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ORIGINAL ARTICLE

# Molecular Phylogeny and Evolutionary Relationships between the Ciliate Genera *Peniculistoma* and *Mytilophilus* (Peniculistomatidae, Pleuronematida)

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## ABSTRACT

*Peniculistoma mytili* and *Mytilophilus pacificae* are placed in the pleuronematid scuticociliate family Peniculistomatidae based on morphology and ecological preference for the mantle cavity of mytiloid bivalves. We tested this placement with sequences of the small subunit rRNA (SSUrRNA) and cytochrome *c* oxidase subunit 1 (*cox1*) genes. These species are very closely related sister taxa with no distinct genetic difference in the SSUrRNA sequence but about 21% genetic difference for *cox1*, supporting their placement together but separation as distinct taxa. Using infection frequencies, *M. pacificae*, like its sister species *P. mytili*, does not interact with *Ancistrum* spp., co-inhabitants of the mantle cavity. On the basis of these ecological similarities, the fossil record of host mussels, and features of morphology and stomatogenesis of these two ciliates, we argue that *M. pacificae* derived from a *Peniculistoma*-like ancestor after divergence of the two host mussels. Our phylogenetic analyses of pleuronematid ciliates includes the SSUrRNA gene sequence of *Sulcigera comosa*, a *Histiobalantium*-like ciliate from Lake Baikal. We conclude: (i) that the pleuronematids are a monophyletic group; (ii) that the genus *Pleuronema* is paraphyletic; and (iii) that *S. comosa* is a *Histiobalantium* species. We transfer *S. comosa* to *Histiobalantium* and propose a new combination *Histiobalantium comosa* n. comb.

SMALL (1967) first recognized the scuticociliates as an order among the "hymenostomes," characterized by their distinct mode of stomatogenesis, which involved proliferation of kinetosomes from the scutica and the adjacent undulating membrane or paroral. The scuticociliates were divided at that time into the philasterine, pleuronematine, and thigmotrich suborders. Since then, the number of families has proliferated, the scuticociliates have been elevated to subclass status, and a new order to include the loxocephalid-related genera has been proposed (Jankowski 2007; Lynn 2008). Jankowski (2007) noted that the families of philasterine scuticociliates are characterized by minor and sometimes questionable differences, and he concluded that perhaps there may be only two or three families.

In discussing the pleuronematine scuticociliates, Jankowski (2007) noted the difficulty in defining this group and suggested that its composition be limited to *Pleuronema* and a few clearly related genera. He included the three traditionally recognized families—Pleuronematidae, Peniculistomatidae, and Histiobalantiidae, which he argued should be a junior synonym of Sulcigeridae Gajewskaja 1928. Jankowski (2007) proposed that the differences in the oral structures of *Mytilophilus* warranted its assignment to a new monotypic family—Mytilophilidae Jankowski 2007, separate from the Peniculistomatidae.

In this report, our objective was to provide gene sequences of *Peniculistoma mytili*, *Mytilophilus pacificae*, and *Sulcigera comosa* to assess the genetic support for the distinctness of the Mytilophilidae. In addition, we construct an evolutionary hypothesis of the relationship between *P. mytili* and *M. pacificae*, by considering the following lines of evidence: differences in stomatogenic pattern, correlation of ecological niche, sexual behavior, and evolution of host mussels.

## MATERIALS AND METHODS

### Sample collection, isolation, characterization, and identification

*Mytilophilus pacificae* (Fig. 1) was collected at low tide from the host mussel *Mytilus californianus* Conrad at Pigeon Point, CA (37°10′06.5″N; 122°23′18.4″W) on March, 2010, Pillar Point, CA (37°29′33.4″N; 122°29′45.1″W) on March, 2010, Depoe Bay, OR (44°48′32.7″N; 124°03.47.0″W) on October, 2008, French Beach, BC (48°23′11″N; 123°56′31″W) on June 2012 and 2013 and Botany Bay, BC (48°31′45″N; 124°27′12″W) in June 2013. Further samples of *M. californianus* were obtained from additional sites to estimate occurrence frequencies. These sites were Pigeon Point CA, Princeton Harbor breakwater, Princeton Harbor, near Pillar Point, CA and King Harbor, Redondo Beach, CA (33°51′00.0″N; 118°24′00.0″W), all sampled in April 1980. During our recent sampling, the range of *M. pacificae* was extended from Cambria, CA to Botany Bay, Vancouver Island, BC.

*Peniculistoma mytili* (Fig. 2) was collected from the host mussel *Mytilus edulis* L. at the Marine Biology Laboratory, Helsingør, Denmark (56°1′50.8″N; 12°35′31.7″E) in September, 2011 and from mussels growing on floating boat docks in the San Francisco Bay (Saint Francis Yacht Harbor, S.F., and Romberg Tiburon Center for Environmental Studies, Tiburon, CA) in April 1980. Further samples of *M. edulis* were obtained from additional sites to estimate occurrence frequencies. These sites were Princeton Harbor, Princeton, CA, Princeton Breakwater, Princeton, CA, and King Harbor, Redondo Beach CA, all sampled in April 1980.

