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The protistan microplankton community along the Kuroshio Current revealed by 18S rRNA gene clone analysis: a case study of the differences in distribution interplay with ecological variability

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Abstract: The distribution of protistan microplankton situated southwest (S09) and northeast (S18) of the Izu Ridge along the Kuroshio Current was revealed by 18S rRNA gene clone analysis. A total of 257 clones were identified, consisting of 65 phylotypes of dinoflagellates, 49 phylotypes of diatoms and 57 phylotypes of other protists affiliated with Ciliophora, Cryptophyta, Cryptophyta nucleomorph, Choanoflagellata, Chlorophyta, Cercozoa, and Heterokonta. The dinoflagellate phylotypes were affiliated with five genera and 14 uncultured groups, with *Gyrodinium* as the most frequently detected genus. The diatoms were also well represented and consisted of 13 genera and six uncultured groups. The clones belonging to the genus *Pseudo-nitzschia* were most frequently detected. The frequencies of dinoflagellate clones and phylotypes were higher at station S09 in the south than at station S18 to the north, with the frequency of diatom phylotypes being higher at the latter. The species richness (number of phylotypes) and diversity (Shannon-Wiener) of the protistan microplankton community were slightly higher at S18 compared to S09. When the Kuroshio Current encountered with the Oyashio Current at northwestern Pacific, it affects the water temperature and nutrients of the Kuroshio Current. The clone analysis results showed a difference in the protistan microplankton community at both stations due to the collision of both currents.

Key words: distribution, ecological variability, Kuroshio Current, protistan microplankton, 18S rRNA gene

Introduction

Protistan microplankton (protistan plankton of size 20–200 μm) play a key role as marine primary producers and consumers because they produce and supply organic matter to the marine ecosystem (Smatacek 1999, Falciatore & Bowler 2002, Han et al. 2002). Furthermore, the genetic diversity of protistan microplankton plays an important role in explaining the interaction of protistan species with the environment, as these interactions will structure the ecosystem (Medlin et al. 2000).

Molecular biological analyses have been carried out to clarify protistan diversity and community structure and how these features are related to ecosystem function. Various studies have been conducted to examine both spatial patterns in protistan communities and their diversity (Edgcomb et al. 2002, Lovejoy et al. 2006, Not et al. 2007, Kok et al. 2012b). These studies showed that protistan taxa differ remarkably over a variety of spatial scales associated with a range of oceanographic features. This indicates the potential of protistan communities to rapidly respond to changes in environmental conditions. However, the 18S rRNA gene catalogues, which require sampling from various environments, are still not well-developed and this is hampering their interpretation (Richards et al. 2005).

The Kuroshio Current, which is a western boundary current in the North Pacific, introduces warm, saline water into the temperate zone from the south. The Kuroshio Current

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transports very large amounts of heat and materials; the variations in the water temperature and nutrient load may be of considerable importance for fisheries and the distribution of plankton (Yasuda 2003). Moreover, the Kuroshio Current also acts as a spawning and nursery ground for many migrating fish species, such as tunas, sardines, anchovies, and mackerels (Nakata et al. 2000, Yasuda 2003). When the Kuroshio Current encounters with the cold and fresher Oyashio Current at the northwestern Pacific, it exerts great influence on the oceanographic conditions including the flow characteristics and water properties of the Kuroshio Current (Qiu 2001). Many studies have been conducted to understand the physical dynamics and physic-chemical properties along the Kuroshio Current; however, the plankton diversity and community structure along the Kuroshio Current and its link with changes in the ecological properties are still not clear.

In this study, we used an 18S rRNA gene clone analysis to reveal the protistan microplankton community along the Kuroshio Current. We also attempted to understand the effect of geographical variables on the spatial distribution of protistan microplankton.

Materials and Methods

Sampling and storage of protistan microplankton cells

The present study was conducted at Station 09 (31°59′59″ N, 136°59′59″ E, depth 4,242 m; henceforth S09) on February 28, and Station 18 (35°59′19″ N, 142°05′46″ E, depth 3,618 m; henceforth S18) on the Pacific Ocean side of Japan on March 4, 2011, during the KH-11-3 cruise (Leg-1) of the R/V *Hakuho-Maru*. Twenty liters of surface seawater was collected using a bucket and screened through 180 μm nylon mesh and collected in a poly tank. The cells remained in the pre-screened seawater were collected on Omnipore membrane filters (pore size, 10 μm, 47 mm; Millipore) and fixed with 5% Lugol's solution in filtered seawater. This is to ensure that the naked DNA will not be captured by the membrane filter. The fixed cells were removed from the filters by vortex-induced vibration and collected by centrifugation (Kubota 3700, AF-2724A) for 3 min at 4℃ and 15,000 rpm. Supernatant was then discarded. This procedure was repeated with additional filtered seawater to ensure that all cells were removed from the membrane filters. The collected cells were stored at −25℃ until further analysis.

