Considerations on the Systematic Position of *Uronychia* and Related Euplotids Based on the Data of Ontogeny and 18S rRNA Gene Sequence Analyses, with Morphogenetic Redescription of *Uronychia setigera* Calkins, 1902 (Ciliophora: Euplotida)

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**Summary.** Characteristics found in morphogenetic process of the *Uronychia setigera* Calkins, 1902 during binary division and the molecular data of several euplotid genera inferred from 18S rRNA gene sequence analyses are compared and discussed. The results indicate that the traditional order Euplotida might comprise 3 paraphyletic groups: the eueuplotids, i.e. euplotids with a 5-cirral anlagen mode and a subcortical origin of the oral primordium during morphogenesis (we call them the “typical” euplotids in the text), gastrocirrhids (the pseudoeuplotids) and discocephalids, the last of which is possibly a convergent lineage. The results suggest also that *Uronychia* represents a primitive type within typical euplotids in phylogeny. Based on these analyses, a conclusion is suggested which consists of essentially the following points: (1) The traditional euplotids are likely a paraphyletic assemblage with differently originated groups. As a suggestion based on the new definition, the euplotids *s. l.* contains at least 2 subgroups: the eueuplotids and the sister group, gastrocirrhids (pseudoeuplotids) with multi-anlagen mode which is a divergent or apomorphic feature. (2) The systematic position of discocephaline remains unclear, but possibly an intermediate group between the euplotids *s. str.* and stichotrichs. We agree, however, that it is presently placed within the order Euplotida, as an outer group of other two assemblages. (3) The morphogenetic feature of 5-cirral-anlagen-mode is highly conservative and should be hence weighted with more phylogenetic value(s) in systematic analysis. (4) We suppose that some features like the origin mode of the leftmost frontal (formed from the UM-anlage), the situation of undulating membranes (e.g. two in number, curved in appearance) represent apomorphic features, and should not be over-evaluated, which caused often the diverse structures of the phylogenetic trees. (5) Euplotids *s. str.* might be derived from the 5-anlagen ancestor-type of hypotrichs *s. l.*, which is possibly also the ancestor of the current stichotrichs.

**Key words:** 18S rDNA gene sequences, Ciliophora, euplotids, morphogenesis and phylogeny, *Uronychia setigera*.

**INTRODUCTION**

Typical euplotid ciliates, as widely accepted for a long time, are regarded as a monophyletic lineage and a sister group with other traditional hypotrichs *s. l.* (or stichotrichs), which contains several well-known taxa, e.g. *Euplotes, Diophrys, Uronychia, Aspidisca, Gastrocirrhus, Discocephalus* (Deroux and Tuffreau 1965, Hartwig 1973, Curds and Wu 1983, Small and Lynn 1985, Song and Wilbert 2000). However, evidence (accumulated recently from molecular investigations) suggests that these ciliates might be a paraphyletic assemblage (Chen and Song 2001, 2002). This result is also partly supported by the interpretation derived from
non-molecular phylogenetic analyses, which indicate that the highly specialized Uronychia branches very early from the main cluster, and represents possibly a marginalized lineage against other euplotids (Song 1995).

The small Uronychia species, U. setigera Calkins, 1902, is commonly found in littoral biotopes (Calkins 1902, Kahl 1932, Bullington 1940, Song and Wilbert 1997). Its morphology and infraciliature were recently redefined by Song and Wilbert (1997), albeit it is often confused with its congener, Uronychia binucleata (under various names, details see Song and Wilbert 1997).

To unravel the phylogenetic position and the systematic relationships of Uronychia among related taxa, the 18S rRNA gene data based on several euplotid genera and morphogenetic characteristics revealed in U. setigera have been re-examined/re-analyzed and, as a new contribution, more detailed descriptions for this species are presented as well in the current paper.

Thus, our paper has two main aims: (1) we will check again the morphogenetical data obtained in those well-known genera of euplotids to find/question hiatuses which are seemingly ignored in last time; (2) Based on both ontogenetical and DNA sequence data, to address the systematic relationships between/among the taxa which are traditionally arranged in euplotids.

MATERIALS AND METHODS

Morphological and morphogenetical studies

Population of Uronychia setigera used for morphogenetic studies was collected from coastal waters near Qingdao (36°08’ N; 120°43’ E), China. After isolation, pure cultures were kept in the Laboratory of Protozoology, OUC. For molecular work, clonal cultures were established and maintained in sterilized seawater at room temperature with rice grains as food source to enrich bacteria. Cells in division were impregnated using protargol method (Wilbert 1975). Drawings were made with the help of a camera lucida at 1250x magnification. For clarity, parental cirri are shown in diagrams of morphogenetic stages only by outline, whereas new ones are shaded black.