*Sulcigera comosa* (Fig. S1) was collected from the waters of Lake Baikal (53°33′36.4″N; 108°9′52.8″E) in April, 2009.

These three species were identified using the following literature: for *M. pacificae* (Antipa and Dolan 1985; Dolan and Antipa 1985); for *P. mytili* (Dolan and Antipa 1985; Fenchel 1965); for *S. comosa* (Obolkina, 1995). See Table S1 for detailed listing of samples and sequences with accession numbers.

The ciliates were rinsed in filtered environmental water and preserved in ≥ 80% ethanol prior to DNA extraction.

### DNA extraction and sequencing

DNA of *M. pacificae* and *S. comosa* was extracted using the DNEasy Tissue kit (Qiagen, Mississauga, ON, Canada), following the manufacturer's protocol, with the exception that only 100 μl of buffer AE were added for the final elution. Primers for the small subunit (SSU) rRNA, ITS1, 5.8S rRNA, and ITS2 gene regions were the forward eukaryote primers A (5′-AACCTGGTTGATCCTGCCAGT-3′; Medlin et al. 1988) and 82F (5′-GAAACTGCGAATGGCTC-3′), and the reverse primer LSUR (5′-GTTAGTTTCTTTTCCTCCGC-3′; Bourland and Strüder-Kypke 2010). The amplification of the mitochondrial *cox1* gene used the primers F388dT (5′-TGTAAAACGACGGCCAGTGGWKCBAAAGATGTWGC-3′)

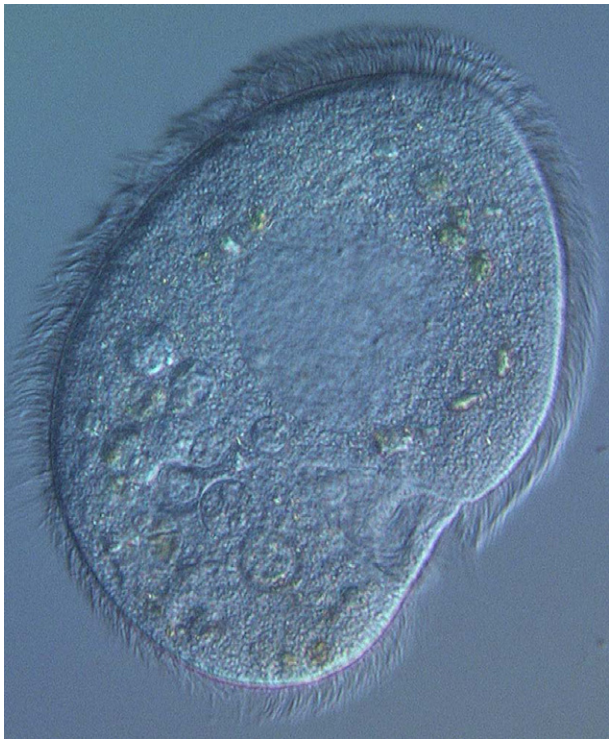

**Figure 1** *Mytilophilus pacificae* collected from its host mussel *Mytilus californianus* on the west coast of Vancouver Island. Differential interference contrast micrograph. Cell length = 140 μm.

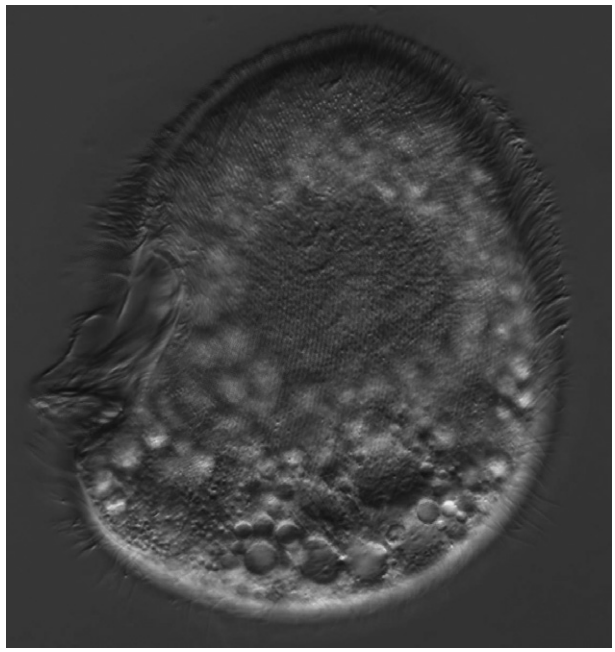

**Figure 2** *Peniculistoma mytili* collected from its host mussel *Mytilus edulis* off Helsingør, Denmark. Differential interference contrast micrograph. Cell length = 145 μm.

and R1184dT (5′-CAGGAAACAGCTATGACTADACYT-CAGGGTGACCRAAAAATCA-3′) (Strüder-Kypke and Lynn 2010). The PCR products were gel-purified with the MinElute kit (Qiagen, Mississauga, ON, Canada). DNA of *P. mytili* was extracted following procedures in Irwin and Lynn (2015). Direct sequencing of both strands using ABI BigDye Terminator v 3.1 (Applied Biosystems, Burlington, ON) was conducted by the Genomics Facility, Advanced Analysis Centre, University of Guelph (Guelph, ON, Canada) for *M. pacificae* and *S. comosa* and by the Nucleic Acid/Protein Service Unit, University of British Columbia (NAPS UBC, BC, Canada) for *P. mytili*.