Environmental DNA extraction and nested PCR amplification

The protistan microplankton cells were resuspended in 100 μL of TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0) buffer containing Triton X-100 (0.2%, w/v) and then boiled at 70℃ for 5 minutes, followed by DNA extraction using a DNA extraction machine (Precision System Science). The extracted DNA was purified using the GFX PCR DNA and Gel Band Purification kit (GE Healthcare) by following the manufacturer's instructions. The purified DNA was then used for the first PCR amplification of the 18S rRNA gene with the primers PP18S-408F (5′- TACCACATC (T/C) AAGGAAGGCAG) and PP18S-1332R (5′- CTCGTTCGT-TAACGGAATTAAC) (Kok et al. 2012a) with *Ex Taq* DNA polymerase (Takara Bio). The cycling conditions were as follows: 3 min at 94.0℃, 35 cycles of 30 sec at 94.0℃, 30 sec at 62.0℃, 90 sec at 72.0℃, and a final extension of 5 min at 72.0℃. Subsequently, nested PCR was performed using 1 μL of the first PCR reactant and the primers PP18S-431F (5′- GGCGCG(C/T)AAATTACCCAAT(C/A)) and PP18S-1133R (5′- TCAGCCTTGCGACCATACTC); the cycling conditions were as follows: 3 min at 94.0℃, 30 cycles of 30 sec at 94.0℃, 30 sec at 62.0℃, 1 min at 72.0℃, and a final extension of 5 min step at 72.0°C. The amplified DNA was purified using the aforementioned GFX kit.

18S rRNA gene clone analysis and phylogenetic tree construction

Cloning protocols were followed for the outgrowth, plat-

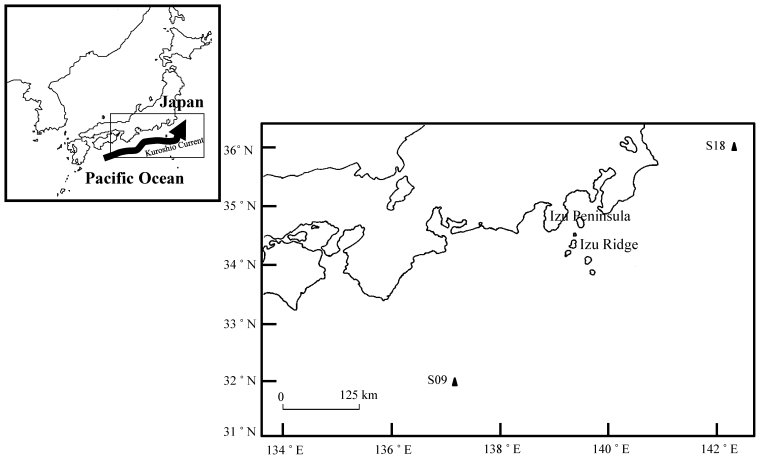

Fig. 1. The location of the sampling sites, Stations 09 (S09) and 18 (S18) along the Kuroshio Current, Japan.

ing and selection of colonies (Kok et al. 2012a). The inserted 18S rRNA gene in each selected colony was amplified by PCR using 1 μL of the culture as the template with the primers PP18S-431F and PP18S-1133R as mentioned above. The PCR procedure was the same as that for nested PCR. The 18S rRNA gene sequences of individual operational taxonomic unit (OTU) representative clones were sequenced and compared with the 18S rRNA gene sequences published in the National Center for Biotechnology Information (NCBI) DNA database using BLAST (BLASTN; http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al. 1990) to identify individual clones. Similarities of more than 98% with known species were considered to indicate the same phylotype, while those from 93.0 to 97.9% were considered to indicate the same genus, those from 87.0 to 92.9% were considered to indicate the same family, and less than 86.9% similarity was considered to indicate the same order. The taxonomic classification of protists in this study followed that of Hausmann et al. (2003). The nucleotide sequences of the 183 phylotypes are available in the DDBJ/EMBL/GenBank databases under the accession numbers AB827444–AB827629.

To analyze the phylogenetic relationships between the clones and previously reported protistan plankton 18S rRNA gene sequences, neighbor-joining trees were constructed for dinoflagellates, diatoms, and others using the CLUSTAL W ver. 1.83 program (Thompson et al. 1994) and GENETYX version 12.1.0 software with the outgroup species *Rhodella violacea* (Kornmann), a member of the Rhodophyta that is situated near the protistan microplankton group on the 18S rRNA gene phylogenetic tree (Adachi 2000). Bootstrap values were estimated from 1,000 replicates.

Diversity coverage and index

The diversity coverage (homologous coverage), *Cx*, was calculated as follows: $Cx=1-N/n$, where N is the number of phylotypes in the sample, and n is the total number of analyzed clones (Good 1953, Singleton et al. 2001). The Shannon-Wiener diversity index, H, was calculated as follows: $H=-\sum (pi)(\ln pi)$, where pi is the proportion of the ith phylotype (Margalef 1958).

Results

Community distribution and diversity of protistan microplankton along the Kuroshio Current according to an 18S rRNA gene clone analysis

Two 18S rRNA gene clone libraries were constructed independently by using water samples collected from S09 and S18. One hundred and twenty-four clones were sequenced for S09, and 133 clones were sequenced for S18. A total of 257 clones were identified, consisting of 117 clones attributed to dinoflagellates, 62 clones attributed to diatoms, and 78 clones affiliated with other protists, such as Ciliophora, Cryptophyta, Cryptophyta nucleomorphs, Choanoflagellata, Chlorophyta, Cercozoa, and Heterokonta (other than diatoms) (Table 1).

Dinoflagellate (Dinoflagellata) community

At S09, 72 clones were affiliated with dinoflagellates and could be classified into 43 phylotypes, whereas 45 clones were affiliated with dinoflagellates and could be classified into 26 phylotypes at S18. All of these phylotypes were affiliated with *Blastodinium*, *Ceratium*, *Gyrodinium*, *Karlodinium*, *Takayama*, and 14 uncultured groups.

