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# Small-Subunit rRNA Phylogenies Suggest That *Epalxella antiquorum* (Penard, 1922) Corliss, 1960 (Ciliophora, Odontostomatida) Is a Member of the Plagyopylea

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ABSTRACT. The odontostomatid ciliates have remained a homogeneous order of ciliates since the 1930s when they were recognized as a monophyletic assemblage. Since that time they have been placed with the heterotrich ciliates, and more recently transferred as incertae sedis to the new ''riboclass'' class Armophorea. We were able to obtain the small subunit rRNA gene sequence of the odontostomatid *Epalxella antiquorum* (Penard, 1922) Corliss, 1960, collected from the meromictic alpine Lake Alat in Germany, in July 2005. An alignment with representatives of all 11 classes of ciliates unambiguously places the *Epalxella* sequence with other representatives of the class Plagiopylea with 100% support in both maximum likelihood and Bayesian analyses. *Epalxella* is the basal lineage with trimyemid and plagiopylid ciliates forming the two terminal sister clades. While this molecular support is strong and unambiguous, there are no obvious morphological features to unite these three clades. Thus, the class Plagiopylea must continue to be referred to as a ''riboclass.'' Using the *Epalxella* sequence as a basal marker, we tentatively identified 20 environmental sequences to the terminal plagiopylean clades: eight to the genus *Trimyema*; four to the genus *Plagiopyla*; and eight to two new species, one of which might represent a new plagiopylean genus.

**Key Words.** Anoxic habitats, Armophorea, Bayesian analysis, ciliates, ciliated protozoa, environmental isolates, *Epalxella*, Epalxellidae, Lake Alat, Plagiopylea, SSrRNA.

OVER 20 years ago, the first sequences of the small subunit rRNA (SSrRNA) genes of ciliates were published by Sogin and Elwood (1986). Since that time, hundreds of SSrRNA gene sequences of ciliate isolates have been deposited in GenBank. These data have permitted the recognition of 11 major clades or classes of ciliates, a number of which were distinguished already based on ultrastructural features and morphogenetic patterns (Lynn 2004). Furthermore, these gene sequences have enabled the placement of a number of problematic genera and higher categories. For example, *Phacodinium* was confirmed as a spirotrich (Shin et al. 2000); metopids and clevelandellids were recognized as a major clade (Affa'a et al. 2004; Van Hoek et al. 1998); an astome was confirmed as an oligohymenophorean (Affa'a et al. 2004); *Licnophora* was shown to be a spirotrich and not a heterotrich (Lynn and Strüder-Kypke 2002); and plagiopylean ciliates were recognized as a new ''riboclass'' of ciliates (Embley et al. 1995; Lynn 2004; Lynn and Small 2002). Of the 17 subclasses and 57 orders recognized by Lynn and Small (1997), there is only one subclass, the Rhynchodia, and one order, the Odontostomatida, for which molecular data would provide substantial support for their current placement.

The odontostomatids were first described in the 19th century, but it was not until the early part of the 20th century that the group was formally recognized as a monophyletic assemblage of three families, placed as a suborder in the order Heterotrichida (Kahl 1932). Over 30 years would pass before another authoritative treatment of the group. Jankowski (1964) provided redescriptions of a number of odontostomatid genera and argued that odontostomatids evolved from a *Metopus*-like ancestor by reducing their body size and numbers of oral polykinetids as they adapted to the anaerobic environments in which they are found. Schrenk and Bardele (1991) provided the first ultrastructural description of an odontostomatid with their detailed study of *Saprodinium dentatum*. While they could show that *Saprodinium* had somatic dikinetids, these dikinetids were clearly not similar to those of the heterotrichs or armophoreans. The oral structures of *Saprodinium* included a series of nine or 10 oral polykinetids, each composed of three rows of kinetosomes, very much resembling the membranelles of other ciliates with adoral zones, but they were definitely not like the heteromembranelles of the clevelandellid armophoreans. Schrenk and Bardele (1991) concluded their discussion by suggesting that a determination of the SSrRNA gene sequence of odontostomatids would be helpful in illucidating their phylogenetic position. Thus, we report here on the SSrRNA gene sequence of *Epalxella antiquorum*, tentatively conclude that the odontostomatids are members of the class Plagipoylea, and tentatively identify a number of plagiopylean environmental clones to genus and species.