## Sequence analysis and alignment

The sequence fragments were assembled into contigs with Sequencher ver. 5.4 (Gene Codes Corp., Ann Arbor, MI), trimmed at the ends, and checked for sequencing errors. Sequences were aligned with Mega ver. 6 (Tamura et al. 2013) and two different alignment files were used for the phylogenetic analyses: SSUrRNA alignment with 33 in-group taxa, (72 taxa, 1,850 positions), *cox1* alignment with all (19) available in-group taxa (25 taxa, 812 positions). Distance data were inferred from complete sequence alignments with only the ends trimmed. Pairwise distances, mean within and mean between groups distances were calculated with Mega 6, based on the Tamura 3-Parameter model (Tamura 1992).

## Phylogenetic analysis

SSUrRNA datasets: SSUrRNA alignments were imported into G-blocks ver. 0.91b (Castresana 2000) and ambiguously aligned, hypervariable regions were removed from the datasets. The final alignment for phylogenetic analyses comprised 1,719 nucleotides (92% of original alignment). The best model for each dataset was calculated by jModeltest 2.1.3 (Darriba et al. 2012; Guindon and Gascuel 2003), AIC criterion. The Transition Model (TIM2) with gamma distribution (G) and proportion of invariable sites (I) was selected for the SSU dataset. Four standard phylogenetic analyses were performed on the alignment of SSUrRNA: Maximum Likelihood (ML), Bayesian Inference (BI), Maximum Parsimony (MP), and Neighbor Joining (NJ). BI and PAUP analyses were performed through the CIPRES Server Portal (Miller et al. 2010). The ML analysis was run via the RAxML BlackBox server (Stamatakis et al. 2008), with 100 rapid bootstrap replicates and a subsequent thorough ML search, using the General-Time-Reversible (GTR) algorithm with gamma distributed substitution rates and invariable sites (GTR+I+G). Bayesian Inference was computed with MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck 2003), using the GTR+I+G model. Two parallel runs were performed and the maximum posterior probability of a phylogeny out of 5,000,000 generations, approximating it with the Markov chain Monte Carlo (MCMC) and sampling every 200th generation, was calculated, discarding the first 25% of trees as burn-in. Average standard deviation of split frequencies (< 0.01) was used to assess convergence of the two runs. The PAUP analysis (PAUP 4.10, Swofford 2002) determined 522 parsimony-informative characters. Species were added stepwise and randomly, the tree bisection-reconnection (TBR) branch-swapping algorithm was used, and the data were bootstrap re-sampled 500 times. PHYLIP ver. 3.6.9, (Felsenstein 2009) was employed to calculate genetic distances with the Kimura-2-parameter model (Kimura 1980) using DNADIST. The distance trees were constructed with NEIGHBOR, using the Neighbor Joining (NJ) algorithm (Saitou and Nei 1987). The data were bootstrap re-sampled 1,000 times.

The *cox1* gene alignment was trimmed at the ends and distance data were calculated based on the F84 algorithm (Felsenstein and Churchill 1996; Kishino and Hasegawa 1989). Neighbor Joining analysis (PHYLIP, Saitou and Nei 1987) was performed with data that were bootstrap resampled 1,000 times.

## Constrained analyses

Several constrained topologies were tested against the best RAxML tree of the SSUrRNA dataset using the Approximate Unbiased (AU) test, as implemented in Consel ver. 1.19 (Shimodaira 2002; Shimodaira and Hasegawa 2001). We tested specifically (1) genus *Pleuronema* monophyletic, (2) family Pleuronematidae monophyletic, (3) *Mytilophilus pacificae* and *Peniculistoma mytili* both monophyletic, (4) *Histiobalantium* monophyletic, and (5) three different branching patterns for the group: (i) (Pleuronematidae + Peniculistomatidae) + Histiobalantiidae; (ii) Pleuronematidae + (Peniculistomatidae + Histiobalantiidae) and (iii) Peniculistomatidae + (Pleuronematidae + Histiobalantiidae).

## Ecological niche analyses

Mussels were examined following the procedures of Antipa and Dolan (1985). Fenchel (1965) reported the co-occurrence of *P. mytili* and *Ancistrum* within *M. edulis*. On the basis of in vitro behavior, he concluded that the two species occupied different areas in the mantle cavity and occupied distinct ecological niches (Fenchel 1965). We have observed the co-occurrence of *M. pacificae* and *Ancistrum* within *M. californianus*. For both mussel species, we tested the hypothesis that infections of resident ciliate species are independent events: that is, the presence of *Ancistrum* does not influence the presence of *P. mytili* in *M. edulis* nor *M. pacificae* in *M. californianus*. For both mussel species, data from several sites sampled in April 1980 were pooled prior to calculating infection frequencies.

