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Composition and temporal variability of picoeukaryote communities at a coastal site of the English Channel from 18S rDNA sequences

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Abstract

We analyzed picoeukaryote assemblages at a French coastal site of the English Channel by sequencing cloned eukaryotic 18S rRNA genes in eight genetic libraries constructed from environmental samples (seven coastal, one estuarine) collected at different periods of the year. Eight hundred clones were examined by amplified restriction fragment length polymorphism (RFLP) using the restriction enzyme *Hae*III. The coverage value calculated from the relative distribution of RFLP types was low, indicating that the library diversity was not entirely recovered. A variable region of the rRNA gene was partially sequenced (550 bases) and analyzed for 397 clones. Thirty-two clones were affiliated with metazoans. Of the remaining clones, 132 were affiliated to algal classes (especially Prasinophyceae, Cryptophyceae, Dinophyceae, and Prymnesiophyceae) and 107 to known heterotrophic groups (Cercozoa, choanoflagellates, stramenopiles, and ciliates). One hundred three sequences fell into uncultivated groups of stramenopiles (43 clones) and alveolates (60 clones). We also found two potentially novel eukaryotic lineages, represented by 9 and 14 clones, respectively, not belonging to any known eukaryotic group. The overall composition of the picoeukaryote community remained fairly stable at the class/division level except during the early summer diatom bloom, when groups such as the Cryptophyceae and the ciliates completely disappeared. However, at a finer taxonomic level (corresponding to 98% sequence identity), the majority of the operational taxonomic units (OTU) were only observed once.

Marine picoplankton (cells with diameter less than 3 μm), both prokaryotic and eukaryotic, contribute significantly to the biomass and primary productivity in all aquatic environments (Stockner 1988) and play an important role in global mineral cycles. These organisms are linked by complex interactions within the microbial food web. In coastal systems, even if the microphytoplankton (diatoms and dinoflagellates) proliferate during a short period of the year, the picophytoplankton can be responsible for more than half of the primary production (Joint and Pomroy 1986).

The introduction of molecular biological approaches into oceanography has widened our knowledge of picoplankton diversity. Molecular methods, such as sequencing rRNA genes directly from DNA obtained from environmental samples, was initially employed to assess prokaryotic diversity (Giovannoni et al. 1990). Recently the same molecular approach has provided spectacular insights into the diversity of picoeukaryotes, either by using the plastid 16S rRNA gene or the nuclear 18S rRNA gene, revealing the presence of unexpected picoeukaryote lineages in widely different ecosystems such as the Antarctic polar front or the equatorial Pacific Ocean (Rappé et al. 1997; López-García et al. 2001; Moon–van der Staay et al. 2001). However, picoeukaryote diversity has not yet been investigated in coastal systems where seasonal fluctuation is important. The aim of the present study was to examine the diversity of picoeukaryotes in coastal waters of the western English Channel as well as their temporal variation during a complete seasonal cycle.

Material and methods

Study site—Picoplankton were collected from surface well-mixed water at Astan (48°45′N; 4°00′W; coastal site) and Dourduff (48°38′N, 3°51′W; estuarine site) off Roscoff, Brittany, France (Table 1). Sampling at Astan was carried out seasonally from April to December 2000 and monthly from April to June 2001 (RAYYMMDD samples). Dourduff was sampled once in May 2001 (RD010517). Hydrological parameters and nutrients were measured by standard methods and are available from the Stations d'Observation du Milieu Littoral (SOMLIT) web site (http://www.obs-vlfr.fr/somlit/data.htm). Diatom concentrations were determined on lugol fixed samples using an inverted microscope by the Utermöhl technique. Picoplankton (*Synechococcus*, photosynthetic picoeukaryote, and bacteria) abundance was measured by flow cytometry according to Marie et al. (1999).

Sample collection and DNA extraction—Each water sample (2.5 liters) was prefiltered through a 3-μm pore size Nuclepore membrane (Whatman). Photosynthetic picoeukaryote abundances measured by flow cytometry before and after filtration were not significantly different (at the 0.05 level), suggesting that filtration did not induce any significant cell loss or cell breakage. The microbial biomass was collected on a 47-mm diameter membrane filter (Supor® 450) with

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Table 1. Temperature and concentrations of nitrates, diatoms (determined using the Utermöhl method), *Synechococcus*, photosynthetic picoeukaryotes, and bacteria (determined by flow cytometry) at the Astan and Dourduff stations. At the Dourduff station, no ancillary data were available for the date at which the library was constructed (17 May 2001) and therefore the data for the next sampling date are also shown.

Station	Date	Temperature (°C)	Nitrates (μmol L^{-1})	Chlorophyll *a* (μg L^{-1})	Diatoms (cell ml^{-1})	*Synechococcus* (cell ml^{-1})	Photosynthetic picoeukaryotes (cell ml^{-1})	Bacteria (cell ml^{-1})
Astan	12 Apr 2000	10.10	10.7	0.30		3272	10,836	449,598
	9 Jun 2000	12.95	5.0	0.42	3.28	2253	20,996	850,895
	7 Sep 2000	15.60	4.4	0.62	1.47	2206	4995	724,803
	19 Dec 2000	11.90	10.9	0.17	1.83	1914	1971	532,057
	12 Apr 2001				1.58	1387	10,014	722,548
	16 May 2001	12.15	6.0	0.53	2.60	832	14,167	573,533
	13 Jun 2001	13.90	0.5	2.50	32.66	2311	10,561	1,115,714
Dourduff	14 Jun 2001	15.50	1.3	1.57		1974	30,638	2,594,765

0.45-μm pore size. The filter was transferred into a cryovial tube containing a DNA lysis buffer (0.75 mol L^{-1} sucrose, 40 mmol L^{-1} ethylenediaminetetraacetic acid [EDTA], 50 mmol L^{-1} Tris-HCl, pH 8), immediately frozen in liquid nitrogen, and stored at −80°C until nucleic acids extraction. DNA was extracted using a 3% cetyltrimethylammonium bromide (CTAB) extraction procedure (Doyle and Doyle 1990). Extracts were purified with the Geneclean II Kit (BIO 101) and then stored at −80°C until used.

Clone library construction—The 18S rRNA gene was amplified by polymerase chain reaction (PCR) using the oligonucleotide primers Euk328f (5′-ACC TGG TTG ATC CTG CCA G-3′) and Euk329r (5′-TGA TCC TTC YGC AGG TTC AC-3′), complementary to regions of conserved sequences proximal to the 5′ and 3′ termini of the 18S rRNA gene and DNA *Taq* Polymerase (Promega) as described (Moon–van der Staay et al. 2000). The PCR reaction was cycled 34 times. Amplification products from four separate reaction mixtures containing the same template were pooled and purified with the Qiaquick PCR purification kit (Qiagen). The purified PCR products were cloned into the pCR 2.1 vector using the TOPO-TA cloning kit (Invitrogen) according to the manufacturer's protocol. A total of 800 positive clones (white colonies) from the eight genetic libraries were transferred to a multiwell plate containing Luria–Bertani medium (50 μg μl^{-1} ampicillin) and 7% glycerol and stored at −80°C.

Clone library screening by RFLP—Clone libraries were screened by restriction fragment length polymorphism (RFLP) as follows. One hundred positive clones from each library were screened by reamplifying the 18S rDNA by PCR as described above, except that 1 μl of culture of *Escherichia coli* containing the insert was used as a template. Ten microliters of PCR product were digested for 2 h at 37°C with 5 U of restriction endonuclease *Hae*III (Promega). Restriction fragments were separated by gel electrophoresis on 2.5% (wt/vol) Metaphor agarose (FMC Bioproducts) in 0.5× Tris-acetate EDTA (TAE) buffer. The gel was stained with ethidium bromide (0.5 μg ml^{-1}) and visualized with UV excitation. Restriction patterns were compared by using the software Fragment Analysis (Amersham BioSciences) and clone diversity analyzed by rarefaction curves (Singleton et al. 2001). Library coverage value was computed as $1 - (N_c/N)$, where N_c is the number of cumulative RFLP types, and N is the total number of clones examined.

Sequencing and phylogenetic analysis—Cloned fragments representative of each RFLP type were partially sequenced (550 bases) by Qiagen Genomics Sequencing Services using the internal primer Euk528f (5′-GCG GTA ATT CCA GCT CCA A-3′) (Elwood et al. 1985). This primer binds upstream of the most variable region of the 18S molecule, which is sufficient for phylogenetic identification at roughly the genus level for eukaryotes. In order to determine the phylogenetic affiliation and the potential chimerical artifacts, all sequences were subjected to a Blast search (July 2002 databases) using the National Center for Biotechnology Information web server (http://www.ncbi.nlm.nih.gov) and to the Check_Chimera program at the Ribosomal Database Project (http://rdp.cme.msu.edu/html/). Sequences were aligned using the automatic alignment tool of the ARB software package (available at http://www.mikro.biologie.tu-muenchen.de) against a database of 3284 complete and partial rRNA eukaryotic sequences. The resulting alignment was checked and corrected manually. Sequence similarity was calculated by constructing a similarity matrix with the ARB tools. Based on these data, sequences were grouped in the following way: sequences with similarities higher than 99.5% were considered to belong to the same phylotype (as defined in Moreira and López-García 2002) and those with similarities higher than 98% to the same operational taxonomic unit or OTU (*see Discussion* for a justification of these limits).

Phylogenetic trees were constructed following the same strategy as Fuller et al. (2003). First, complete 18S rDNA sequences (1670 positions, except for Cercozoa, for which only 1100 unambiguously aligned positions were considered) most similar to our cloned sequences were used to construct trees using maximum-likelihood (ML) and neighbor-joining (NJ, with Jukes-Cantor correction) distance algorithms provided in the ARB program. Bootstraps values were computed for NJ with 1000 replicates and reported on the ML tree. In a second step, a single sequence representative from each OTU detected in our samples was chosen. These partial sequences (540 positions) were inserted into

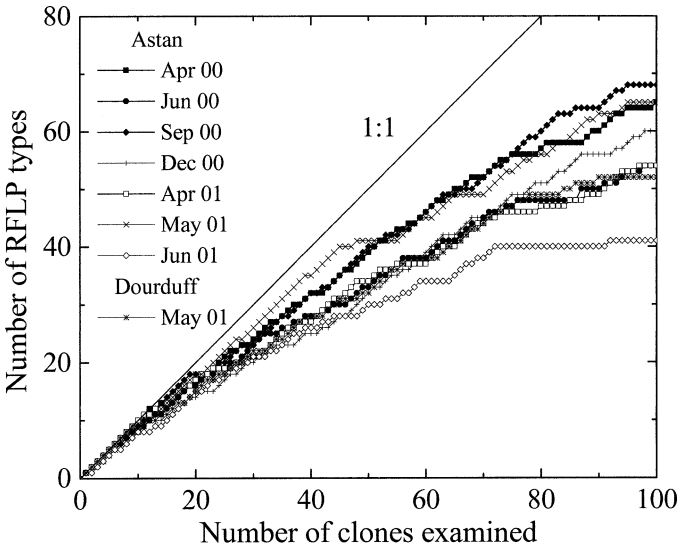


Fig. 1. Rarefaction curves for the different RFLP types of 18S rDNA clones in eight clone libraries. The number of different RFLP types in each library was determined with one restriction endonuclease (*Hae*III). RFLP types versus number of clones screened.

the ML tree using a special ARB parsimony tool that does not affect the initial tree topology and that provides upper estimates of bootstrap values.