## MATERIALS AND METHODS

**Isolation and culturing.** *Epalxella antiquorum* (Penard, 1922) Corliss, 1960 was isolated from a meromictic alpine lake (Alat, Germany) in July 2005. The lake is 35 m deep and has a surface area of 18 ha, and is characterized by a permanent chemocline at 15–18 m (Fig. 1). Below the chemocline, the lake has been permanently anoxic and sulfidic for most likely several thousands of years (Brümmer, pers. commun.). At the chemocline, purple sulfur bacteria form a 1-m thick, reddish layer in the water column. As this layer may at times extend to the lake's surface, Lake Alat is also called the ''bleeding lake.'' Scuba divers collected undisturbed samples of microbial mats from the bottom of the lake below the chemocline using special sampling devices which could be sealed under water and returned by the divers to the surface. The samples were kept for several hours at ambient bottom water temperature (6.1 °C) until isolation of the organism in the laboratory. Cells were studied in vivo using an oil immersion objective and differential interference contrast optics. The ciliary and nuclear patterns were revealed by protargol and silver nitrate impregnation (Foissner 1991), using raw cultures grown on Lake Alat water enriched with some squashed wheat grains.

**DNA extraction, PCR amplification, and sequencing.** For analysis of SSrDNA sequences, 10–20 individual cells were picked into 180 µl of ATL buffer (Animal Tissue Lysis buffer, a component of Qiagen's DNEasy Tissue Kit, Qiagen, Hildesheim, Germany) and 20 µl Proteinase K (20 mg/ml). Genomic DNA was extracted using the protocol for cultured animal cells of the

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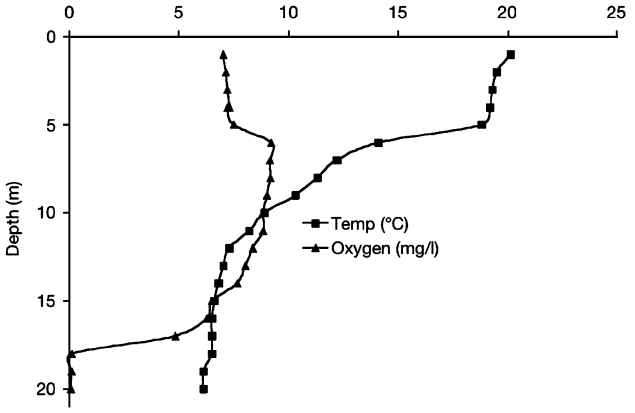


Fig. **1.** Oxygen and temperature profiles in Lake Alat at the time of sampling.

DNEasy Tissue Kit (Qiagen) according to the manufacturer's instructions. Amplification of the SSrRNA gene was performed via PCR using the universal eukaryotic primers EukA and EukB (Medlin et al. 1988), amplifying nearly the full-length gene. Each PCR reaction contained 10–20 ng of DNA template, 2.5 U HotStar Taq DNA polymerase (Qiagen) in the manufacturer-provided reaction buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 0.5 μM of each oligonucleotide primer. The final volume was adjusted to 50 μl with sterile distilled water. The PCR protocol for SSrRNA gene amplification consisted of an initial hot start incubation of 15 min at 95 °C followed by 30 identical amplification cycles (i.e. denaturating at 95 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 2.5 min), and a final extension at 72 °C for 7 min. Negative control reactions included *Escherichia coli* DNA as a template. The resulting PCR products were cleaned with the PCR MinElute Kit (Qiagen) and cloned into a vector using the TA-Cloning kit (Invitrogen, Carlsbad, CA). Plasmids were isolated with Qiaprep Spin Miniprep Kit (Qiagen) from overnight cultures and PCR-reamplified using M13F and M13R primers to screen for inserts of the expected size (ca 1.8 kb in case of the SSrDNA fragment).

The *E. antiquorum* SSrRNA gene was sequenced bidirectionally from random positively screened plasmids by MWG Biotech (Ebersberg, Germany) using an Applied Biosystems (ABI, Foster City, CA) 3730 DNA Stretch Sequencer, with the XL Upgrade and the ABI Prism BigDye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit. The sequence was assembled using CodonCode Aligner (CodonCode Corp., Dedham, MA).

