

Phylogenetic Relationships among Trichodinidae (Ciliophora: Peritricha) Derived from the Characteristic Values of Denticles

Yingchun GONG^{1, 2}, Yuhe YU¹, Weisong FENG¹ and Yunfen SHEN¹

¹Laboratory of Taxonomy and Ecology of Protozoa, Institute of Hydrobiology, The Chinese Academy of Sciences; ²Graduate School of the Chinese Academy of Sciences, Wuhan, P. R. China

Summary. The phylogenetic relationships among trichodinids remain obscure. As an important diagnostic marker, the morphology of the denticles in the adhesive disc as well as the adoral spiral has been widely used in generic discrimination and species identification of trichodinids. We studied the characters of denticles of the ten genera of Trichodinidae and the sole genus *Urceolaria* of Urceolariidae by using a quantitative method. The characteristic values were used to generate Manhattan distance, on which the dendrogram was based to construct with the Unweighted Paired Group Method using the Arithmetic mean (UPGMA). The investigations show that all the genera of the family Trichodinidae were clearly separate from the outgroup *Urceolaria*, and within the Trichodinidae: (i) *Dipartiella* grouped with *Trichodinella* and *Tripartiella* and lay in the closest position to the outgroup with a low dissimilarity, suggesting *Dipartiella* might be the most primitive genus in the family; (ii) *Hemitrichodina* clustered in a single clad and lay in the farthest position to the outgroup with the highest dissimilarity, indicating that it might be the most advanced genus; and (iii) the other 6 genera, *Trichodina*, *Paratrichodina*, *Semitrichodina*, *Vauchomia*, *Pallitrichodina* and *Trichodoxa* clustered in a big clad with very low dissimilarity, showing that they are closely related to each other. We discuss the evolutionary trend of the denticle and conclude that the denticles of the adhesive disc should be an apomorphic feature of the trichodinids and their changes could reflect the evolutionary tendencies of these ciliates.

Key words: denticle, phylogenetic relationships, quantitative method, Trichodinidae.

INTRODUCTION

Trichodinids (Ciliophora: Peritrichida) are one of the most common parasitic ciliates that are well characterized by two main features: the morphology of the denticles in the adhesive disc and the development of the adoral ciliary spiral (Lom 1958, Small and Lynn 1985, Basson and Van As 1989). Since the original

description by Ehrenberg (1838) of *Trichodina pediculus*, many protozoologists paid a lot of attention to the taxonomy (Lom 1958, 1959, 1962, 1963; Lom and Haldar 1976, 1977; Van As and Basson 1989, 1992, 1993; Xu *et al.* 1999a, b, 2002) and systematics (Raabe 1963, Corliss 1979, Small and Lynn 1985). So far, more than 260 species representing ten genera have been described from the skins, gills and urinary bladder of fishes and amphibians and also from the integument of invertebrates (Kazubski 1958, Sirgel 1983, Basson and Van As 1989, Van As and Basson 1993, Gong *et al.* 2004). At the present time, studies on trichodinids still concern mainly with their taxonomy especially with re-investigating or

Address for correspondence: Yuhe Yu, Laboratory of Taxonomy and Ecology of Protozoa, Institute of Hydrobiology, The Chinese Academy of Sciences, 430072 Wuhan, P. R. China; Fax: (8627)-68780773; E-mail: yhyu@ihb.ac.cn

re-describing the old species using modern techniques (Özer 2003, Mitra and Haldar 2004).

However, very little attention has been given to the phylogenetic relationships of these ciliates, consequently these studies were fragmentary. Raabe (1963) was the first worker to discuss the phylogeny of the genera of Trichodinidae according to the length of the adoral ciliary spiral and the shape of the denticles (Fig. 1). Xu (1999) carried on the cluster analysis of 11 genera in Mobilina including ten genera of Trichodinidae based on eight main morphological characters (Fig. 2). However, there is as yet no firm conclusion on the phylogenetic relationships among trichodinids because of the confusion of the phylogenetic markers with high systematic values.

The morphology of the denticles in the adhesive disc has been widely used in generic discrimination and species identification of trichodinids (Van As and Basson 1989, 1990). It was supposed that the evolution of these complex ciliates would be made clear by studies on the ontogeny of the denticles of all the genera in the family Trichodinidae (Kruger *et al.* 1995). However, the phylogenetic relationships among trichodinids remain unresolved because data on the ontogeny of the denticles are available only from the genus *Trichodina* (Kazubski 1967, Feng 1985, Kruger *et al.* 1995), and the application of the ontogeny studies to the other genera were postponed since it is difficult to collect the materials for narrow host-specificity and special geographical distribution of some genera, such as *Hemitrichodina*, *Trichodoxa*, and *Dipartiella*. Since the ontogeny of the denticles of each genus of Trichodinidae is presumably similar, and their differences mainly lie in the extent of the development of the mature denticle, we attempted to study their phylogenetic relationships based on the characteristic values of the mature denticles which can indicate the developmental extent of the blade, central part, and thorn using the quantitative method described by Gong *et al.* (2004). The method can be applied to material already published, provided clear micrographs or drawings are available and based on silver impregnated specimens. Because we have access to literature with micrographs that include all the genera of Trichodinidae and one genus of Urceolariidae, it was feasible to study the phylogeny of trichodinids with this numerical method.

