

## Phylogenetic Positions of Two Ciliates, *Paranophrys magna* and *Mesanophrys carcini* (Ciliophora: Oligohymenophorea), within the Subclass Scuticociliatia Inferred from Complete Small Subunit rRNA Gene Sequences

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**Summary.** The complete small subunit rRNA gene sequences of two scuticociliates, *Paranophrys magna* Borror, 1972 and *Mesanophrys carcini* Grolière & Leglise, 1977, were determined. The results show that each comprises 1759 nucleotides. The phylogenetic positions of both species within the subclass Scuticociliatia were deduced using distance matrix and maximum parsimony methods. The trees indicate that the order Philasterida is probably a monophyletic group, within which *Mesanophrys carcini* is allied in a clade with *Anophryoides haemophila* that branches basally to other four species: *Paranophrys magna*, *Uronema marinum*, *Pseudocohnilembus marinus* and *Cohnilembus verminus*, while the clade including *Paranophrys magna* and *Uronema marinum* is grouped with that of *Pseudocohnilembus marinus* and *Cohnilembus verminus*.

**Key words:** Ciliophora, *Mesanophrys carcini*, *Paranophrys magna*, phylogeny, scuticociliates, SSrRNA.

### INTRODUCTION

The subclass Scuticociliatia is regarded by most taxonomists as a monophyletic group within the phylum Ciliophora (Corliss 1979, Lynn 1979, Puytorac *et al.* 1984, Lynn and Sogin 1988, Lynn and Small 1997, Strüder-Kypke *et al.* 2000). According to Lynn and Small (1997) this subclass, which belongs to the class Oligohymenophorea, is divided into three orders: Philasterida, Pleuronematida and Thigmotrichida. Taxo-

nomic and systematic studies on scuticociliates are traditionally based morphological and morphogenetic characters. Over the past two decades, numerous studies have been carried out in this field. Nevertheless, there is still some confusion concerning the phylogenetic relationships among many taxa within the group (Borror 1972; Grolière and Leglise 1977; Grolière 1980; Song 1993, 2000; Morade and Small 1994; Hu *et al.* 1996; Song and Wei 1998; Song and Wilbert 2000).

Molecular methods, in particular the determination of small subunit rRNA (SSrRNA), have been used to re-evaluate the systematics of various ciliate groups in recent years. However, sequence data for scuticociliates remains comparatively rare and incomplete. To date, SSrRNA gene sequences have been determined for only

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about 10 species within the subclass Scuticociliatia (Greenwood *et al.* 1991, Ragan *et al.* 1996).

*Paranophrys magna* and *Mesanophrys carcini* are two common scuticociliates that are frequently found in coastal or saprobic mariculture environments near Qingdao, China. Their morphologies were redescribed recently by Song (2000). The aims of the present paper were to sequence the SSrRNA gene of both species and to compare these with sequences of other ciliates in order to gain a better understanding of the phylogenetic relationships among various taxa within the Scuticociliatia.

## MATERIALS AND METHODS

### Ciliate collection and culture

*Paranophrys magna* Borror, 1972 and *Mesanophrys carcini* Grolière & Leglise, 1977 were collected from two off-shore mariculture ponds near Qingdao, China. Clonal cultures were established and maintained in sterilized seawater at room temperature with rice grains as food source to enrich bacteria.

### Identification of species

Observations on living cells were carried out using Nomarski differential interference contrast microscopy. Protargol and Chatton-Lwoff silver impregnation techniques were used in order to reveal the infraciliature and the silverline system respectively. Terminology is based mainly on Song (2000).

### Extraction of genomic DNA

Cells were starved overnight, rinsed three times with sterile artificial marine water and then pelleted by centrifugation. 0.5 ml lysis buffer (10mM Tris-HCl, pH 8.3; 50mM KCl; 2.5mM MgCl<sub>2</sub>; 0.6% Tween 20; 0.6% Nonidet P40; 60µg/ml Proteinase K) was added and the cells were incubated at 56°C for 2 h. After incubation, DNA was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with 70% alcohol. DNA was stored at -20 °C (Kusch and Heckmann 1996, Chen *et al.* 2000).

### PCR amplification

Amplifications by PCR were carried out in a total volume of 100 µl containing 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.1% Triton X-100; 3 mM MgCl<sub>2</sub>; 0.2 mM dNTP; 0.5 mM of each oligonucleotide primer (16s-like F: 5' AACCTGGTTGATCCTGCCAGT-3'; 16s-like R: 5'-TGATCCTTCTGCAGGTTACCTAC-3'); 50 ng of genomic DNA and 5U Taq Ex DNA polymerase (TaKaRa, Japan). The reaction mixtures were denatured at 94°C for 5 min before the polymerase was added (hot start), followed by the first 5 cycles consisting of denaturation for 1 min at 94°C, primer annealing for 2 min at 56°C, and extension for 2 min at 72°C. In the subsequent 35 cycles, the annealing temperature was increased to 62°C. This was followed by

a final extension step for 5 min at 72°C (Elwood *et al.* 1985, Medlin *et al.* 1988, Chen and Song 2001).

### Cloning and sequencing the SSrRNA gene

The amplified products were extracted with UNIQ-5 DNA Cleaning Kit (Sangon Bio. Co., Canada) and inserted into a pUCm-T vector. The plasmid mini-prep spin column kit (Sangon Bio. Co., Canada) was used to harvest and purify plasmid DNA. DNA sequencing for *Paranophrys magna* and *Mesanophrys carcini* was accomplished using the ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc.) with three forward and three modified reverse 16S sequencing primers (Elwood *et al.* 1985, Medlin *et al.* 1988) as well as the RV-M and M13-20 primers. All sequences were confirmed from both strands.

