

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/250084211>

Ujiié, Y., and J. H. Lipps. Cryptic diversity in planktic foraminifera in the northwest Pacific Ocean. Journal of Foraminiferal...

Article in The Journal of Foraminiferal Research · August 2009

DOI: 10.2113/gsjfr.39.3.145

CITATIONS

23

READS

98

2 authors:



Yurika Ujiié

Kochi University

43 PUBLICATIONS 707 CITATIONS

SEE PROFILE



Jere H. Lipps

University of California, Berkeley

145 PUBLICATIONS 2,388 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



A Rota dos Fósseis - The Route of the Fossils [View project](#)



Organization of the RCANS 2017 (6th Regional Committee on Neogene Atlantic Stratigraphy), 10-13 July, 2017, Ponta Delgada, AZORES [View project](#)

CRYPTIC DIVERSITY IN PLANKTIC FORAMINIFERA IN THE NORTHWEST PACIFIC OCEAN

YURIKA UJIIÉ^{1,*} AND JERE H. LIPPS²

ABSTRACT

Recent molecular studies show multiple cases of cryptic diversification in planktic foraminifera; however, the geographic distribution of the cryptic species is poorly known. Herein, we present the first insight into the cryptic diversity of planktic foraminifera from Northwest Pacific samples. We obtained 97 new SSU rDNA sequences representing 13 planktic foraminiferal morphospecies, including the first sequence data for *Candeina nitida*. Bayesian phylogenetic analysis of 192 sequences (97 new and 95 known sequences) from 21 morphospecies identified 36 genetic types, including three new ones. Thirty of the genetic types have transoceanic distributions that can be explained by global oceanic circulation patterns, particularly around the Cape of Good Hope.

INTRODUCTION

The biogeography of planktic foraminiferal morphospecies generally correlates with hydrographic conditions, and the approximately 50 modern morphospecies are distributed among five major ecological provinces (Bé, 1977). The 27 warm-water species are classified into four groups whose biogeographic distributions in the Northwest Pacific are restricted to four different water masses: the Kuroshio Current, Central Water, coastal water (continental shelf), and relatively cold water (Ujiié and Ujiié, 2000).

Based on molecular phylogenetic analyses of the partial nuclear small subunit ribosomal RNA gene (SSU rDNA), high cryptic diversity occurs in planktic foraminifera with at least 54 genetic types occurring among 18 planktic foraminiferal morphospecies (Darling and Wade, 2008). Many genetic types have been identified in cold-water species: *Neogloboquadrina pachyderma* (Ehrenberg), *N. incompta* (Cifelli), *Globigerina bulloides* d'Orbigny, and *Turborotalita quinqueloba* (Natland) (Darling and others, 1999, 2000, 2003, 2004, 2007; Darling and Wade, 2008). These were assumed to have restricted distributions in water masses mainly in high-latitude areas around the Arctic, Antarctic, and North Pacific oceans, and in the cold Benguela and California currents. Bipolar distributions of *N. incompta* and *G. bulloides* genetic types indicate that gene flow and speciation patterns in the pelagic microplankton are driven by a combination of geographic barriers and ecological constraints (Darling and others, 2000, 2007).

Cryptic speciation was also recognized in warm-water species, notably *Globigerinoides ruber* (d'Orbigny), *Globigerinella siphonifera* (d'Orbigny), *Orbulina universa* d'Or-

bigny, *Neogloboquadrina dutertrei* (d'Orbigny), and *Globorotalia truncatulinoides* (d'Orbigny) (Darling and others, 1997, 1999, 2003; de Vargas and others, 1999, 2001, 2002; Huber and others, 1997). The biogeographic isolation of genetic types in *G. siphonifera*, *O. universa*, and *G. truncatulinoides* is associated with the hydrographic characteristics of their habitats along extensive transects in the North to South Atlantic, eastern Pacific, and eastern Indian oceans (de Vargas and others, 1999, 2001, 2002, 2004; Darling and Wade, 2008).

Most previous molecular work on planktic foraminifera, especially warm-water species, used few data from the Pacific Ocean (Darling and Wade, 2008). In particular, molecular data from the Northwest Pacific is absent (Fig. 1a). This area is hydrographically variable because of the subtropical gyre and the marginal seas affected by continental shelf waters like the East and South China seas (e.g., Ujiié and Ujiié, 2000; Xu and others, 2005). In the Northwest Pacific, thermohaline circulation (i.e., Global Oceanic Circulation), which starts in the North Atlantic, upwells to the surface and proceeds southwesterly. Therefore, additional data on the geographic distribution of planktic foraminiferal genetic types in the northwestern Pacific might enhance our understanding of the gene flow of warm-water species between the Atlantic and Pacific oceans, which has been a significant biogeographic problem.