The uncultured groups derived from both stations belonged to the Marine Alveolates Group (MALV) (Díez et al. 2001, López-Gracía et al. 2001, Moon-van der Staay et al. 2001), uncultured Blastodiniales, uncultured Gymnodiniales, uncultured *Gymnodinium* sensu stricto, uncultured Kareniaceae, uncultured Peridiniales, uncultured Prorocentrales, uncultured Suessiales and uncultured Kuroshio Dinoflagellate groups I to VI (Table 1, Fig. 2). Within the MALV group, two phylotypes (HP09-83 and HP18-15) affiliated with the Syndiniales Group II were detected from both stations and showed 91–99% similarity with uncultured Syndiniales clone PROSOPE.EM-5 m.186 (Guillou et al. 2008). The other two phylotypes that were identified as belonging to the same group were HP09-87, which showed 90% similarity with the uncultured marine Syndiniales clone RA080215T.008 isolated from the English Channel (Marie et al. 2010), and HP09-71, which showed 93% similarity with the uncultured marine Syndiniales clone BIO9_E2 isolated from the deep sea (Sauvadet et al. 2010).

Six clusters of uncultured Kuroshio Dinoflagellates were found. All the phylotypes in the uncultured Kuroshio Dinoflagellate I group showed significant similarity with clone PA28 isolated from Sagami Bay, Japan (Kok et al. 2012b). The three phylotypes affiliated with the uncultured Kuroshio Dinoflagellate II group showed 92–94% similarity with clone SCM37C27, which was isolated from Sargasso Sea Eddies (DNA database Acc. No. AY664962). Most of the phylotypes recognized as members of the uncultured Kuroshio Dinoflagellate III group showed 94–99% similarity with an uncultured marine dinoflagellate that was detected offshore of southeastern North Carolina (DNA database Acc. No. FJ914412). Conversely, all the phylotypes affiliated with the uncultured Kuroshio dinoflagellates IV group showed 93–99% similarity with the uncultured eukaryote clone CC02A175.085 detected in the South China Sea (DNA database Acc. No. JX188356). The uncultured Kuroshio Dinoflagellate V group and the uncultured Kuroshio Dinoflagellate VI group each contained four phylotypes, showing 94–98% similarity with the uncultured eukaryote clone SCM15C83 detected in the Sargasso Sea (DNA database Acc. No. AY664957) and 95–98% similarity with the uncultured dinoflagellate clone W159H8 detected in the Ross Sea in Antarctica (DNA database Acc. No. AY429071).

Diatom (Bacillariophyceae) community

Nineteen diatom clones derived from S09 were classifiable into 17 phylotypes, and 43 diatom clones from S18

Table 1. Protistan microplankton detected by the 18S rRNA gene clone analysis at Stations 09 and 18.

Phyla Subphyla Classes	Affiliation	Number of clones (Number of phylotypes)		Total clones (Total phylotypes)
		Station 09	Station 18	
Alveolata				
Dinoflagellata	*Blastodinium*	2 (1)		2 (1)
	Ceratium	1 (1)		1 (1)
	Gyrodinium	4 (3)	23 (9)	27 (10)
	Karlodinium	2 (2)		2 (2)
	Takayama	1 (1)		1 (1)
	Syndiniales Group II (MALV)	2 (2)	1 (1)	3 (3)
	MALV	1 (1)		1 (1)
	Uncultured Blastodiniales	1 (1)	2 (1)	3 (2)
	Uncultured Gymnodiniales		1 (1)	1 (1)
	Uncultured *Gymnodinium* sensu stricto	17 (1)	3 (1)	20 (1)
	Uncultured Kareniaceae	5 (4)		5 (4)
	Uncultured Peridiniales	2 (1)	1 (1)	3 (2)
	Uncultured Prorocentrales		1 (1)	1 (1)
	Uncultured Suessiale	1 (1)		1 (1)
	Uncultured Kuroshio Dinoflagellate I	5 (5)	1 (1)	6 (6)
	Uncultured Kuroshio Dinoflagellate II	1 (1)	2 (2)	3 (3)
	Uncultured Kuroshio Dinoflagellate III	21 (12)	4 (2)	25 (14)
	Uncultured Kuroshio Dinoflagellate IV	2 (2)	2 (2)	4 (3)
	Uncultured Kuroshio Dinoflagellate V	2 (2)	2 (2)	4 (4)
	Uncultured Kuroshio Dinoflagellate VI	2 (2)	2 (2)	4 (4)
	Subtotal for Dinoflagellata	72 (43)	45 (26)	117 (65)
Ciliophora	*Laboea*		1 (1)	1 (1)
	Parastrombidinopsis		1 (1)	1 (1)
	Salpingella	3 (3)	1 (1)	4 (4)
	Strombidium	1 (1)	5 (5)	6 (6)
	Varistrombidium		1 (1)	1 (1)
	Uncultured Kuroshio Ciliophora I	1 (1)	11 (4)	12 (5)
	Uncultured Kuroshio Ciliophora II	8 (7)	2 (2)	10 (9)
	Uncultured Kuroshio Urostylida	4 (4)	2 (2)	6 (5)
Heterokonta				
Chromista				
Bacillariophyceae	*Arcocellulus*	1 (1)		1 (1)
	Chaetoceros		1 (1)	1 (1)
	Cerataulina		2 (2)	2 (2)
	Detonula		1 (1)	1 (1)
	Ditylum	1 (1)		1 (1)
	Eucampia		1 (1)	1 (1)
	Hemiaulus		2 (2)	2 (2)
	Navicula		1 (1)	1 (1)
	Odontella		1 (1)	1 (1)
	Pseudo-nitzschia	7 (7)	1 (1)	8 (8)
	Skeletonema	1 (1)	4 (2)	5 (3)
	Stephanopyxis		3 (1)	3 (1)
	Thalassiosira	1 (1)	1 (1)	2 (2)
	Uncultured Kuroshio Hemiaulales I	3 (2)	5 (4)	8 (6)
	Uncultured Kuroshio Hemiaulales II		9 (6)	9 (6)
	Other Uncultured Hemiaulales		1 (1)	1 (1)
	Uncultured Kuroshio Thalassiosirales I	2 (2)	4 (4)	6 (6)
	Uncultured Kuroshio Thalassiosirales II	1 (1)	2 (2)	3 (3)
	Uncultured Melosirales	2 (1)	4 (2)	6 (2)
	Subtotal for Bacillariophyceae	19 (17)	43 (33)	62 (49)
Others	*Florenciella*	1 (1)		1 (1)
	Pelagomonas	1 (1)	1 (1)	2 (1)
	Pelagococcus		1 (1)	1 (1)
	Uncultured Pelagomonadales	1 (1)		1 (1)