### Sequence availability

The nucleotide sequences used in this paper are available from the GenBank/EMBL databases under the following accession numbers: *Anophyroides haemophila* U51554, *Cyclidium plouneouri* U27816, *Cohnilembus verminus* Z22878, *Cyclidium glaucoma* Z22879, *Cyclidium porcatum* Z29517, *Ichthyophthirius multifiliis* U17354, *Obertruria georgiana* X65149, *Ophryoglena catenula* U17355, *Paramecium bursaria* AF100314, *Paramecium tetraurelia* X03772, *Paramecium nephridiatum* AF100317, *Platyophrya vorax* AF060454, *Pseudomicrothorax dubius* X65151, *Pseudocohnilembus marinus* Z22880, *Pseudoplatyophrya nana* Af060452, *Tetrahymena corlissi* U17356, and *Uronema marinum* Z22881. A karyorelictid ciliate, *Loxodes striatus* L24248 was selected as the outgroup species.

### Phylogenetic analyses

The sequences were aligned with other SSrRNA gene sequences using a computer assisted procedure, Clustal W, ver. 1.80 (Thompson *et al.* 1994), and refined by considering the conservation of primary structures. PHYLIP package, version 3.57c (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 least-squares [LS] and the neighbor-joining [NJ] methods (Fitch and Margoliash 1967, Saitou and Nei 1987). The DNAPARS program in PHYLIP was used to find the most parsimonious tree (Kluge and Farris 1969). Both parsimony and distance data were bootstrap resampled 1,000 times (Felsenstein 1985).

## RESULTS

### Sequences and comparisons (Fig. 1)

The complete SSrRNA gene sequences of *Paranophrys magna* (GenBank/EMBL accession number AY103191) and *Mesanophrys carcini* (GenBank/EMBL accession number AY103189) are the same length at 1759 nucleotides. The GC contents (*Paranophrys magna* 43.89%, *Mesanophrys carcini* 43.15%) are in the same range as most other ciliates

<i>P. mag</i>	AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCCTGCATGCTAAGTATAAAATAGTAT	75
<i>U. mar</i>	-----TGCATGCTAAGTATAAAATAGTAT	24
<i>M. car</i>	AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCCTGCATGCTAAGTATAAAATAGTAT	75
<i>A. hae</i>	AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCCTGCATGCTAAGTATAAAATAGTAT	75
<i>P. mag</i>	ACAGTGAAACTGCGAATGGCTCATTAAAACAGTTATATTTTATTGATAAATGAAATATTACATGGATAACCGTGC	150
<i>U. mar</i>	ACAGTGAAACTGCGAATGGCTCATTAAAACAGTTATATTTTATTGATAAATGAAAGCTACATGGATAACCGTGC	99
<i>M. car</i>	ACAGTGAAACTGCGAATGGCTCATTAAAACAGTTATATTTTATTGATAAATGAAAGCTACATGGATAACCGTGC	150
<i>A. hae</i>	ACAGTGAAACTGCGAATGGCTCATTAAAACAGTTATATTTTATTGATAAATGAAAGCTACATGGATAACCGTGC	150
<i>P. mag</i>	TAATTCTAGCGCTAATACATGCTGTCAACCCGACTTTGCGAAGGGCTGTATTTATTAGATATGAA-GCCAATA	224
<i>U. mar</i>	TAATTCTAGCGCTAATACATGCTGTCAACCCGACTTTGCGAAGGGCTGTATTTATTAGATATGAA-GCCAATA	173
<i>M. car</i>	TAATTCTAGCGCTAATACATGCTGTCAACCCGACTTTGCGAAGGGCTGTATTTATTAGATATGAAAGCCAATA	225
<i>A. hae</i>	TAATTCTAGCGCTAATACATGCTGTCAACCCGACTTTGCGAAGGGCTGTATTTATTAGATATGAA-GCCAATA	224
<i>P. mag</i>	FTCCTT-CTGTCTATTGTGTGATCATAGTAACTGATCGAACCCTTTTTC-GGTAAATCATTCAAGTTTCG	297
<i>U. mar</i>	FTCCTT-CTGTCTATTGTGTGATCATAGTAACTGATCGAACCCTTCATTTTC--GATAAATCATTCAAGTTTCG	246
<i>M. car</i>	FTCCTT-CTGTCTATTGTGTGATCATAGTAACTGATCGAACCCTTTTTC-AGATAAATCATTCAAGTTTCG	299
<i>A. hae</i>	FTCCTT-CTGTCTATTGTGTGATCATAGTAACTGATCGAACCCTTTTTCG-GATAAATCATTCAAGTTTCG	298
<i>P. mag</i>	CCCTATCAGCTTTTCGATGGTAGTGTATTGCTGACTACCATGGCACTGACGGGTAAAGGAGAATTAGGGTTCCGTT	372
<i>U. mar</i>	CCCTATCAGCTTTTCGATGGTAGTGTATTG--GACTACCATGGCACTGACGGGTAAAGGAGAATTAGGGTTCCGTT	318
<i>M. car</i>	CCCTATCAGCTTTTCGATGGTAGTGTATTG--GACTACCATGGCACTGACGGGTAAAGGAGAATTAGGGTTCCGTT	372
<i>A. hae</i>	CCCTATCAGCTTTTCGATGGTAGTGTATTG--GACTACCATGGCACTGACGGGTAAAGGAGAATTAGGGTTCCGTT	371
<i>P. mag</i>	CCGGAGAGGGAGCCTGAGAAACCGCTACCACATCTAAGGAAGGCAGCAGGGGGTAAATTACCCAATCCCGATTG	447
<i>U. mar</i>	CCGGAGAGGGAGCCTGAGAAACCGCTACCACATCTAAGGAAGGCAGCAGTGGTAAATTACCCAATCCCGATTG	393
<i>M. car</i>	CCGGAGAGGGAGCCTGAGAAACCGCTACCACATCTAAGGAAGGCAGCAGGGGGTAAATTACCCAATCCCGATTG	447
<i>A. hae</i>	CCGGAGAGGGAGCCTGAGAAACCGCTACCACATCTAAGGAAGGCAGCAGGGGGTAAATTACCCAATCCCGATTG	446
<i>P. mag</i>	AGGGAGGTAGTGACAAGAAATAACAACCTGGGCACTTGGT--GTTACGGATTGCAATGAGAACAATTAAGAA	521
<i>U. mar</i>	AGGGAGGTAGTGACAAGAAATAGAAACTGGGCACCTTGGT--GTTACGGATTGCAATGAGAACAATTAAGAA	466
<i>M. car</i>	AGGGAGGTAGTGACAAGAAATAACAACCTGGGCACTTGGT--GTTACGGATTGCAATGAGAACAATTAAGAA	520
<i>A. hae</i>	AGGGAGGTAGTGACAAGAAATAACAACCTGGGCACTTGGT--GTTACGGATTGCAATGAGAACAATTAAGAA	521
<i>P. mag</i>	CTTTATCGAG-AACGATTTGGAGGGCAAGTCTGGTCCAGCAGCCCGGGTAAATCCAGCTCCAATAGCGTATAT	595
<i>U. mar</i>	ACTTATCGAGAAACGATTTGGAGGGCAAGTCTGGTCCAGCAGCCCGGGTAAATCCAGCTCCAATAGCGTATAT	540
<i>M. car</i>	ACTTATCGAGAAACGATTTGGAGGGCAAGTCTGGTCCAGCAGCCCGGGTAAATCCAGCTCCAATAGCGTATAT	594
<i>A. hae</i>	ACTTATCGAGAAACGATTTGGAGGGCAAGTCTGGTCCAGCAGCCCGGGTAAATCCAGCTCCAATAGCGTATAT	595

