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Stomatogenesis of the marine ciliate *Paranophrys magna* (Protozoa: Ciliophora: Scuticociliatida) from Qingdao, China

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The morphology and stomatogenesis of the marine scuticociliate *Paranophrys magna*, collected from a crab-culturing pond in Qingdao, China, were studied. It possessed typical characteristics of the genus *Paranophrys*. The stomatogenesis of this species corresponds basically with the results provided in previous studies for this genus, the main sequence develops can be generalized as follows according to the Qingdao population: (i) in the proter: the remnant of the parental paroral membrane generates the new paroral membrane and the scutica; and (ii) in the opisthe: both the paroral membrane and scutica originate from the proliferation of the anterior part of the secondary primordial field, membranelles 1&2 derive from the posterior part of the secondary primordial field, while membranelle 3 from the proliferation of the parental scutica which form the primary primordial field.

Some differences between our results and previous descriptions are compared and discussed.

INTRODUCTION

Thompson & Berger (1965) established the genus *Paranophrys*, without giving clear diagnosis for this new taxon, though most subsequent descriptions are based on the 'modern' method which revealed generally the infraciliature and the silverline system, disagreements consistently occurred with identification and arrangement of some *Paranophrys*-like ciliates (Borror, 1972; Czapik & Wilbert, 1986; Didier & Wilbert, 1976a,b; Grolière & Leglise, 1977; Kahan et al., 1987; Puytorac & Grolière, 1979; Puytorac et al., 1974; Small & Lynn, 1985; Song & Wilbert, 1989; Strüder & Wilbert, 1992).

Because of this conflicting information, Song & Wilbert (2000) published an improved diagnosis: 'with pointed anterior end; well-developed membranelle 1 (M_1) and 2 (M_2), of which the M_1 consists of two or more longitudinal rows of basal bodies and is about the same length as M_2; paroral membrane uniformly, extending anteriorly to the apical end of M_2; single caudal cilium present'; and several synonyms of *Paranophrys magna* Borror, 1972 have been given: *Anophryoides salmacida* Puytorac & Grolière, 1979; *Anophryoides puytoraci* Small & Lynn, 1985; *Anophryoides soldoi* Small & Lynn, 1985; *Paranophrys carnivora* Czapik & Wilbert, 1986.

As to the morphogenesis of the genus, at least two descriptions have been reported (Czapik & Wilbert, 1986; Didier & Wilbert, 1976a; Puytorac & Grolière, 1979). Since some details about the developmental pattern are still lacking, especially the developing sequences of the oral structure, the present paper provides the results of our observations on *P. magna* during binary fission.

MATERIALS AND METHODS

Ciliates were collected from the offshore crab-culturing pond in May 1998, Qingdao (Tsingtao, 36°08′N 120°43′E), China. The salinity was 20 psu, pH 7.9–8.1. After isolation, specimens were maintained as pure or raw cultures in Petri dishes in the laboratory for days to weeks with rice grains as a food source for bacteria.

Synchronized cultures were obtained with beef extract in fresh seawater (3 g l^{-1}) at 24–28°C. Cells usually begin to divide after 2–3 h in these cultures.

Living observations were made under a microscope equipped with phase-contrast optical lens. Protargol (Wilbert, 1975), Chatton-Lwoff impregnation method (Corliss, 1953) and pyridinated silver carbonate impregnation (Fernández-Galiano, 1976) were used to reveal the infraciliature and silverline system in different morphogenetic stages respectively.

All measurements were made under oil immersion (×1250). Drawings were performed with the help of a camera lucida. Terminology mainly according to Morado & Small (1994) and Pérez-Uz et al. (1996).

RESULTS

Infraciliature of the non-dividing cells (Figures 1A–C & 3A–C; Table 1)

Morphology, infraciliature and behaviour in Qingdao population of *Paranophrys magna* are similar to those described previously (Borror, 1972; Czapik & Wilbert, 1986; Mugard, 1949; Puytorac & Grolière, 1979; Song & Wei, 1998; Song & Wilbert, 2000). A mature, non-dividing cell is characterized by its elongated bag- or cylinder-like body shape and (usually) dark grey colour under low magnification. Cells ~50–80×20–30 μm in life with pointed apical end and generally rounded posterior end. As in some other congeners, body shape varied in mass-culture condition. Cilia densely arranged, 8–10 μm long, one single caudal cilium (CC) ~15 μm. One small contractile vacuole (CV) terminally placed at