The test for independence of co-occurrence consisted of comparing measured frequencies of mono, dual, and zero infection with frequencies calculated using only individual species infection rates. Thus, if species A is found to occur with "*X*" frequency and species B with "*Y*" frequency, the predictions possible are: (1) for dual infection = (*XY*); (2) for zero infection = [(1 − *X*)(1 − *Y*)]; (3) for

mono species infection = 1 − {(XY) + [(1 − X)(1 − Y)]}. The calculated values assume no interaction between the ciliate species within a host mussel.

## RESULTS

### Phylogenetic analyses and taxonomic status of Mytilophilidae

The phylogenetic analyses based on the SSUrRNA gene demonstrate that *P. mytili* and *M. pacificae* are very closely related sister species (genetic difference < 0.1%) that form a terminal branch on the radiation of *Pleuronema* species (Fig. 3). The SSUrRNA gene sequences only differ in five positions among all *M. pacificae* and *P. mytili* samples, none of those differences are species specific (Table S2). All European and North American isolates are almost identical (genetic divergences 0.0–0.1%). However, the SSUrRNA sequence of the Chinese isolate of "*M. pacificae*" differs in three nucleotide positions (genetic divergence 0.18–0.24%); Z. Zhan (pers. commun.) confirms that this should properly be referred to as an "unidentified peniculistomatid," which was isolated from *Mytilus coruscus* (Fig. 3). The ITS region was sequenced for nine *M. pacificae* and eight *P. mytili* samples, and shows five differences—three of which are distinct between the two species (Table S2). While we do not have any *cox1* sequences for *Pleuronema* species, *P. mytili* and *M. pacificae* show significant divergence (genetic difference 21–22%) (Fig. 4). The *Mytilophilus* isolates cluster in two well-supported groups that are 0.89% divergent. The two more northern isolates (i.e., Depoe Bay and French Beach) possess a nine nucleotide long insert region (Table S2). *Peniculistoma* isolates show only minor variation in the *cox1* gene sequence, with three nucleotide differences among all 11 isolates (0.0–0.18% divergence). Divergence values of *P. mytili* samples collected from the same or from different bivalves do not differ significantly from each other (0.0–0.43%). Divergence values of *M. pacificae* samples collected from the same or from different bivalves also do not differ significantly from each other (0.0–0.29%).

In our constrained analyses, we tested the monophyly and branching patterns of the Histiobalantiidae, Pleuronematidae, and Peniculistomatidae. The constrained analyses using the SSUrRNA dataset and AU tests based on the TIM2+I+G model provided log-likelihood and *p*-values for the AU tests as follows: (1) −16094.710 for monophyly of the genus *Pleuronema*, $p < 0.001$; (2) −16212.558 for monophyly of the family Pleuronematidae, $p < 0.001$; (3) −15261.130 for the species *M. pacificae* and *P. mytili* each forming a monophyletic taxon, $p < 0.003$; and (4) −15239.521 for monophyly of the genus *Histiobalantium*, $p = 0.053$; (5) Since monophyly of the genus *Pleuronema* was rejected, all tested branching patterns of Pleuronematidae + Peniculistomatidae + Histiobalantiidae were also rejected (−16393.332 to −16467.409; $p < 0.001$). All values are in reference to the best tree with log-likelihood −15233.917.

*Sulcigera comosa* falls within the histiobalantiid clade, and is sister to *Histiobalantium minor* based on the SSUrRNA gene (Fig. 3). As the *cox1* sequence for *S. comosa* is the only one for histiobalantiids, we can only observe that *S. comosa* is very distantly related to *P. mytili* and *M. pacificae* (Fig. 4).

### Ecological niche analyses

Our analyses of predictions based on infection frequencies of single ciliate species *P. mytili* and *Ancistrum* spp. in *M. edulis* (Table 1) and of *M. pacificae* and *Ancistrum* spp. in *M. californianus* (Table 2) showed that the frequency of co-habitation does not differ significantly from a prediction based on the occurrence of each species alone. These findings support the conclusion of Fenchel (1965) that *Ancistrum* and *P. mytili* do not interact and extend the pattern to *Ancistrum* and *M. pacificae*.

## DISCUSSION

### Phylogeny and taxonomy

Our objectives were to test the following: (1) status of the Mytilophilidae; (2) genetic distinctiveness of *Peniculistoma* from *Mytilophilus*; and (3) genetic distinctiveness of *Sulcigera*.

Similarities in gross morphology, habitat, and stomatogenesis lead to the assignment of these two species to the same family (Antipa and Dolan 1985; Dolan and Antipa 1985). We add here our observations on similarities in conjugation of these two genera. Kidder (1933) observed that conjugating *P. mytili* formed a cytoplasmic bridge between the anterior end of the dorsal surface of one cell and the anterior end of the ventral surface of the partner cell and that the cell joined by its ventral surface was consistently the larger of the two cells. Our measurements of conjugating *M. pacificae* indicate that it conjugates in a similar fashion: the cells joined by their dorsal surface measured 119 ± 6.3 μm ($n = 38$) while those joined by their ventral surface measured 123 ± 9.0 μm ($n = 38$) (GAA and JRD, unpubl. observ.).