Genomic DNA extraction, PCR amplification, sequencing and phylogenetic analyses

Nine species with original sequence data are concerned in the present studies, which were identified based on both in vivo observation and silvered (protargol and silver nitrate methods) specimens. DNA extraction, isolation and PCR reaction were performed as previously described (Chen and Song 2002).

Sequences analyzed are accessible at GenBank/EMBL with the following codes: Aspidisca steini AF305625 (Chen and Song 2002), Diophys appendiculata AY004773 (Chen and Song 2001), Euplotidium arenarium Y19166 (Petroni et al. 2000), Euplotes charon AF492705 (Chen and Song, unpubl), E. crusius AJ310492 (Bernhard et al. 2001), E. eurystomus AF52707 (Chen and Song, unpubl), E. magnicirratus AJ549210 (Petroni et al. 2002), E. minuta AJ310490 (Bernhard et al. 2001), E. parawoodruffi AF452708 (Chen and Song, unpubl), E. rariseta AF492706 (Chen and Song, unpubl), E. vanus AJ004772 (Chen and Song 2002), E. woodruffi AF492707 (Chen and Song, unpubl), E. raikovi AJ305251 (Petroni et al. 2002), Holosticha multistylata AJ277876 (Shin et al. 2000), Laboeca strobiola AF399151 (Snoeyenbos-West et al. 2002), Oxytricha ferruginea X53486 (Chen and Song, unpubl), Phacodinium metchnikoffii AJ277877 (Shin et al. 2000), Protoeuglena sp. AF194409 (Shin et al. 2000), Sterkiella nova (= Oxytricha nova) X03948 (Elwood et al. 1985), Strombidium purpureum U97112 (Hirt et al. unpubl), Stylonychia lemucae AJ310497 (Bernhard et al. 2001), Uronychia transfuga AF260120 (Chen and Song 2001).

The 18S rRNA gene sequences were aligned with a computer assisted procedure, Clustal W (Version 1.80) (Thompson et al. 1994), and refined by considering the conservation of both primary and secondary structures (Elwood et al. 1985). PHYLIP (Phylogeny Inference Package, Version 3.57) (Felsenstein 1995) was used to calculate the sequence similarity and evolutionary distances between pairs of nucleotide sequences using the Kimura (1980) two-parameter model. Distance-matrix trees were then constructed using the Fitch and Margoliash (1967) least-squares (LS) method and the neighbor-joining (NJ) method (Saitou and Nei 1987). PAUP v4.0b1 was used for the maximum parsimony (MP) analysis (Swofford 1998). Of the 2037 aligned sites, 540 phylogenetically informative characters were used to find the most parsimonious tree using the heuristic search method. Both parsimony and distance data were bootstrap resampled 1,000 times (Felsenstein 1985).

Terminology and systematics

Basically, the terminology and systematics used in the present paper are according to Corliss (1979) and Lynn and Small (2002). However, the higher systematic classification or contents have changed greatly since Corliss’ review and are still in argument. Thus we use mainly a vernacular terminology in the text, e.g. stichotrichs, euplotids, hypantrids or hypotrichs and add sensu lato (s. l) stricto (s. str.) to the taxa. Some terms are recalled to avoid possible confusion:

Cirral anlagen. This word is used basically for stichotrichs and euplotids to describe the primordia which will generate late frontoventral transverse cirri, including the undulating membrane anlage (UM-anlage) and streaks which appear ventrally to the right of the buccal field. In these primordia, the UM-anlage usually gives rise to either near the UM-anlage either in the UM-anlage (in the opisthe), or completely isolated (in the protist); hence, this term is defined in the present work, only for these 5 streak-like primordia (the UM-anlage is excluded). The cirral anlagen I-V here are equivalent to the anlagen II-VI by some other researchers (i.e., UM-anlage is the No. (1) (Foissner 1996, Eigner 1997, Berger 1999).

Epicortical (opposite to subcortical). This term refers to the most common mode of primordia formation in morphogenesis, of which the basal bodies of primordia occur on the cell surface rather than subsurface (beneath the cortex).
**RESULTS**

**Morphology and infraciliature of *Uronychia setigera***

(Figs 1-7, 9-10)

The morphology of living cells is slightly different from that previously described (Song and Wilbert 1997, Chen *et al*. 2003); hence it is only briefly redescribed here.