Table 1. (Continued.)

Phyla Subphyla Classes	Affiliation	Number of clones (Number of phylotypes)		Total clones (Total phylotypes)
		Station 09	Station 18	
Cryptophyta	*Geminigera*	1 (1)		1 (1)
	Plagioselmis		3 (2)	3 (2)
	Teleaulax		1 (1)	1 (1)
Cryptophyta nucleomorph	*Rhinomonas*	1 (1)	1 (1)	2 (2)
Opisthokonta Choanozoa Choanoflagellata	*Stephanoeca*	2 (1)		2 (1)
Viridiplantae Chlorophyta	*Micromonas*	8 (4)	5 (3)	13 (5)
	Ostreococcus		1 (1)	1 (1)
	Uncultured Mamiellales	1 (1)		1 (1)
Cercozoa	*Cryothecomonas*		3 (2)	3 (2)
	Uncultured Cercomonadida		1 (1)	1 (1)
	Uncultured Thecofilosea		2 (2)	2 (2)
Other protist	MAST-3		1 (1)	1 (1)
			1 (1)	1 (1)
Total of all clones		124 (87)	133 (93)	257 (171)

were classifiable into 33 phylotypes. These phylotypes were affiliated with *Arcocellulus*, *Chaetoceros*, *Corataulina*, *Detonula*, *Ditylum*, *Eucampia*, *Hemiaulus*, *Navicula*, *Odontella*, *Pseudo-nitzschia*, *Skeletonema*, *Stephanopyxis*, *Thalassiosira*, and uncultured groups affiliated with Hemiaulales, Thalassiosirales and Melosirales (Table 1, Fig. 3).

All phylotypes affiliated with uncultured Kuroshio Hemiaulales I group showed 89–93% similarity with the uncultured marine diatom clone RA080215N.027 isolated from the English Channel (Marie et al. 2010). The uncultured Kuroshio Hemiaulales II group only consisted exclusively of phylotypes derived from Station 18 and showed 89–91% similarity with the uncultured marine diatom clone ANT_16 (DNA database Acc. No. JX840887). The Kuroshio Thalassiosirales I group consisted of six phylotypes which showed 93–99% similarity with the uncultured eukaryote clone C4_G08 isolated from the anoxic Cariaco Basin (Edgcomb et al. 2011), whereas the three phylotypes clustering with the uncultured Kuroshio Thalassiosirales II group showed 93–96% similarity to the uncultured diatom clone PM43 isolated from Sagami Bay, Japan.

Other members of the protistan community

Clones other than dinoflagellates or diatoms were affiliated with Ciliophora (32 phylotypes), Cryptophyta (four phylotypes), Cryptophyta nucleomorphs (two phylotypes), Choanoflagellata (one phylotype), Chlorophyta (seven phylotypes), Cercozoa (five phylotypes), and Heterokonta (four phylotypes other than diatoms) (Table 1, Fig. 4).

In the Cryptophyta, the phylotype H09-62 affiliated with the genus *Geminigera*, was detected at S09. Two phylotypes detected at S18, HP18-33 and HP18-36, were affiliated with *Plagioselmis* and the phylotype HP18-32 was affiliated with the genus *Teleaulax*. Conversely, two cryptophyte nucleomorph phylotypes, HP09-90 and HP18-7, were affiliated with the genus *Rhinomonas*. The Choanoflagellata group consisted of only one phylotype (HP09-59), affiliated with the genus *Stephanoeca*.

In the Chlorophyta, seven phylotypes were detected from both stations. Five phylotypes were affiliated with *Micromonas*, and one phylotype each was affiliated with *Ostreococcus* and uncultured Mamiellales. Most phylotypes in the Ciliophora were affiliated with uncultured groups. The most frequent phylotype in the uncultured Kuroshio Ciliophora I group, HP18-3, showed 98% similarity with the uncultured eukaryote clone ENI47297.00250 (Kim et al. 2011). Most of the phylotypes affiliated with the uncultured Kuroshio Ciliophora II group were significant similar to the uncultured marine ciliate clone DH114_3A07 isolated from the South Atlantic Ocean (Marande et al. 2009). All phylotypes affiliated with the uncultured Kuroshio Urostylida group showed 94–98% similarity to the uncultured eukaryote clone D3P05G10 isolated from the Arctic Ocean (DNA database Acc. No. EF100290).