The object of the paper is to attempt to provide a new way to study the phylogenetic relationships for trichodinids by taking advantage of the existing material and technology.

MATERIALS AND METHODS

Materials. Thirty-five species representing ten genera of Trichodinidae and one genus of Urceolariidae were investigated in this study. These include 2 out of 16 species of *Urceolaria* described; 1 *Hemitrichodina* species; 20 out of over 200 species of *Trichodina*; 2 out of 11 species of *Paratrichodina*; 1 out of 2 species of *Semitrichodina*; 1 out of 2 species of *Vauchomia*; 2 *Pallitrichodina* species; 1 out of 2 species of *Trichodoxa*; 2 out of 15 species of *Tripartiella*; 2 out of 8 species of *Trichodinella*; and 1 *Dipartiella* species. The drawings of all ciliates studied in the paper were made from those of various researchers (Table 1). Among 11 genera studied, *Trichodina* is one of the largest and most widely distributed ciliate genera (Xu *et al.* 2000b), so we tried to select many species representing different types. In contrast, few were selected from the other genera because the data available in the literature are very scant.

Quantification of denticles. The quantitative method has been put forward in our early paper (Gong *et al.* 2004) and the description was based on the denticulate ring of *Trichodina nobilis* Chen, 1963, but can also be applied to other genera of Trichodinidae. In the case of representatives of the Urceolariidae, we regarded the denticles as made up of only the central part, having no blade and thorn. The method required photomicrography to enlarge denticles and the software Axionvision of Zeiss to calculate the area of denticles.

Quantitative descriptions for the denticle were achieved by measuring the percentages of the areas of the blade, central part and thorn respectively to that of a single denticle and the percentage of the total area of all the denticles in the adhesive disc to that of the loop of the denticulate ring (Figs 3, 4). The formulae were as follows: Pbd (Percentage of the area of blade to that of denticle) = S_b/S_d ; Pcd (Percentage of the area of central part to that of denticle) = S_c/S_d ; Ptd (Percentage of the area of thorn to that of denticle) = S_t/S_d ; Pdr (Percentage of the area of all the denticles to that of the loop of the denticulate ring) = $n \cdot S_d/S_r$.

In the formulae, "n" represents the number of the denticles in the adhesive disc; "Sd", "Sb", "Sc", "St" and "Sr" represent the area of denticle, blade, central part, thorn and the loop of the denticulate ring, respectively. Though the blade, central part and thorn are irregular, "Sb", "Sc" and "St" can be computed by the software Axionvision of Zeiss or similar software; "Sd" is calculated according to the formula: $S_d = S_b + S_c + S_t$; "Sr" can be calculated using the areas of the excircle and the central circle of denticulate ring which can be computed using the diameters of the excircle and the central circle of denticulate ring (Fig. 3).

When calculating the areas of the blade, central part, and thorn in a drawing of a trichodinid, a denticle is selected as a reference marker, and each three or four denticles are then used for the calculations.

Data analysis. The mean characteristic values of the denticles of all the representative species of each genus were regarded as the characteristic values of the corresponding genus and analyzed by the program NTSYSpC (Numerical Taxonomy System, Version 2.0). A range standardized Manhattan distance was used to generate a dissimilarity matrix between the individual operational taxonomic units based on the characteristic values. The resulting distance matrix was analyzed using UPGMA (Unweighted Paired Group Method using the Arithmetic mean) to construct the dendrogram. We took *Urceolaria* as outgroup, since representatives of the Trichodinidae are considered to be more advanced than representatives of the Urceolariidae in Mobilina (Kruger *et al.* 1995).

RESULTS

Comparison of the characteristic values of denticles among genera of Mobilina

Nearly all the genera have their own fluctuation range of the characteristic values, for example, Pbd (Percentage of the area of blade to that of denticle) and Ptd (Percentage of the area of thorn to that of denticle) of the genus *Trichodina* range from 47 to 66% and 16 to 30%, respectively, but those of *Trichodinella* range from 79 to 80% and 0 to 4%, respectively (Table 1). Overlapping occurs in the values of some genera, as shown in Table 1. For example, the Ptd of *Pallitrichodina rogenae* and those of *Trichodina compacta* and *Trichodina raabei* share 51% which, however, can be differentiated with the other three values.