Newly sampled cells show about the same size, *ca* 50-60 × 40-50 µm *in vivo*, while after culture for weeks their size becomes rather variable; from 50 to maximum 90 µm in length with body shape generally oval (as shown in Fig. 1). Buccal field enormous, extending over 60% of body length. Lateral spur-like protrusions on left margin often less conspicuous in this population than the forms described by Song and Wilbert (1997) and Chen *et al*. (2003) (Fig. 2, arrow). Ventrally 2 concaves in posterior portion of cell, where transverse (TC) and left marginal (MC) cirri insert, whereas on dorsal side, a third depression corresponding position of caudal cirri (Fig. 3, CC). Cytoplasm colourless to greyish, usually comprising many to numerous granules (2-8 µm across). No food vacuoles detected (Fig. 1). Two macronuclear nodules (Ma), connected by funiculus (Figs 2-4). Macronucleus not observed. Movement typical of *Uronychia*: rapidly jumping sideways or backwards, swimming very fast while rotating around its longitudinal axis. During pause, all cirri stiffly spread as shown in Fig. 1.

Structure of adoral zone of membranelles (AZM) genus typical (Figs 2, 3, 6, 7). Invariably 11 adoral membranelles in anterior part (AZM₁); cilia of membranelles about 15 µm long. Posterior part (AZM₂) composed always of 4 membranelles (bases about 10 µm in length; Fig. 7, arrowhead), in which basal bodies are irregularly arranged (not in rows) like those in primordia. One small cirrus-like membranelle (named buccal cirrus here, BC) apart from AZM₂ and inconspicuous, with the base about 3 µm long (Figs 2, 7). Paroral membrane (PM) mighty and surrounding buccal field, with right end near buccal cirrus, often slightly crook-like (Fig. 2); cilia of PM about 30 µm long (Fig. 1).

Somatic ciliature as described before (Song and Wilbert 1997) except the number of basal body pairs in

![Figs 1-3. *Uronychia setigera* *in vivo* (1) and after protargol impregnation (2, 3). 1 - ventral view of a typical individual; 2 - ventral view, to show the general infraciliature; arrow marks the spine which is often lower positioned, whereas double-arrowheads indicate the fragment-like part at the posterior end of the leftmost two dorsal kineties; 3 - dorsal view of the same specimen as in Fig. 2, to show the dorsal kineties and the nuclear apparatus; arrow marks the 3rd kinety which curves and extends to the posterior margin. AZM₁ - anterior and posterior parts of the adoral zone of membranelles, BC - buccal cirrus, CC - caudal cirri, DK - dorsal kinety, FC - frontal cirri, MC - marginal cirri, Ma - macronuclei, PM - paroral membrane, TC - transverse cirri, VC - ventral cirri. Scale bars - 40 µm.]

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**Eueuplotids** (opposite to **pseudoeuplotids**). This term indicates euplotids with a 5-cirral anlagen mode and subcortical origin of the oral primordium during morphogenesis; it excludes the pseudoeuplotids which do not have such features.
portion of which one short anlage (for the leftmost along the right edge of each opening pouch, at mid the UM-anlage begins to lengthen and then develops surface of cell (Figs 11, 14, 15, 17, 32, 34). Meanwhile, in the AOP are differentiated and finally migrate onto the membranelles, and two groups both with 5 membranelles quently, two groups in the POP, each with 11 and 5 subcortical pouch, which is located opposite to the posterior end of the AOP and anlage, appears within the subcortical pouch, which is portion of which one short anlage (for the leftmost frontal cirrus) occurs de novo in both proter and opisthe (Figs 15; 32, arrowheads). In late stages, two groups of membranelles in both oral primordia are separated, and among them the first one migrates anteriorly. As in its congeners, the first part in the proter will replace the posterior 5 membranelles of AZM, while the anterior (parental) 6 are retained (Fig. 35, arrow). In the second group of AZM in both dividers, the most posterior membranelle (the smallest one) moves apart from the other 4 and then to the final position as the “buccal cirrus” (Figs 34, 35).