**Phylogenetic analyses of *Epalxella antiquorum*.** The *E. antiquorum* SSrDNA sequence was aligned to 45 representative ciliate sequences from GenBank, representing the 11 described ciliate classes recognized to date (Lynn 2004) using CLUSTAL-X (Thompson et al. 1997). The alignment was refined manually in MacClade (Maddison and Maddison 2003) using phylogenetically conserved regions. Subsequently, 1,132 conserved and unambiguously aligned positions were used in phylogenetic analyses. The program Modeltest (Posada and Crandall 1998) was applied to choose the model of DNA substitution that best fit our data set from among 56 possible models. We calculated both an evolutionary distance tree using the PAUP software package 4.0b10 PAUP (Swofford 2002) and a Bayesian inference tree using Mr. Bayes (Ronquist and Huelsenbeck 2003).

The evolutionary distance tree was constructed under maximum-likelihood (ML) criteria by using a GTR+I+G DNA substitution model with the variable-site γ distribution shape parameter at 0.6758, the proportion of invariable sites at 0.3447, and base frequencies and a rate matrix for the substitution model as suggested by Modeltest. We assessed the relative stability of tree topologies using 1,000 bootstrap replicates. Heuristic searches for bootstrap analyses employed stepwise addition, starting trees with simple addition of sequences and followed by TBR branch swapping. Settings for bootstrap analyses were chosen according to the Modeltest output as described above.

For the Bayesian tree we ran two simultaneous, completely independent analyses starting from different random trees. The analysis also employed GTR+I+G as the DNA substitution model with the γ distribution shape parameter, the proportion of invariable sites, base frequencies and a rate matrix for the substitution model as assessed by Mr. Bayes. Metropolis coupling with three heated chains and one ''cold'' chain was employed to improve the Markov Chain Monte Carlo sampling of the target distribution. We ran 1,000,000 generations and sampled every 1,000th generation, resulting in 1,001 samples from the posterior probability distribution. All data are available from T. Stoeck upon request.

**Phylogenetic analyses of plagiopylean environmental isolates.** Since the SSrRNA gene sequence of *E. antiquorum* provided a basal branch in the class Plagiopylea (see ''Results''), we made a phylogenetic analysis of all published environmental sequences with a plagiopylean affinity (except for AY835674 which was too short to be included in the analyses). Phylogenetic analyses included both an evolutionary distance analysis under ML criteria with the substitution model as suggested by Modeltest (TrN+I+G with the proportion of invariable sites = 0.44451, and the γ distribution shape parameter = 0.6300) and with 1,000 bootstraps, and a Bayesian inference analysis with 1,000,000 generations as described above.

## RESULTS

For a morphological characterization of *E. antiquorum* see Pénard (1922) and Foissner, Berger, and Kohmann (1992).

**Primary structure of *Epalxella* SSrRNA gene sequence.** The length, GC content, and GenBank Accession number of the PCR-amplified SSrRNA gene of *E. antiquorum* are as follows: 1,764 nucleotides; GC = 42%; and Accession no. EF014286.

**Phylogenetic position of *Epalxella*.** Both phylogenetic analyses provided unambiguous support for placing this odontostomatid with other species in the class Plagiopylea. Both bootstrap support in the ML analysis and the posterior probability for the Bayesian analysis were 100 (Fig. 2). Thus, there can be no doubt that this genus of odontostomatids, at least, is strongly related to the plagiopylean ciliates. This is supported by an analysis of Plagiopylea class-specific sequence signatures (Table 1). In our

Table **1.** Specific 18S rDNA sequence signatures that are exclusive to the class Plagiopylea and to *Epalxella antiquorum* compared to all other ciliate classes (ciliate outgroup).

| Nucleotide position | Nucleotide in class Plagiopylea | Nucleotide in *E. antiquorum* | Nucleotide in ciliate outgroup[c] | Secondary structure helix |
|---|---|---|---|---|
| 50[a]/630[b] | T | T | A | 23_1 (paired) |
| 402[a]/941[b] | A | A | T | 24 (paired) |
| 562[a]/1096[b] | A | A | T | 31 (paired) |

We only considered positions 580–1316 of the reference sequence (*E. antiquorum*).