The nucleotide sequences reported in this study have been deposited in GenBank under accession numbers AY295352–AY295760.

Results

Study site—In the western English Channel, the water column is permanently mixed under the combined effect of strong tidal currents and wind action (Pingree and Griffiths 1978), while the euphotic zone seldom becomes exhausted of nutrients (Wafar et al. 1983). Phytoplankton off Roscoff is characterized by a strong seasonal cycle with a *Guinardia* diatom bloom in late spring/early summer (Sournia et al. 1987). During the 2 yr sampled in the present study, the bloom was offset by a full month, occurring in late July in 2000 and late June in 2001. Chlorophyll concentration varied from below 0.2 μg L^{-1} in winter to over 2.5 μg L^{-1} during the bloom (Table 1). Photosynthetic picoeukaryote concentration also displayed a clear seasonal cycle with a minimum in December/January at 2000 cell ml^{-1} and a maximum in summer at around 20,000 cell ml^{-1} (Table 1). At the Dourduff estuarine station, picoeukaryotes were up to three times more abundant than at Astan (Table 1; Not and Marie pers. comm.). Marine *Synechococcus* concentration was much lower than that of the photosynthetic picoeukaryotes, varying between 800 and 3000 cell ml^{-1} (Table 1).

RFLP and phylogenetic analysis—Clone libraries were screened by RFLP using the *Hae*III restriction enzyme, which has previously been shown to cut 18S rDNA sequences into fragments that allow optimal resolution (Díez et al. 2001*b*). In order to determine how the sampling of clones covered the diversity of the picoeukaryotic 18S rDNA in the libraries, the cumulative number of RFLP types was plotted versus the total number of clones examined to generate a so-called rarefaction curve (Fig. 1). Except for the June 2001 library corresponding to the diatom bloom (RA010613), none of the curves showed saturation. Coverage values (Table 2) were low, ranging from 32% (September 2000) to 59% (June 2001).

A total of 397 partial sequences were used for this study. Blast analyses showed that all clones were eukaryotic, confirming the high specificity of the eukaryotic PCR primers. All metazoan sequences (32), mostly from cnidarians, ascidians, and mollusks corresponding probably to small gametes, were excluded from further phylogenetic analyses. The phylogenetic affiliations and similarity values of the most closely related GenBank sequences for the remaining sequences obtained in this study are presented in Web Appendix 1 at http://www.aslo.org/lo/toc/vol_49/issue_3/0784a1.pdf.

Overall, 86.5% of sequences had more than 90% similarity to known 18S rDNA sequences belonging to the following taxa: Prasinophyceae, Prymnesiophyceae, Cryptophyceae, stramenopiles, Dinophyceae, alveolates group I and group II, ciliates, Cercozoa, choanoflagellates, Mesomycetozoa, and fungi (Fig. 2). Two sets of 9 clones and 14 clones formed independent lineages (Rosko I and II, respectively) not belonging to any of the phylogenetic groups described to date.

Chlorophyta—Seventy clones were affiliated to the class Prasinophyceae (division Chlorophyta) falling into nine OTUs and 21 different phylotypes. The majority of sequences were related to genera belonging to the order Mamiellales (Fig. 2). The genus *Ostreococcus* was represented by three

Table 2. Summary of RFLP analysis and sequenced clones of the eight genetic libraries.

Station	Clone library	Date	Number of clones	Number of RFLP types	Coverage value (%)	Number of clones sequenced
Astan	RA000412	12 Apr 2000	100	65	35	84
	RA000609	9 Jun 2000	100	54	46	46
	RA000907	7 Sep 2000	100	68	32	48
	RA001219	19 Dec 2000	100	60	40	45
	RA010412	12 Apr 2001	100	54	46	48
	RA010516	16 May 2001	100	65	35	48
	RA010613	13 Jun 2001	100	41	59	46
Dourduff	RD010517	17 May 2001	100	52	48	43

OTUs, the sequences of which had 95% to 99% similarity with *Ostreococcus tauri.* One OTU, represented by clone RA000412.85, was distributed in all clone libraries except in the September and December Astan libraries. The genus *Bathycoccus* was represented by one OTU (24 clones) found in all clone libraries except in September 2000. The sequence of the representative clone (RA000412.35) shared 100% similarity with *Bathycoccus prasinos.* Three OTUs represented by clones RA000412.97, RA000412.36, and RA000412.37 were closely related to the three *Micromonas* strains CCMP 489, CCMP 490, and RCC 434, respectively, with sequence similarities ranging from 99% to 100%. These OTUs were detected at least three times at Astan. At Dourduff, we only found the OTU related to CCMP 490.

Among the other Prasinophyceae sequences, we detected two clones belonging to the same phylotype that clustered with *Pyramimonas australis* (order Pyramimonadales, 98.6% similarity). Finally, a unique OTU found only in the December clone library was closely related (99.5% similarity) to a yet undescribed clade represented by the coccoid green algal strain CCMP 1205.

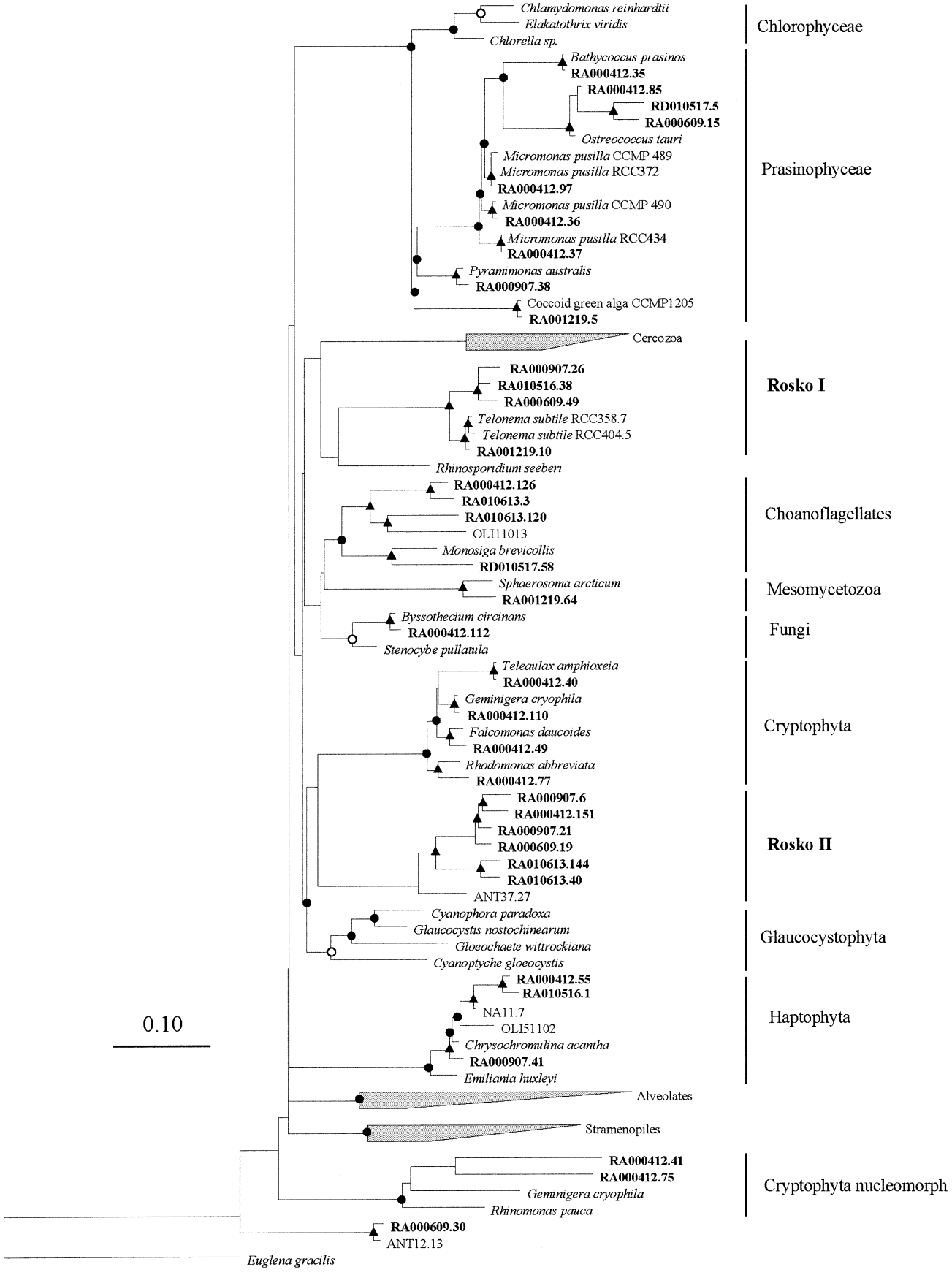
Cryptophyta—Twenty-eight clones were affiliated with nuclear sequences of Cryptophyceae and six with Cryptophyceae nucleomorph sequences. Cryptophyceae nucleus sequences were gathered into four OTUs, which were closely similar to *Geminigera cryophila* (99.3%), *Teleaulax amphioxeia* (99.6%), *Falcomonas daucoides* (98.5%), and *Rhodomonas abbreviata* (97.1%; Web Appendix 1). The OTU RA000412.110 related to *Geminigera* was the most abundant, with nine different phylotypes distributed in all libraries.