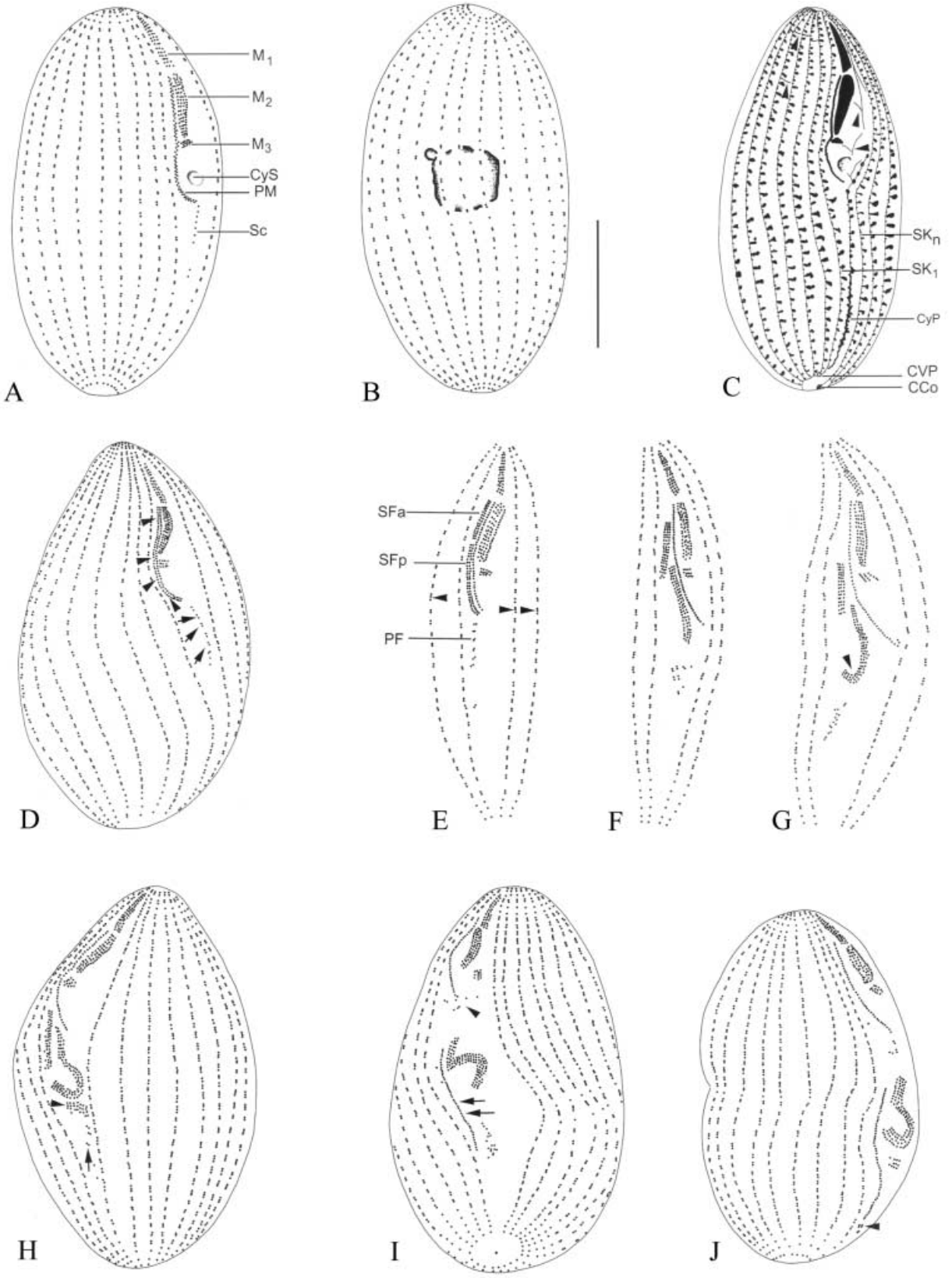

Figure 1. Early stomatogenetic stages in *Paranophrys magna*. (A–C) Non-dividing stages (arrowheads show intermediate silverline); (D) initial morphogenetic stage (arrows and arrowheads show kinetosomal proliferation in the scutica and longitudinal splitting of paroral membrane respectively); (E) the proliferation and dividing of SF (arrowheads show proliferation of somatic kinetosomes); (F,G) downward migration of SFa and SFp (arrowhead shows the J-shape SFp); (H) showing rearrangement of PF; (I,J) SFa rearranged linearly (arrows), arrowheads showing sparsely distributed kinetosomal pairs. CCo, caudal cilium complex; CVP, contractile vacuole pore; CyP, cytopyge; CyS, cytostome; M_1, membranelle 1; M_2, membranelle 2; M_3, membranelle 3; PF, primary field of proliferation; PM, paroral membrane; Sc, scutica; SFa, the anterior secondary field of proliferation; SFp, posterior secondary field of proliferation; SK_1, the first somatic kinety; SKn, the n-th somatic kinety. Scale bar: 20 μm.

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Table 1. *Biometrical characterization of* Paranophrys magna. *Data based on protargol impregnated specimens.*

Character	Minimum	Maximum	Mean	SD	SE	CV	N
Body length	42	76	52.2	17.27	3.45	33.1	25
Body width	17	41	28.0	8.25	1.65	29.5	25
Length of buccal field	18	32	23.8	3.57	0.71	14.9	25
Number of macronucleus	1	1	1	0	0	0	>100
Number of micronucleus	1	1	1	0	0	0	>100
Length of macronucleus	6	16	13.3	4.36	0.87	32.7	25
Width of macronucleus	5	14	10.8	3.80	0.76	35.2	25
Length of membranelle 1	5	9	6.9	1.15	0.23	16.7	24
Length of membranelle 2	6	11	8.9	1.20	0.24	13.5	24
Number of somatic kineties	20	22	21.1	0.72	0.12	3.4	32

CV, coefficient of variation; Mean, arithmetic mean; N, sample size; SD, standard deviation; SE, standard error of the mean. Measurements in μm.