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<i>P. mag</i>	AAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCAACATCTGGTGGCGGCTCCTGTCCATCGAAGAT	670
<i>U. mar</i>	AAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCAACATCTGGTGGCGGCTCCTGTCCATCGAAGAT	615
<i>M. car</i>	AAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCAACATCTGGTGGCGGCTCCTGTCCATCGAAGAT	669
<i>A. hae</i>	AAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGCAACATCTGGTGGCGGCTCCTGTCCATCGAAGAT	670
<i>P. mag</i>	GGTACATCCGCTTACAAASTTAGCCGGTCTTCATTTGGTCCACTAAGGAGTAGGCCCTTTACTTTGAAAAAAT	745
<i>U. mar</i>	TCTGCATCCGCTTACAAAGCTTTCGGGCTTAACTGGTCCCTACTAGAGTAGGCCCTTTACTTTGAAAAAAT	690
<i>M. car</i>	TCCACATCCGCTTACAAAGCTTTCGGGCTTAACTGGTCCCTACTAGAGTAGGCCCTTTACTTTGAAAAAAT	744
<i>A. hae</i>	TGGCATCCGCTTACAAATCTCGACCGGCTTAACTGGTCCCTACTAGAGTAGGCCCTTTACTTTGAAAAAAT	745
<i>P. mag</i>	AGAGTGTTCAGCCAGGCCATGGCTCGAATACATTAGCATGGAATAATGCAATAGGACTTCTCCATTTCGGTTC	820
<i>U. mar</i>	AGAGTGTTCAGCCAGGCCATGGCTCGAATACATTAGCATGGAATAATGCAATAGGACTTCTCCATTTCGGTTC	765
<i>M. car</i>	AGAGTGTTCAGCCAGGCCATGGCTCGAATACATTAGCATGGAATAATGCAATAGGACTTCTCCATTTCGGTTC	819
<i>A. hae</i>	AGAGTGTTCAGCCAGGCCATGGCTCGAATACATTAGCATGGAATAATGCAATAGGACTTCTCCATTTCGGTTC	820
<i>P. mag</i>	GTTATTGACCTTAGTAATGATTAAAGGGACAGTTGGGGGCATTAGTACTTAAAGATCAGAGGTAAATTCCTG	895
<i>U. mar</i>	GTTATTGACCTTAGTAATGATTAAAGGGACAGTTGGGGGCATTAGTACTTAAAGATCAGAGGTAAATTCCTG	840
<i>M. car</i>	GTTATTGACCTTAGTAATGATTAAAGGGACAGTTGGGGGCATTAGTACTTAAAGATCAGAGGTAAATTCCTG	894
<i>A. hae</i>	GTTATTGACCTTAGTAATGATTAAAGGGACAGTTGGGGGCATTAGTACTTAAAGATCAGAGGTAAATTCCTG	895
<i>P. mag</i>	GATTTGTTAAAGACTAACTTATGCGAAAGCATTGCCAAGGATGTTTTCAATTAATCAAGAACGAAAGTTAGGGG	970
<i>U. mar</i>	GATTTGTTAAAGACTAACTTATGCGAAAGCATTGCCAAGGATGTTTTCAATTAATCAAGAACGAAAGTTAGGGG	915
<i>M. car</i>	GATTTGTTAAAGACTAACTTATGCGAAAGCATTGCCAAGGATGTTTTCAATTAATCAAGAACGAAAGTTAGGGG	969
<i>A. hae</i>	GATTTGTTAAAGACTAACTTATGCGAAAGCATTGCCAAGGATGTTTTCAATTAATCAAGAACGAAAGTTAGGGG	970
<i>P. mag</i>	TCAAAGACGATAGATACCGTCTAGTCTTAACTATAAACTATAACCGACTCCGATCCCGAGGCTTAAACT	1045
<i>U. mar</i>	TCAAAGACGATAGATACCGTCTAGTCTTAACTATAAACTATAACCGACTCCGATCCCGAGGCTTAAACT	990
<i>M. car</i>	TCAAAGACGATAGATACCGTCTAGTCTTAACTATAAACTATAACCGACTCCGATCCCGAGGCTTAAACT	1044
<i>A. hae</i>	TCAAAGACGATAGATACCGTCTAGTCTTAACTATAAACTATAACCGACTCCGATCCCGAGGCTTAAACT	1045
<i>P. mag</i>	TGGCCGGCCCGTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGG	1120
<i>U. mar</i>	ATTCCGGCCCGTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGG	1065
<i>M. car</i>	TATACGGCCCGTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGG	1119
<i>A. hae</i>	TGGCCGGCCCGTATGAGAAATCAAAGTCTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGG	1120
<i>P. mag</i>	ATTGACGGAAGGGCACCACAGCGTGGACCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACAGGTCGA	1195
<i>U. mar</i>	ATTGACGGAAGGGCACCACAGCGTGGACCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACAGGTCGA	1139
<i>M. car</i>	ATTGACGGAAGGGCACCACAGCATGCTGGACCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACAGGTCGA	1194
<i>A. hae</i>	ATTGACGGAAGGGCACCACAGCGTGGACCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACAGGTCGA	1195