Members of the Cercozoa were only detected at S18. Of the five phylotypes that were detected, two were affiliated with the genus *Cryothecomonas*, one phylotype was affiliated with uncultured Cercomonadida and two phylotypes were affiliated with uncultured Thecofilosea. Three genera affiliated with the Heterokonta (excluding diatoms) were detected: *Florenciella*, *Pelagomonas*, and *Pelagococcus*,

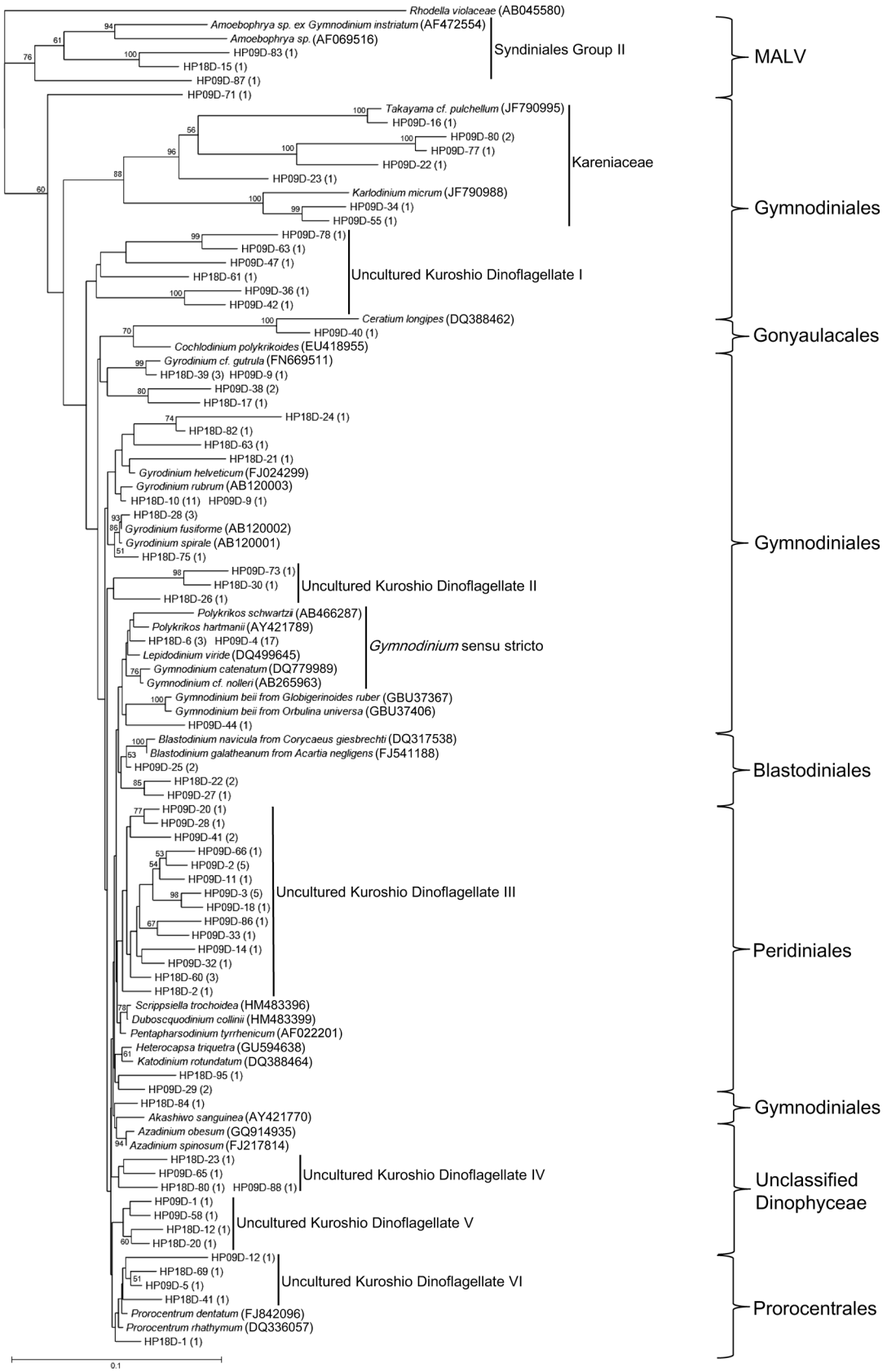

Fig. 2. Neighbor-joining (NJ) tree for dinoflagellate clones. The sequences obtained in this study are indicated by "HP09, HP18 and numbers". HP09 and HP18 represent stations 09 and 18, respectively. The number of clones of each phylotype is indicated in parentheses. Bootstrap values derived from 1,000 replicates are given at the respective nodes (values less than 50% are not shown).