Development of the somatic ciliature. At about the same time as the oral primordia are formed, basal bodies for FVT-cirral primordia (CA) develop on the cell surface as first 4, and then 5 streaks (anlagen I-V) anterior to the transverse cirri (Figs 19, 23, 24). Clearly no parental ciliary organelles are involved in the formation of these new primordia. Then each of 5 anlagen extends to its maximum length before they divide into two sets (Figs 19, arrow; 26, double-arrowheads). Subsequently, the later events within the cirral anlagen in both dividers undergo in the same mode as in previous descriptions (Song 1996, Shi and Song 1999): streaks broaden, break apart and then migrate developing as distinct cirri (Figs 18, 20, 22, 30, 32, 34, 35).

According to the segmentation of cirri, the cirral anlagen I-V give rise to the pattern of 3:3:2:2:3, respectively, while the leftmost cirrus is formed de novo near the UM-anlage as described above. Nevertheless, only 11 cirri in total are formed in non-divisional stage following the pattern of 3:2:1:1:3, that is, one segment from each of anlagen II-IV will be resorbed before/after cell division is finished.

The anlagen of left marginal cirri for proter and opisthe are formed also de novo and separately on the cell surface near the old AZM, and the marginal cirri (Figs 13, arrowheads; 24, arrows), which are then enlarge and segmented to form the cirri for daughter cells (Fig. 28, double-arrowheads).

On dorsal side, the proliferation of new basal bodies occurs at two levels within each of the six old kineties. The development of these anlagen seems to follow a
Figs 14-22. Photomicrographs of *Uronychia setigera* in morphogenesis. 14, 15 - ventral views of the same specimen focusing on different levels; arrow in Fig. 14 marks the newly build AZM, which just replaces the old structure, arrow in Fig. 15 marks the AZM, of the opisthe, while arrowhead indicates the leftmost frontal cirrus derived from the paroral membrane anlage; 16, 17 - ventral views of the same specimen at different focus levels; arrows in Fig. 16 indicate the two inconspicuous ventral cirri, double-arrowheads in Fig. 17 mark the AZM, of the proter (note that the paroral membrane, arrow, is positioned at different level); 18 - ventral view, arrowheads mark the AZM, in both dividers, whereas arrow indicates the segmented cirral anlagen; 19 - ventral view, arrow marks the newly formed caudal cirrus in the rightmost dorsal kinety, which is just as a slender patch of kinetosomes, while arrowhead indicates the 5 “primary” cirral anlagen; 20 - ventral view, arrow marks the AZM, in the opisthe; 21 - dorsal view, arrows mark the caudal cirri of the proter; 22 - ventral view, arrow marks the posterior portion of the oral primordium in the proter, where the last membranelles of the AZM, are still in formation.
Table 1. Summary of data on morphogenesis in five well-known typical euplotid genera, with emphasis on the features excluding other non-euplotidous spirotrichs. (AZM - adoral zone of membranelles, FVT - frontoventral transverse, UM - undulating membrane).

<table>
<thead>
<tr>
<th>Characters</th>
<th>Uronychia</th>
<th>Aspidisca</th>
<th>Euplotes</th>
<th>Diophrys</th>
<th>Certesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVT-cirral anlagen</td>
<td>5 primary streaks</td>
<td>5 primary streaks</td>
<td>5 primary streaks</td>
<td>5 primary streaks</td>
<td>5 primary streaks</td>
</tr>
<tr>
<td>Oral primordium in the proter</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Origin of the oral primordium in the opisthe</td>
<td>subcortically</td>
<td>subcortically</td>
<td>subcortically</td>
<td>subcortically</td>
<td>subcortically</td>
</tr>
<tr>
<td>Fate of the parental adoral zone of membranelles</td>
<td>invariably 5 proximal membranelles will be replaced by the OP in the proter</td>
<td>completely retained will be renewed by de-differentiating and rebuilding mode</td>
<td>completely retained proximal portion</td>
<td>completely retained</td>
<td></td>
</tr>
<tr>
<td>Formation of the 2nd part of AZM</td>
<td>present</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Undulating membrane in the proter</td>
<td>rebuilt by the <em>de novo</em> newly-formed UM-anlage</td>
<td>old one retained no UM-anlage</td>
<td>old one retained, no UM-anlage</td>
<td>completely renewed the new UM-anlage*</td>
<td>completely renewed by the new UM-anlage*</td>
</tr>
<tr>
<td>Formation of the par- and endoral membranes from the UM-anlage</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>Origin of the leftmost frontal cirrus</td>
<td><em>de novo</em>, near the UM-anlage</td>
<td><em>de novo</em></td>
<td><em>de novo</em></td>
<td>derived from the UM-anlage</td>
<td>derived from the UM-anlage</td>
</tr>
<tr>
<td>Formation of the left marginal cirri</td>
<td><em>de novo</em>, from MC-anlage</td>
<td>absent</td>
<td><em>de novo</em>, from MC-anlage</td>
<td><em>de novo</em>, from MC-anlage</td>
<td>from MC-anlage, <em>de novo</em></td>
</tr>
<tr>
<td>No. of membranelles formed by oral primordia</td>
<td>invariable</td>
<td>variable</td>
<td>variable</td>
<td>variable</td>
<td>variable</td>
</tr>
<tr>
<td>Origin of dorsal kinety anlagen</td>
<td>intrakinetically, secondary**</td>
<td>as left</td>
<td>as left</td>
<td>as left</td>
<td>as left</td>
</tr>
<tr>
<td>Formation of caudal cirri</td>
<td>from rightmost two anlagen, with a complex mode***</td>
<td>no caudal cirri formed</td>
<td>from rightmost one anlage, with a complex mode</td>
<td>from rightmost two anlagen, with a complex mode</td>
<td>no caudal cirri formed</td>
</tr>
<tr>
<td>Arrangement of basal bodies in cirri/membrane/membranelles in non-division stage</td>
<td>irregular and anarchic, in “anlage” state</td>
<td>well-developed, in rows</td>
<td>well-developed, in rows</td>
<td>well-developed, in rows</td>
<td>well-developed, in rows</td>
</tr>
</tbody>
</table>

