlagen (DKA). **47.** Right marginal anlagen (RMA) and DKA. **48.** Right portions of the FVT anlagen in both dividers. **49–52.** Ventral views of a late divider. **49.** Showing the migration of the frontoterminal cirri (arrowhead). **50.** Showing the dividing macronuclear nodules. **51, 52.** Demonstrating that the buccal cirri, which are in the buccal cavity cross, with FVT anlagen (arrows). FVT, frontoventral-transverse. Scale bar = 25 μm.

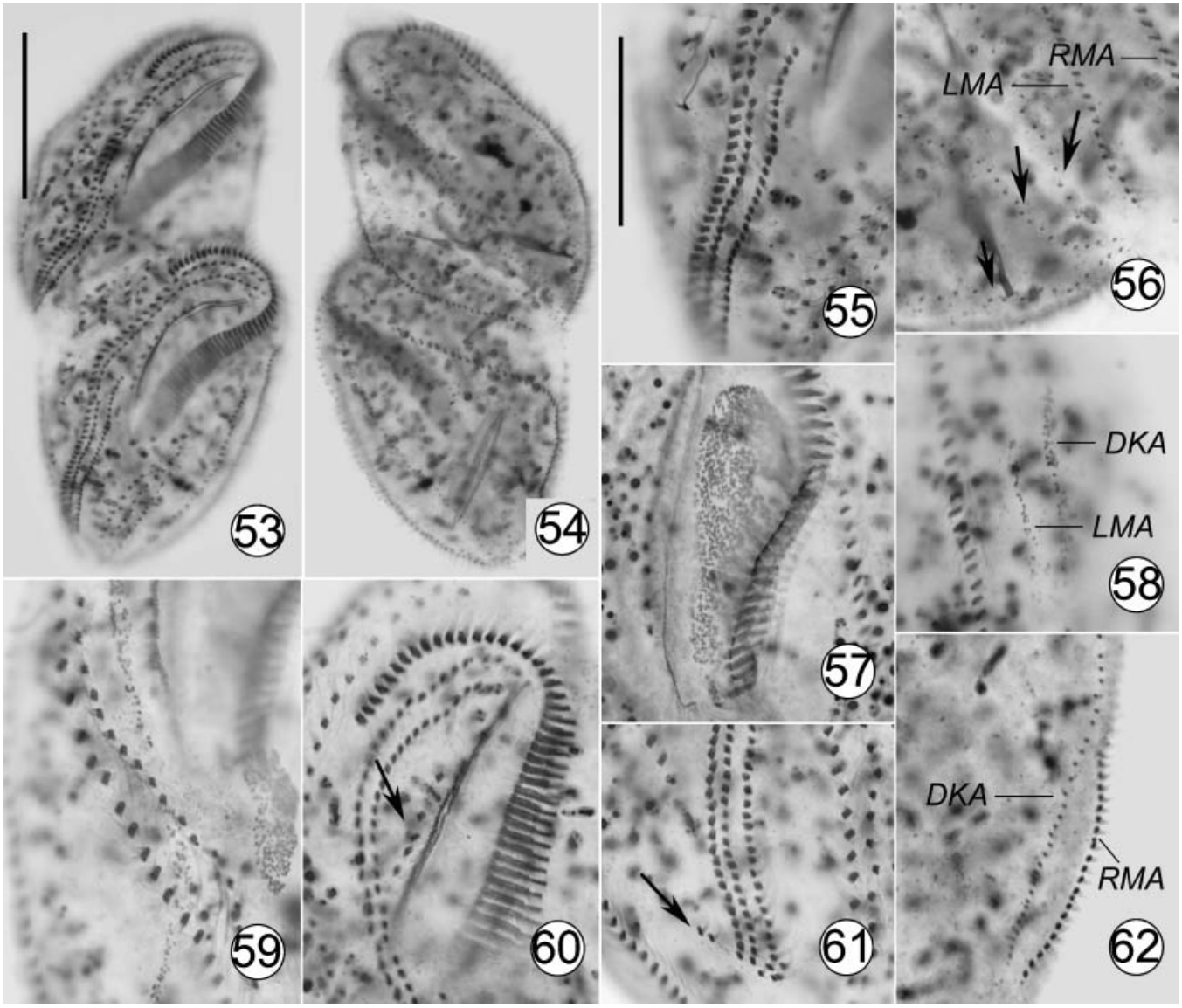


Fig. **53–62.** Photomicrographs of *Apokeronopsis crassa* n. comb. in the late stage of morphogenesis (Fig. 53–56) and undergoing physiological regeneration (Fig. 57–62). **53–56.** Ventral (Fig. 53, 55) and dorsal (Fig. 54, 56) views of a very late divider. **53, 54.** Whole cell. **55.** Right portion of the FVT anlagen. **56.** Showing the left marginal anlagen (LMA) in the proter and the right marginal anlagen (RMA) in the opisthe. Arrows mark the dorsal kineties anlagen (DKA). **57–59.** Ventral views of the same specimen at an early stage of physiological regeneration. **57.** Showing the oral primordium. **58.** Demonstrating that the marginal anlagen (LMA) and DKA are formed de novo. **59.** Depicting FVT anlagen. **60–62.** Ventral and dorsal views of the same specimen at a late stage of physiological regeneration, demonstrating that the general developmental pattern is similar to that in the proter during morphogenesis. Arrow in Fig. 60 points to the buccal cirri, arrow in Fig. 61 marks the frontoterminal cirri. FVT, frontoventral-transverse. Scale bars in Fig. 53, 54 = 100 μm; in Fig. 55–62 = 50 μm.

(Foissner 1996; Hu et al. 2004a; Petz 1995; Wicklow 1981). Of the five known *Uroleptopsis* species, only two of them, i.e. *Uroleptopsis citrina* and *Uroleptopsis ignea*, have been morphogenetically studied by modern methods (Berger 2004; Mihailowitsch and Wilbert 1990). These studies demonstrated that the genera *Pseudokeronopsis*, *Uroleptopsis*, and *Thigmokeronopsis* have very stable morphogenetic characters, which can be compared with those of *A. crassa*.

The morphogenetic events in *A. crassa* and *A. antarctica* have several features in common with those of *Uroleptopsis* and *Thigmokeronopsis*: (1) the parental oral apparatus is completely renewed by the independently formed oral primordium; (2) the parental basal bodies do not participate in the formation of the FVT cirri of the daughter cells; and (3) the macronuclear nodules do not fuse into a single round to elliptical mass as in other urostylids, as noted by Gruber (1884), Mihailowitsch and Wilbert (1990), Berger (2004) and Hu et al. (2004a). Members of *Pseudokeronopsis* share points 1 and 2 with species of *Apokeronopsis* (Hu and Song 2001; Hu et al. 2004b; Shi and Xu 2003; Shi et al. 2007; Wirnsberger 1987).