Haptophyta—The class Prymnesiophyceae (division Haptophyta) was represented by six clones defining three OTUs, only detected in clone libraries from Astan. Two OTUs represented by clones RA000412.55 (three phylotypes) and RA010516.1 (one phylotype) belonged to a clade containing only environmental sequences (Fig. 2) with 97.6% and 96.7% similarity, respectively, to NA11-7 recovered from the North Atlantic Ocean (Díez et al. 2001*a*). The third OTU (RA000907.41) showed high sequence similarity (98.5%) with *Chrysochromulina acantha.*

Stramenopiles—Fifty sequences, comprising 26 OTUs, were affiliated to the stramenopiles (Fig. 3; Web Appendix 1): 25 OTUs at Astan and one (RD010517.56) at Dourduff. Very surprisingly, among these 26 OTUs, none could be assigned to classes containing photosynthetic picoplankton species such as the Pelagophyceae or the Bolidophyceae. Clone RA001219.6 showed high sequence similarity (99.6%) with *Cafeteria* strain EWM2. Clones RA010613.1 and RA010613.4 were related to *Hypochytrium catenoides* and *Pythium monospermum* with relatively low similarities of 92% and 87.5%, respectively. The OTU represented by clone RA000907.36 did not clearly affiliate with any known species. It occupies an isolated and uncertain position and emerges as a basal branch of the photosynthetic stramenopiles.

The 21 remaining OTUs belonged to seven clades that contain only environmental sequences from various oceanic areas and have been called novel marine stramenopiles (NMS, Massana et al. 2002). We have used the extended clade nomenclature recently proposed by Massana et al. (unpubl. data). Clade NMS-3 was represented in our libraries by five OTUs detected only at the coastal site (Web Appendix 1). Two subclusters can be further distinguished (Fig. 3), with OTUs related to the environmental clones NA11-5 and ANT12-7 recovered, respectively, from the North Atlantic and Antarctic (Díez et al. 2001*b*). Clade NMS-6 was represented by two OTUs showing high sequence similarity (95.2–98.5%) with the environmental clone ME1-24 recovered from the Mediterranean Sea (Díez et al. 2001*b*). Clade NMS-2 was represented by a unique clone (RA010516.13), which showed high sequence similarity (98.4%) with the environmental clone DH148-5-EKD53 recovered at 3000 m depth in the Antarctic (López-García et al. 2001). Clade NMS-1 is represented by five OTUs. The OTU RA001219.48 was closely similar to the environmental clone DH144-EKD10 found at 250 m depth in the Antarctic (López-García et al. 2001). The two other OTUs (RA000412.91 and RA000609.22) were related to the environmental clone OLI11008 recovered from the Pacific Ocean (Moon–van der Staay et al. 2001) with 93.2% and 94.3% sequence similarity, respectively. The phylogenetic position of RA010613.38 and RA010613.136 was unstable (Fig. 3), rendering their correct affiliation uncertain. Clades NMS-12, 7, and 4 emerge as basal branches of the heterokont tree. Clade NMS-12 was represented by three OTUs. One of them showed high sequence similarity (96.1%) with the environmental clone OLI51105, to whom the two other ones were only weakly related (83% sequence similarity). Clade NMS-7 comprised four OTUs. Two OTUs were closely related to the environmental clones ANT12.6 and ANT12.10 recovered from the Antarctic with high sequence similarity (99.3–99.5%). Clade NMS-4 was represented by two OTUs showing 97% sequence similarity with the environmental clone NA11.4 detected in the North Atlantic (Díez et al. 2001*b*).

Alveolates—With 138 out of 365 picoeukaryote sequences (excluding metazoans), alveolates represented the largest group recovered from all our clone libraries (Fig. 4; Web Appendix 1). They can be grouped into 60 OTUs and 100 phylotypes (Web Appendix 1). The alveolate sequences were distributed across dinoflagellates, alveolates group I and II (López-García et al. 2001), and ciliates, with the exception of one OTU (RA010412.48), which clustered with moderate bootstrap support with the oyster parasite *Perkinsus marinus* (88% sequence similarity). Dinoflagellates were represented by 17 clones in seven OTUs. The dinoflagellate sequences identified were very closely related to known genera such as *Gymnodinium* sp., *Dinophysis norvegica,* and *Amphidinium longum,* with similarity between 97.7% and 99.3%, except for OTUs RA000609.61 and RA000609.43, which were closely related, respectively, to dinoflagellate clone sequences OLI11005 from the equatorial Pacific Ocean (Moon–van der Staay et al. 2001) and DH147-KD21 from the Antarctic polar front (López-García et al. 2001). No dinoflagellate sequence was detected in December 2000 and June 2001. In contrast, all OTUs appeared in June 2000.


Chlamydomonas reinhardtii
Elakatothrix viridis
Chlorella sp.
Chlorophyceae
Bathycoccus prasinos
RA000412.35
RA000412.85
RD010517.5
RA000609.15
Ostreococcus tauri
Micromonas pusilla CCMP 489
Micromonas pusilla RCC372
RA000412.97
Micromonas pusilla CCMP 490
RA000412.36
Micromonas pusilla RCC434
RA000412.37
Pyramimonas australis
RA000907.38
Coccoid green alga CCMP1205
RA001219.5
Prasinophyceae
Cercozoa
RA000907.26
RA010516.38
RA000609.49
Telonema subtile RCC358.7
Telonema subtile RCC404.5
RA001219.10
Rosko I
Rhinosporidium seeberi
RA000412.126
RA010613.3
RA010613.120
OLI11013
Monosiga brevicollis
RD010517.58
Choanoflagellates
Sphaerosoma arcticum
RA001219.64
Mesomycetozoa
Byssothecium circinans
RA000412.112
Stenocybe pullatula
Fungi
Telecaulax amphioxeia
RA000412.40
Geminigera cryophila
RA000412.110
Falcomonas daucoides
RA000412.49
Rhodomonas abbreviata
RA000412.77
Cryptophyta
RA000907.6
RA000412.151
RA000907.21
RA000609.19
RA010613.144
RA010613.40
ANT37.27
Rosko II
Cyanophora paradoxa
Glaucocystis nostochinearum
Gloeochaete wittrockiana
Cyanoptyche gloeocystis
Glaucocystophyta
RA000412.55
RA010516.1
NA11.7
OLI51102
Chrysochromulina acantha
RA000907.41
Emiliania huxleyi
Haptophyta
0.10
Alveolates
Stramenopiles
RA000412.41
RA000412.75
Geminigera cryophila
Rhinomonas pauca
Cryptophyta nucleomorph
RA000609.30
ANT12.13
Euglena gracilis

Ciliophora constitute the second major alveolate group represented in our clone libraries, with 23 OTUs (16% of all OTUs) appearing in all clone libraries except in June 2001 (Fig. 4; Web Appendix 1). Dourduff clone library contained the greatest number of ciliates with 13 OTUs. Three of them were present only at Dourduff and were loosely related to known species such as *Prorodon viridis, Cohnilembus vermimus,* and *Steinia sphagnicola.* Nineteen ciliate OTUs could not be related to any known species for which sequences are available. These OTUs showed high sequence similarity with environmental clones ANT37-24, NA11-3, and C1_E019 recovered, respectively, from Antarctic waters, North Atlantic waters (Díez et al. 2001*b*), and marine sediment of the Guaymas basin hydrothermal vents (Edgcomb et al. 2002).

The alveolate group I (López-García et al. 2001) contained four OTUs with seven different phylotypes. These OTUs exhibited high sequence similarity with clones recovered from both the equatorial Pacific and Antarctic Ocean (López-García et al. 2001; Moon–van der Staay et al. 2001). Alveolate group I was represented only in September and December 2000 and June 2001.

Twenty-five OTUs (20% of OTUs) were related to alveolate group II (Web Appendix 1). This group appeared to be highly diversified. In contrast to stramenopiles (Fig. 3), the phylogenetic tree, constructed mainly from partial sequences, showed in general very low bootstrap support (Fig. 4) and could not be used to infer phylogenetic relationships. Two OTUs (RA000907.29 and RA010613.9) had high sequence similarity to *Amoebophrya* sp. infecting *Dinophysis* (97.5%) and formed a well-supported cluster together with this organism (Fig. 4). Some of the other OTUs had high similarity to environmental sequences from the equatorial Pacific Ocean (Moon–van der Staay et al. 2001) and the Antarctic Ocean at 3000 m depth (López-García et al. 2001).

Choanoflagellates, Mesomycetozoa, and Cercozoa—Choanoflagellates were only represented by four OTUs (Fig. 2; Web Appendix 1). One OTU, RD010517.58, showed low similarity with the aloricate species *Monosiga brevicolis,* while the other three were loosely related to the environmental clone OLI11013 (Moon–van der Staay et al. 2001). One sequence RA001219.64 was closely related to the Mesomycetozoa *Sphaerosoma arcticum* (96.5% similarity). Cercozoa were represented by 18 OTUs (Web Appendix 1) with clones distributed in all coastal libraries but absent in the estuarine one (Dourduff). Based on the phylogenetic analysis (Fig. 5), four OTUs were related to the genus *Cryothecomonas,* while the other clustered with environmental sequences NA12-14 and SIC.7235, recovered from North Atlantic water (Díez et al. 2001*b*) and Antarctic sea ice (Brown and Bowman 2001), respectively.

Novel eukaryotic lineages—Two distinct sets of sequences could not be placed within currently recognized eukaryotic divisions (Web Appendix 1). Each group was strongly supported by bootstrap analysis (Fig. 2).

The first novel lineage (Rosko I) detected in our clone libraries was represented by four OTUs and eight phylotypes. One OTU (RA001219.10) was closely related to the species of uncertain affiliation, *Telonema subtile* (strain RCC 358, 99.5% identity). It appeared at Astan three times. In contrast, OTUs RA000609.49, RA000907.26, and RA010516.38 were detected only once. No clone related to this division was found in the Dourduff clone library.

The second unknown lineage (Rosko II) was represented by six OTUs and 12 phylotypes that shared low similarity to the environmental clone ANT37-27 (89.7–92.6% similarities). The phylogenetic position of this lineage cannot be assessed reliably from partial sequences alone, since bootstrap values are very low (Fig. 2). Four OTUs (RA000412.151, RA000907.21, RA000609.19, and RA000907.6) were highly similar to each other (>95%) but showed low similarity (<92%) to RA010613.40 and RA010613.144, which suggests that this lineage may have several representatives at the generic level. This group appeared most diverse in September 2000 with three OTUs (six phylotypes).

Temporal and spatial patterns of picoeukaryote diversity—Although clone library diversity does not reflect the actual environmental diversity (*see Discussion*) and the number of analyzed clones was insufficient to cover the full diversity of the libraries (Table 2), some patterns emerged when comparing the composition of the libraries established at different seasons. Only three groups, Prasinophyceae, stramenopiles, and alveolates group II, were found in all clone libraries. In contrast, alveolates group I and choanoflagellates were most rarely observed but they were still present in at least three libraries. No strong seasonal trend could be detected since even the most sporadic groups were observed at all seasons (e.g., alveolates group I were observed in early summer, early fall, and winter). In fact, differences in composition can be very marked between samples taken at the same season 1 yr apart as demonstrated for the June 2000 and 2001 libraries (Fig. 6). The Rosko I lineage, ciliates, Dinophyceae, and Cryptophyceae were only present in June 2000, while alveolates group I and choanoflagellates were only observed in 2001. The restriction of photosynthetic lineages to the Prasinophyceae in June 2001 is particularly interesting, since this date coincided with the diatom bloom.