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*P. mag* AACATGGGTGGATTGACAGATTGAGAGCTCTTTCTTGATCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGG 1270  
*U. mar* AACTAGGGTGGATTGACAGATTGAGAGCTCTTTCTTGATCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGG 1214  
*M. car* AACATGGGTGGATTGACAGATTGAGAGCTCTTTCTTGATCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGG 1269  
*A. hae* AACATGGGTGGATTGACAGATTGAGAGCTCTTTCTTGATCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGG 1270

*P. mag* TGGAGTGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTAACCTGCTAAATAGTAGACTTATCTCCAATGG 1345  
*U. mar* TGGACTGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTAACCTGCTAAATAGTAGACTTATCTCCAATGG 1289  
*M. car* TGGAGTGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTAACCTGCTAAATAGTAGACTTATCTCCAATGG 1344  
*A. hae* TGGAGTGATTGTCTGGTTAATTCGGTTAACGAACGAGACCTTAACCTGCTAAATAGTAGACTTATCTCCAATGG 1345

*P. mag* CCGTTACTTCTTAGAGGGACTATGCTATGCAACCCATGGAAAGTTGAGGCAATAACAGGTCGTGTATGCCCTT 1420  
*U. mar* GCGTTACTTCTTAGAGGGACTATGCTATGCAACCCATGGAAAGTTGAGGCAATAACAGGTCGTGTATGCCCTT 1364  
*M. car* CCGTTACTTCTTAGAGGGACTATGCTATGCAACCCATGGAAAGTTGAGGCAATAACAGGTCGTGTATGCCCTT 1419  
*A. hae* GCGTTACTTCTTAGAGGGACTATGCTATGCAACCCATGGAAAGTTGAGGCAATAACAGGTCGTGTATGCCCTT 1420

*P. mag* AGATGTGCTGGCCCGCAGCGCGCTCAATGATTAATTCAGAAAGT-TTACCTGCTGGAAAGGTA-CCGTAA 1494  
*U. mar* AGATGTGCTGGCCCGCAGCGCGCTCAATGATTAATTCAGAAAGT-TTACCTGCTGGAAAGGTA-CCGTAA 1438  
*M. car* AGATGTGCTGGCCCGCAGCGCGCTCAATGATTAATTCAGAAAGT-TTACCTGCTGGAAAGGTA-CCGTAA 1494  
*A. hae* AGATGTGCTGGCCCGCAGCGCGCTCAATGATTAATTCAGAAAGT-TTACCTGCTGGAAAGGTA-CCGTAA 1495

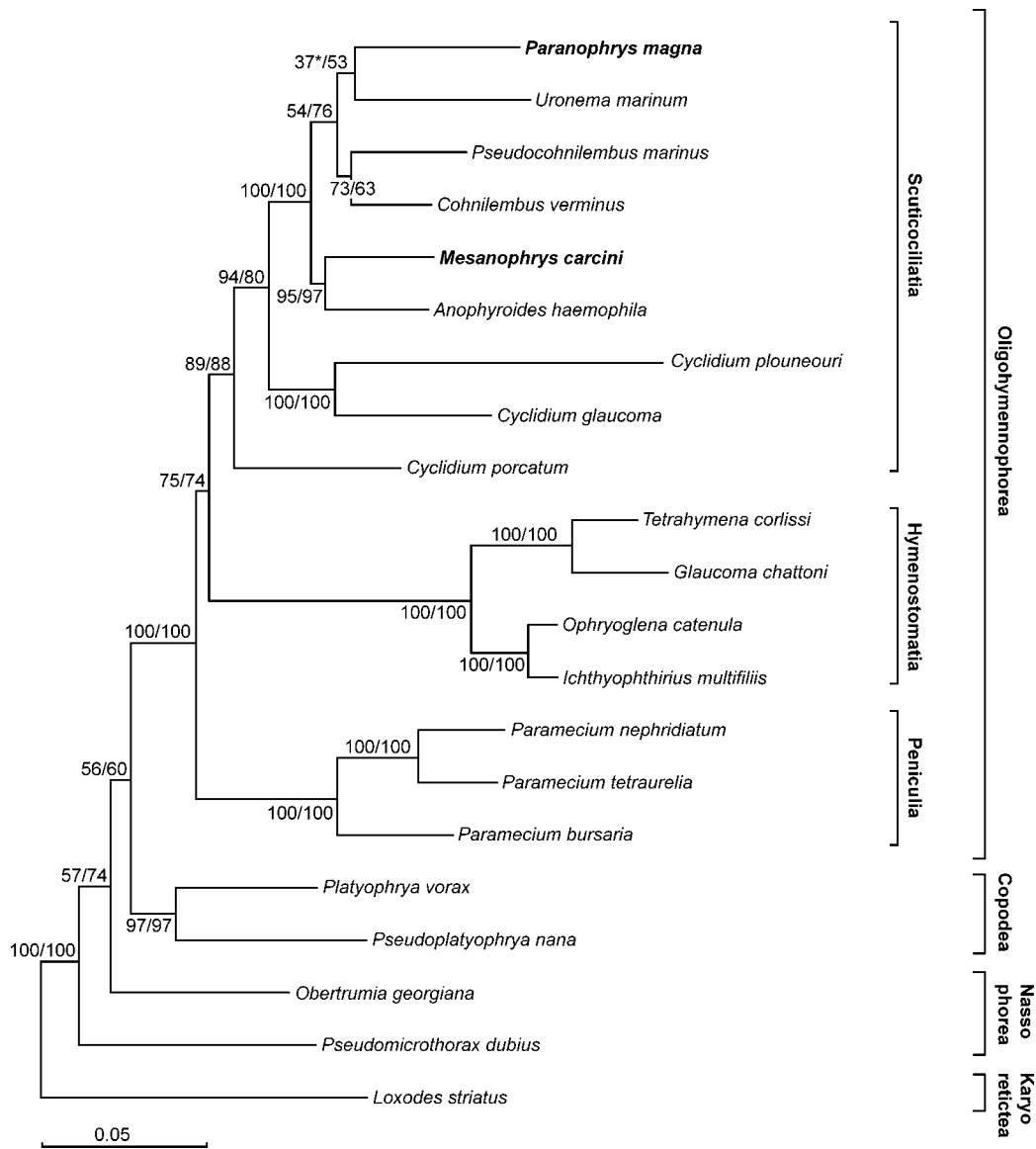
*P. mag* TCGTTGTAATATGCTCGTGTAGGGATCGATCTTTGCAATTATAGATCTTGAACGAGGAATGCTAGTAAGTGC 1569  
*U. mar* TCGTTGTAATATGCTCGTGTAGGGATCGATCTTTGCAATTATAGATCTTGAACGAGGAATGCTAGTAAGTGC 1513  
*M. car* TCGTTGTAATATGCTCGTGTAGGGATCGATCTTTGCAATTATAGATCTTGAACGAGGAATGCTAGTAAGTGC 1569  
*A. hae* TCGTTGTAATATGCTCGTGTAGGGATCGATCTTTGCAATTATAGATCTTGAACGAGGAATGCTAGTAAGTGC 1570