Comparing to the four characteristic values, Pbd and Ptd vary significantly among genera (Table 2), such as Pbd decreasing from 84% in *Dipartiella* to 20% in *Hemitrichodina* and Ptd increasing from 0% in *Dipartiella* to 47% in *Hemitrichodina*; Pcd (Percentage of the area of central part to that of denticle) and Pdr (Percentage of the area of all the denticles to that of the loop of the denticulate ring) change a little, only from 14 to 33% and 43 to 81% respectively. From the Table 2, we also found the characteristic values of the denticles of *Dipartiella*, *Trichodinella*, *Tripartiella*, and *Hemitrichodina* can be clearly differentiated from others, however that of *Trichodina*, *Paratrichodina*, *Semitrichodina*, *Vauchomia*, *Pallitrichodina*, and *Trichodoxa* were similar with little difference.

Phylogenetic analysis

From the UPGMA dendrogram (Fig. 5), the ten genera of Trichodinidae are clearly separate from the outgroup *Urceolaria* and are grouped into three clusters: *Dipartiella*, *Trichodinella* and *Tripartiella* clustered in a small clad; *Dipartiella* lay in the nearest position to *Urceolaria* with a low dissimilarity of 0.219 (Table 3) and *Trichodinella* and *Tripartiella* grouped together showing high similarity; *Hemitrichodina* clustered in a single clad and lay in the farthest position to the outgroup with the highest dissimilarity of 0.486 (Table 3); the other six genera including *Trichodoxa*, *Pallitrichodina*, *Vauchomia*, *Semitrichodina*, *Paratrichodina*, and *Trichodina* clustered in a big clad with very low dissimilarity (Table 3), and *Semitrichodina* and *Paratrichodina* constituted a sister group showing their close relation to each other.

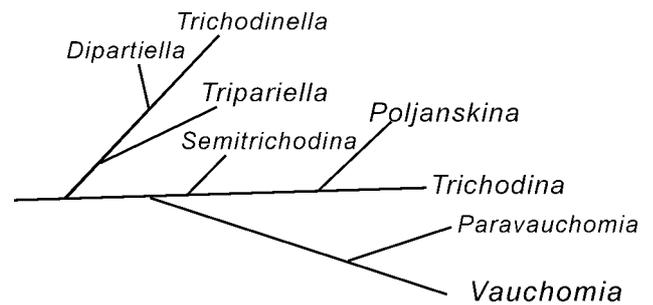


Fig. 1. Phylogenetic diagram of Trichodinidae (redrawn from Raabe 1963). *Paravauchomia* for *Trichodina urinaria* Dogiel, 1940; *Poljanskina* for *Trichodina oviducti* Poljansky, 1955.

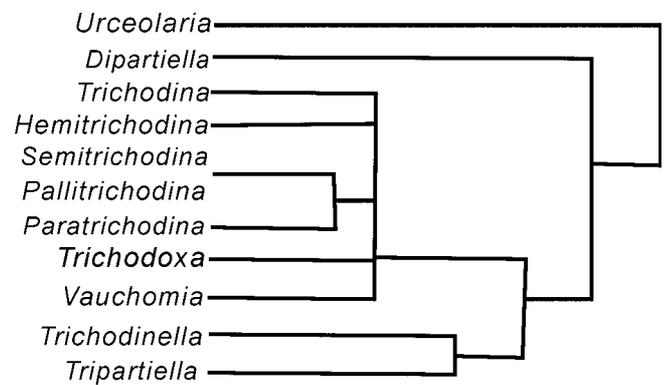


Fig. 2. Cluster analysis of the morphological similarities of 11 genera of Mobilina (redrawn from Xu 1999).

DISCUSSION

Phylogeny within the family Trichodinidae

Which is the most primitive genus in the family Trichodinidae? According to the dendrogram (Fig. 5) based on the characteristic values of the denticles, *Dipartiella* was confirmed to be the most primitive genus in the existing genera of Trichodinidae for it lay nearest to the outgroup. However, our results showed contrary to the theory put forwarded by Raabe (1963) who considered *Trichodina* with the adoral ciliary spiral of an arc over 360° (400°) was initial and fundamental form in Trichodinidae mainly based on the evolutive trend of the adoral ciliary spiral. Though both the adoral ciliary spiral and the denticle of the adhesive disc are