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Fig. 2. 18S rDNA phylogenetic tree showing the position of the picoeukaryote sequences obtained from Astan (RA) and Dourduff (RD) clone libraries (bold). The topology of the tree was obtained by maximum-likelihood analysis of complete sequences onto which partial sequences were added using the ARB maximum parsimony tool. Circles correspond to bootstrap values (1000 replicates) from neighbor-joining analysis of full sequences, while triangles correspond to maximum parsimony placement of short sequences. Full and empty symbols correspond to bootstrap values equal to or above 90% and between 75% and 90%, respectively. *Euglena gracilis* was used as outgroup. Collapsed clusters are expanded in the next three figures. The bar indicates 10% sequence divergence.

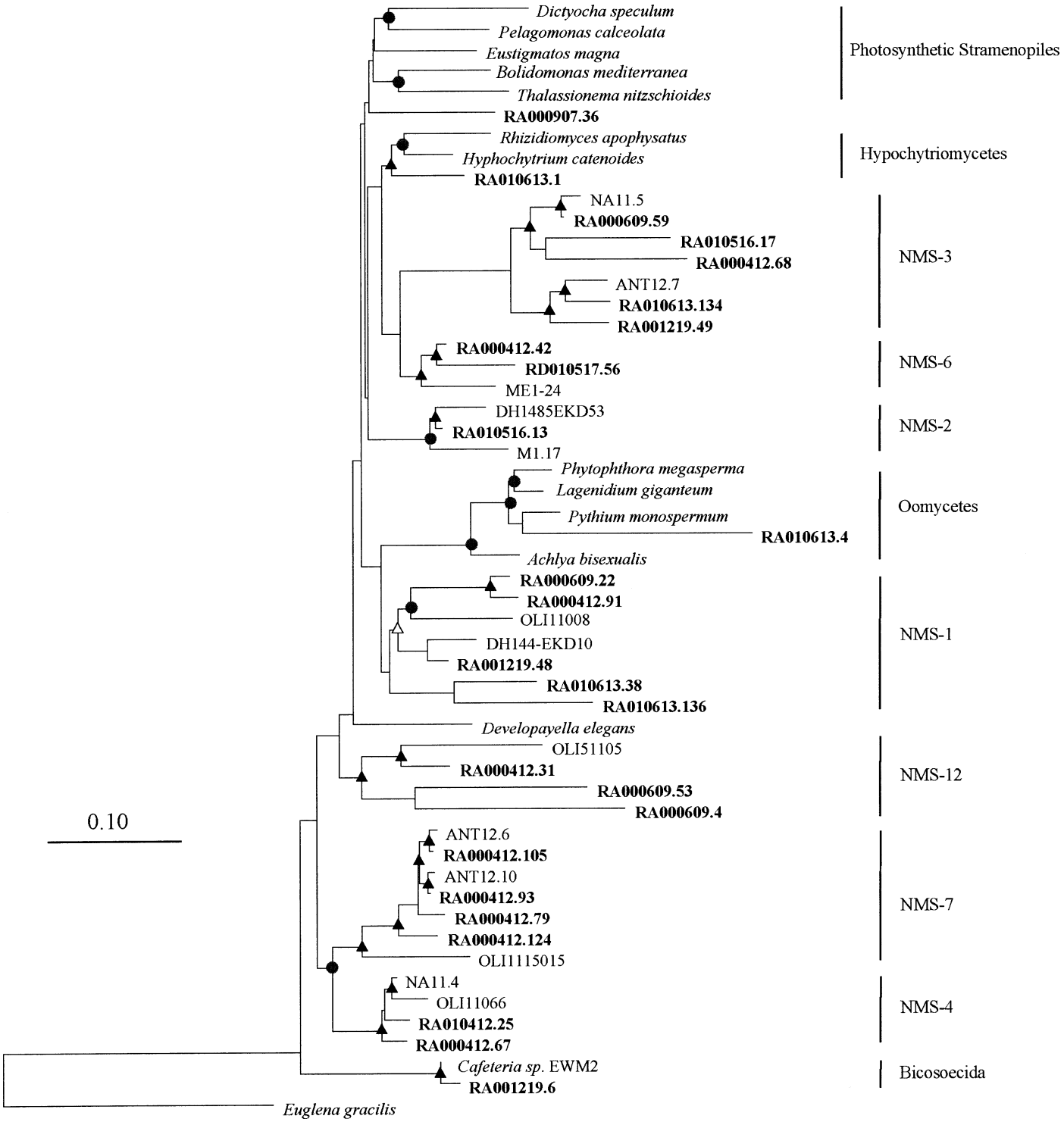

Fig. 3. Phylogenetic tree for stramenopiles. Nomenclature of novel marine stramenopiles clades (NMS) follows that of Massana et al. (unpubl. data). Symbols as in Fig. 2.

At a lower taxonomic level (Web Appendix 1), quite surprisingly, not a single OTU was observed in all libraries, only three related to *Micromonas*, *Bathycoccus*, and *Geminigera*, respectively, were found in six out of the seven coastal libraries and two (related to *Cryothecomonas* and *Ostreococcus*) in five out of seven. This suggests that, despite the persistence of broad groups throughout the different seasons, individual taxa occur much more sporadically. This is confirmed by looking at the number of OTUs observed only in a single library. Restricting ourselves to the Astan coastal station, 84 out of 128, i.e., 66% of the OTUs have only been observed once (Table 3). Among these, OTUs

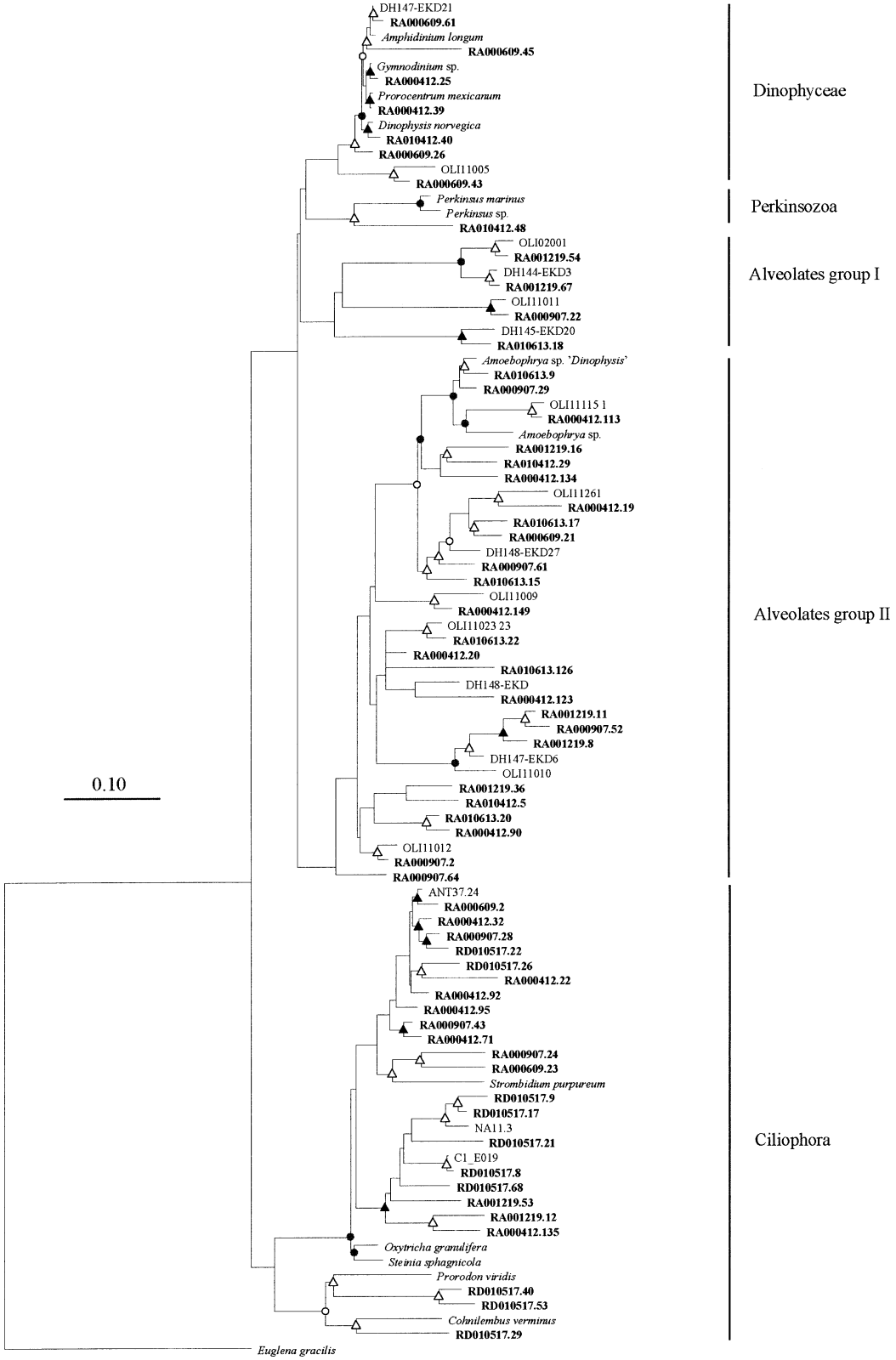

Fig. 4. Phylogenetic tree for alveolates. Symbols as in Fig. 2.