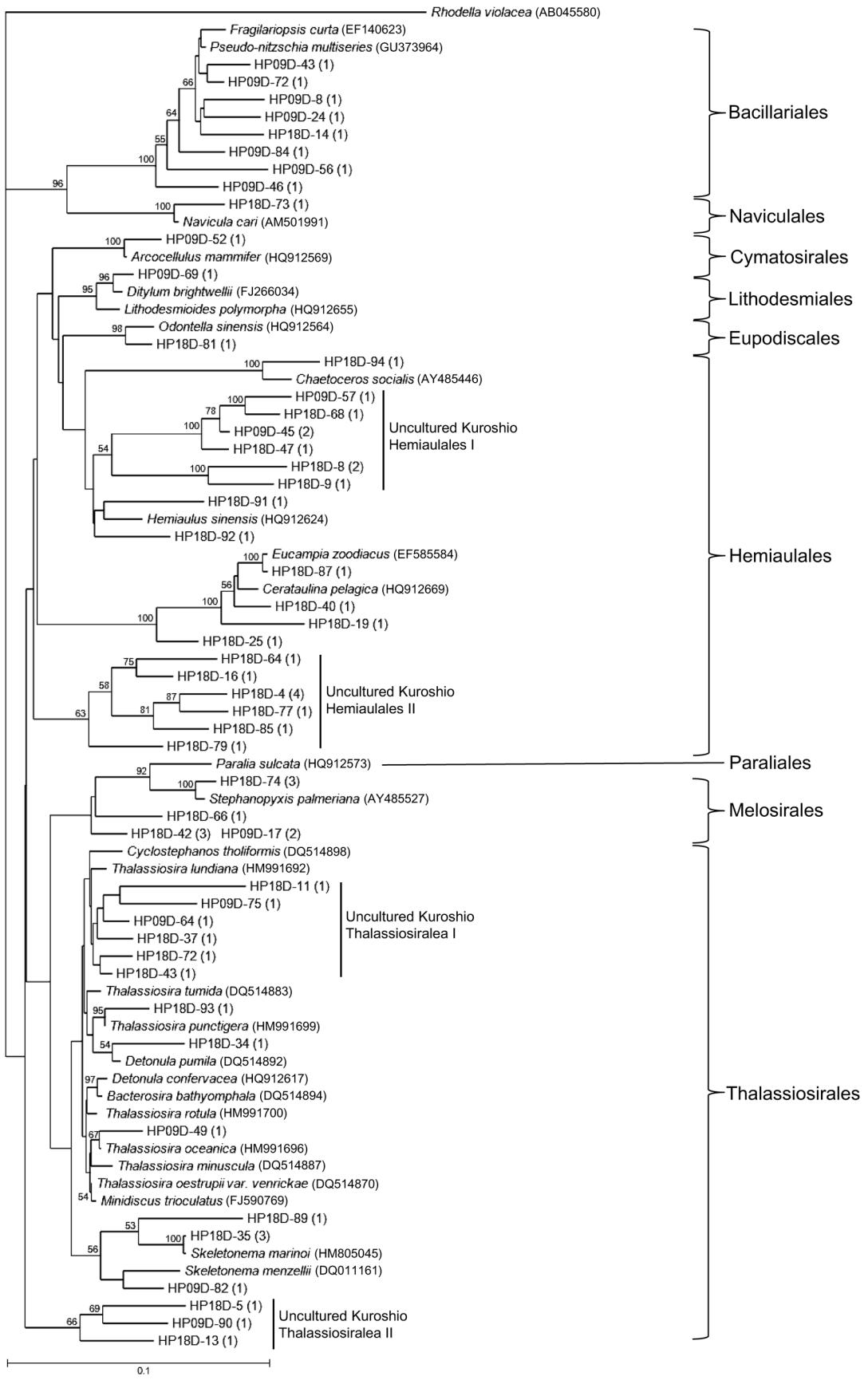

Fig. 3. Neighbor-joining (NJ) tree for diatom clones. See Fig. 2 for further explanation.