Our phylogenetic analyses based both on sequences of the SSU rRNA and *cox1* genes clearly support the monophyly of the Peniculistomatidae, and show *Peniculistoma* and *Mytilophilus* as sister taxa, differentiated from other pleuronematid species. The AU tests further support (−15233.917, $p = 0.975$) their monophyly and therefore the taxonomic recognition of the family Peniculistomatidae. The genetic difference in the SSUrRNA gene sequences of these two genera do not support the genetic distinctness of the family Mytilophilidae Jankowski 2007, and it is our opinion (see below) that other features of these two genera are also not distinct enough to separate them at the family level. While our AU tests refuted the monophyly of the genus *Pleuronema* and therefore the family Pleuronematidae, these are results based only on the SSUrRNA gene. We prefer to retain the family Pleuronematidae as morphologically conceived until a

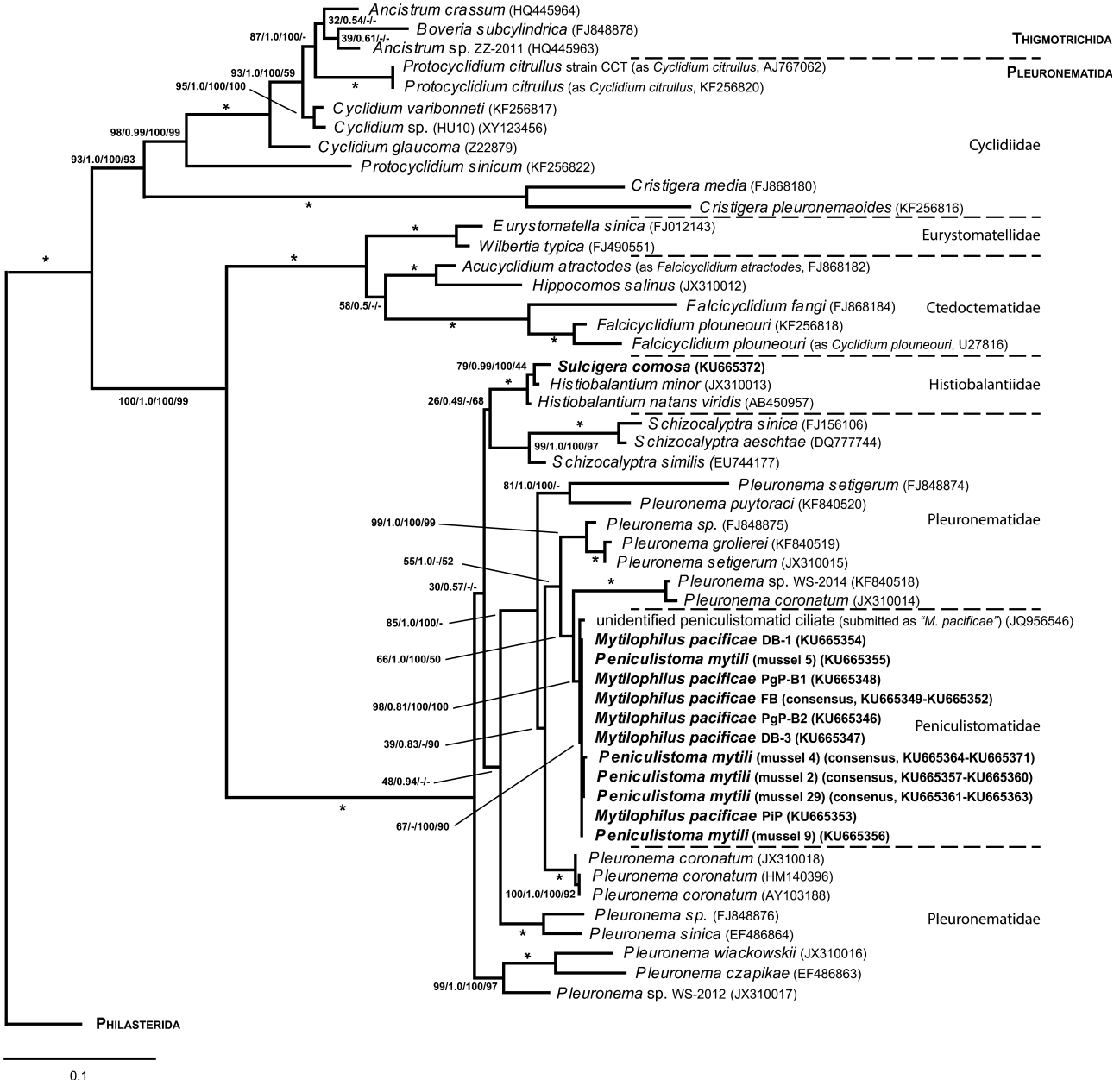


**Figure 3** Small subunit (SSU) rRNA phylogenetic reconstruction of phylogeny of peniculistomatids computed with RAxML (Stamatakis et al. 2008), based on the General-Time-Reversible model (GTR) with gamma distribution and an estimate of invariable sites. Note that the Chinese isolate of "*Mytilophilus pacificae*" should be considered an "unidentified peniculistomatid" (Z. Zhan, pers. commun.). The first number at the nodes represents the bootstrap support for RAxML (ML); the second number represents posterior probability values of the Bayesian Inference analysis (BI); and the third and fourth numbers represent bootstrap values for maximum parsimony (MP) and neighbor joining (NJ), respectively. Asterisks indicate full support in all analyses; dashes indicate support values below 30%. The scale bar represents 10 substitutions per 100 nucleotides.

larger genetic database is able to resolve the genetic diversification of pleuronematids.