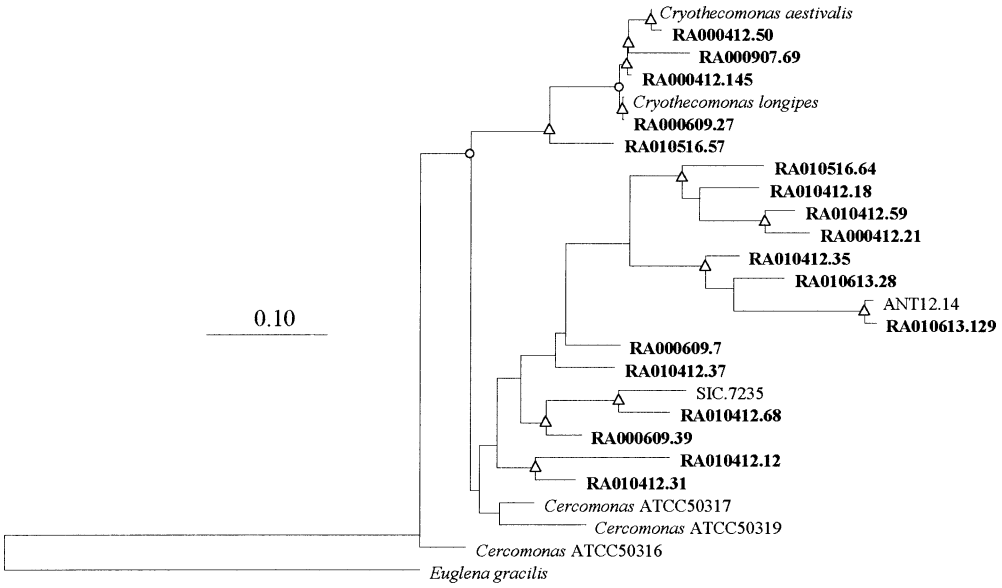

Fig. 5. Phylogenetic tree for Cercozoa. Symbols as in Fig. 2.

belonging to the Prasinophyceae and Cryptophyceae were the most recurrent, while in contrast for alveolates group II and Cercozoa, more than 80% of the OTUs only occurred once. From a temporal point of view, the June 2001 library displayed the highest proportion of unique OTUs (especially among choanoflagellates and alveolates group II). This fits with its overall composition, which differs from the other clone libraries (*see above*). However, no other clear temporal trend can be observed in the appearance of unique OTUs (Table 3).

The comparison of the clone library composition at the coastal Astan and estuarine Dourduff station, done in May 2001 1 d apart, reveals that environmental conditions (nutrient, salinity) have probably more influence than seasonal changes. At this particular period (May 2001), diversity was lower at the estuarine station, as evidenced by the higher RFLP coverage values (Table 2) and the lower number of divisions observed (Fig. 6), three groups being restricted to the coastal station (Rosko I, Prymnesiophyceae, and Cryptophyceae). At the estuarine station, the ciliates dominated the library with over 50% of the clones. At the OTU level (Web Appendix 1), the difference is even more striking since only five OTUs out of a total of 43 were common to both stations. The clones in common were related to the two Prasinophyceae *Micromonas* and *Bathycoccus* and to three ciliates similar to an Antarctic clone (ANT37-24).

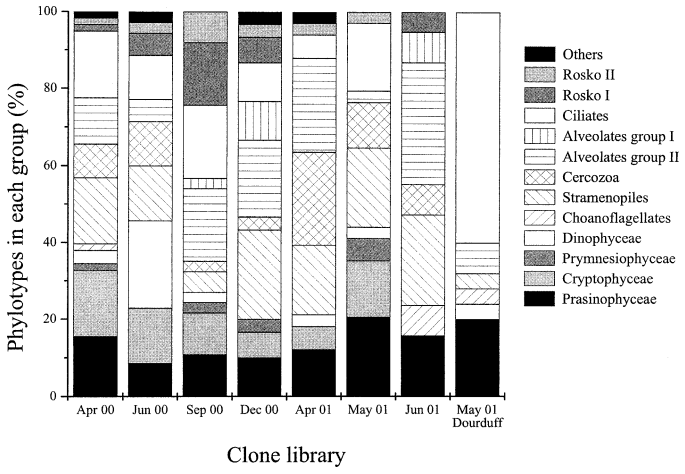

Fig. 6. Seasonal variation of the different picoeukaryote groups at the Roscoff coastal sites expressed as a percentage of total phylotypes.

Discussion

Picoeukaryote diversity—In general, our data confirm and extend recent studies, which showed that picoeukaryote assemblages displayed a surprisingly high diversity (Díez et al. 2001*b;* López-García et al. 2001; Moon–van der Staay et al. 2001). Rarefaction curves based on RFLP profiles did not saturate even after the analysis of 100 clones per library (Fig. 1), resulting in low coverage values of diversity for all clone libraries (Table 2). In order to assess the validity of diversity estimates derived from RFLP patterns, we related RFLP patterns and 18S rDNA partial sequences. In some rare cases (two), two clones having two different RFLP patterns showed 100% partial sequence similarity. This could be due to point substitutions produced by the *Taq* DNA polymerase, which is known to have an intrinsic misincorporation rate during strand synthesis (Gelfand 1989). However, according to the manufacturer's data, the *Taq* used in this study has a very low error rate (about one per 10^5 nucleotides). Moreover, some clones sequenced in the present study had sequences identical to those of cultivated strains (e.g., clone RA000412.35 and *Bathycoccus prasinos* share 100% similarity), supporting further a low overall error rate. Alternatively, and more likely, sequences similar in the sequenced region (roughly from *E. coli* position 528 to 1028) but dissimilar outside could yield different RFLP patterns. More

Table 3. Number of OTUs appearing only once in clone libraries from the Astan site.

Group	Total number of OTUs observed	Number of OTUs observed only in a single clone library								Total	% of all OTUs
		Apr 2000	Jun 2000	Sep 2000	Dec 2000	Apr 2001	May 2001	Jun 2001			
Prasinophyceae	8		1		1					2	25
Cryptophyta (includes nucleomorph)	6	1								1	17
Haptophyta	3			1			1			2	67
Cercozoa	18	2	3			7	2	2		16	89
Choanoflagellates	3							2		2	66
Mesomycetozoa	1				1					1	
Stramenopiles	26	5	3		2	1	1	5		17	65
Perkinsozoa	1					1				1	
Dinophyceae	7		4			1				5	71
Alveolate group I	4				1			1		2	50
Alveolate group II	25	4	1	3	4	2		6		20	80
Cillates	14	1		2	2		1			6	43
Rosko I	6			2				2		4	67
Rosko II	4		1	1			1			3	75
Fungi	1	1								1	
RA000609.30	1		1							1	
Total number of OTUs observed only once		14	14	9	11	12	6	18		84	66
Total number of OTUs	128	44	31	28	26	29	27	30			
% of OTUs observed once		32	45	32	42	41	22	60			

frequently (13 cases), as observed in bacterial communities (Dunbar et al. 1999), identical RFLP patterns could correspond to different sequences such as RA000907.38 (Prasinophyceae) and RA000907.36 (stramenopile). This result indicated that the diversity in the clone libraries was underestimated by RFLP patterns generated using a single restriction enzyme.

Clearly, sequence data provide a sharper image of the actual diversity of the community than RFLP patterns. However, in order to synthesize the data obtained, it is necessary to group sequences based on their similarity. Choosing adequate thresholds is far from trivial because sequence identities vary widely with the taxa considered. Inspection of the public 18S rDNA sequence database reveals that, for example, the two *Prymnesium* species, *P. parvum* and *P. patilleferum*, share 99.8% identity. In contrast, within the species *Micromonas pusilla*, the two strains CCMP 489 and CCMP 490 share only 97.7% identity, while two different Pelagophyceae genera, *Pelagococcus* and *Pelagomonas*, have 98.7% identity. In this paper we used two operational thresholds. The higher one (99.5% = phylotype) takes into account all the potential errors due to *Taq* amplification, sequencing, as well as minor differences in rRNA operon sequences within a given organism. The lower threshold (98% = OTU) roughly corresponds to the genus/species level. For example, among Mamiellales, one group very well represented in our clone libraries, sequences with similarities above this threshold correspond to organisms belonging to the same genus.

The total number of OTUs obtained over the eight clone libraries was quite high (139) and is in the same range, for example, as the number of diatom species that can be identified over the year in Roscoff (Ristori unpubl. data), suggesting that picoplankton diversity is as high or even higher than that of microphytoplankton. Of these OTUs, only 34 had similarity over 98% (the OTU threshold) with sequences available in databases and even fewer (17) with described genera or species (Web Appendix 1). Seven major lineages were represented: Prasinophyceae, Cryptophyceae, Prymnesiophyceae, Cercozoa, choanoflagellates, stramenopiles, and alveolates. Moreover, some groups of sequences did not correspond to any known lineage (Rosko I and Rosko II). Thirty-six percent of the OTUs fell into taxonomic groups with no cultivated representative but previously characterized by molecular criteria within the stramenopiles and alveolates (Massana et al. 2002). Photosynthetic lineages (Prasinophyceae, Cryptophyceae, Prymnesiophyceae, and Dinophyceae) had the highest fraction of OTUs related to existing taxa or undescribed cultures: 18 out of 23 with 93.7% to 100% sequence similarity (excluding Cryptophyceae nucleomorph sequences). Interestingly, it was among these OTUs that the number of phylotypes was the highest. In particular, OTU RA000412.110 related to the cryptophyte *Geminigera* contained nine phylotypes. This may point to a high diversity of species or ecotypes within photosynthetic genera. In contrast, heterotrophic groups had very few OTUs that could be related to described taxa, with the notable exception of Cercozoa for which a few sequences matched those of *Cryothecomonas* species. This difference between autotrophic and heterotrophic groups probably has several

causes. First, as pointed out earlier (Vaulot et al. 2002), heterotrophic eukaryotes are probably much more diverse than photosynthetic ones because the roles they play in the microbial food web, and therefore their ecological niches, are much more diversified: they may feed on a specific type of prey, degrade a specific substrate, or be linked to a specific organism through parasitic relationships, e.g., *Pirsonia*, which parasites diatoms, appear to be species specific (Schnepf and Schweikert 1997). Second, photosynthetic lineages have probably benefited from more scientific attention because of the larger community of phycologists. Third, available sequences from cultures do not cover all described taxa. On the one hand, some groups have been less studied by molecular phylogeny approaches than other. For example, more than 45 species belonging to the ciliate genus *Strombidium* have been described (Anonymous 1999) but only one species (*Strombidium purpureum*) has been sequenced. On the other hand, marine heterotrophic protists are in general more difficult to isolate in culture than autotrophic ones because they often rely on specific food species. In fact, attempts to culture heterotrophic protists often result in the recurring appearance of ''weed'' species such as the chrysophyte *Paraphysomonas* (Lim et al. 1999), while most species present in the initial sample escape cultivation. A notorious example is the heterotrophic toxic dinoflagellate *Dinophysis* that has escaped all culture attempts to date because it may require specific picophytoplanktonic preys (Imai and Nishitani 2000). Direct acquisition of sequences from single cells identified taxonomically by their morphology and separated from fixed marine samples (e.g., Guillou et al. 2002) will have to be used extensively to circumvent this shortage of molecular data.