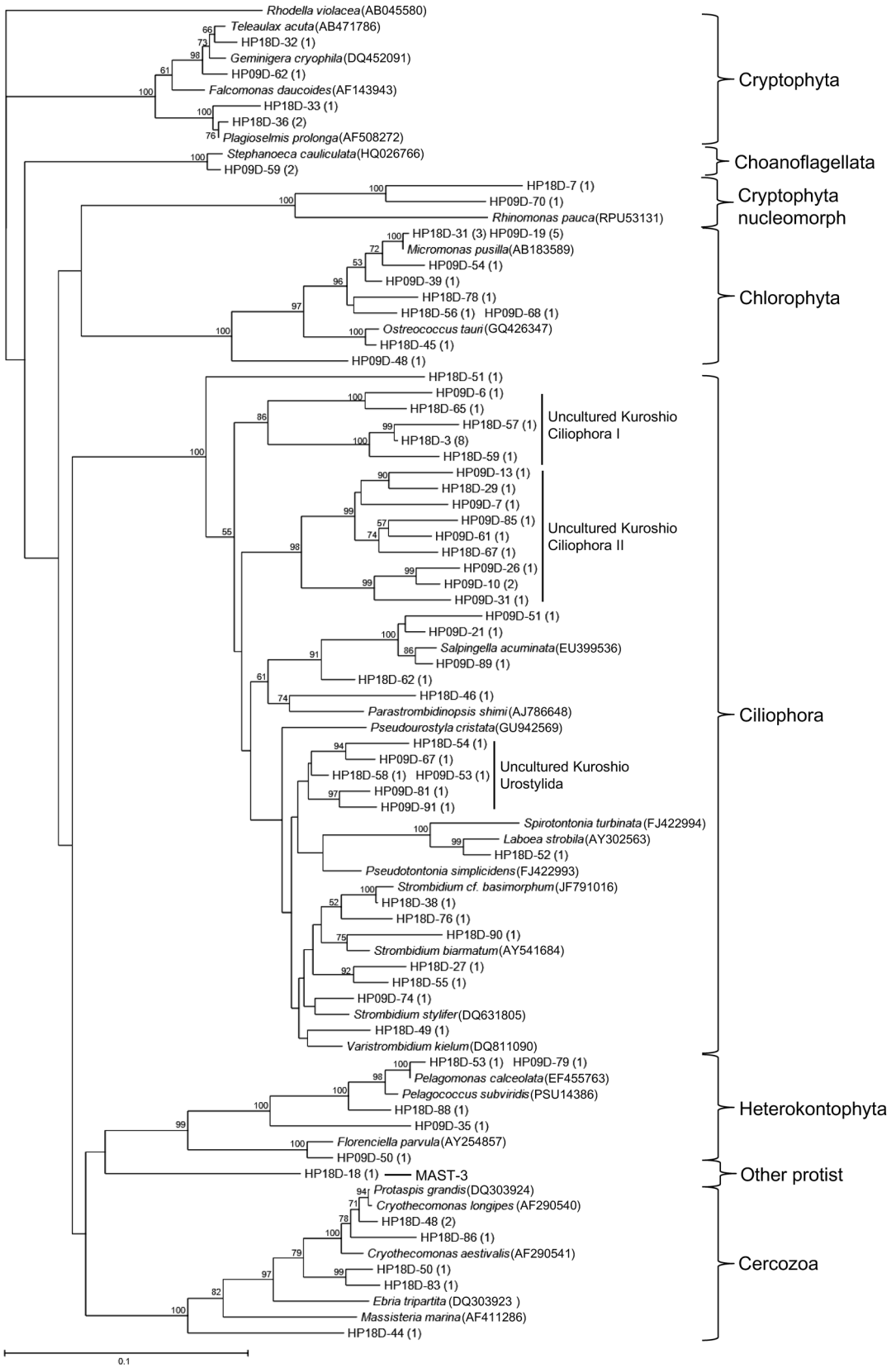

Fig. 4. Neighbor-joining (NJ) tree for clones other than dinoflagellates and diatoms. The phylotypes were grouped on the basis of phylum. See Figs. 2 and 3 for further explanation.

along with an uncultured group - uncultured Pelagomonadales. One phylotype was found to be affiliated with Marine Stramenopiles-3 (MAST-3, Massana et al. 2002), whereas the phylotype HP18-51, which showed no significant similarity with any known species, had a 92% similarity with the uncultured marine eukaryote clone MO010_1.00296 isolated from the North Western Pacific (Caron et al. 2009).

Protistan microplankton richness and distribution

The number of phylotypes (richness) in each clone library was evaluated by using a variety of standard diversity indices (Table 2). According to the richness values (S) obtained with the 18S rRNA gene clone libraries, both stations have similar phylotypes richness, where 87 and 93 phylotypes were obtained from S09 and S18, respectively. A similar result was obtained for the Shannon-Wiener index (H) analysis, where S09 recorded a slightly lower diversity (H= 4.6) and S18 recorded a higher diversity with a value of 5.2. The clone analyses, derived from both stations, pointed to a homologous coverage of 0.3.

Discussion

Protistan microplankton community structure and diversity along the Kuroshio Current

The diversity in the protistan microplankton along the Kuroshio Current was revealed by the 18S rRNA gene clone analysis. We detected a total of 171 protistan phylotypes based on 257 clones derived from surface seawater at S09 and S18. The community consisted of 18 genera of dinoflagellates and diatoms, 16 genera of other protists, and many uncultured groups.

In the dinoflagellate community, we identified phylotypes that were affiliated with *Karlodinium* (HP09-34 and HP09-55) and *Takayama* (HP09-16), which are both toxin producing dinoflagellates (Deeds et al. 2002, Tang et al. 2012). Various species of tunas, sardines and anchovies, mackerels, squids and many other commercially important species are found along the Kuroshio Current, particularly populations of the Japanese sardine (Yasuda 2003). Therefore, the detection method for these harmful plankton hopefully will allow monitoring in order to minimize losses if these plankton are carried into coastal areas where harmful algal blooms could happen. On the other hand, one phylotype was found to be affiliated with *Blastodinium*, which is known to live as parasites in the gut of marine planktonic copepods (Skovgaard et al. 2012). In addition, phylotypes that were affiliated with uncultured Blastodiniales (HP18-22 and HP09-27) are believed to be parasitic plankton, while the phylotype that was affiliated with uncultured Suessiales (HP09-44) is believed to be a symbiotic plankter as it was situated in the same cluster as *Pelagodinium bei* (Spero), which is a symbiotic dinoflagellate from planktonic foraminifera (Gast & Caron 1996). These dinoflagellates have not been reported previously in any studies on plankton carried out along the Kuroshio Current (Furuya & Marumo 1983, Gómez 2007). Three phylotypes (HP09-83, HP18-15 and HP09-87) that were affiliated with Syndiniales Group II, which is known to parasitize dinoflagellates and ciliates (Coats & Park 2002, Chambouvet et al. 2008, Guillou et al. 2008). The detection of these phylotypes was likely to have been correlated with their hosts as a high number of dinoflagellates and ciliates were detected in this study.