In this study we report the nuclear SSU rDNA sequences of 13 planktic foraminiferal morphospecies collected in the Northwest Pacific Ocean. Our main purpose is to identify the genetic types and to examine their distributional ranges by analyzing the new Northwest Pacific sequences together with all available sequence data for planktic foraminifera from other areas.

MATERIAL AND METHODS

SAMPLING AND TAXONOMIC IDENTIFICATION

Living planktic foraminifera were collected at nine sites in the Northwest Pacific Ocean (Fig. 1b) with a NORPAC net (63 µm mesh size) towed vertically from 150 m up to the sea surface (water temperature: ~18.5–27.5°C). Six sites are effectively in the North Equatorial and Kuroshio currents, which transfer heat in the subtropical gyre. Three other sites are in the northwestern Central Water mass of the subtropical gyre.

All planktic foraminiferal specimens were picked from a Petri dish under a stereoscopic microscope. Digital images were taken of each specimen after cleaning in filtered seawater. Based on the taxonomic descriptions of Ujiié and Ujiié (2000), 38 specimens were placed into 13 morphospecies representing nine genera (Appendix 1), and all of them were subjected to nucleotide sequence analyses.

¹UMR CNRS 7144 Evolution du Plancton et PaleOceans, Station Biologique, BP 74, 29682 Roscoff, France.

²Museum of Paleontology and Department of Integrative Biology, University of California, Berkeley, CA 94720-4780, USA