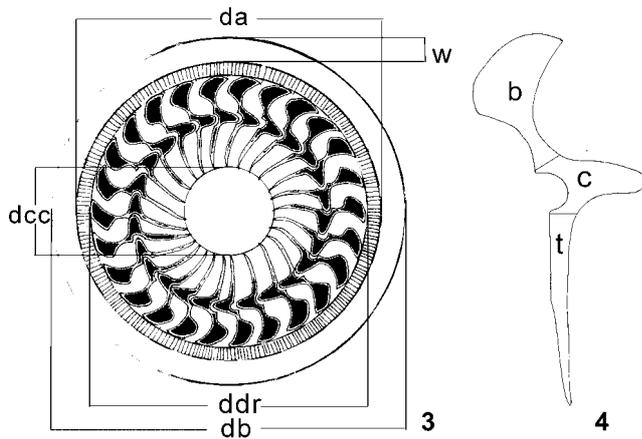
Table 1. Comparison of characteristic values of denticles of 33 species of trichodinids and 2 species of *Urceolaria*. (1) Xu and Song 1998; (2) Laird 1961; (3) Xu *et al.* 1999a; (4) Lom 1963; (5) Lom and Haldar 1977; (6) Sirgel 1983; (7) Van As and Basson 1993; (8) Lom and Haldar 1976; (9) Kazubski 1971; (10) Kazubski and Migala 1968; (11) Van As and Basson 1989; (12) Basson *et al.* 1983; (13) Van As and Basson 1992; (14) Lom and Laird 1969; (15) Lom 1962; (16) Lom 1970a; (17) Loubser *et al.* 1995; (18) Basson and Van As 1991; (19) Lom 1970b (20) Asmat and Haldar 1998; (21) Basson and Van As 1993; (22) Lom 1959; (23) Xu *et al.* 1999b; (24) Lom 1960; (25) Basson and Van As 1989. N- the number of the drawings used for calculations. Pbd - percentage of the area of blade to that of denticle, Pcd - percentage of the area of central part to that of denticle, Ptd - percentage of the area of thorn to that of denticle, Pdr - percentage of the area of all the denticles to that of the loop of the denticulate ring.

Species	Pbd	Pcd	Ptd	Pdr	N	References
<i>Urceolaria cheni</i> Xu <i>et al.</i> 1998	0%	100%	0%	100%	1	(1)
<i>U. karyodactyla</i> Laird, 1961	0%	100%	0%	100%	1	(2)
<i>Dipartiella simplex</i> Stein, 1961	84%	16%	0%	43%	1	(3)
<i>Trichodinella epizootica</i> Raabe, 1950	82%	17%	1%	67%	2	(4) (5)
<i>T. lomi</i> Xu, Song <i>et al.</i> Warren, 1999	79%	18%	3%	49%	3	(3)
<i>Tripatiella copiosa</i> Lom, 1959	72%	18%	10%	64%	1	(5)
<i>T. obtusa</i> Ergens <i>et al.</i> Lom, 1970	75%	16%	9%	56%	1	(4)
<i>Trichodoxa genitilis</i> Sirgel, 1983	65%	25%	10%	81%	1	(6)
<i>Pallitrichodina rogenae</i> Van As <i>et al.</i> Basson, 1993	51%	33%	16%	60%	5	(7)
<i>P. stephani</i> Van As <i>et al.</i> Basson, 1993	47%	31%	21%	59%	3	(7)
<i>Vauchomia renicola</i> Mueller, 1938	61%	14%	25%	53%	1	(8)
<i>Semitrichodina sphaeronuclea</i> Kazubski, 1958	63%	17%	20%	68%	1	(9)
<i>Paratrichodina degiustii</i> Lom <i>et al.</i> Haldar, 1976	64%	15%	21%	54%	1	(8)
<i>P. phoxini</i> Lom, 1963	60%	18%	22%	78%	2	(10)
<i>Trichodina acuta</i> Lom, 1961	53%	25%	22%	70%	3	(11) (12)
<i>T. compacta</i> Van As <i>et al.</i> Basson, 1989	51%	27%	23%	63%	4	(11) (13)
<i>T. cottidarum</i> Dogiel, 1940	58%	16%	26%	53%	3	(14)
<i>T. domerguei</i> Wallengren, 1897	61%	20%	19%	66%	3	(15) (10)
<i>T. heterodontata</i> Ducan, 1977	47%	23%	30%	62%	4	(11) (12)
<i>T. jadrana</i> Lom <i>et al.</i> Laird, 1969	61%	23%	16%	65%	3	(16) (17)
<i>T. maritinkae</i> Basson <i>et al.</i> Van As, 1991	49%	24%	27%	68%	5	(18) (13)
<i>T. modesta</i> Lom, 1970	61%	22%	17%	71%	3	(19)
<i>T. murmanica</i> Poljansky, 1955	53%	24%	23%	60%	4	(3)
<i>T. mutabilis</i> Kazubski <i>et al.</i> Migala, 1968	58%	21%	21%	61%	4	(12) (19)
<i>T. mystusi</i> Asmat <i>et al.</i> Halder, 1998	55%	22%	23%	57%	5	(20)
<i>T. nigra</i> Lom, 1960	54%	24%	22%	65%	4	(19)
<i>T. oviducti</i> Poljansky, 1955	57%	19%	24%	54%	1	(8)
<i>T. polycirra</i> Lom, 1960	52%	20%	58%	59%	1	(8)
<i>T. puytoraci</i> Lom, 1962	60%	18%	22%	53%	4	(15) (20)
<i>T. raabei</i> Lom, 1962	51%	19%	30%	65%	3	(15)
<i>T. rectuncinata</i> Raabe, 1958	66%	17%	17%	56%	3	(15) (4)
<i>T. reticulata</i> Hirschman <i>et al.</i> Partsch, 1955	55%	20%	25%	58%	3	(21) (22)
<i>T. sinonovaculae</i> Xu, Song <i>et al.</i> Warren, 1999	60%	20%	20%	63%	3	(23)
<i>T. urinaria</i> Dogiel, 1940	58%	13%	29%	55%	1	(24)
<i>Hemitrichodina robusta</i> Basson <i>et al.</i> Van As, 1989	20%	33%	47%	68%	3	(25)