*P. mag* AAGTCATACCGTACTGATTACGTCCCTGCTTTGTACACACCCCGCTCGCTCTACCGATTTCCGATGAT 1644  
*U. mar* GCTTCAT-AGAGCGTACTGATTACGTCCCTGCTTTGTACACACCCCGCTCGCTCTACCGATTTCCGATGAT 1587  
*M. car* AAGTCATACCGTACTGATTACGTCCCTGCTTTGTACACACCCCGCTCGCTCTACCGATTTCCGATGAT 1644  
*A. hae* AAGTCATACCGTACTGATTACGTCCCTGCTTTGTACACACCCCGCTCGCTCTACCGATTTCCGATGAT 1645

*P. mag* CCGGTGAACCTCTGGACTGACACCGCAAGTTT--CGGGAAGTTAGTAAACTTAAAGCTAGAGGAGGGAGT 1717  
*U. mar* CCGGTGAACCTCTGGACTGACACCGCAAGTTT--CGGGAAGTTAGTAAACTTAAAGCTAGAGGAGGGAGT 1660  
*M. car* CCGGTGAACCTCTGGACTGACACCGCAAGTTT--CGGGAAGTTAGTAAACTTAAAGCTAGAGGAGGGAGT 1717  
*A. hae* CCGGTGAACCTCTGGACTGACACCGCAAGTTT--CGGGAAGTTAGTAAACTTAAAGCTAGAGGAGGGAGT 1720

*P. mag* AGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA- 1759  
*U. mar* AGTCGTAACAAGGTTTCCGT----- 1680  
*M. car* AGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA- 1759  
*A. hae* AGTCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCA-NN 1763

**Fig. 1.** Small subunit ribosomal RNA gene sequences of the scuticociliates *Paranophrys magna* (*P. mag*) and *Mesanophrys carcini* (*M. car*) aligned with the sequences from *Uronema marinum* (*U. mar*) and *Anophryoides haemophila* (*A. hae*). Numbers at the end of lines indicate the number of nucleotides. The differences in sequence length were compensated for by introducing alignment gaps (-) in the sequences. Matched sites are highlighted in black.



**Fig. 2.** A small subunit ribosomal RNA tree derived from evolutionary distances showing the phylogenetic positions of *Paranophrys magna* and *Mesanophrys carcini*. The consensus tree of 1000 bootstrap resamplings of the data set was constructed using the Fitch and Margoliash (1967) least-squares [LS] method. The numbers at the nodes represent the bootstrap percentages of 1000 for the LS method followed by the bootstrap values for the neighbor-joining [NJ] method of Saitou and Nei (1987). Asterisks indicate bootstrap values less than 50%. Evolutionary distance is represented by the branch length to separate the species in the figure. The scale bar corresponds to five substitutions per 100 nucleotide positions. The new sequences are represented in boldface.