*Correspondence author. E-mail: ujiié@sb-roscoff.fr

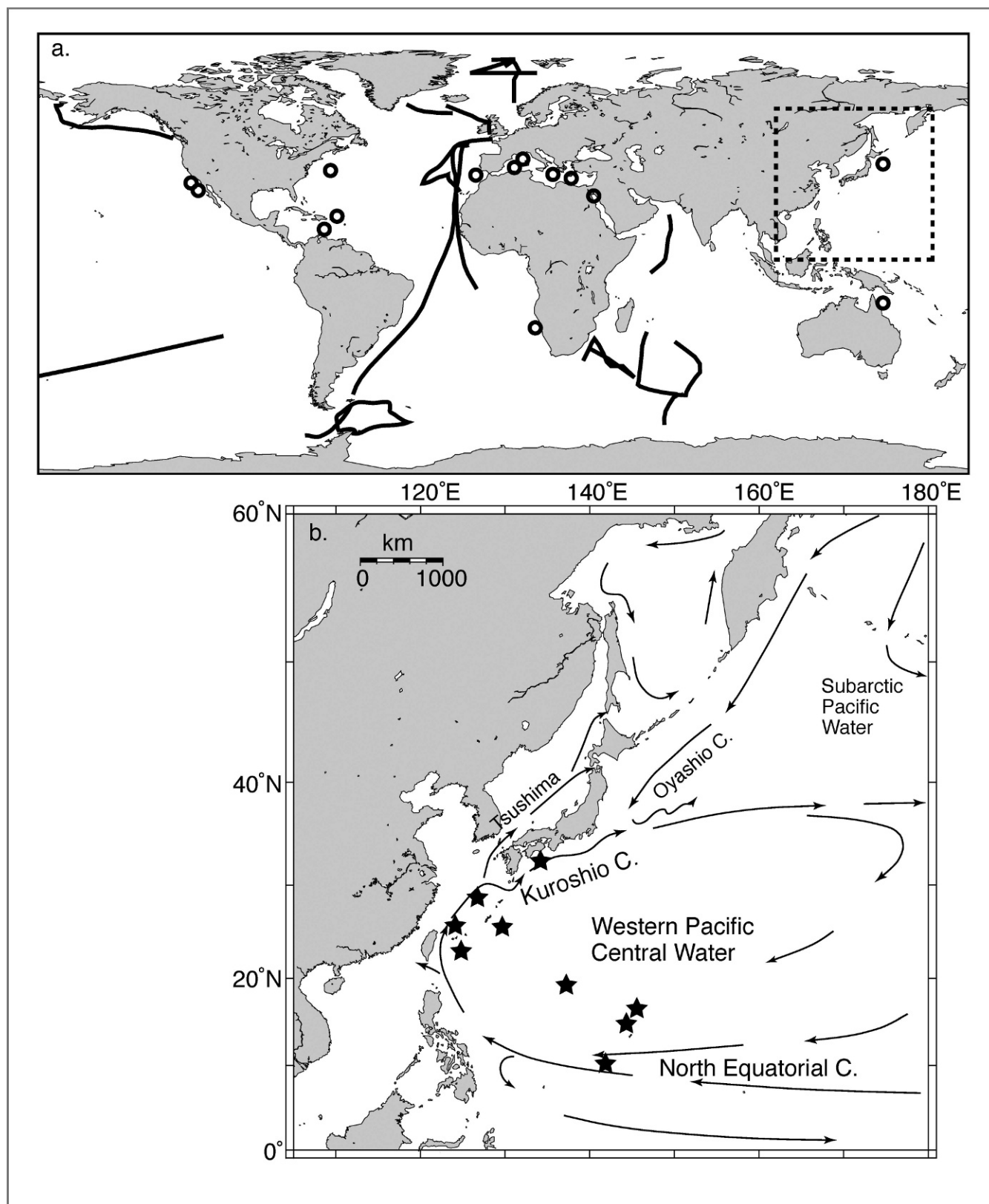


FIGURE 1. Map showing sampling sites (stars) for living planktic foraminifera and the surface currents in the NW Pacific Ocean.

DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

DNA extraction and PCR amplification were performed as described by de Vargas and others (1997). PCR amplification of ~1,200 bp (base pair) region of the terminal 3' end of the foraminiferal rRNA gene was carried out, using the foraminiferal specific primers S14p (5'-AAG GGC ACC ACA AGM GCG-3') or S14fl (5'-AAG GGC ACC ACA AGA ACG C) coupled with universal primer SBf (5'-TGA TCC ATC RGC AGG TTC ACC TAC-3'). PCR products were cloned by a PCR vector system with TOP 10 *E. coli* cell of the Zero Blunt TOPO PCR Cloning Kit (Invitrogen, Carlsbad, CA, USA) and sequenced using primers T3 (5'-ATT AAC CCT CAC TAA AGG GA-3') and T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') with an ABI 377 automated sequencer (Applied Biosystems, Carlsbad, CA, USA) at the Molecular Phylogenetics Laboratory of the Museum of Paleontology, University of California, Berkeley, CA, USA.

Individual sequences were edited and analyzed using Sequence Navigator v. 1.0.1. In this study, we obtained 97 new sequences, which are deposited in the DDBJ/EMBL/Genbank with accession numbers from AB263433 to AB263466 and AB468826 to AB468888.

PHYLOGENETIC ANALYSIS

We analyzed two datasets of foraminiferal partial SSU rDNA in order to avoid bias due to the high heterogeneity of evolutionary rates between the Globigerinidae and two other planktic families (see details in DISCUSSION; Ujiie and others, 2008). The first dataset was composed of 105 sequences that included 45 new sequences of species in the Candeinidae and Globorotaliidae taken from the Northwest Pacific Ocean, 46 sequences of species from the same planktic foraminiferal families from other geographic areas, and 14 sequences of species in the benthic rotaliids and textulariids as an outgroup (Appendix 1). The second dataset comprised 118 sequences including 52 new sequences of species in the Globigerinidae and Hastigerinidae, 49 sequences of the same species from other areas, three sequences of the Globorotaliidae, and 14 sequences of the same benthic foraminifera as in the first dataset. The two sequence datasets were manually aligned in BioEdit (Hall, 1999). The 598 and 528 unambiguously aligned nucleotide sites for the first and second datasets were used for each phylogenetic analysis. In order to obtain detailed phylogeny for the first dataset, we analyzed three sub-trees for *Neogloboquadrina* species, *Globorotalia truncatulinoides*, and *Globigerinita glutinata* (Egger), increasing nucleotide sites to 883, 900, and 899 bp, respectively.