Photosynthetic lineages—Prasinophyceae constituted the most conspicuous photosynthetic group and was detected in all clone libraries. In particular, it was the only photosynthetic group remaining during the June 2001 diatom bloom. The importance of Prasinophyceae in marine waters has been previously suggested in particular from microscopy observations (e.g., Thomsen and Buck 1998) as well as from the presence of characteristic pigments such as chlorophyll *b* and prasinoxanthin, in particular in Atlantic waters (Gibb et al. 2000). Chlorophyll *b* was also observed off Roscoff in the early study of Klein and Sournia (1987). Pigment analyses performed during the present study suggest that most chlorophyll *b* and all of prasinoxanthin are associated with the picoplanktonic size fraction. Moreover chlorophyll *b* concentration is always higher than chlorophyll *c* in this fraction (Latasa pers. comm.). Among the Prasinophyceae, most of the sequences corresponded to three genera *Micromonas, Bathycoccus,* and *Ostreococcus*, belonging to the order Mamiellales. *Micromonas pusilla* was actually the first picoplankton species ever to be described as *Chromulina pusilla* (Butcher 1952). Despite the fact that *Micromonas* is considered ubiquitous, only a limited number of field studies have actually recorded its presence, probably because it can only be identified either in live samples or in transmission electron microscopy preparations (Thomsen and Buck 1998). Cottrell and Suttle (1991) have been able to isolate viruses specific to this species from both coastal waters off North America and in the oligotrophic central Gulf of Mexico. More recently, the common presence of *Micromonas* in Italian coastal waters was deduced based on the occurrence of its specific viruses and on serial dilution cultures (Zingone et al. 1999). Sequences related to *Micromonas* have been detected in the North Atlantic near 60°N (Díez et al. 2001*b*), pointing out the ubiquity of this genus in temperate waters. *Bathycoccus* was initially described from a culture isolated at the bottom of the euphotic zone in the Mediterranean Sea (Eikrem and Throndsen 1990) and has been little recorded since. *Ostreococcus,* the smallest free living eukaryote (Courties et al. 1994), was initially isolated from a coastal Mediterranean Sea lagoon. Since, *Ostreococcus* sequences have been recorded from the open Mediterranean Sea (Díez et al. 2001*b*), and strains from a wide range of environments, such as the Atlantic Ocean and Red Sea, are now available in culture collections (Vaulot et al. 2004). These three genera have been repeatedly isolated off Roscoff (Vaulot et al. 2004). These data suggest that Mamiellales constitute probably one of the key photosynthetic eukaryotic groups in temperate waters. Their importance in several oceanic systems (e.g., Red Sea) has been very recently confirmed by environmental *psbA* sequence data (Zeidner et al. 2003). The other Prasinophyceae sequences correspond to *Pyramimonas,* a very complex genus that can form blooms in cold waters (e.g., Rodriguez et al. 2002), and to a clade for which the cultured representatives (e.g., strain CCMP 1205) have not been formerly described to date. Interestingly, a sequence from this clade was obtained from the equatorial Pacific (Moon–van der Staay et al. 2001), which suggests it could be fairly ubiquitous, like the Mamiellales.

Cryptophyceae, which are so well represented in Roscoff libraries, are often observed in coastal waters (Jochem 1990). Surprisingly, Díez et al. (2001*b*) did not find any Cryptophyceae sequences in North Atlantic and Antarctic waters, but only in the Mediterranean Sea. However, observation of alloxanthin, a pigment only found in Cryptophyceae, confirms their presence in coastal Atlantic as well as Antarctic waters (Gibb et al. 2001; Rodriguez et al. 2002). Off Roscoff, this pigment has also been found during the spring bloom (Klein and Sournia 1987). During the present study, alloxanthin was observed all year round and was mostly restricted to the less than 3-μm fraction (Latasa pers. comm.), as also observed in the North Pacific (Suzuki et al. 2002). The characteristic cellular fluorescence of cryptomonads originating from phycoerythrin allows their detection by flow cytometry, and recent studies using this technique have shown that they are present all year round in Bedford Basin coastal waters, at concentrations varying between 100 and 1000 cell ml^{-1} with a maximum in summer (Li and Dickie 2001), and throughout the North Atlantic down to the latitude of Bermuda where they abruptly disappear (Cavender-Bares et al. 2001). Surprisingly in our study, Cryptophyceae sequences were not recovered at the estuarine station, which indicates that small members of this group may either be sensitive to the slightly lower salinity encountered there or to the higher nutrient load. To date, a single Cryptophyceae species of picoplanktonic size has been described, *Hillea marina* (Butcher 1952), for which no sequence is available, while most genera closely related to our sequences

(*Geminigera, Rhodomonas*) are nanoplanktonic. The sequences fall into three of the seven lineages (B, E, F) recently described by Deane et al. (2002). One OTU (RA000412.110), related to *Geminigera,* appears particularly interesting because of all OTUs observed off Roscoff, it is the one that harbors most phylotypes, which suggests probably a wide diversity at the species or ecotype level.

Prymnesiophyceae are considered the most important group in the open ocean eukaryotic picoplankton because their carotenoid 19′ hexanoyloxyfucoxanthin (19HF) is the dominant pigment in the <2–3-μm fraction (e.g., Moon–van der Staay et al. 2000). Off Roscoff, 19HF is, like alloxanthin, mostly restricted to the <3-μm fraction but is on average less important than fucoxanthin in this fraction (Latasa pers. comm.). Prymnesiophyceae sequences obtained are related either to environmental sequences or to *Chrysochromulina*, a very diversified genus, of which several species are of a size below 3 μm, e.g., *Chrysochromulina leadbeateri* (Eikrem and Throndsen 1998).

Although no dinoflagellate of picoplanktonic size has been described to date, we recovered several sequences from this important phytoplankton class, most of them related to genera present in coastal waters such as *Prorocentrum.* In particular, one OTU closely related to the toxic genus *Dinophysis* appeared in spring, a time of the year when *Dinophysis* is present in French coastal waters (Gailhard et al. 2002). The dinoflagellate sequences obtained could either correspond to yet undescribed picoplanktonic species of these genera or to life stages of existing species. Peridinin, a pigment characteristic of some dinoflagellates, was always a minor complement of carotenoids in the picoplankton size fraction (Latasa pers. comm.), suggesting that some of the sequences could correspond to heterotrophic dinoflagellates, which constitute half of known species (Larsen and Sournia 1991).

Surprisingly, we did not recover sequences from species that are often considered typical of picoplankton and easily brought in culture. Among Chlorophyta, notoriously missing were sequences related to *Nannochloris* and to the Prasinococcales (*Prasinococcus, Prasinoderma*). Among the non-Chlorophyta, entire classes that harbor mainly picophytoplankton species were missing. This was the case in particular for the Bolidophyceae, Eustigmatophyceae, and Pelagophyceae. These missing lineages may be restricted to more open ocean waters, since sequences related to them have been recovered from the Mediterranean Sea, Antarctic waters, and the Equatorial Pacific Ocean (Díez et al. 2001*b;* Moon–van der Staay et al. 2001). Finally, the absence of any diatom sequence in our clone libraries despite their importance off Roscoff (Sournia et al. 1987) demonstrates the effectiveness of our filtration procedure.

Nonphotosynthetic lineages—A number of sequences could be related to heterotrophic groups that are known to be well represented in marine waters, even though most of them do not harbor species in the picoplankton size range. Among these, the most important are the ciliates, the Cercozoa, and the choanoflagellates.

Ciliates constitute probably the best studied heterotrophic group in marine waters because they are easily preserved and therefore well represented in collected samples. The number of sequences recovered is quite surprising because their DNA is estimated to be a very small fraction of the total microbial DNA (less than 0.3%, Arin et al. 1999), which suggests an amplification or cloning bias. They are usually more abundant in eutrophic waters, since their concentration has a tendency to be correlated with chlorophyll (Dolan et al. 1999). This may explain why more ciliate sequences were obtained off Roscoff, especially at the estuarine site (Fig. 6), than in oceanic waters (e.g., Díez et al. 2001*b*). None of the sequences obtained matched known species. In contrast, some of them displayed relatively high similarity (Web Appendix 1) to environmental sequences from the Antarctic and Atlantic and from sediments near hydrothermal vents (Edgcomb et al. 2002), suggesting that novel groups of ciliates of very small size may be present in oceanic waters.

Cercozoa are small flagellates present in many different environments. While the majority of the sequences obtained were only very distantly related to the genus *Cercomonas* or to environmental sequences (Web Appendix 1), a few were affiliated to *Cryothecomonas,* a typically marine genus for which concentrations can reach up to 100 cell ml^{-1} in Antarctic waters (Thomsen et al. 1990). Interestingly, the OTU with most phylotypes (RA000412.50) was similar to *Cryothecomonas aestivalis,* which feeds on the diatom *Guinardia delicatula* (Kuhn et al. 2000), the major blooming species off Roscoff (Sournia et al. 1987). It is possible that some of the other Cercozoa, detected by their sequence, could play a similar role on other important diatom species. As for the ciliates, Cercozoa sequences have also been recovered from the Antarctic, Mediterranean Sea, and North Atlantic, but not from the Equatorial Pacific (Díez et al. 2001*b;* Moon–van der Staay et al. 2001).

Choanoflagellates are flagellated cells that display a collar of cilia and may possess a lorica that allows their identification by optical or electron microscopy. Some species can be very small, such as *Monosiga micropelagica,* which is 3–4 μm in length and 1–2 μm in width (Throndsen 1974). Their concentration usually ranges between 10 and 100 cell ml^{-1} but has been shown to reach 3900 cell ml^{-1} in coastal waters (Buck et al. 1991). In contrast to both ciliates and Cercozoa, their sequences have only been detected in equatorial waters (Moon–van der Staay et al. 2001), not in the temperate Atlantic and Antarctic, despite numerous records in these waters (e.g., Throndsen 1974). In our seasonal survey, they appear much more sporadic than Cercozoa and ciliates.

Stramenopiles and alveolates group II dominate the sequences obtained off Roscoff (Fig. 6). A recent phylogenetic analysis of environmental sequences has defined 12 different clades located at the base of the stramenopiles (Massana et al. unpubl. data) in a region already containing many small heterotrophic protists, such as the bicosoecids (*Cafeteria*), the oomycetes, and *Developayella elegans.* Heterotrophic stramenopiles also contain genera like *Pirsonia* that are parasitic of diatoms (Schnepf and Schweikert 1997). Environmental sequences have been mostly recorded from Antarctic and Mediterranean Sea waters (Díez et al. 2001*b*) but much less from Atlantic and equatorial ocean waters. Massana et

al. (2002) have established using oligonucleotide probes that the organisms belonging to two of these environmental clades (NMS-3 and 4) are picoplanktonic in size and feed on bacteria. The group II of alveolates remains much more enigmatic, since the only known organism affiliated to it is *Amoebophrya* (Gunderson et al. 1999), a strange parasite of dinoflagellates that displays a complex life cycle (Coats and Park 2002) and has been historically classified as a dinoflagellate. Although all sequences belonging to alveolates group II could correspond to parasites of dinoflagellates resembling *Amoebophrya,* it is unlikely since similar sequences have been recovered from deep Antarctic waters (López-García et al. 2001) where dinoflagellates are probably not very abundant.