[a]Position in conserved region of our alignment.

[b]Position in *E. antiquorum* sequence as reference (Genbank Accession No. EF014286).

[c]Based on at least three representative sequences of each class.

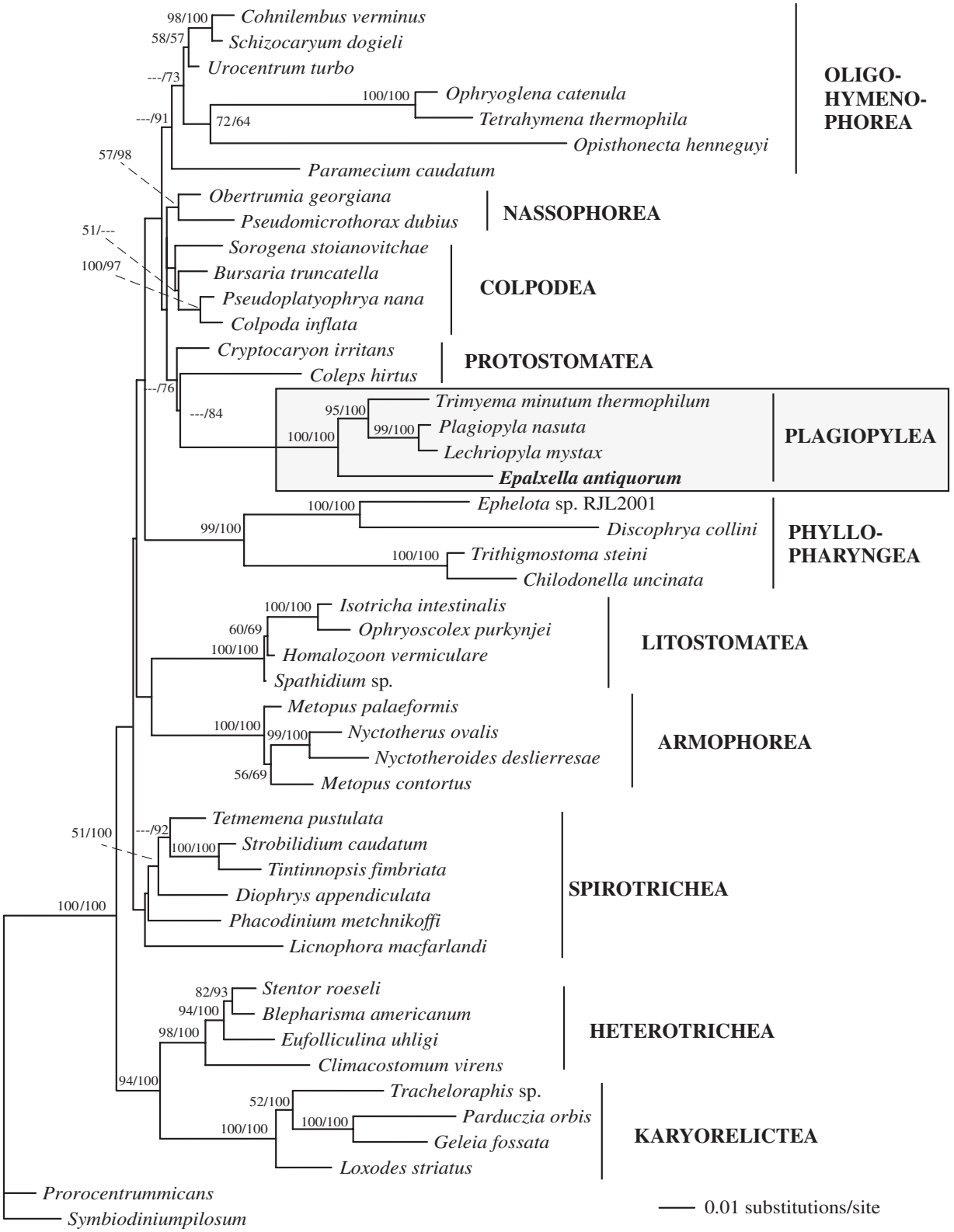


Fig. **2.** Phylogenetic tree based on small subunit rRNA gene sequences demonstrating the position of the odontostomatid *Epalxella antiquorum* (bold letters) among the 11 classes of ciliated protozoa. This evolutionary distance tree demonstrates that the trimyemid *Trimyema*, the plagiopylids *Plagiopyla* and *Lechriopyla*, and the odontostomatid *Epalxella* (gray shaded area) share a relatively long branch that is supported at 100% (first number at node) based on 1,000 bootstraps and at a level of 100 posterior probability based on 1,000,000 generations and 1,001 sampled trees (second number at node) in a Bayesian analysis.