The genus *Sulcigera*, with type species *S. comosa* Gajewskaja 1928, was proposed by Gajewskaja (1928) for a planktonic ciliate found in Lake Baikal. Obolkina (1995) redescribed this species with silver impregnation, but did not provide sufficient details on the oral structures. Foissner et al. (2009) argued that *Sulcigera* should be recognized as a junior synonym of *Histiobalantium* Stokes, 1886, based on morphological features. Our genetic characterization of *S. comosa* supports this decision. However, *S. comosa* is morphologically distinct enough from other *Histiobalantium* species to be considered a separate species: it typically has more somatic kineties, is larger in

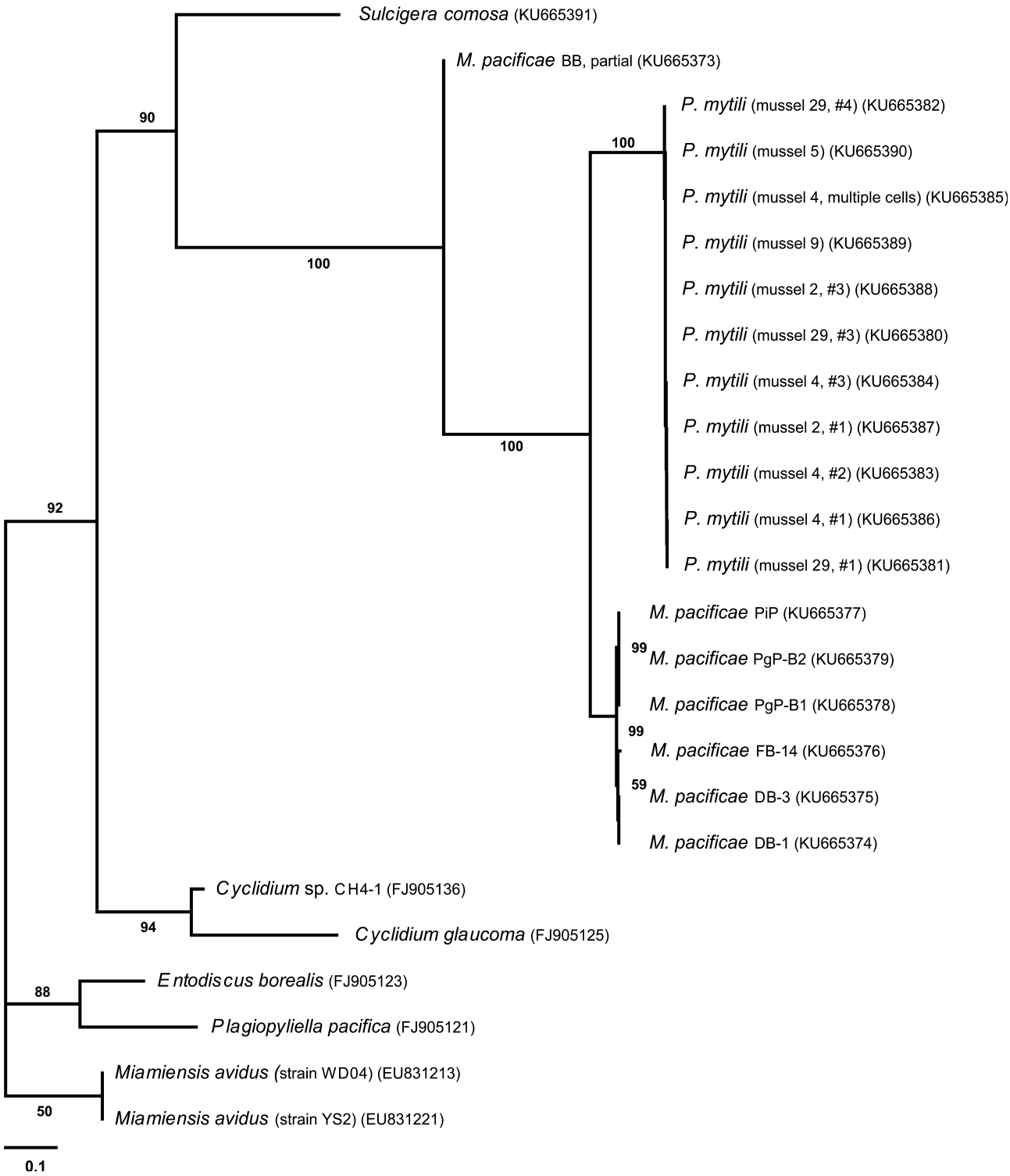


**Figure 4** Phylogenetic reconstruction of oligohymenophorean ciliates inferred using the cytochrome *c* oxidase subunit 1 (*cox1*) barcode, computed with Neighbor Joining (Saitou and Nei 1987), based on the F84 model (Felsenstein and Churchill 1996; Kishino and Hasegawa 1989). The numbers at the nodes represent the bootstrap support out of 1,000 replicates. The scale bar represents 10 substitutions per 100 nucleotides.