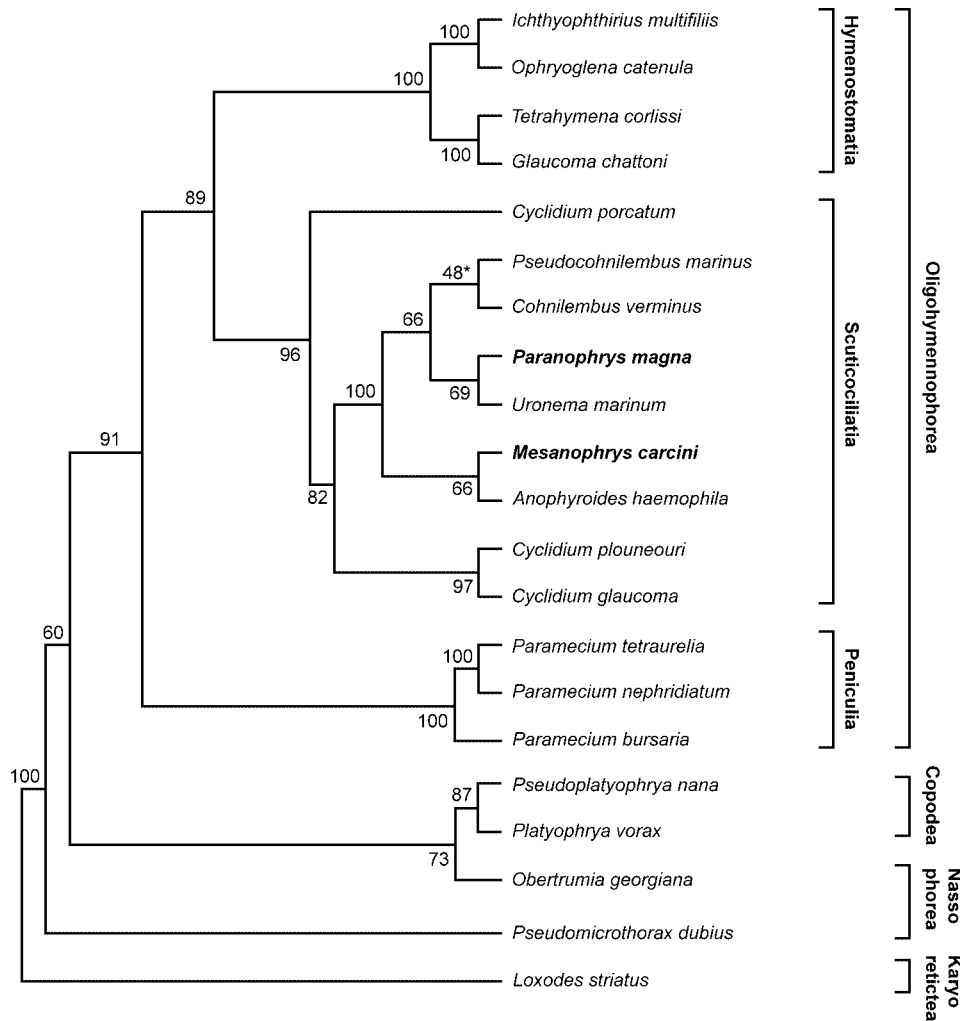
(Elwood *et al.* 1985, Sogin and Elwood 1986). The sequence of *P. magna* differed in 181 nucleotides from that of *M. carcini* with the similarity 89% between them. After the removal of ambiguous sites in the alignment, a total of 1712 nucleotides remained for the subsequent analysis (Fig. 1). Sequence data were reduced from 1712 to 809 phylogenetically informative sites for the maximum parsimony (MP) analysis.

Table 1 shows the structural similarity and evolutionary distance values that were calculated pairwise for 20 ciliate species including the two scuticociliates treated in the present work. From these data it can be seen that the evolutionary distance value for *Mesanophrys carcini* and *Anophyroides haemophila* is only 0.0611, suggesting that these two species are closely related, while the most closely related species to *Paranophrys magna* is

**Table 1.** 16s-like SSrRNA structural similarity (upper half) and evolutionary distance (lower half) for scuticociliates and ciliates from other classes for which relevant sequence data are available. Data analysed by the Elwood *et al.* (1985) and Jukes and Cantor (1969) formulas for conversion of structural similarity.