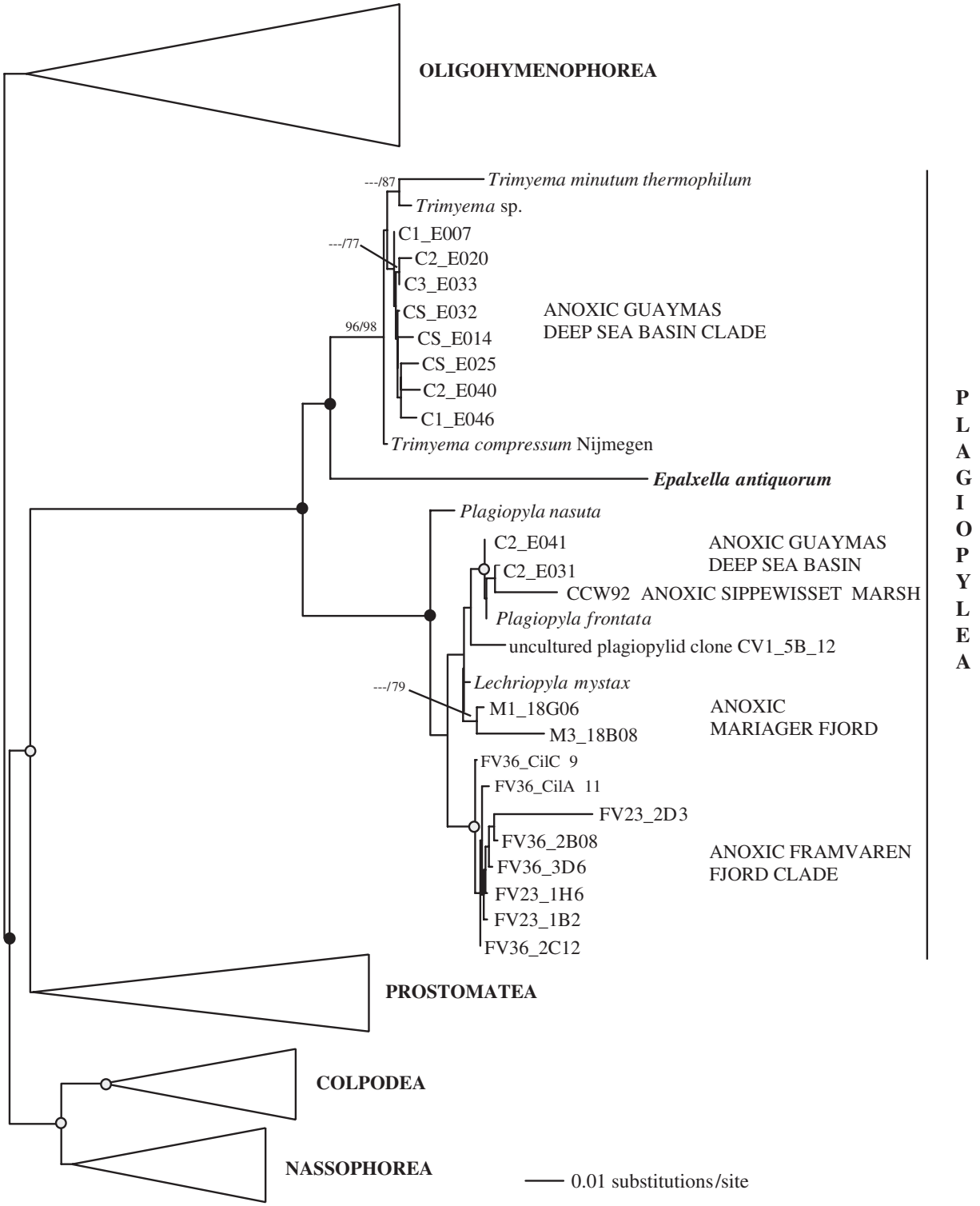


Fig. **3.** Phylogenetic tree based on small subunit rRNA gene sequences of all named plagiopylean sequences from GenBank and including cloned environmental isolates from several anoxic environments (Table 2). Bootstraps and posterior probabilities $\geq$ 75% are given at the respective nodes. In cases where both analyses resulted in a 100% support the node is a black dot while in cases where at least one of the analyses gave a 100% support, the dot is gray.