However, distinct differences exist among them. (1) Frontoventral-transverse streak I contributes one coronal and one (in *Pseudokeronopsis* and *Uroleptopsis*) or one to two (in *Thigmokeronopsis*) buccal cirrus whereas in *Apokeronopsis* it forms one coronal cirrus and a row of buccal cirri. (2) In *Pseudokeronopsis* and *Uroleptopsis*, the parental structures contribute to the construction of the anlagen of the marginal rows and dorsal

kineties whereas in *Apokeronopsis* and *Thigmokeronopsis* species these anlagen form apokinetally. (3) In *Pseudokeronopsis* and *Uroleptopsis*, the midventral rows remain closely juxtaposed forming a zig-zag pattern whereas in *Apokeronopsis* and *Thigmokeronopsis* species the midventral rows become distinctly separated at interphase. (4) In *Uroleptopsis*, cirral streaks only develop into midventral complex but no transverse cirrus is formed. In *Thigmokeronopsis*, *Pseudokeronopsis*, and *Apokeronopsis*, the last FVT cirral streaks contribute one transverse cirrus each. (5) In *Thigmokeronopsis*, all cirral streaks (except for streak I and posterior several ones), each of which develops additional several fine cirri, form the thigmotactic files whereas in *Apokeronopsis*, *Uroleptopsis*, and *Pseudokeronopsis* no such thigmotactic cirri are formed. (6) The macronuclear segments fuse into many masses in all members of *Apokeronopsis* and *Uroleptopsis* as well as most *Pseudokeronopsis* species before division (vs. a single branched mass in *Thigmokeronopsis* or a single spherical mass in *P. similis* (Hu and Song 2001; Hu et al. 2004a, b; Petz 1995; Shi and Xu 2003; Shi et al. 2007; Sun and Song 2005; Wicklow 1981; Wirnsberger 1987).

Using the system proposed by Wicklow (1981), Borror and Wicklow (1983) united *Pseudokeronopsis* and *Thigmokeronopsis* in the Pseudokeronopsidae. Eigner and Foissner (1992) also considered *Thigmokeronopsis* and *Pseudokeronopsis* as sister genera. Subsequently, a different and more detailed foundation of the relationships within the Pseudokeronopsidae was given by Berger (2004, 2006). Based on his new system, we therefore conclude that *Apokeronopsis* is a transitional form between the Pseudokeronopsinae and *Thigmokeronopsis* as it shares morphogenetic features with both. However, based on the findings reported here and from previous studies, we could not estimate whether *Apokeronopsis* has a closer relationship with Pseudokeronopsinae or *Thigmokeronopsis* as well as whether *Apokeronopsis* should be placed in the Pseudokeronopsinae.

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