evolutionary, the latter is a peculiarity of the Mobilina peritrichs not occurring in Sessilina, while the former exist in both Mobilina and Sessilina. Since the Mobilina is derived from the Sessilina (Lom 1964), the adoral ciliary spiral should be a plesiomorphic feature for trichodinids, and the denticle of the adhesive should be an apomorphic feature. However, only the apomorphic feature could be used for constructing phylogenetic research (Bardele 1989), therefore the denticle characters were more suitable to be the phylogenetic marker of

trichodinids than the adoral ciliary spiral. Consequently we argue that the denticles have a very high systematic value and therefore its changes ought to reflect the evolutionary tendencies of trichodinids.

From the topological position of *Hemitrichodina* in the dendrogram (Fig. 5), it was difficult to establish its status within the Trichodinidae. However, the dissimilarity distance between *Hemitrichodina* and the outgroup *Urceolaria* was higher than that between any other genus and the outgroup (Table 3), therefore



Figs 3, 4. The diagram to illustrate the adhesive disc and the denticles from the adoral view (redrawn from Gong *et al.* 2004). b - blade, c - central part, da - adhesive diameter, db - body diameter, dcc - central circle diameter, ddr - excircle of denticle ring diameter, t - thorn, w - border membrane width.

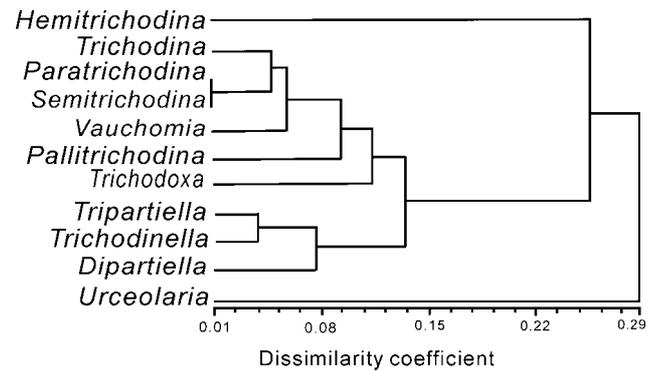


Fig. 5. Dendrogram of the family Trichodinidae based on characteristic values of denticles using UPGMA.

Table 2. Character values of denticles of ten genera of Trichodinidae and the outgroup *Urceolaria*. Abbreviations as in Table 1.

Genera	Pbd	Pcd	Ptd	Pdr
<i>Urceolaria</i> Stein, 1867	0%	100%	0%	100%
<i>Dipartiella</i> Stein G., 1961	84%	16%	0%	43%
<i>Trichodinella</i> Raabe, 1950	80%	18%	2%	58%
<i>Tripartiella</i> Lom, 1959	74%	17%	9%	60%
<i>Trichodoxa</i> Siregel, 1983	65%	25%	10%	81%
<i>Pallitrichodina</i> Van As <i>et</i> Basson, 1993	49%	32%	19%	59%
<i>Vauchomia</i> Mueller, 1938	61%	14%	25%	53%
<i>Semitrichodina</i> Kazubski, 1958	63%	17%	20%	68%
<i>Paratrichodina</i> Lom, 1963	62%	17%	21%	66%
<i>Trichodina</i> Ehrenberg, 1838	56%	21%	23%	61%
<i>Hemitrichodina</i> Basson <i>et</i> Van As, 1989	20%	33%	47%	68%

Table 3. Manhattan distance matrix of the characteristic values of denticles among 11 genera of Mobilina. 1 - *Hemitrichodina*, 2 - *Trichodina*, 3 - *Paratrichodina*, 4 - *Semitrichodina*, 5 - *Vauchomia*, 6 - *Pallitrichodina*, 7 - *Trichodoxa*, 8 - *Tripartiella*, 9 - *Trichodinella*, 10 - *Dipartiella*, 11 - *Urceolaria*.