*This process is undertaken yet not *de novo*, but by a “de-differentiating and then rebuilding in situ” mode, basal bodies of old structures join the formation of the new anlage;
** Sometimes inconspicuously or difficult to outline the new anlagen because newly proliferated basal bodies appear within the parental structures; *** In this mode, two or more caudal cirri are developed from the posterior end of the rightmost anlage.
gradient from right to left (Figs 25, 31, 33, arrows). Caudal cirri are generated as in other congeners (and even as in Diophrys) (Song and Packroff 1993): two caudal cirri are formed at the posterior end of the rightmost anlage in both proter an opisthe, while the second primordium (from right) gives rise to the third one (Figs 8, 12, 21, 27, 29, 36).

As a conclusion, the morphogenesis in Uronychia setigera, as in its congeners, can be summarized as follows: (1) The oral primordium (OP) in both proter and opisthe develops de novo in a subcortical pouch, respectively, in which the new membranelles formed in the proter’s OP will replace the leftmost 5 parental ones; 6 old membranelles will be retained for the proter. (2)
Highly specialized undulating membrane generates from the isolated UM-anlage which is formed and develops independently from the OP within the same subcortical pouch. (3) Five primary FVT-cirral anlagen appear *de novo* on the cell surface, which divide and give rise to 2 sets of cirral anlagen for the proter and opisthe. (4) Three frontal, 2 ventral and 5 transverse cirri derive from the 5 FVT-cirral anlagen in both daughter cells, usually 2 to 3 extra ventral cirri generated from these anlagen will be resorbed; the left marginal cirri develop from the marginal-anlage; all these primordia are formed *de novo*. (5) The leftmost frontal cirrus develops *de novo* on the cell surface in both dividers, and has no connection with the UM-anlage. (6) Origination of 3 caudal cirri is involved in the 2 rightmost dorsal kineties with a multi-segmentation mode.

**Molecular phylogenetic trees constructed from complete 18S rRNA gene sequences**

The bootstrap trees constructed from 18S rRNA sequences are demonstrated in Figs 37, 38. The maximum-parsimony analyses in our work provide strong
support for the monophyly of typical taxa in Euplotina while the genus Euplotidium, the only Gastrocirrhidae, of which the sequences data are available, branches from them at low level (Fig. 38). The distance-matrix tree gives slightly different result (Fig. 37), i.e. the 18S rRNA data locate Uronychia and Diophrys within the Stichotrichia clade (the hypotrichs s. l.) though with very lower (lower than 50%) bootstrap support. Euplotidium is also grouped with them. All Euplotes species and Aspidisca are clustered together in a separate clade. In agreement with Borror and Hill (1995) who concluded that Diophrys and Uronychia are more closely related to each other than to Euplotes (hence as a different family, Uronychiidae), both trees support this arrangement and indicate also that the genus Aspidisca might separate from them at family level as well. All results obtained reflect consistently that Euplotidium (or Gastrocirrhidae) is a sister group to all of them.