	<i>P. mag</i>	<i>P. mar</i>	<i>U. mar</i>	<i>C. ver</i>	<i>M. car</i>	<i>A. hae</i>	<i>C. plo</i>	<i>C. gla</i>	<i>C. por</i>	<i>T. cor</i>	<i>G. cha</i>	<i>O. cat</i>	<i>I. mul</i>	<i>P. nep</i>	<i>P. tet</i>	<i>P. bur</i>	<i>P. vor</i>	<i>P. nan</i>	<i>O. geo</i>	<i>P. dub</i>	<i>L. str</i>
<i>P. mag</i>	-	0.9056	0.8916	0.9072	0.8969	0.9032	0.8126	0.8588	0.8490	0.8061	0.7951	0.8218	0.8139	0.8240	0.8293	0.8448	0.8418	0.8347	0.8393	0.8110	0.7775
<i>P. mar</i>	0.0944	-	0.8900	0.9334	0.9131	0.9227	0.8303	0.8734	0.8659	0.7933	0.7821	0.8116	0.8039	0.8300	0.8244	0.8492	0.8499	0.8361	0.8398	0.8211	0.7705
<i>U. mar</i>	0.1084	0.1100	-	0.9159	0.8916	0.9064	0.8252	0.8555	0.8537	0.8062	0.7968	0.8109	0.8164	0.8115	0.8146	0.8256	0.8353	0.8236	0.8388	0.8065	0.7618
<i>C. ver</i>	0.0928	0.0666	0.0841	-	0.9215	0.9283	0.8374	0.8830	0.8820	0.8135	0.8051	0.8267	0.8282	0.8364	0.8409	0.8370	0.8598	0.8424	0.8610	0.8321	0.7837
<i>M. car</i>	0.1031	0.0869	0.1084	0.0785	-	0.9389	0.8322	0.8779	0.8827	0.8220	0.8164	0.8374	0.8366	0.8271	0.8416	0.8548	0.8340	0.8446	0.8464	0.8174	0.7749
<i>A. hae</i>	0.0968	0.0773	0.0936	0.0717	0.0611	-	0.8416	0.8840	0.8765	0.8138	0.8038	0.8308	0.8354	0.8331	0.8368	0.8507	0.8381	0.8517	0.8525	0.8303	0.7861
<i>C. plo</i>	0.1874	0.1697	0.1748	0.1626	0.1678	0.1584	-	0.8567	0.7234	0.7694	0.7662	0.7801	0.7867	0.7835	0.7783	0.7910	0.7906	0.7720	0.8058	0.7682	0.7314
<i>C. gla</i>	0.1412	0.1266	0.1445	0.1170	0.1221	0.1160	0.1433	-	0.8689	0.8042	0.7949	0.8160	0.8114	0.8278	0.8269	0.8425	0.8365	0.8194	0.8439	0.8214	0.7803
<i>C. por</i>	0.1510	0.1341	0.1463	0.1180	0.1173	0.1235	0.1766	0.1311	-	0.8101	0.8048	0.8233	0.8210	0.8324	0.8392	0.8416	0.8530	0.8398	0.8548	0.8356	0.7696
<i>T. cor</i>	0.1939	0.2067	0.1938	0.1865	0.1780	0.1862	0.2306	0.1958	0.1899	-	0.9630	0.9385	0.9337	0.7974	0.8016	0.8079	0.7928	0.7780	0.7908	0.7900	0.7249
<i>G. cha</i>	0.2049	0.2179	0.2032	0.1949	0.1846	0.1962	0.2338	0.2051	0.1952	0.0370	-	0.9326	0.9285	0.7753	0.7804	0.7894	0.7915	0.7790	0.7837	0.7745	0.7137
<i>O. cat</i>	0.1782	0.1884	0.1891	0.1733	0.1626	0.1692	0.2199	0.1840	0.1767	0.0615	0.0674	-	0.9815	0.8013	0.8110	0.8123	0.8104	0.7997	0.8004	0.8053	0.7370
<i>I. mul</i>	0.1861	0.1861	0.1836	0.1718	0.1634	0.1646	0.2133	0.1886	0.1790	0.0663	0.0715	0.0185	-	0.8012	0.8099	0.8089	0.8080	0.7996	0.8004	0.8012	0.7338
<i>P. nep</i>	0.1760	0.1700	0.1885	0.1636	0.1729	0.1669	0.2165	0.1722	0.1676	0.2026	0.2247	0.1987	0.1988	-	0.9425	0.9111	0.8362	0.8265	0.8232	0.8094	0.7703
<i>P. tet</i>	0.1707	0.1756	0.1854	0.1591	0.1584	0.1632	0.2217	0.1731	0.1608	0.1984	0.2196	0.1890	0.1901	0.0575	-	0.9132	0.8315	0.8225	0.8293	0.8132	0.7642
<i>P. bur</i>	0.1552	0.1508	0.1744	0.1430	0.1452	0.1493	0.2090	0.1575	0.1584	0.1921	0.2106	0.1877	0.1911	0.0889	0.0868	-	0.8378	0.8294	0.8340	0.8244	0.7795
<i>P. vor</i>	0.1582	0.1501	0.1647	0.1402	0.1460	0.1419	0.2094	0.1635	0.1470	0.2072	0.2085	0.1896	0.1920	0.1638	0.1685	0.1622	-	0.8126	0.8823	0.8608	0.7953
<i>P. nan</i>	0.1653	0.1639	0.1764	0.1576	0.1554	0.1483	0.2280	0.1806	0.1602	0.2220	0.2210	0.2003	0.2004	0.1735	0.1775	0.1706	0.1074	-	0.8736	0.8473	0.7933
<i>O. geo</i>	0.1607	0.1602	0.1612	0.1390	0.1546	0.1475	0.1942	0.1561	0.1452	0.2092	0.2163	0.1996	0.1996	0.1768	0.1707	0.1660	0.1177	0.1264	-	0.8725	0.8034
<i>P. dub</i>	0.1890	0.1789	0.1935	0.1679	0.1726	0.1697	0.2318	0.1786	0.1644	0.2100	0.2255	0.1947	0.1988	0.1906	0.1868	0.1756	0.1392	0.1527	0.1275	-	0.8048
<i>L. str</i>	0.2225	0.2295	0.2382	0.2163	0.2251	0.2139	0.2686	0.2197	0.2304	0.2751	0.2863	0.2630	0.2662	0.2297	0.2358	0.2205	0.2047	0.2067	0.1966	0.1952	-

Abbreviation: *P. mag* - *Paranophrys magna*; *P. mar* - *Pseudocohlembus marinus*; *U. mar* - *Uronema marinum*; *C. ver* - *Cohlembus verinus*; *M. car* - *Mesanophrys carini*; *A. hae* - *Anophryoides haemophila*; *C. plo* - *Cyclidium plouneouri*; *C. gla* - *Cyclidium glaucoma*; *C. por* - *Cyclidium porcatum*; *T. cor* - *Tetrahymina corlissi*; *G. cha* - *Glaucoma chattoni*; *O. cat* - *Ophryoglena catenula*; *I. mul* - *Ichthyophthirus multifiliis*; *P. nep* - *Paramecium nephridiatum*; *P. tet* - *Paramecium tetraurelia*; *P. bur* - *Paramecium bursaria*; *P. vor* - *Platyophrya vorax*; *P. nan* - *Pseudoplathyophrya nana*; *O. geo* - *Obertrumia georgians*; *P. dub* - *Pseudomicrothorax dubius*; *L. str* - *Loxodes striatus*



**Fig. 3.** A consensus bootstrap tree based on maximum parsimony analysis constructed from small subunit ribosomal RNA sequences. The systematic positions of *Paranophrys magna* and *Mesanophrys carcini*, and phylogenetic relationships among those scuticociliates for which small subunit ribosomal RNA sequences are available, are shown. The numbers at the forks give the percentage of times the group occurred out of the 1,000 trees. No significance is placed on branch lengths connecting the species. The new sequences are represented in boldface.

*Uronema marinum* (evolutionary distance value 0.1084). *Pseudocohnilembus marinus* and *Cohnilembus verminus*, which are members of the same clade, are both more closely related to *P. magna* (evolutionary distance values 0.0944 and 0.0928 respectively) than is *U. marinum* (0.1084).

**Distance matrix analysis (Fig. 2)**

Both least-squares (LS) and neighbor-joining (NJ) analyses give strong bootstrap support for the monophyly of the class Oligohymenophorea (100% LS, 100% NJ, Fig. 2). The monophyly of the class Colpodea is also well supported (100% LS, 100% NJ), whereas according

to these analyses the class Nassophorea is probably paraphyletic (Figs 2, 3). Within the class Oligohymenophorea, the subclass Peniculia branches basally with a maximum bootstrap support (100% LS, 100% NJ); the clades for the Hymenostomata and the Scuticociliatia branch later and appear to be sister groups with moderate support (75% LS, 74% NJ).