Genera	1	2	3	4	5	6	7	8	9	10	11
1	0.000	0.196	0.214	0.216	0.243	0.166	0.255	0.288	0.322	0.383	0.478
2	0.196	0.000	0.042	0.054	0.057	0.062	0.115	0.092	0.125	0.187	0.317
3	0.214	0.042	0.000	0.012	0.053	0.095	0.093	0.075	0.116	0.169	0.275
4	0.216	0.054	0.012	0.000	0.064	0.101	0.083	0.075	0.116	0.167	0.264
5	0.243	0.057	0.053	0.064	0.000	0.110	0.146	0.097	0.131	0.150	0.313
6	0.166	0.062	0.095	0.101	0.110	0.000	0.134	0.124	0.155	0.217	0.357
7	0.255	0.115	0.093	0.083	0.146	0.134	0.000	0.094	0.128	0.190	0.223
8	0.288	0.092	0.075	0.075	0.097	0.124	0.094	0.000	0.041	0.095	0.232
9	0.322	0.125	0.116	0.116	0.131	0.155	0.128	0.041	0.000	0.061	0.207
10	0.383	0.187	0.169	0.167	0.150	0.217	0.190	0.095	0.061	0.000	0.219
11	0.478	0.317	0.275	0.264	0.313	0.357	0.223	0.232	0.207	0.219	0.000

Hemitrichodina should be the most advanced in Trichodinidae. The results support the view that *Hemitrichodina* might be *Trichodina* in an advanced form (Xu *et al.* 2000a).

Phylogenetic relationships of the six genera of *Trichodina*, *Paratrachodina*, *Semitrichodina*, *Vauchomia*, *Pallitrichodina* and *Trichodoxa* need further research for their low dissimilarity distances (Table 3), which was caused by their similar type of denticles.

The evolutive trend of denticles within Trichodinidae

The obliquely positioned simple plate of Urceolariidae should be the plesiomorphic feature of the denticle of Mobilina ciliates (Raabe 1963). In Trichodinidae, the denticle of *Dipartiella* which has simple blade and undeveloped central part should be considered as the initial form. With the evolution of the denticle, the thorn appeared gradually, like in the denticle of *Trichodinella* which has weakly developed short thorn curved along the delicate central part; then like in that of *Tripartiella* which bears developed straight ray besides the delicate central part. *Trichodinella* and *Tripartiella* are closely related to each other for their denticles are wedged together by a double system of central conical parts and anterior projection of the blades (Lom and Haldar 1977). The denticle continued to evolve to make the blade, central part and thorn all well developed to form the denticle of the type similar to *Trichodina*. Finally the denticle degenerated, like that of *Hemitrichodina* with triangular blade and robust central part and robust thorn. In a word, it can be concluded from Table 2 and Fig. 5 that with the evolution of trichodinids, the blades degenerated gradually and the thorn developed gradually, but the central part did not change much.

We tried to explain the evolutive trend of the denticles from the view of the function of their components. The thorn is relatively inflexible and doubtless this enhanced the action of maintaining constancy of shape of the central part. On the other hand the blade is very flexible (Sandon 1965), which allows enough flexibility for adhesive disc. Therefore the adhesive disc maintains its basic shape whilst allowing enough flexibility to attach to an uneven surface by the blade and thorn (Van As and Basson 1990). Perhaps with the evolution of the denticle, that the blade degrades gradually and thorn develops gradually is to adapt to special niche, in which, the adhesive disc needed stronger stability and less flexibility. Further study of the behavior of each genus of Trichodinidae and the special environment they exist in

will contribute to access this explanation. The central parts which revealed a remarkable analogy with the spinal column of the vertebrates (Van As and Basson 1990), are indispensable for the denticles in that they play an important role not merely in connecting both the blade and thorn, but also in integrating all the denticles. This might be the reason for the central parts not changing such a lot during the evolution of the denticles.

Conclusions

In this paper, we investigated the phylogeny of the trichodinids based only on the morphology of the denticles by making full use of the existing material and knowledge of trichodinids. However, purely morphological characters are inadequate for phylogenetic studies, and molecular technology provides a much-needed alternative method to resolve problematic phylogeny. Therefore, we will continue the study of the systematic evolution of the family Trichodinidae inferred from molecular information such as the small subunit rRNA (SSrRNA), large subunit rRNA (LSrRNA) and Hsp70, etc. Up till now, we have successfully sequenced the SSrRNA of *Trichodina heterodentata* (AY788099) and *Trichodina reticulata* (AY741748). More molecular information of other genera will hopefully shed light on the evolution of these complex ciliates and aid in assessing the validity of our quantitative method.

Acknowledgements. The authors are much grateful to Prof. W. Song of Ocean University of China for offering literature on trichodinids, and to Dr K. Xu of Universität Salzburg for helpful suggestions and revising the text, and to Dr C. Fu of Institute of Hydrobiology, The Chinese Academy of Sciences, for helping with analysis program. We would like also to express our deep appreciation for the helpful comments of two anonymous reviewers. The work was supported by NSFC (the National Natural Science Foundation of China) grant No. 30270164 to Yuhe Yu.