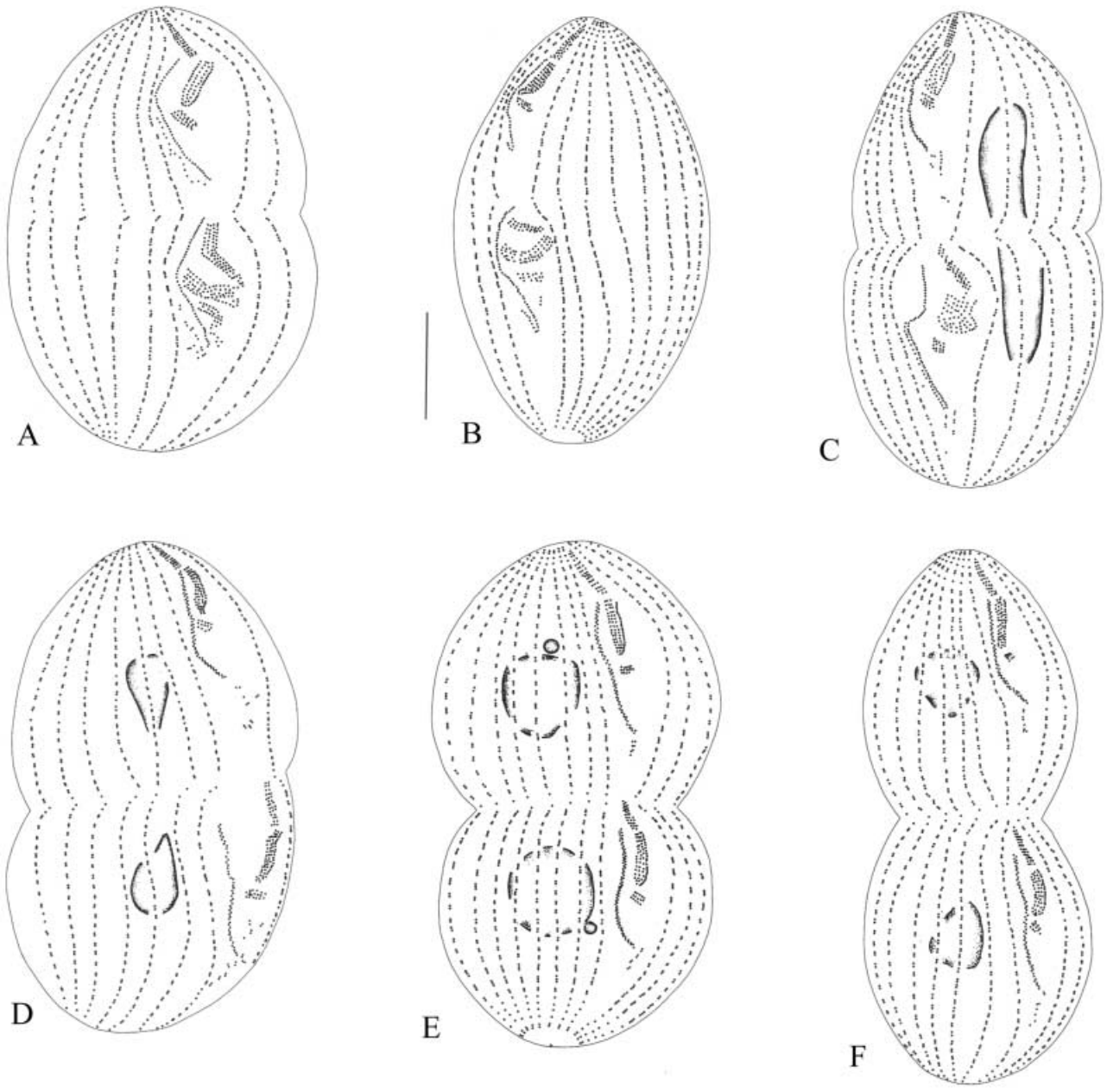

Figure 2. Late stomatogenetic stages in *Paranophrys magna*; reorganization of opisthe