unambiguous alignment based on conserved secondary structures (positions 580–1,316 of the *E. antiquorum* sequence) we detected three specific nucleotide positions that are exclusively specific to all available plagiopylean sequences (including environmental sequences) and that do not occur in the ciliate outgroup. At these positions *E. antiquorum* displays the same nucleotide as all other plagiopylean ciliates. Sequence similarities between *E. antiquorum* and its closest named plagiopylean relatives were as follows: 88.05%—*Lechriopyla mystax*; 87.76%—*Plagiopyla frontata*; 88.30%—*Trimyema* spp.

**Taxonomic identity of plagiopylean environmental isolates.** Because *E. antiquorum* represents a basal lineage in the class Plagiopylea, we undertook to identify a number of cloned environmental sequences of plagiopylean affinity (Table 2). The sequences from these five anoxic environments grouped into four clades, in all cases better supported by Bayesian analysis than by ML (Fig. 3). Eight clones from the anoxic Guaymas Basin form a clade with a number of *Trimyema* species (Table 2). Two clones from the anoxic Guaymas deep-sea basin clade and one clone from the anoxic Sippewisset salt marsh possibly represent isolates of *P. frontata* (Table 2). Six clones from the anoxic Framvaren Fjord probably represent a novel candidate plagiopylean genus (Table 2). Finally, two clones from the anoxic Mariager Fjord show close affinities to the sea urchin endosymbiont *L. mystax* (Table 2).

## DISCUSSION

**Phylogenetic position of *Epalxella*.** As noted in the introduction, the odontostomatids have been assigned to the class Heterotrichea and more recently to the class Armophorea. If we presume that the somatic kinetid of *Epalxella* is similar to that reported for the odontostomatid *Saprodinium* (Schrenk and Bardele 1991), the absence of strongly overlapping postciliodesmata now excludes odontostomatids from the class Heterotrichea as defined by Lynn and Small (1997, 2002). Association with the class

Table **2.** Tentative assignments of plagiopylean small subunit rRNA (SSrRNA) clones of environmental samples based on sequence similarity criteria to identified plagiopylean species in the SSrRNA database.

| Clone name (Accession No.) | Source of environmental samples | Closest named genus in the GenBank data base | Sequence similarity to closest named species in the GenBank data base (%) |
|---|---|---|---|
| C1_E007 (AY046611) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 96.12 (*T. compressum*) |
| C2_E020 (AY046810) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.09 (*T. compressum*) |
| CS_E023 (AY046656) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.36 (*T. compressum*) |
| CS_E025 (AY046651) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.53 (*T. compressum*) |
| CS_E040 (AY046825) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 96.04 (*T. compressum*) |
| CS_E014 (AY046644) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.27 (*T. compressum*) |
| C1_E046 (AY046640) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.44 (*T. compressum*) |
| C3_E033 (AY046861) | Guaymas Basin, Gulf of California, Mexico | *Trimyema* | 97.27 (*T. compressum*) |
| C2_E041 (AY046826) | Guaymas Basin, Gulf of California, Mexico | *Plagiopyla* | 99.05 (*P. frontata*) |
| C2_E031 (AY046821) | Guaymas Basin, Gulf of California, Mexico | *Plagiopyla* | 99.23 (*P. frontata*) |
| CCW92 (AY180039) | Sippewisset Salt Marsh, Cape Cod MA, USA | *Plagiopyla* | 98.07 (*P. frontata*) |
| CV15B12 (AY821931) | Unnamed pond, Paris, France | *Plagiopyla* | 96.88 (*P. frontata*) |
| FV36_CilC9 (DQ310292) | Framvaren Fjord, Norway | *Lechriopyla* | 96.86 (*L. mystax*) |
| FV36_CilA11 (DQ310288) | Framvaren Fjord, Norway | *Lechriopyla* | 96.61 (*L. mystax*) |
| M1_18G06 (DQ103835) | Mariager Fjord, Denmark | *Lechriopyla* | 97.40 (*L. mystax*) |
| M3_18B08 (DQ103876) | Mariager Fjord, Denmark | *Lechriopyla* | 97.31 (*L. mystax*) |
| FV23_2D3 (DQ310253) | Framvaren Fjord, Norway | Novel candidate genus I | 94.44 (*L. mystax*) |
| FV36_2B08 (DQ310234) | Framvaren Fjord, Norway | Novel candidate genus I | 94.05 (*L. mystax*) |
| FV23_1H6 (DQ310222) | Framvaren Fjord, Norway | Novel candidate genus I | 94.40 (*L. mystax*) |
| FV23_1B2 (DQ310215) | Framvaren Fjord, Norway | Novel candidate genus I | 93.45 (*P. frontata*) |
| FV36_3D6 (DQ310330) | Framvaren Fjord, Norway | Novel candidate genus I | 94.91 (*P. frontata*) |
| FV36_2C12 (DQ310208) | Framvaren Fjord, Norway | Novel candidate genus I | 94.42 (*L. mystax*) |