**DISCUSSION**

**Identification of Uronychia setigera**

Morphogenesis and systematics of *Uronychia* and euplotids

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The small form, *Uronychia setigera*, as defined by Song and Wilbert (1997), differs from the closely-related *U. binucleata* in lower number of basal bodies in the leftmost dorsal kineties (ca 20 vs. ca 30), conspicuously smaller size in natural water (ca 60 µm vs. about 100 µm in length) and presence of the lateral spur or spine at cell margin, *Uronychia multicirrus*, was added (Song 1997).

The small form, *Uronychia setigera*, as defined by Song and Wilbert (1997), differs from the closely-related...
about anterior 2/3 of cell on left margin (vs. absent or inconspicuous in *U. binucleata*) (Calkins 1902, Kahl 1932, Curds and Wu 1983, Song and Wilbert 1997). This conclusion was recently confirmed by researches using DNA-fingerprinting and ARDAR riboprinting methods (Chen et al. 2003).

Some noticeable morphogenetic features in *Uronychia*

The morphogenesis in all three well-known morphospecies of *Uronychia*, *U. transfuga*, *U. binucleata* and *U. setigera* has been repeatedly investigated even in last decades using silver methods. Among them, the largest form with beaded macronuclei, *U. transfuga* was studied by Wilbert and Kahan (1981) and Wilbert (1995), although only the main stages were documented. The species described by Hill (1990) under the name *Uronychia transfuga* and by Song (1995) under the name of *U. uncinata* would be populations of *U. binucleata* (see Song and Wilbert 1997). The small one, *U. setigera* was likely only once described (Shi and Song 1999), which matches completely the results of the present work.

All the hitherto data demonstrate that fissional events in this genus are highly stable and conservative, hence, the general morphogenetic pattern is shared by all known congeners. These data also suggest that *Uronychia*, compared with other related taxa, e.g. *Euplotes*, *Aspidisca*, *Diophrys*, possesses more primitive features: (1) UM-primordium forms only a single anlage-like membrane; (2) all ciliary organelles are present, and (3) most ciliary organelles appear to be at early developing stage considering the arrangement of basal bodies which are uniquely distributed in an anar-chic mode. In addition, 2 caudal cirri developing from the posterior end of a single kinety anlage (the leftmost one) is possibly also a plesiomorphic characteristic, for the anterior one might represent the non-loosened kinety corresponding to some oxytrichids, in which the rightmost DK-anlage (of the dorsal group) is usually segmented to form the 4th kinety and the newly formed kinety bears the caudal cirrus (Eigner 1997, Foissner 1996, Berger 1999). This mode is seen also in *Euplotes* and *Diophrys* (Tuffrau et al. 1976, Hill 1981, Song and Packroff 1993, Song and Wilbert 1994), and implying that it is a conservative feature having a long evolutionary history.

Considering the general process of the development of the oral apparatus, *Uronychia* exhibits the combination of 3 unusual stomatogenetic characteristics: (1) oral primordium in the proter develops de novo and subcor-

tically as well; (2) AZM divided into 2 parts, and (3) the piece-together-mode of the AZM, in the proter. Among these, the most noticeable event is the point 3, namely the formation of AZM, in the proter: it is (invariably !) composed of 6 parental (retained) and 5 newly formed membranelles. This phenomenon is unique even in all spirotrichids, though a “similar” process is seen also in several other non-euplotids, e.g. *Hemigastrostyla* and some urosylids. In these taxa, nevertheless, the proter’s oral primordium develops unexceptionally on the cell surface (epicortically) and forms a variable number of membranelles (Hemberger 1982, Song and Hu 1999), i.e. very likely a convergent similarity.

As given in the morphogenetic descriptions (see Results), some newly formed ventral cirri will be re-sorbed before/after cell division is completed, i.e. one cirrus from the each of cirral anlagen II-IV will be dissolved. Clearly, this process might be individual-de-pendent: in some cases, those to-be-dissolved cirri could be retained for a short time after division as we observed in some individuals.

Another event needs to be clarified: the leftmost frontal cirrus in both proter and opisthe is formed de novo rather than generated from the UM-primordium like that in most hypotrichs s. l. This situation is seen also in at least two other morphologically rather specialized genera, *Aspidisca* (Diller 1975, Hill 1979, Song 2003) and *Euplotes* (Washburn and Borror 1972, Tuffrau et al. 1976), this imlys that this might be a plesiomorphic character.