and proter. Scale bar: 20 μm.

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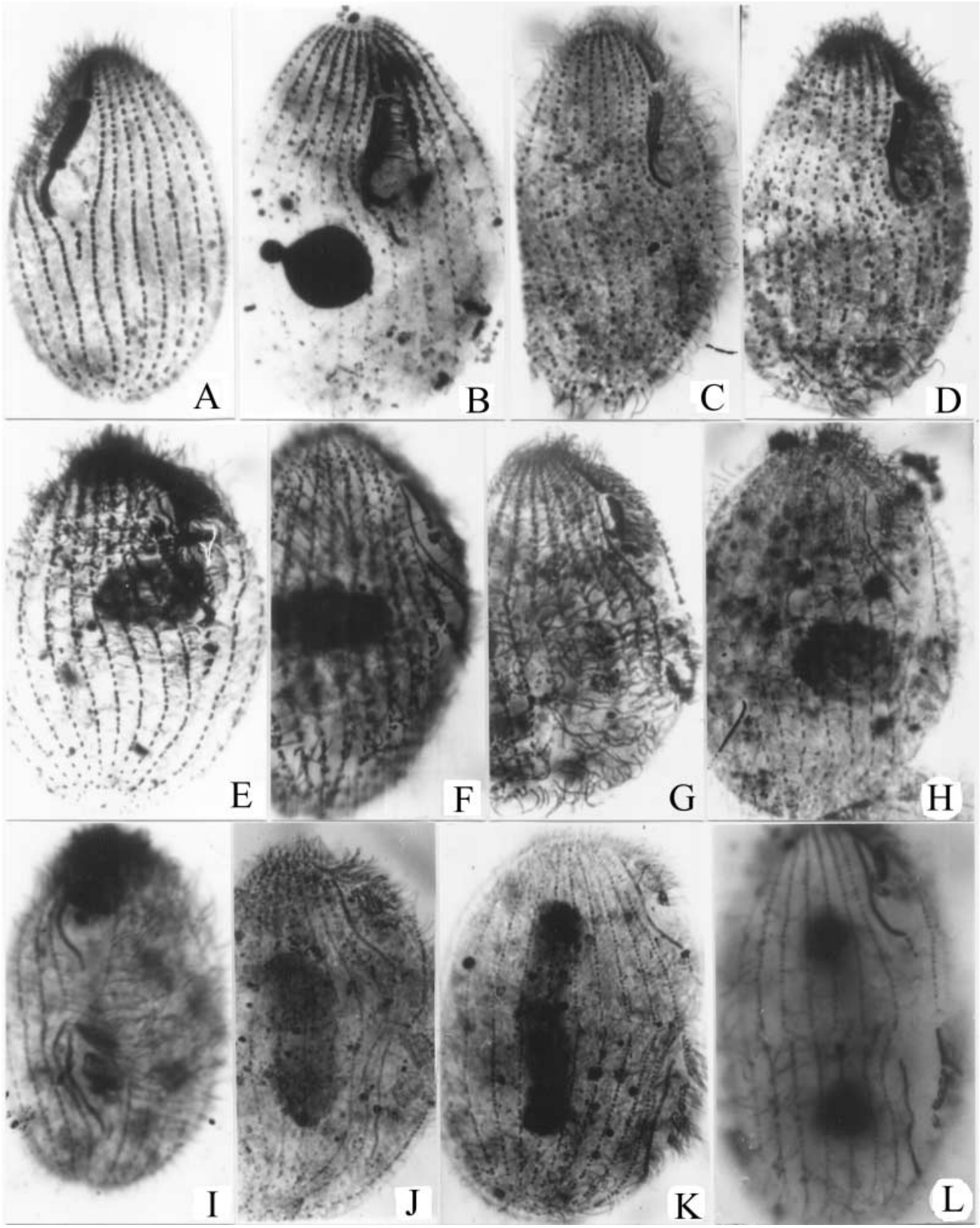