Armophorea has been suggested primarily on the basis of the hypothesis of Jankowski (1964) that odontostomatids evolved from an armophorid ancestor similar to *Metopus*. However, the somatic kinetids of both armophorean clades, the clevelandellids and armophorids, have conspicuous kinetodesmal fibrils (Foissner and Agatha 1999; Puytorac and Grain 1969), which the somatic kinetid of the odontostomatid *Saprodinium* lacks (Schrenk and Bardele 1991). Furthermore, armophoreans have been established as a class using only molecular phylogenetic analyses. Removal of the odontostomatids from the class on the basis of molecular phylogenetic analysis is thus consistent with its establishment in the first place.

Thus, our molecular phylogenetic analysis refutes assignment of the odontostomatids to either the heterotrichs or the armophoreans. We have carefully re-examined our alignment to determine if there might be sequence irregularities and artifacts in the SSrRNA of *Epalxella* and we can find none. Thus, we are confident that at least the odontostomatid *Epalxella* belongs to the plagiopylean clade. We note that the branching position of *Epalxella* within this clade is still uncertain as there are discrepancies between different phylogenetic analyses (Fig. 2, 3). It is a well-known fact that taxon sampling may have a significant influence on the branching position of taxa, specifically if the taxon sampling is low as in present case (Poe 1998). Thus, concerted efforts should be in order to increase taxon sampling within the Plagiopylea to enable a more detailed phylogeny within this class.

Our result is surprising for several reasons. The class Plagiopylea is already unusual in that it is one of two ''riboclasses'' in the phylum (the other one being the class Armophorea)—classes established only on the basis of very strong support derived from SSrRNA gene sequences (Lynn 2004). Thus, at the level of gross morphology, there are already few similarities among plagiopylid and trimyemid ciliates. The somatic ciliation of plagiopylids is holotrichous with a typically high density of monokinetids (Lynch 1930; Sola et al. 1988). On the other hand, the somatic ciliation of trimyemids forms a series of apparently spiralling rows, generated by the ''spiralled'' distribution of somatic monokinetids between adjacent somatic kineties (Augustin, Foissner, and Adam 1987; Baumgartner, Stetter, and Foissner 2002; Serrano, Martín-González, and Fernández-Galiano 1988). The odontostomatids provide yet another variation on this somatic ciliary pattern by having kineties, which are typically restricted to the anterior and posterior ends of the cells (Jankowski 1964; Tuffrau 1992), and which are probably composed of dikinetids (Schrenk and Bardele 1991).

There is also no unity among these three groups in relation to the ultrastructure of the somatic cortex. The kinetids of trimyemids and plagiopylids are quite similar, being monokinetids with a well-developed divergent postciliary ribbon, anteriorly directed kinetodesmal fibril, and a weakly developed, somewhat radial transverse ribbon (Berger and Lynn 1992; Detcheva, de Puytorac, and Groliere 1981). On the other hand, the somatic dikinetids of the odontostomatid *Saprodinium* have a divergent postciliary ribbon associated with the posterior kinetosome, a tangential transverse ribbon associated with the anterior kinetosome, and no kinetodesmal fibril (Schrenk and Bardele 1991).