The other groups, which are potentially heterotrophic, were of lesser importance. The sequences from alveolates group I were much less abundant than those of alveolates group II and often absent from clone libraries. Three sequences were distantly related, respectively, to the oyster parasite *Perkinsus,* to the fungi, and to the Mesomycetozoa, a sister group of the choanoflagellates containing in particular parasites of fish and marine invertebrates (Mendoza et al. 2002). Another sequence was quite similar to an environmental sequence recovered from the Antarctic (Díez et al. 2001*b*), both forming an isolated group (Fig. 2) with no clear phylogenetic affinity.

One of the novel lineages we observed, Rosko I, is quite interesting. While in the process of establishing cultures from the same Roscoff samples from which we acquired molecular data, we found that sequences cloned from several of the cultures had a high similarity to Rosko I environmental sequences. Examination of the cultures revealed it contained a biflagellated protist, *Telonema subtile,* first described in 1913 (actually from Roscoff samples, Griessmann 1913). This small predatory organism is often observed in the plankton (Backe-Hansen and Throndsen 2002). Its phylogenetic affiliation remains unresolved, and studies are currently under way both at the molecular and ultrastructural level to address this question (Shalchian-Tabrizi pers. comm.).

Finally, we have no idea about the morphology of the typical cells from the Rosko II lineage, making this group quite intriguing (Fig. 2). Defining its exact phylogenetic position will require detailed analysis of full-length sequences, which is currently under way (Valentin pers. comm.).

The present study confirms and extends molecular work published recently that demonstrated the wide diversity of marine eukaryotic picoplankton and the importance of yet undescribed groups. The use of quantitative approaches such as fluorescent in situ hybridization (FISH; Not et al. 2002) is required to determine the distribution and seasonal dynamics of each group, and representatives of uncultivated groups, especially heterotrophic ones, must be brought into culture to assess their biology and role in the ecosystem.

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Composition and temporal variability of picoeukaryote communities at a coastal site of the English Channel from 18S rDNA sequences

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Web Appendix 1.

Appendix 1. List of all the OTUs (grouping sequences with similarities >98%) found in this study. For each OTU, a representative sequence is provided with its phylogenetic affiliation, its closest relative from GenBank with accession number, and their percent similarity based on aligned base pairs. The remaining columns indicate the total number of phylotypes (99.5% similarity) found in all Astan clone libraries and the number of phylotypes from each clone library. Note that the total number of phylotypes found at Astan can be lower than the sum of the phylotypes found in each of the Astan libraries, if the same phylotype has been found in two or more libraries (it is then counted only once in the total).

OTUs							Number of phylotypes								
		Phylogenetic affiliation					Astan								Dourduff
Library	Clone	Division	Class	Closest relative	Accession	Identity (%)	Total	12 Apr 2000	9 Jun 2000	7 Sep 2000	19 Dec 2000	12 Apr 2001	16 May 2001	13 Jun 2001	17 May 2001
RA000412	35	Chlorophyta	Prasinophyceae	*Bathycoccus prasinos*	AY425314	100.0	3	1	1	0	1	1	2	2	1
RA000412	85			*Ostreococcus tauri*	Y15814	99.3	3	3	1	0	0	1	2	1	2
RA000609	15			*Ostreococcus tauri*	Y15814	95.2	1	0	1	0	0	0	0	0	0
RD010517	5			*Ostreococcus tauri*	Y15814	95.4	0	0	0	0	0	0	0	0	1
RA000412	97			*Micromonas* CCMP489	AJ010408	99.5	3	2	0	1	1	1	2	1	0
RA000412	37			*Micromonas* RCC 434	AY425316	100.0	4	2	0	2	0	0	1	1	0
RA000412	36			*Micromonas* CCMP490	AY425320	99.3	2	1	0	0	0	1	0	0	1
RA000907	38			*Pyramimonas australis*	AJ404886	98.6	1	0	0	1	0	0	0	1	0
RA001219	5			strain CCMP 1205	U40921	99.5	1	0	0	0	1	0	0	0	0
RA000412	110	Cryptophyta	Cryptophyceae	*Geminigera cryophila*	U53124	99.3	9	3	4	2	1	2	2	0	0
RA000412	40		(nucleus)	*Teleaulax amphoxeia*	AJ007287	99.6	2	1	1	2	0	0	1	0	0
RA000412	49			*Falcomonas daucoides*	AF143943	98.5	3	2	0	0	1	0	1	0	0
RA000412	77			*Rhodomonas abbreviata*	U53128	97.1	1	1	0	0	0	0	1	0	0
RA000412	75		Cryptophyceae	*Geminigera cryophila*	U53123	79.2	1	1	0	0	0	0	0	0	0
RA000412	41		(nucleomorph)	*Rhinomonas pauca*	U53131	83.0	2	2	0	0	0	0	0	0	0
RA000412	55	Haptophyta	Prymnesiophyceae	NA11–7 North Atlantic	AF363164	97.6	3	1	0	0	1	0	1	0	0
RA010516	1			NA11–7 North Atlantic	AF363164	96.7	1	0	0	0	0	0	1	0	0
RA000907	41			*Chrysochromulina acantha*	AJ246278	98.5	1	0	0	1	0	0	0	0	0
RA000412	50	Cercozoa		*Cryothecomonas aestivalis*	AF290539	99.3	4	3	1	0	1	1	1	0	0
RA000412	145			*Cryothecomonas longipes*	AF290540	98.5	1	1	0	0	0	0	0	0	0
RA000609	27			*Cryothecomonas longipes*	AF290540	98.5	1	0	1	0	0	0	0	0	0
RA000907	69			*Cryothecomonas longipes*	AF290540	93.3	2	0	0	1	0	0	1	0	0
RA010516	57			*Cryothecomonas longipes*	AF290540	92.0	1	0	0	0	0	0	1	0	0
RA010412	59			*Cryothecomonas longipes*	AF290540	85.1	1	0	0	0	0	1	0	0	0
RA00609	39			*Cryothecomonas longipes*	AF290540	91.4	1	0	1	0	0	0	0	0	0
RA010412	31			*Cercomonas* ATCC50317	U42449	91.1	1	0	0	0	0	1	0	0	0
RA010412	37			*Cercomonas* ATCC50317	U42449	87.7	1	0	0	0	0	1	0	0	0
RA010412	12			*Cercomonas* ATCC50317	U42449	87.0	1	0	0	0	0	1	0	0	0
RA000412	21			*Cercomonas* ATCC50317	U42449	86.9	1	1	0	0	0	0	0	0	0
RA000609	7			SIC.7235 Antarctic sea-ice	AF277495	86.1	1	0	1	0	0	0	0	0	0
RA010412	18			SIC.7235 Antarctic sea-ice	AF277495	84.9	1	0	0	0	0	1	0	0	0
RA010516	64			SIC.7235 Antarctic sea-ice	AF277495	85.2	1	0	0	0	0	0	1	0	0
RA010412	68			SIC.7235 Antarctic sea-ice	AF277495	91.1	1	0	0	0	0	1	0	0	0
RA010613	28			ANT12–14 Antarctic	AF363225	86.3	2	0	0	0	0	0	0	2	0
RA010412	35			ANT12–14 Antarctic	AF363225	86.4	1	0	0	0	0	1	0	0	0
RA010613	129			ANT12–14 Antarctic	AF363225	98.8	1	0	0	0	0	0	0	1	0
RA00412	126	Choanoflagellida		OLI11013 Equatorial Pacific	AJ402331	89.3	2	1	0	0	0	0	0	1	0
RA010613	3			OLI11013 Equatorial Pacific	AJ402331	89.6	1	0	0	0	0	0	0	1	0
RA010613	120			OLI11013 Equatorial Pacific	AJ402331	90.8	1	0	0	0	0	0	0	1	0
RD010517	58			*Monosiga brevicollis*	AF084618	90.6	0	0	0	0	0	0	0	0	1

Appendix. Continued.