The best-fit models of nucleotide substitution for two datasets were selected by MrModelTest ver. 2.2 (Nylander, 2004). The general time-reversible model (GTR; Yang, 1994a) with variable sites assumed to follow a gamma distribution approximated by four discrete categories (Γ ; Yang, 1994b) was chosen for all analyses. Bayesian phylogenetic analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) for all datasets under the optimal model. The Markov chain Monte Carlo (MCMC) process was set so that four chains (three heated and one cold) ran simultaneously. Two independent runs

were subsequently conducted, and the runs were continued for 1.0×10^6 cycles after reaching stationarity. We pooled all trees after confirming agreement of the estimated parameters between the two independent runs and after a burn-in period from both runs. Posterior probabilities (PP) were estimated based on the pooled trees. Maximum-likelihood (ML) analyses were performed with Treefinder (Jobb and others, 2004) with the same model of evolution. Bootstrap support was based on 100 replicates.

In order to estimate the sequence divergences between genetic types, sequence distances were calculated for pairwise sequence comparisons between the clades within each morphospecies in all trees and sub-trees following a maximum likelihood model within PAUP* 4.0b10 (Swoford, 2002).

RESULTS

PHYLOGENETIC TREE TOPOLOGY

The two planktic families Globorotaliidae and Candeinidae form monophyletic clades with high posterior probabilities (PP) and bootstrap values (BV), based on the analysis of the first dataset (Fig. 2a). The Bayesian topology is almost identical to that of the ML tree, except for the nodes between planktic and benthic foraminifera, the external nodes of *Pulleniatina obliquiloculata* (Parker and Jones), and the basal node of the Candeinidae. The Candeinidae, composed of *Globigerinita glutinata*, *Gt. uvula* (Ehrenberg), and *Candeina nitida* d'Orbigny, nests within the clade including the Discorbiidae, Rosalinidae, and Uvigerinidae in the Bayesian tree. The Globorotaliidae, including the genera *Neogloboquadrina* (*N. dutertrei*, *N. pachyderma*, and *P. obliquiloculata*) and *Globorotalia* [*Gl. inflata*, *Gl. crassaformis* (Galloway and Wissler), *Gl. hirsuta* (d'Orbigny), *Gl. truncatulinoides*, and *Gl. menardii* (Parker, Jones, and Brady)], is sister to the benthic rotaliids *Epistominella vitrea* Parker and *Bulimina marginata* d'Orbigny, though these basal nodes are supported by low PP. In the ML tree, the monophyletic group of the Candeinidae nests in the 14 benthic taxa. The Globorotaliidae is sister to the clade of the Candeinidae + 14 benthics (figure is not shown).

The phylogenetic position of *Candeina nitida*, analyzed here for the first time, branches close to *Globigerinita uvula* and *Gt. glutinata* (Fig. 2a). Although support for the clade that groups these three species is strong, the position of *C. nitida* as sister to *Gt. uvula* is very weakly supported. In the Globorotaliidae clade, all morphospecies form monophyletic clades, except for *N. pachyderma* (Fig. 2a). *Neogloboquadrina pachyderma* forms two clades corresponding to the coiling direction of the shell with high PP and BV. However, exceptions in both clades include two sequences of left-coiled specimens (JP-L-3 and -4) in the right-coiled clade and one sequence of a right-coiled individual (JP-R-3) in the left-coiled clade. These specimens were collected from the temperate area off northeastern Japan (Hokkaido). The phylogenetic relationships between the *Neogloboquadrina* and the other Globorotaliidae are multidivergent (Fig. 2a). The sub-tree of *Neogloboquadrina* shows monophyly of both coiling types of *N. pachyderma* as well as the sister