Figure 3. Ventral views of non-dividing stage and stomatogenetic sequences with protargol (A,C–L) and silver carbonate impregnation (B). (A–C) Non-dividing stage; (D) early stomatogenetic stage, to show the proliferation of basal bodies in scutica; (E–H) proliferation of kinetosomes and downward migration of the anlagen in opisthe; (I–L) reconstruction of the oral apparatus in the proter and opisthe.

posterior end of cell. One large oval macronucleus centrally located, which is often irregularly shaped after impregnation. Single round micronucleus tightly attaching to macronucleus.

Buccal field ~2/5 cell length, oral apparatus consisting of three *Parauronema*-like membranelles, membranelle 1 (M_1) ~6–9 μm long, slightly away from apex, consisting of 2–3 longitudinal rows of kinetids of unequal length;

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membranelle 2 (M_2) ~9–11 μm long, 4–5 rowed, of which the leftmost row usually has more densely arranged basal bodies; membranelle 3 (M_3) small, with ~3–4 short obliquely arranged rows of basal bodies. Paroral membrane (PM), beginning on right of the anterior end of M_2, usually described one or two curves along right side, composed of zigzag rows of basal bodies. Scutica (Sc), usually arranged in a short line with several pairs of kinetosomes.

Twenty to 22 bipolar somatic kineties arranged longitudinally, which are basically 'mixed' di- and monokinetid and all kineties forming apically a cilia-free area.

Silverline system as shown in Figure 1C, kinety n crossing over caudal area and continued on to caudal cilium complex. Extrusomes located between kineties. Intermediate silverlines observed at anterior portion of the trophont, between kinety n and membranelles (Figure 1C, arrowheads). Cytopyge (CyP) as a long argentophilic slit between kinety 1 and n, irregularly shaped. Contractile vacuole pore (CVP) located near posterior end of kinety 2.

Morphogenesis during binary fission (Figures 1D–J, 2 & 3D–L)

The first sign of stomatogenesis is the proliferation of kinetosomes that comprise the Sc segments. Initially, more basal body pairs appear in the Sc area, which are aligned in a longitudinal line (Figures 1D & 3D, arrows). This area is designated the primary primordial field (PF) because it appears first and early in stomatogenesis. Meanwhile, the 'zigzag' structure of PM begins to split longitudinally from the anterior to the posterior part into two, of which the right one serves as the secondary primordial field (SF) (Figure 1D, arrowheads).

Meanwhile, a parallel uniform proliferation of SF kinetosomes occurs along the entire length of the PM. Shortly afterwards, SF divides into two parts, the anterior (SFa) ~1/3 and the posterior (SFp) ~2/3 of SF (Figure 1E). The somatic kinetosomes proliferation may be recognized at this time, of which three basal bodies grouped together at equatorial area were observed (Figure 1E, arrowheads).