OTUs							Number of phylotypes								
		Phylogenetic affiliation					Astan								Dour-duff
Library	Clone	Division	Class	Closest relative	Accession	Identity (%)	Total	12 Apr 2000	9 Jun 2000	7 Sep 2000	19 Dec 2000	12 Apr 2001	16 May 2001	13 Jun 2001	17 May 2001
RA001219	64	Mesomycetozoa		*Sphaerosoma arcticum*	Y16260	96.5	1	0	0	0	1	0	0	0	0
RA000412	79	Stramenopiles		ANT12–10 Antarctic	AF363196	97.4	1	1	0	0	0	0	0	0	0
RA000412	93			ANT12–10 Antarctic	AF363196	99.5	4	2	1	0	0	0	1	0	0
RA000412	105			ANT12–6 Antarctic	AF363192	99.3	1	1	0	0	0	0	0	0	0
RA000412	124			ANT12–6 Antarctic	AF363192	96.1	1	1	0	0	0	1	1	0	0
RA000609	59			NA11–5 North Atlantic	AF363204	99.5	1	0	1	0	0	1	0	0	0
RA010516	17			NA11–5 North Atlantic	AF363204	92.2	2	0	0	0	0	0	1	1	0
RA000412	68			NA11–5 North Atlantic	AF363204	91.3	1	1	0	0	0	0	0	0	0
RA001219	49			ANT12–7 Antarctic	AF363193	93.8	1	0	0	0	1	0	0	0	0
RA10613	134			ANT12–7 Antarctic	AF363193	95.2	1	0	0	0	0	0	0	1	0
RA00412	67			NA11–4 North Atlantic	AF363203	97.1	2	1	0	0	1	1	0	1	0
RA010412	25			NA11–4 North Atlantic	AF363203	97.6	1	0	0	0	0	1	0	0	0
RA000907	36			ME1–24 Mediterranean	AF363207	91.9	2	0	0	2	0	1	0	0	0
RA010613	136			ME1–24 Mediterranean	AF363207	89.8	1	0	0	0	0	0	0	1	0
RA000412	42			ME1–24 Mediterranean	AF363207	98.5	1	1	0	0	0	0	0	0	0
RD010517	56			ME1–24 Mediterranean	AF363207	95.2	0	0	0	0	0	0	0	0	1
RA010613	38			ME1–24 Mediterranean	AF363207	90.9	1	0	0	0	0	0	0	1	0
RA000609	4			OL151105 Equatorial Pacific	AF167414	83.9	1	0	1	0	0	0	0	0	0
RA000412	31			OL151105 Equatorial Pacific	AF167414	96.1	1	1	0	0	0	0	0	0	0
RA000609	53			OLI51105 Equatorial Pacific	AF167414	83.2	1	0	1	0	0	0	0	0	0
RA001219	6			*Cafeteria* sp. EWM	AF174365	99.6	1	0	0	0	1	0	0	0	0
RA001219	48			DH144-EKD10 Antarctic	AF290063	99.3	4	0	0	0	4	1	0	0	0
RA000412	91			OLI11008 Equatorial Pacific	AJ402350	93.2	4	1	0	0	0	0	3	0	0
RA000609	22			OLI11008 Equatorial Pacific	AJ402350	94.3	1	0	1	0	0	0	0	0	0
RA010613	4			*Pythium monospermum*	AJ238653	87.5	2	0	0	0	0	0	0	2	0
RA010613	1			*Hyphochytrium catenoides*	X80344	92.0	2	0	0	0	0	0	0	2	0
RA010516	13			DH148–5-EKD53 Antarctic	AF290083	98.4	1	0	0	0	0	0	1	0	0
RA000412	25	Alveolates	Dinophyceae	*Gymnodinium* sp.	AF274260	99.3	2	1	1	0	0	0	1	0	0
RA010412	40			*Dinophysis norvegica*	AF239261	99.3	2	0	1	0	0	1	0	0	1
RA000412	39			*Prorocentrum mexicanum*	Y16232	99.4	2	1	1	1	0	0	0	0	0
RA000609	26			*Prorocentrum mexicanum*	Y16232	97.6	2	0	2	0	0	0	0	0	0
RA000609	61			DH147-KD21 Antarctic	AF290050	98.9	1	0	1	0	0	0	0	0	0
RA000609	43			OLI11005 Equatorial Pacific	AJ402349	95.8	1	0	1	0	0	0	0	0	0
RA000609	45			*Amphidinium longum*	AF274254	93.7	1	0	1	0	0	0	0	0	0
RA000412	20	Alveolate	group II	OLI11023 Equatorial Pacific	AJ402335	95.0	5	1	0	2	0	0	1	1	0
RA010613	22			OLI11023 Equatorial Pacific	AJ402335	97.8	2	0	0	0	0	0	0	2	0
RA010613	126			OLI11023 Equatorial Pacific	AJ402335	87.8	1	0	0	0	0	0	0	1	0
RA010412	5			OLI11012 Equatorial Pacific	AJ402330	88.9	1	0	0	0	0	1	0	0	0
RA001219	36			OLI11012 Equatorial Pacific	AJ402330	89.8	1	0	0	0	1	0	0	0	0
RA010613	20			OLI11012 Equatorial Pacific	AJ402330	90.2	2	0	0	0	0	0	0	2	0
RA00907	2			OLI11012 Equatorial Pacific	AJ402330	97.8	1	0	0	1	0	0	0	0	0
RA000412	90			OLI11012 Equatorial Pacific	AJ402330	88.3	1	1	0	0	0	0	0	0	0

Appendix. Continued.

OTUs							Number of phylotypes								
		Phylogenetic affiliation					Astan								Dour-duff
Library	Clone	Division	Class	Closest relative	Accession	Identity (%)	Total	12 Apr 2000	9 Jun 2000	7 Sep 2000	19 Dec 2000	12 Apr 2001	16 May 2001	13 Jun 2001	17 May 2001
RA000907	64			OLI11012 Equatorial Pacific	AJ402330	92.3	5	0	0	1	2	3	0	2	0
RA000609	21			DH148-EKD27 Antarctic	AF290077	93.4	1	0	1	0	0	0	0	0	0
RA010613	17			DH148-EKD27 Antarctic	AF290077	92.9	1	0	0	0	0	0	0	1	0
RA000907	61			DH148-EKD27 Antarctic	AF290077	95.4	1	0	0	1	0	0	0	0	0
RA010613	15			DH148-EKD27 Antarctic	AF290077	93.4	1	0	0	0	0	0	0	1	0
RA000412	113			OLI11115 Equatorial Pacific	AJ402326	98.4	4	1	0	0	0	3	0	0	0
RA000907	29			*Amoebophrya* (*Dinophysis*)	AF239260	97.5	1	0	0	1	0	0	0	0	0
RA010613	9			*Amoebophrya* (*Dinophysis*)	AF239260	97.5	1	0	0	0	0	0	0	1	0
RA001219	16			*Amoebophrya* (*Dinophysis*)	AF239260	91.2	1	0	0	0	1	0	0	0	0
RA010412	29			*Amoebophrya* (*Dinophysis*)	AF239260	89.8	1	0	0	0	0	1	0	0	0
RA000412	19			OLI11261 Equatorial Pacific	AJ402338	91.3	1	1	0	0	0	0	0	0	2
RA000412	134			*Amoebophrya* (*Dinophysis*)	AF239260	90.0	1	1	0	0	0	0	0	0	0
RA000412	80			OLI11009 Equatorial Pacific	AF402348	94.3	2	1	1	0	0	0	0	0	0
RA001219	8			DH147-EDK6 Antarctic	AF290068	96.8	1	0	0	0	1	0	0	0	0
RA001219	11			DH147-EKD6 Antarctic	AF290068	97.4	1	0	0	0	1	0	0	0	0
RA000907	52			DH147-EDK6 Antarctic	AF290068	95.8	2	0	0	1	0	0	0	1	0
RA000412	123			DH148-EKD14 Antarctic	AF290079	90.2	1	1	0	0	0	0	0	0	0
RA001219	54	Alveolates	group 1	OLI02001 Equatorial Pacific	AF167413	99.5	3	0	0	0	1	0	0	2	0
RA001219	67			DH144-EKD3 Antarctic	AF290064	98.5	1	0	0	0	1	0	0	0	0
RA000907	22			OLI11011 Equatorial Pacific	AJ402354	97.8	2	0	0	1	1	0	0	0	0
RA010613	18			DH145-EKD20 Antarctic	AF290067	96.5	1	0	0	0	0	0	0	1	0
RA010412	48	Alveolates	Perkinsozoa	*Perkinsus marinus*	AF324218	88.4	1	0	0	0	0	1	0	0	0
RD010517	29	Alveolates	Ciliophora	*Cohnilembus vermimus*	Z22878	87.5	0	0	0	0	0	0	0	0	1
RD010517	40			*Prorodon viridus*	U97111	89.8	0	0	0	0	0	0	0	0	1
RD010517	53			*Prorodon viridus*	U97111	88.6	0	0	0	0	0	0	0	0	1
RA000412	71			ANT37–24 Antarctic	AF363221	96.2	1	1	0	0	0	0	0	0	1
RA000907	43			ANT37–24 Antarctic	AF363221	96.3	2	0	0	1	0	0	1	0	0
RA000412	95			ANT37–24 Antarctic	AF363221	96.5	2	2	0	1	0	0	0	0	0
RA000609	2			ANT37–24 Antarctic	AF363221	98.0	2	0	1	1	0	0	0	0	0
RA000412	92			ANT37–24 Antarctic	AF363221	97.8	2	1	0	0	0	0	1	0	0
RA000907	28			ANT37–24 Antarctic	AF363221	97.3	1	0	0	1	0	0	0	0	0
RA000412	32			ANT37–24 Antarctic	AF363221	97.6	3	2	2	0	0	0	0	0	0
RD010517	22			ANT37–24 Antarctic	AF363221	96.5	0	0	0	0	0	0	0	0	2
RD010517	26			ANT37–24 Antarctic	AF363221	95.4	1	0	0	0	0	0	1	0	1
RA000907	24			ANT37–24 Antarctic	AF363221	89.0	1	0	0	1	0	0	0	0	0
FA000609	23			ANT37–24 Antarctic	AF363221	89.2	3	0	1	0	0	1	1	0	1
RA000412	22			ANT37–24 Antarctic	AF363221	91.4	4	3	0	1	0	0	2	0	1
RD010517	17			NA11–3 North Atlantic	AF363220	97.3	0	0	0	0	0	0	0	0	1
RD010517	9			NA11–3 North Atlantic	AF363220	95.1	0	0	0	0	0	0	0	0	1
RD010517	21			NA11–3 North Atlantic	AF363220	90.5	0	0	0	0	0	0	0	0	1
RA000412	135			NA11–3 North Atlantic	AF363220	89.4	4	1	0	1	1	1	0	0	0
RD010517	68			C1 E019 Sediments	AY046621	92.9	0	0	0	0	0	0	0	0	1
RD010517	8			C1 E019 Sediments	AY046621	99.5	0	0	0	0	0	0	0	0	2

Appendix. Continued.

OTUs							Number of phylotypes								
		Phylogenetic affiliation					Astan								Dour-duff
Library	Clone	Division	Class	Closest relative	Accession	Identity (%)	Total	12 Apr 2000	9 Jun 2000	7 Sep 2000	19 Dec 2000	12 Apr 2001	16 May 2001	13 Jun 2001	17 May 2001
RA001219	53			C1 E019 Sediments	AY046621	91.3	1	0	0	0	1	0	0	0	0
RA001219	12			*Steinia sphagnicola*	AJ310494	90.8	1	0	0	0	1	0	0	0	0
RA001219	10	Rosko I		*Telonema subtile* RCC358	AY295352	99.5	3	1	0	0	1	1	0	0	0
RA000907	26			*Telonema subtile* RCC358	AY295352	93.2	3	0	0	3	0	0	0	0	0
RA010516	38			*Telonema subtile* RCC358	AY295352	93.8	1	0	0	0	0	0	1	0	0
RA000609	49			*Telonema subtile* RCC358	AY595352	94.2	1	0	1	0	0	0	0	0	0
RA000412	151	Rosko II		ANT37–27 Antarctic	AF363179	91.7	3	1	1	2	0	0	0	0	0
RA010613	40			ANT37–27 Antarctic	AF363179	89.7	1	0	0	0	0	0	0	1	0
RA010613	144			ANT37–27 Antarctic	AF363179	90.1	1	0	0	0	0	0	0	1	0
RA000907	21			ANT37–27 Antarctic	AF363179	92.6	2	0	0	2	0	0	0	0	0
RA000609	19			ANT37–27 Antarctic	AF363179	92.4	3	0	1	0	2	0	0	0	0
RA000907	6			ANT37–27 Antarctic	AF363179	91.6	2	0	0	2	0	0	0	0	0
RA000412	112	Fungi		*Bissothecium circinans*	AY016339	98.5	1	1	0	0	0	0	0	0	0
RA000609	30	Unknown		ANT12–13 Antarctic	AF363228	99.3	1	0	1	0	0	0	0	0	0