As shown in Fig. 2, the subclass Scuticociliatia is monophyletic (89% LS, 88% NJ). The species within Philasterida also form a monophyly with maximum bootstrap support (100% LS, 100% NJ) and are separate from the three *Cyclidium* species which belong to the order Pleuronematida. Within the Philasterida,



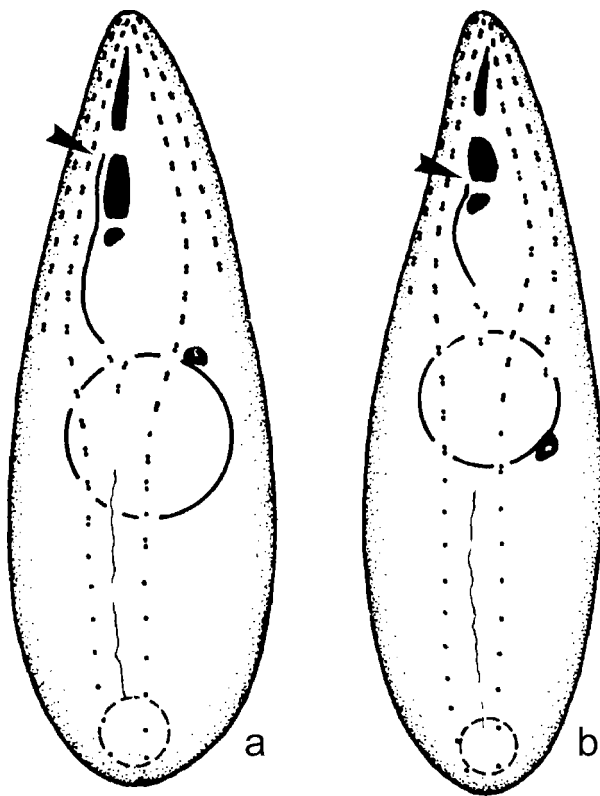


Fig. 4. Schematic comparison of buccal apparatus of *Paranophrys magna* (a) and *Mesanophrys carcini* (b). Arrowhead marks the anterior end of paroral membrane (after Song and Wilbert 2000).

*Mesanophrys carcini* and *Anophyroides haemophila* branch as a monophyletic clade (95% LS, 97% NJ), while *Paranophrys magna* forms a clade with *Uronema marinum* (53% LS, 37% NJ) which is a sister group to a lineage that includes *Pseudocohnilembus marinus* and *Cohnilembus verminus* (54% LS, 76% NJ).

#### Maximum parsimony analysis (Fig. 3)

The maximum parsimony tree (MP), as shown in Fig. 3, is generally similar to that inferred from the distance matrix analysis. The only significant difference is the position of *Obertrumia georgiana*, which groups with *Pseudoplatyophrya nana* and *Platyophrya vorax* in the MP tree (Fig. 3).

## DISCUSSION

Comparisons of the complete SSrRNA gene sequences supports the monophyly of the Scuticociliatia

which is consistent with other recent molecular studies (Bernhard *et al.* 1995, Strüder-Kypke *et al.* 2000). Our results also confirm that the Scuticociliatia and Hymenostomatia are sister taxa, as has been previously suggested following analysis both of morphological and molecular data (Lynn 1979, 1981; Bardele 1981; Beran 1990; Strüder-Kypke *et al.* 2000).

According to the SSrRNA gene sequence data obtained in the present study, the monophyly of the order Philasterida is confirmed with maximum bootstrap support (100% LS, 100% NJ, 100% MP).

Corliss (1979) placed *Paranophrys*, *Mesanophrys* and *Uronema* in the family Philasteridae on account of their similar morphologies. The molecular data reported here, however, suggest that *Paranophrys* and *Uronema* form a clade, the sister group of which includes *Pseudocohnilembus* and *Cohnilembus* of the family Cohnilembidae (according to the Corlissian system). Nevertheless, it should be noted that these four taxa exhibit at least three distinct patterns of morphogenesis and arrangements of the buccal apparatus; one for *Paranophrys* and *Uronema*, a second one for *Pseudocohnilembus* and a third for *Cohnilembus* (Song and Wilbert 2000, Ma *et al.* 2003). *Mesanophrys*, by contrast, groups with *Anophyroides* rather than with *Paranophrys* and *Uronema*.

Song and Wilbert (2000) suggested that the genus *Anophyroides* should be synonymized with *Paranophrys*. However, in the present study, *Anophyroides haemophila* converged with a morphospecies of the genus *Mesanophrys* (*M. carcini*), which differs from *Paranophrys* only in the terminal position of the anterior end of the paroral membrane (i. e. adjacent to the posterior end of  $M_2$  vs. adjacent to the anterior end of  $M_2$ ) (Fig. 4). The SSrRNA of *M. carcini* differs by only 107 nucleotides from that of *A. haemophila* with a similarity 93.89%, while the SSrRNA of *Paranophrys magna* differs by 170 nucleotides from that of *Anophyroides haemophila* (similarity 90.32%), indicating that *Anophyroides* might be phylogenetically closer to *Mesanophrys* than to *Paranophrys*. Previous studies of scuticociliates and other ciliate groups have similarly reported that phylogenetic trees based on molecular data may be incongruent with those based on morphological data (Ragan *et al.* 1996, Chen *et al.* 2000, Strüder-Kypke *et al.* 2000). As noted recently, there at least two reasons for this phenomenon: (a) the morphological and morphogenetic characters do not necessarily reflect evolutionary relationships between taxa since

some morphological similarities might be due to phenetic adaptation; (b) the information from the SSrRNA gene sequence does not always accurately reflect the actual phylogeny (Bernhard *et al.* 1995, Hirt *et al.* 1995, Wright and Lynn 1995, Strüder-Kypke *et al.* 2000).

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