A marked proliferation of SF kinetosomes continued and was again followed by the realignment of formed structures. Three rows of basal bodies in SFa and SFp emerged longitudinally and, comparing with the former structure a slight winding of the posterior in SFp appeared. A partial migration of the primary kinetosomal field begins subsequently (Figure 1F). The posterior of SFa migrates to lie right at the anterior of SFp. The kinetosomes of PF rearranged irregularly (Figures 1F & 3E). Somatic kinetosome proliferation showed obviously in three basal bodies grouped together in the middle area (Figure 1F). Then, the posterior of SFp curved and SFp adopts a J-shape configuration (Figures 1G & 3F, arrowhead).

The SFa moves downward externally to SFp, which bends obviously. At the same time, most of the proliferated pairs of basal bodies originating from Sc move closer together (Figures 1H & 3G, arrowhead), whereas beneath it several other pairs arrange loosely (arrow).

The next stage in the process is shown in Figures 1I,J & 3H. The kinetosomes originating from proliferation of Sc are now arranged as a structure that will give rise to M_3 in the opisthe. A marked SFa stretches as a long row in the opisthe (Figure 1I, arrows). In the proter, beneath the remnant of parental PM there are pairs of kinetosomes observed, although their origin is not completely clear. Then, in the opisthe, pairs of kinetosomes beneath the linear SFa are also observed afterwards (Figure 1J, arrowhead).

Figures 2A,B & 3I show four fields of kinetosomes that are the primordia of the buccal structure for the new trophont. SFp splits into two parts, the anterior small and the posterior large portion, which will give rise to M_1 and M_2, respectively. The posterior kinetosomes of both the SFa in opisthe and the remnant of PM in proter proliferate to give rise to a kinetosomal hook that later differentiates into the Sc, respectively.

Figures 2C & 3J show the reorganization of the oral apparatus and the appearance of the kinetosomes of Sc.

The final phases of stomatogenesis, just before cytokinesis takes place, are shown in Figures 2D–F & 3K–L. PM is gradually closer to membranelles, several kinetosomes will eventually be reorganized or reabsorbed to produce the final oral apparatus.

Based on the data obtained from the Qingdao-population, the developmental sequence of *Paranophrys magna* can be summarized as follows: both the paroral membrane and scutica in the opisthe originate from the proliferation of the anterior part of the secondary primordial field (SFa), while membranelles 1–2 and membranelle 3 from the posterior part of the secondary primordial field (SFp) and the primary primordial field (PF) respectively.

DISCUSSION

According to present investigations, the morphogenetic progresses of *Paranophrys magna* are generally similar to many other related scuticociliates, i.e. *Uronema*, *Philasterides*, *Parauronema*, *Mesanophrys* (Grolière, 1974, 1980; Grolière & Leglise, 1977; Morado & Small, 1994; Pèrez-Uz et al., 1996; Puytorac et al., 1974; Song, 1991). The paroral membrane and scutica in the opisthe originate from the proliferation of anterior portion of the parental paroral membrane; the posterior portion of the parental paroral membrane develops into two or three fields that will form the membranelles 1 and 2, while the proliferated scutica develops into the membranelle 3.

The stomatogenesis of *P. magna* had been, more or less insufficiently in our opinion, described previously but called *Paranophrys carnivora* and *Anophryoides salmacida* respectively (Czapik & Wilbert, 1986; Puytorac & Grolière, 1979). According to them, the stomatogenetic developing pattern for the opisthe is: PM→PM, M_1, M_2, Sc; Sc→M_3, which corresponds well with our observations. Czapik & Wilbert (1986) described up to three fields originating from the anterior portion of the parental PM gave rise to PM and scutica of the opisthe. However, in our observations, only one fragment was formed, which developed into the same structure.

Apart from *P. magna*, *Paranophrys thompsoni* is the only congener, of which the morphogenesis was observed (Didier & Wilbert, 1976a). Compared with that, our question is related to the fate of the parental scutica. Didier & Wilbert gave only a brief description about the developing

process, which appears distinctly different from *P. magna*, i.e. the parental scutica give rise to M_2 and M_3 in the opisthe rather than the M_3 only in *P. magna*. If this interpretation is correct (unfortunately many stages in their work were lacking), then this somatogenetic pattern difference might indicate that the two taxa do not belong to the same genus. But further studies are definitely necessary.

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