REFERENCES

- Asmat G. S. M., Haldar D. P. (1998) *Trichodina mystusi*, a new species of trichodinid ciliophoran from Indian estuarine fish, *Mystus gulio* (Hamilton). *Acta Protozool.* **37**: 173-177
- Bardele C. F. (1989) From ciliate ontogeny to ciliate phylogeny: a program. *Boll. Zool.* **56**: 235-243
- Basson L., Van As J. G. (1989) Differential diagnosis of the genera in the family Trichodinidae (Ciliophora; Peritricha) with the description of a new genus ectoparasitic on freshwater fish from southern Africa. *Syst. Parasitol.* **15**: 153-160
- Basson L., Van As J. G. (1991) Trichodinids (Ciliophora: Peritrichia) from a calanoid copepod and catfish from South Africa with notes on host specificity. *Syst. Parasitol.* **18**: 147-158
- Basson L., Van As J. G. (1993) First record of the European Trichodinids (Ciliophora: Peritrichida), *Trichodina acuta* Lom, 1961 and *T. reticulata* Hirschmann et Patsch, 1955 in South Africa. *Acta Protozool.* **32**: 101-105

- Basson L., Van As J. G., Paperna I. (1983) Trichodinid ectoparasites of cichlid and cyprinid fishes in South Africa and Israel. *Syst. Parasitol.* **5**: 245-257
- Corliss J. O. (1979) *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*. 2nd ed., Pergamon Press, New York
- Ehrenberg C. G. (1838) *Die Infusionstierchen als volkommene Organismen*. Leipzig
- Feng S. (1985) A biological investigation of asexual production of *Trichodina nobillis* Chen. *Acta Hydrobiol. Sin.* **9**: 331-342
- Gong Y., Yu Y., Shen Y. (2004) Quantitative analysis of *Trichodina* denticulating characters and phylogenetic relationship studies on interspecies and intraspecies. *Acta Hydrobiol. Sin.* **28**: 225-233
- Kazubski S. L. (1958) *Semitrichodina* gen. nov. *sphaeronuclea* (Lom, 1956) (*Peritricha-Urceolariidae*) in *Schistophallus orientalis* Cless. (*Plumonata-Zonitidae*) in Poland. *Bull. Acad. Polon. Sci.* **6**: 109-112
- Kazubski S. L. (1967) Study on the growth of skeletal elements in *Trichodina pediculus* Ehrbg. *Acta Protozool.* **5**: 37-48
- Kazubski S. L. (1971) Morphological variability of *Semitrichodina sphaeronuclea* (Lom, 1956). *Acta Protozool.* **8**: 251-259
- Kazubski S. L., Migala K. (1968) *Urceolariidae* from breeding carp-*Cyprinus carpio* L. in Zabieniec and remarks on the seasonal variability of trichodinids. *Acta Protozool.* **6**: 137-170
- Kruger J., Van As J. G., Basson L. (1995) Observations on the adhesive disc of *Trichodina xenopodus* Fantham, 1924 and *T. heterodentata* Duncan, 1977 (Ciliophora: Peritricha) during binary fission. *Acta Protozool.* **34**: 203-209
- Laird M. (1961) *Urceolaria karyodactyla* n. sp. (Ciliata: Peritricha) from *Ischnochiton rubber* (L.) at Saint Andrews, New Brunswick. *Can. J. Zool.* **39**: 827-832
- Lom J. (1958) A contribution to the systematics and morphology of endoparasitic trichodinids from amphibians with a proposal of uniform specific characteristics. *J. Protozool.* **5**: 251-263
- Lom J. (1959) *Trichodina reticulata* Hirschmann and Partsch 1955 from Crucian Carp, and *T. domerguei* f. *latispina* Dogel 1940 from *Diaptomus*. *Vest. Ėsl. Spol. Zool.* **24**: 246-257
- Lom J. (1960) On two endozoic trichodinids, *Trichodina urinaria* Dogel, 1940 and *Trichodina polycirra* sp. n. (Contribution to the knowledge of Trichodinids, III) *Acta Parasit. Pol.* **8**: 169-180
- Lom J. (1962) Trichodinid ciliates from fishes of the Rumanian Black Sea coast. *Parasitology* **52**: 49-61
- Lom J. (1963) The ciliates of the family Urceolariidae inhabiting gills of fishes (The *Trichodinella*-group). *Vest. Ėsl. Spol. Zool.* **27**: 7-19
- Lom J. (1964) The morphology and morphogenesis of the buccal ciliary organelles in some peritrichous ciliates. *Arch. Protistenk.* **107**: 131-162
- Lom J. (1970a) Trichodinid ciliates (Peritrichida: Urceolariidae) from some marine fishes. *Folia Parasit.* **14**: 113-125
- Lom J. (1970b) Observations on trichodinid ciliates from freshwater fishes. *Arch. Protistenk.* **112**: 153-177
- Lom J., Haldar D. P. (1976) Observations on trichodinids endocommensal in fishes. *Trans. Am. Micros. Soc.* **95**: 527-541