size, has only one contractile vacuole, and does not have tactile bristles, compared to *Histiobalantium bodamicum* (LAO, unpubl. observ.). As it is also genetically distinct from *Histiobalantium natans viridis* and *Histiobalantium minor*, we propose the new combination *Histiobalantium* (*Sulcigera*) *comosa* n. comb.

**Table 1.** The measured and predicted frequencies of *Peniculistoma mytili* and *Ancistrum* sp. in *Mytilus edulis* in California. Numbers of host mussels given with frequencies in parentheses

| Occurrence | Measured | Predicted | Difference | Dif$^2$/ Pred.[a] |
|---|---|---|---|---|
| *Ancistrum* present | 43 (0.8269) | | | |
| *P. mytili* present | 11 (0.2115) | | | |
| Both present | 10 (0.1923) | 9.09 | 0.91 | 0.091 |
| One present | 34 (0.6538) | 35.81 | 1.81 | 0.092 |
| Neither present | 8 (0.1538) | 7.09 | 0.91 | 0.117 |
| Total | 52 | 52 | | |

[a]For 2 degrees of freedom $X^2 = 0.189$, $p > 0.05$.

**Table 2.** The measured and predicted frequencies *Mytilophilus pacificae* and *Ancistrum* spp. in *Mytilus californianus* in California. Numbers of host mussels given with frequencies in parentheses

| Occurrence | Measured | Predicted | Difference | Dif$^2$/ Pred.[a] |
|---|---|---|---|---|
| *Ancistrum* present | 28 (0.4179) | | | |
| *M. pacificae* present | 36 (0.5373) | | | |
| Both present | 17 (0.2537) | 15.04 | 1.95 | 0.253 |
| One present | 30 (0.4478) | 33.92 | 3.92 | 0.453 |
| Neither present | 20 (0.2985) | 18.04 | 1.96 | 0.218 |
| Total | 67 | 67 | | |

[a]For 2 degrees of freedom $X^2 = 0.554$, $p > 0.05$.

## Ancestor-descendant relationship between *Peniculistoma* and *Mytilophilus*

Our final objective was to provide an argument to support the hypothesis that *Mytilophilus* is likely a species descended from an ancestor species, related to *Peniculistoma*, which colonized mytiloid bivalves.

Both species appear to be ecologically similar. Fenchel (1965) concluded that *P. mytili* did not appear to compete with *Ancistrum* spp. within the mantle cavity of *M. edulis*. On the basis of settling behavior when presented with a choice of tissues in vitro, he believed that *Ancistrum* and *Peniculistoma* likely occupied different areas in the mantle cavity with *Ancistrum* on the gills and *Peniculistoma* around the foot. Our analyses of predictions based on the occurrence/co-occurrence of these species, as one measure of ecological niche, confirmed Fenchel's observation of independence and extend it also to *M. pacificae*: there is also no evidence of interaction of *M. pacificae* with *Ancistrum* spp.

The mussels *M. californianus* and *M. edulis*, hosts of *M. pacificae* and *P. mytili*, respectively, are members of the *Mytilus* complex of species. In general, *M. californianus* is only found along the Northeast Pacific coast and is restricted to hard substrates subjected to surf. Conversely, *M. edulis* is found in bays and estuaries of various salinities in temperate regions around the world (Ricketts et al. 1969). However, juveniles as well as adults of the two species can exist microsympatrically (Harger 1967; Petraitis 1978). The genus *Mytilus* likely originated in the Northern Pacific (Durham and MacNeil 1967). *Mytilus edulis* first appears in Miocene deposits with a primary range of the Northeast Pacific coast from Alaska to Southern California (Grant and Gale (1931) in Dodd 1965); *M. californianus* is particularly common in Pleistocene deposits with a primary range of Northern California to Baja California (Ellen Goodman, United States Geological Survey (USGS), pers. commun.; Grant and Gale (1931) in Dodd 1965). Based on several mitochondrial genes, Ort and Pogson (2007) estimated that *M. californianus* diverged from the *M. edulis* species complex about 7.6 million years ago. Clearly, *M. edulis* represents the ancestral stock, appearing earlier in the fossil record and having a very broad geographic distribution, while *M. californianus* is a descendant species with a more restricted ecological distribution. This provides support for the notion that *M. pacificae* derived from *P. mytili*-like ancestors, which possibly diverged as their host mussels diverged.