Table 2. Statistical analysis of the protistan microplankton community at Stations 09 and 18.

Index	Kuroshio Current community	
	Station 09	Station 18
Phylotype richness *S*	87	93
Shannon-Wiener diversity index *H*	4.6	5.2
Homologous coverage C_x	0.3	0.3

The Kuroshio Current is a warm current entering Japanese waters from the south. However, our clone analysis results revealed phylotypes related to cold water genera, such as *Geminigera* (Cryptophyta) (Scott & van den Hoff 2005). In addition, we identified diatom phylotypes affiliated with the genus *Skeletonema*, which has been reported to be abundant in coastal waters (Sarno et al. 2005) and three phylotypes of *Stephanopyxis*, which is a common diatom genus in tropical waters but has also been found to be carried into colder waters by currents (Guiry & Guiry 2013). These findings suggest that the Kuroshio Current might be a transporter of tropical plankton that helps them intrude into different water columns when the Kuroshio Current collides and mixes with other currents. The clones that were affiliated with *Pelagomonas*, *Pelagococcus*, *Micromonas*, *Ostreococcus* are thought to be nano- or pico-sized plankton, like their affiliated genera. These protistan plankton cells are sometimes found dissolved or attached to other bigger plankters.

The protistan microplankton diversity along the oligotrophic Kuroshio Current is, surprisingly, higher than that determined for a coastal area in our previous study. In our previous study, 191 phylotypes were detected from Sagami Bay based on 1,076 clones (Kok et al. 2012b), whereas 171 phylotypes were detected in this study based on 257 clones. The values of homologous coverage suggest that the number of phylotypes detected in Sagami Bay represented approximately 80% of the real diversity. However, the number of phylotypes detected along the Kuroshio Current represented only approximately 30% of the diversity. The 70% of protistan phylotypes that are assumed to occur through this analysis have not yet been positively identified in the surface waters along Kuroshio Current. This study might not be sufficiently comprehensive to elaborate on the diversity of protistan microplankton along the Kuroshio Current, but it nevertheless, succeeded in detecting highly diverse pro-

tists distributed in the current.

Protistan microplankton community with surface ecological variability

The distribution and diversity of protistan microplankton are strongly affected by ecological variations; for example, temperature is an important physical factor, whereas nutrients are important chemical factors. Moreover, the ecological variability is known to vary considerably in response to the encounters of the Kuroshio Curent and Oyashio Current (Yasuda 2003). Station S09, to the south, was diverse and was dominated by dinoflagellates. S18, to the north, showed a higher diversity of diatoms. Only nine similar phylotypes common to both stations were found among the detected 171 phylotypes. The difference in the protistan microplankton community is believed to be due to differences in the ecology of the surface waters.

The distribution of protistan microplankton most likely reflects their differing nutrient requirements. In this study, we assessed the combinations of elements that may serve as potential factors affecting the growth of protistan microplankton. Dinoflagellates normally bloom during the summer (Ara & Hiromi 2008, Ara et al. 2011), and they are widely known to be more common in warmer rather than colder environments. Indeed, dinoflagellates were found to be more abundant at S09 with a higher surface water temperature (Table 3). Conversely, intrusion of cold water and increases in the nutrient content along the Kuroshio Current has been was recorded when it passes the Izu Ridge. This increase is believed to be caused by the intrusion of the cold Oyashio Current flowing from the north (Qiu 2001). S18 was recorded to have a lower surface seawater temperature and higher nutrient concentration. Therefore, diatoms, which normally dominate the microplankton during spring (Ara et al. 2011), are believed to prevail in the environment at S18.

As shown in Table 3, a low NO_3 : NH_4 ratio was recorded at S09, whereas a high abundance of dinoflagellates was detected at this station, with most of the detected clones affiliated with *Gyrodinium* and *Gymnodinium* sensu stricto. Dinoflagellates uptake and utilize mostly ammonium for synthesis of amino acids and other macromolecules (Collos & Slawyk 1980, Syrett 1981), and the tolerance level of ammonium is species-specific (Dortch et al. 1984). The results of this study suggest that dinoflagellates affiliated with *Gyrodinium* and *Gymnodinium* sensu stricto assimilate more ammonium than other dinoflagellate genera. Conversely, a high SiO_2 : PO_4 ratio was recorded at S18 along with comparatively high diatom abundance. The cell wall of diatoms consists of silica, which is important for diatom biology and ecology (Becker 1996). The diatom community tends to thrive in the presence of silica (Montsant et al. 2005). Diatoms affiliated with Thalassiosirales and Hemiaulales were frequently detected at S18, suggesting that these diatoms utilize the silica from the seawater.

Table 3. Physicochemical parameters of the surface seawater measured at Stations 09 and 18.

Parameters	Kuroshio Current	
	Station 09	Station 18
Temperature (°C)	20.1	17.6
NO_3 : PO_4	10.2	11.7
SiO_2 : PO_4	3.0	11.3
NO_3 : NH_4	3.5	15.7

This study is the first example of a molecular biological analysis of a protistan microplankton community along the Kuroshio Current. The results clearly showed that the diversity of protistan microplankton was surprisingly high along the Kuroshio Current. Moreover, the distribution of protistan microplankton was a function of ecological variables and was significantly impacted by the currents. This study likely contributes to understanding the food resources of migrating fish along the Kuroshio Current.

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