This diversity of the somatic cortex is further complicated by diversity among these groups in relation to oral structures. The plagiopylids apparently have simple oral structures: essentially the somatic kineties extend into a funnel-shaped oral cavity or vestibulum (Berger and Lynn 1992; Lynch 1930; Sola et al. 1988). Trimyemids have a semicircle of kinetosomes in loose pairs bordering a shallow oral region, possibly enclosing some small adoral organelles (Baumgartner et al. 2002; Serrano et al. 1988). Odontostomatids may have a quite complex oral cavity that sometimes contains a dozen oral polykinetids or membranelles and possibly other oral ciliature. A paroral has been observed by Tuffrau (1992) in *Discomorphella*, while it has not been observed in other odontostomatid species (Jankowski 1964; Schrenk and Bardele 1991).

Another tentative support of the Plagiopylea and the assignment of *Epalxella* to this class may be found in the common life strategy of the taxa that are united in this riboclass. The genera *Epalxella*, *Trimyema*, *Plagiopyla*, and *Lechriopyla* clustering within this class (Fig. 2, 3) are characterized by an anaerobic life style and endosymbiotic methane bacteria (Fenchel and Finlay 1995). Possibly, this anaerobic mode of life is a ''physiological'' apomorphy evolving in the last common ancestor of this group supporting our molecular phylogenetic finding.

There can be no doubt about the robustness of the molecular signal provided by the SSrRNA gene sequence of *Epalxella*, which relates it strongly to the plagiopyleans (phylogeny and specific sequence signature). However, as the *E. antiquorum* sequence is the as yet only available odontostomatid ciliate sequence, we can only tentatively conclude that the odontostomatids as a group ought to be placed in the class Plagiopylea, and we await additional odontostomatid gene sequences to test this hypothesis.

**Assignment of plagiopylean environmental isolates.** With the discovery that *Epalxella* branches in the class Plagiopylea, we undertook to ''identify'' 20 cloned isolates from anoxic environments that had affinities to this class. As ciliate species are clearly defined by a set of morphological and ultrastructural characters, we do not want to attempt to assign generic species or genus names to environmental sequences. Yet, if we consider the close sequence similarities of eight environmental clones from the anoxic Guaymas deep-sea basin to different *Trimyema* species (Table 2) as well as the branching of these sequences inside a well-supported *Trimyema* clade (Fig. 3), it is reasonable to assume that the respective organisms may indeed be tentatively assigned to the genus *Trimyema*. The other two clones from the Guaymas deep-sea basin, one clone from the anoxic Sippewisset salt marsh, and one anoxic freshwater clone from France are closely related to *P. frontata* ($>96\%$ sequence similarity).

Four clones, two of which are from the anoxic Mariager Fjord and two from the anoxic Framvaren Fjord are similar to an SSrRNA sequence of *L. mystax* ($>96.5\%$ sequence similarity). However, this species has only ever been observed as an endocommensal in the intestinal tract of strongylocentrotid echinoids (Berger and Lynn 1992; Lynch 1930). While we do not know precisely how these endocommensal ciliates disperse from one sea urchin to the next, it seems highly probable that they would have a free-swimming stage to migrate between hosts. Could it be that these clonal isolates from the Mariager Fjord represent dispersing *L. mystax*? Recollection from this habitat with the aim of isolating living organisms will be necessary to test this hypothesis.

Finally, six clones from the anoxic Framvaren Fjord are all $<95\%$ similar to any other plagiopylean in the database. In addition to *Lechriopyla* and *Plagiopyla* (Lynn 2003), there are two other genera assigned to the Family Plagiopylidae, *Paraplagiopyla* and *Pseudoplagiopyla*, which have not yet been sequenced nor has any species in the Family Sonderiidae. The Framvaren Fjord clade could represent one of these described genera or may represent a new genus and species.

There are many more environmental clones of ciliates in GenBank that remain to be identified. Now that the sampling density of ciliate taxa has reached a significant level and all the major ''morphological'' lineages have sequence representation in the SSrRNA database, we can proceed with some confidence to assign the unidentified environmental clones to lineages within the phylum Ciliophora.

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