Hill (1990) described that the paroral membrane in *Uronychia binucleata* (called *U. transfuga*) divides and overlaps at the anterior end before completion of division, thus forming the right and left oral membranes. This description is, however, neither supported by the morphological (Song and Wilbert 1997) nor by the morphogenetic observations (Song 1996, called *U. uncinata*). In *U. setigera*, the present work and a previous report (Shi and Song 1999) substantiate the same conclusion: UM-primordium gives rise to only a single structure. Similar results are also obtained in *U. transfuga* (Wilbert and Kahan 1981, Wilbert 1995).

Systematic relationships and position of *Uronychia* and related euplotids

Euplotids traditionally comprise of at least 12 traditional genera assigned by Corliss (1979) to 3 families, Aspidiscidae, Euplotidae and Gastrocirrhididae. They are characterized by reduced somatic ciliature, rigid body and mostly oval/flattened shape, absence of right mar-
ginal cirri, dominant AZM, and subcortical mode of stomatogenesis (Borror 1972, Tuffrau et al. 1976, Corliss 1979, Curds and Wu 1983, Dragesco and Dragesco-Kernéis 1986). This classification is generally accepted by most taxonomists, though some related or “sister groups” were subsequently added into this increasingly comprehensive complex, and its systematic arrangement was often slightly redefined (Small and Lynn 1985, Hill and Borror 1992, Borror and Hill 1995). The updated system is suggested by Lynn and Small (2002), in which the order Euplotida consists of 2 suborders: the Euplotina with “typical” euplotids (5-anlagen-mode) plus gastrocirrhids, while another suborder, Discocephalina, is composed of some cephalized marine groups (Wicklow 1982).

In euplotids, most genera in Aspidiscidae and Euplotidae belong to the type of 5-cirral anlagen (5 transverse cirri in non-dividing stage) or “typical” euplotids (eueuplotids, see terminology) though only 5 of them, Aspidisca, Euplotes, Certesia, Diophrys and Uronychia, have been both morphologically and morphogenetically studied (Washburn and Borror 1972, Wicklow 1983, Hill 1990, Song and Packroff 1993, Song and Wilbert 1994). Different from these euplotids, the discocephalins with the representative genus Discocephalus, demonstrate distinctly diverse morphology and a highly different mode with multi-cirral anlagen and epicortical stomatogenesis (Wicklow 1982) (Table 2). In Gastrocirrhadae, the genus Gastrocirrhus is likely the only one, in which the morphogenesis is partly known (Hu and Song 2003). Based on the description by Hu and Song, 5 features can be recognized: (1) the oral primordium develops on cell surface; (2) FVT-cirri generate from many (more than 5) cirral anlagen; (3) no marginal and caudal cirri are formed; (4) paroral membrane is a single structure, (5) one streak-like UM-anlage develops de novo in both proter and opisthe, from which the leftmost frontal cirrus is generated. This reflects that Gastrocirrhus is an intermediate type between Discocephalus and other typical euplotids mentioned above (Table 2).

The main morphogenetic features found in 5 “typical” euplotid genera are tabulated (Table 1). Among these, the most significant characters are: (1) oral primordium develops subcortically; (2) five de novo formed primary FVT-cirral anlagen; (3) single undulating membrane generates from the isolated UM-anlage (yet presence of two membranes in Diophrys is clearly an apomorphic character); (4) the leftmost frontal cirrus develops completely separated from the UM-anlage (again, it is exceptional in Diophrys which exhibits a stichotrichous mode); (5) multi-segmentation of caudal cirri (when present); (6) no right marginal cirri generated. Considering so many morphological and morphogenetic features shared by diverse taxa, it is unlikely that these euplotids develop from different ancestors convergently, so that combination of these characters should be, in our opinion, regarded to be critical criteria for a monophyletic complex. The multi-anlagen-mode (non-5-cirral anlagen) is seemingly an apomorphic character, which derives divergently from (?) the 5-anlagen type as revealed by investigations on Amphisiella annulata by Berger (2004). The morphogenesis of this stichotrich ciliate supports that, apart from 5 normal cirral streaks, there is always an inconspicuous, extra anlage being formed which develops to the 6th transverse cirrus. The epicortical origin of the oral primordium is definitely a primary character (vs. subcortical mode) for it occurs in numerous highly diverse stichotrichs/hypotrichs, which indicates it is impossible due to convergent evolution. Hence a reasonable surmise is that, within the order Euplotida, the gastrocirrhids are indeed a closely related group to the euplotids, which are as pseudoeuplotids belonging to the same suborder as suggested by Lynn and Small (2002). This view is basically supported also by molecular data (Chen and Song 2001, 2002; Chen et al. 2002), based on which gastrocirrhids are almost always clustered with other euplopid clade (Figs 37, 38). As to the discocephalids/discocephaline, they could be, considering their morphological and morphogenetic features (no molecular data in this group available yet), possibly a marginalized or even a paraphyletic group to the euplotine, which are even more closely related to oxytrichids, e.g. grouped frontoventral cirri, 2 UM-membranes, epicortical origin of oral primordium, elongated body shape and generally presence of both marginal rows (Table 2). Nevertheless, further evidences are needed to indicate in which position this group should be placed.