As the hosts diverged, both genetically and ecologically, into separate habitats, it is likely that their peniculistomatid symbionts did also. This genetic differentiation has been accompanied by morphological divergence of the two genera. Corliss (1968) argued that ontogenetic features could be useful in reconstructing protozoan phylogenies. We argue below that features of peniculistomatid stomatogenesis suggest that the stomatogenesis of *M. pacificae* is derived from that of *P. mytili*.

During stomatogenesis, *P. mytili* and *M. pacificae* exhibit two stages that may be used as "markers" to correlate the developmental process in the two species. The first "marker" is the development of an array of three oral primordia, longitudinally oriented and of increasing length from right to left, a phenomenon that appears to be unique to these two species (Fig. 2b, 3d in Dolan and Antipa 1985). The second "marker" is the partitioning of basal bodies from a single primordium to form the bipartite membranelle 2 (M2) or oral polykinetid 2 (OPk2) (Fig. 3d, 6c in Dolan and Antipa 1985).

Using these two "markers" of stomatogenic development along with the more objective determinations of onset and termination of stomatogenesis, *M. pacificae* appears to show a more complex development as the membranellar primordia undergo additional or more complex developmental stages relative to *P. mytili*. In *M. pacificae*, there is greater disparity between the array of membranellar primordia and the final trophic buccal structure (Fig. 3e, 4 in Dolan and Antipa 1985), much more so than in *P. mytili* (Fig. 2c, 1 in Dolan and Antipa 1985).

The undulating membrane or paroral develops in a similar way in both species with the appearance of a narrow band of basal bodies roughly parallel to the posterior curve of the developing undulating membrane (Fig. 2d, 5d in Dolan and Antipa 1985). In both stomatogenic sequences, the band of basal bodies appears just after formation of OPk2. However, the band of basal bodies appears in *P. mytili* just before completion of buccal development

(Fig. 3d in Dolan and Antipa 1985), while the corresponding stage appears in *M. pacificae* well before stomatogenesis is complete (Fig. 6a in Dolan and Antipa). These differences in stomatogenic pattern suggest a more complex development of both membranellar and undulating membrane primordia in *M. pacificae* and allow us to tentatively identify *M. pacificae* as the relatively derived form. We can only speculate as to what selective advantage the distinctly different mouth structure of *M. pacificae* might have relative to that of *P. mytili*. However, there are likely differences in the environments of the two ciliate species as the host mussels differ with regard to gill size and pumping rate, both of which are greater in *M. edulis* compared to *M. californianus*, the host of *M. pacificae* (Fankboner et al. 1978).

In conclusion, similarities in morphology, stomatogenesis, ecological niche, sexual behavior, and gene sequences support the family Peniculistomatidae as a divergent assemblage within the Pleuronematida. Moreover, differences in stomatogenesis and in the evolutionary history of their host mussels support the hypothesis that *M. pacificae* is derived from a *P. mytili*-like ancestor likely following the speciation of the *Mytilus* host mussels.

## TAXONOMIC SUMMARY

Class Oligohymenophorea de Puytorac et al., 1974
Subclass Scuticociliatia Small 1967
Order Pleuronematida Fauré-Fremiet in Corliss, 1956
Family Histiobalantidiidae de Puytorac and Corliss in Corliss, 1979
Genus *Histiobalantium* Stokes, 1886
*Histiobalantium (Sulcigera* Gajewskaja 1928*) comosa* n. comb.

**Diagnosis.** Species, **without** tactile bristle cilia, polymorphic, with three morphs ranging in body length from 45 to 115 μm.

**Description.** Freshwater histiobalantiid ciliate with broadly oval body, dorsal side hemispherical, ventral side flat; with three morphs, ranging in body length from 45 to 115 μm and body width 33–90 μm; macronucleus, reniform, 25–30 μm long by 10–12 μm wide; micronuclei, numbering 4–5, 2.5–3.0 μm in diameter; 44–116 somatic kineties; somatic cilia 10–13 μm long; tactile bristle cilia, absent; extrusomes, spindle-shaped; one contractile vacuole, in posterior right; oral region occupying almost entire ventral surface; histiobalantiid paroral with cilia ~ 20 μm long; oral polykinetid (OPk) 1 and OPk 2 parallel to each other, oblique to the main body axis, in the anterior of the oral region; OPk 3 in the middle of the oral region, almost parallel to OPk 1 and OPk 2; oral cilia, 24–55 μm long.

**Ecology.** Stenothermic, cold-water planktonic species, appearing in early Spring, under the ice, preferring depths 0–50 m and temperatures 0–4 °C; disappearing in the end of June to first half of July as Spring plankton bloom sinks; swims rapidly, rotating clockwise around the main body axis, remains motionless and resumes swimming without jumping; feeds on phytoplankton, dinoflagellates, and perhaps bacteria.

**Type locality.** Lake Baikal, Russia (53°36′N, 108°7′E)

**Gene sequence.** The small subunit rRNA gene sequence is deposited as Gen Bank Accession No. KU665372.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Phase micrograph of *Sulcigera comosa* collected from Lake Baikal.
**Table S1.** Sampling.
**Table S2.** Bp differences.