- Lom J., Haldar D. P. (1977) Ciliates of the genera *Trichodinella*, *Tripartiella* and *Paratrichodina* (Peritricha, Mobilina) invading fish gills. *Folia Parasit.* **24**: 193-210
- Lom J., Laird M. (1969) Parasitic protozoa from marine and euryhaline fish of Newfoundland and New Brunswick. I. Peritrichous ciliates. *Can. J. Zool.* **47**: 1367-1380
- Loubser G. J. J., Van As J. G., Basson L. (1995) Trichodinid ectoparasites (Ciliophora: Peritrichida) of some fishes from the Bay of Dakar, Senegal (West Africa). *Acta Protozool.* **34**: 211-216
- Mitra A. K., Haldar D. P. (2004) First record of *Trichodinella epizootica* (Raabe, 1950) Šramek-Hušek, 1953, with description of *Trichodina notopteridae* sp. n. (Ciliophora: Peritrichida) from freshwater fishes of India. *Acta Protozool.* **43**: 269-274
- Özer A. (2003) The occurrence of *Trichodina domerguei* Wallengren, 1987 and *Trichodina tenuidens* Fauré-Fremiet, 1944 (Peritrichia) on three-spined stickleback, *Gasterosteus aculeatus* L., 1758 found in a brackish and freshwater environment. *Acta Protozool.* **42**: 41-46
- Raabe Z. (1963) Systematics of the family *Urceolariidae* Dujardin 1841. *Acta Protozool.* **1**: 121-138
- Sandon H. (1965) Some species of *Trichodina* from South Africa. *Acta Protozool.* **15**: 39-56
- Sirgel W. F. (1983) A new ciliate genus *Trichodoxa* n. g. (Ciliate, Peritricha, Mobilina, Trichodinidae) with two new species from the genital system of terrestrial pulmonates. *J. Protozool.* **30**: 118-125
- Small E. B., Lynn D. H. (1985) Phylum Ciliophora Doflein, 1901. In: *An Illustrated Guide to the Protozoa* (Eds. J. J. Lee, S. H. Hutner, E. C. Bovee). Allen Press, Kansas, 393-575
- Van As J. G., Basson L. (1989) A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritricha) and a review of the taxonomic status of some fish ectoparasitic trichodinids. *Syst. Parasitol.* **14**: 157-179
- Van As J. G., Basson L. (1990) An articulated internal skeleton resembling a spinal column in a ciliated Protozoan. *Naturwissenschaften* **77**: 229-231
- Van As J. G., Basson L. (1992) Trichodinid ectoparasites (Ciliophora: Peritrichida) of freshwater fishes of the Zambesi River System, with a reappraisal of host specificity. *Syst. Parasitol.* **22**: 81-109
- Van As J. G., Basson L. (1993) On the biology of *Pallitrichodina rogenae* gen. n., sp. n. and *P. stephani* sp. n. (Ciliophora: Peritrichida), mantle cavity symbionts of the giant African snail *Achatina* in Mauritius and Taiwan. *Acta Protozool.* **32**: 47-62
- Xu K. (1999) Parasitic and commensal ciliated Protozoa from marine molluscs and fishes off the coast of the Yellow Sea and the Bohai Bay, with the review of Mobiline peritrichous ciliates (Protozoa, Ciliophora). Doctoral dissertation, Ocean University of Qingdao, Qingdao, China
- Xu K., Song W. (1998) A morphological study on a new species of gill parasitic ciliate, *Urceolaria cheni* nov. spec. from the clam *Scapharca subcrenata*. *J. Fish. Sc. China* **5**: 13-17
- Xu K., Song W., Warren A. (1999a) Trichodinid ectoparasites (Ciliophora: Peritrichida) from the gills of cultured marine fishes in China, with the description of *Trichodinella lomi* n. sp. *Syst. Parasitol.* **42**: 219-227
- Xu K., Song W., Warren A. (1999b) Trichodinid ectoparasites (Ciliophora: Peritrichida) from the gills of mariculture molluscs in China, with the descriptions of four new species of *Trichodina* Ehrenberg, 1838. *Syst. Parasitol.* **42**: 229-237
- Xu K., Song W., Lei Y., Choi J. K., Warren A. (2000a) Diagnoses and probable phylogenetic relationships of the genera in the family Trichodinidae (Ciliophora, Peritrichia). *The Yellow Sea* **6**: 42-49
- Xu K., Song W., Warren A. (2000b) Observations on trichodinid ectoparasites (Ciliophora: Peritricha) from the gills of mariculture molluscs in China, with descriptions of three new species of *Trichodina* Ehrenberg, 1838. *Syst. Parasitol.* **45**: 17-24
- Xu K., Song W., Warren A. (2002) Taxonomy of trichodinids from the gills of marine fishes in coastal regions of the Yellow Sea, with descriptions of two new species of *Trichodina* Ehrenberg, 1830 (Protozoa: Ciliophora: Peritrichia). *Syst. Parasitol.* **51**: 107-120

Received on 10th December, 2004; revised version on 23rd March, 2005; accepted on 14th April, 2005