With reference to the AZM in the proter, at least 4 basic patterns can be recognized in all spirotrichs (Hemberger 1982, Foissner 1996, Eigner 1997, Berger 1999, Hu et al. 2003): (1) The parental structure is intact and retained for the proter, e.g. in many euplotids, oxytrichids, many amphisiellids, discocephalids and kahlidiellids. (2) Posterior portion of AZM is partly renewed after de-differentiation of old membranelles and then a re-building process, in which no new OP is involved, e.g. Diophrys and some urostylids. (3) The old AZM will be completely replaced by a new one which
is formed by a de novo appeared OP, like in most urostylids. (4) (Only in the posterior portion) partly renewed by the newly formed structure, hence piecing together with the retained old part (the distal part), e.g. Uronychia, Hemigastrotyla. Thus, euplotids (both eueuplotids and pseudoeuplotids) exhibit 3 different patterns of the formation/rebuild of the oral structure. This suggests that this diversity is likely due to divergent evolution (from an ancestor type) though evidence could not be traced in the morphogenesis.

Finally, an interesting point of discussion is the relationship between euplotids and hypotrichs s. l. (stichotrichs) or the origin of the euplotids if we consider that two assemblages have close connection as most hitherto information has revealed. Our opinion is that the euplotids are not the most primitive forms, though there is still no firm evidence showing that stichotrichs is the forerunner of euplotids. The most “critical” reasons are from morphogenetic data, e.g. 5-anlagen pattern and the subcortical origin of the oral primordium. The former feature is shared by both euplotids and many highly dedifferentiated stichotrichs (e.g. oxytrichids, kahlilids vs. the primitive Kii. tricha), hence not the most primitive taxa. This indicates clearly that this similarity does not derive from parallel evolution. Whereas the latter feature, the oral primordium originating subcortically, should be also an apomorphic characteristic because in most other lower ciliates (non-spirotrichs) oral primordium develops on the cell surface (Foissner 1996). Other evidence comes from the morphological features: (1) many euplotids have only left marginal cirri or simply absent (divergent from predecessors with both left and right ones); (2) presence of bipartite AZMs is surely an apomorphic feature. However, considering some primitive characteristics in euplotids, e.g. most genera have only a single undulating membrane, 2 caudal cirri develop from a single dorsal kinety anlage, the ancestor of euplotids is also less likely any current type of stichotrichs. We presume that, especially because of the fact that 5-anlagen pattern is widely seen in many taxa in oxytrichids as well, euplotids (at least the eueuplotids) derive from a forerunner possessing the 5-anlagen mode, which might be also the predecessor of many present hypotrichs s. l.

In conclusion, our new points of views on taxonomy of euplotids are: (1) The traditional euplotids could be a paraphyletic assemblage consisting of “true” and outer groups. As a suggestion based on the new definition, the order Euplotida s. l. contains at least 2 subgroups: the eueuplotids or “typical” euplotids, a monophyly consisting of the taxa with the pattern of 5-cirral-anlagen as well as the subcortical origin of the oral primordium, and the sister group, gastrocirrhids (or pseudoeuplotids), with multi-anlagen feature which is a divergent, or apomorphic feature. (2) The systematic position of discocephaline remains unclear, but possibly an intermediate group between the euplotids s. str. and stichotrichs; However, we agree that it is presently placed within the order Euplotida, as an outer group of other two assemblages. (3) The morphogenetic feature of 5-cirral-anlagen-mode is highly conservative and should be hence weighted with more phylogenetic value(s) in systematic analysis. (4) We suppose that some features like the origin mode of the leftmost frontal (formed from the UM-anlage), and the situation of undulating membranes (e.g., two in number and curved in appearance) represent apomorphic features, and should not be over-evaluated. (5) Euplotids s. str. might be derived from the 5-anlagen ancestor-type of hypotrichs s. l., which is possibly also the ancestor of the current stichotrichs.

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