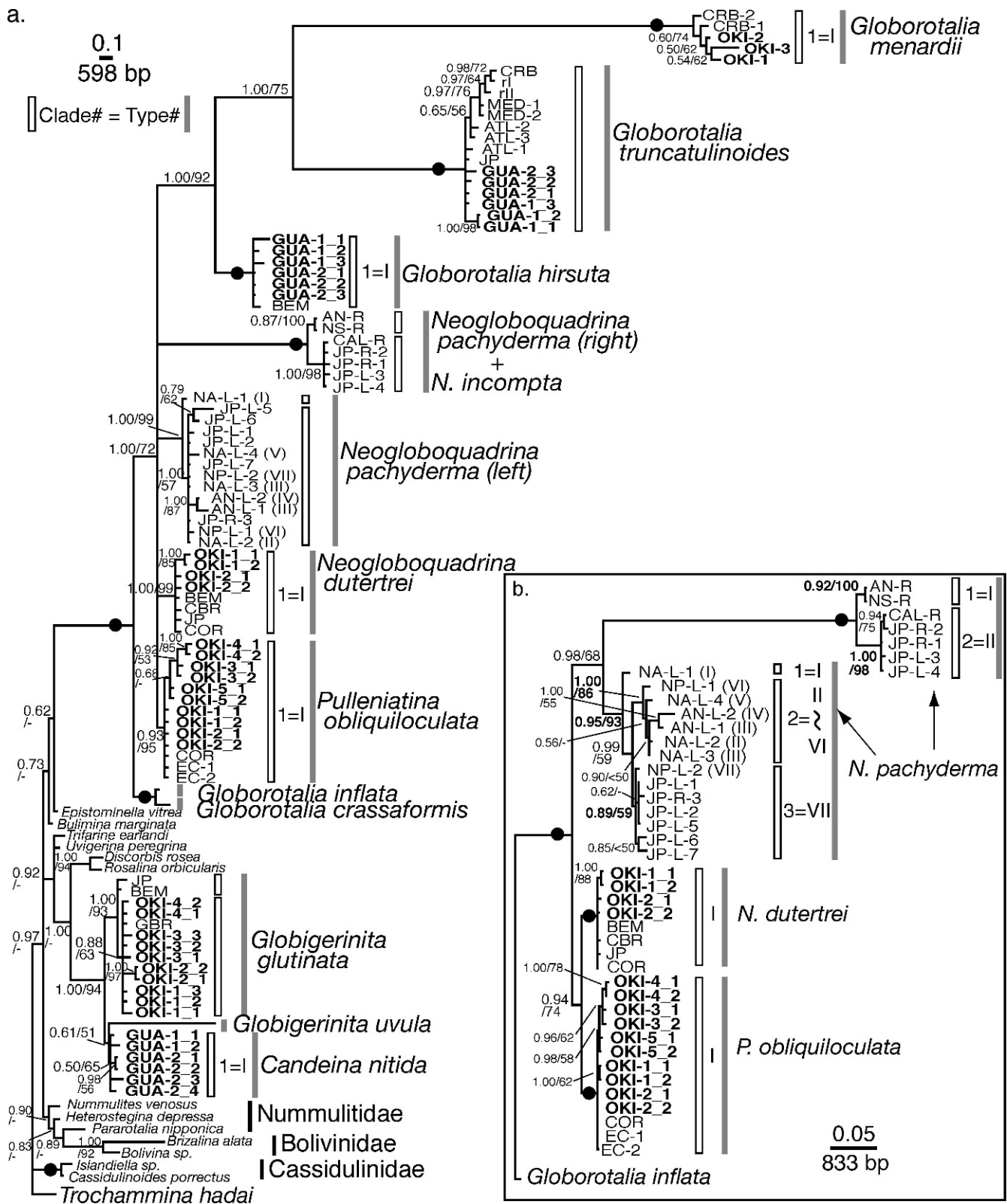


FIGURE 2. The 50% majority consensus trees from the Bayesian analyses of partial SSU rDNA based on (a) 105 sequences of the Globorotaliidae + Candeinidae and benthic foraminifera, (b) 43 sequences of the *Neogloboquadrina* species with *Globorotalia inflata* as an outgroup. Nucleotide sequence codes are named by abbreviations of regions sampled with numerals if multiple sequences were discovered in the same region; bold type denotes novel sequences from the NW Pacific. Shaded bars refer to adjacent morphospecies name; unshaded bars and adjacent Roman numerals denote their genetic types, and hatch marks are aligned with available code names. Numbers above or below each inter-node express Bayesian posterior probability and Bootstrap value of Maximum-Likelihood analysis.

group of *N. dutertrei* + *Pulleniatina obliquiloculata* (Fig. 2b). Three *Globorotalia* species (*Gl. hirsuta*, *Gl. truncatulinoides*, and *Gl. menardii*) are monophyletic, while *Gl. inflata* + *Gl. crassaformis* branches as a sister group to all Globorotaliidae.

The second dataset shows the phylogenetic relationships of the families Globigerinidae and Hastigerinidae (Fig. 3). The Hastigerinidae [i.e., *Hastigerina pelagica* (d'Orbigny)] branches as a sister group to all Globigerinidae species with high PP and BV. The clade of Globigerinidae + Hastigerinidae is sister to the Globorotaliidae, though the PP and BV are low (0.70 PP and 52% in Fig. 3). Each of the eight morphospecies in the Globigerinidae + Hastigerinidae forms a monophyletic group, except for *Globigerinella calida* (Parker) and *Globigerinoides ruber*. *Globigerinella calida* nested in the clade of *Glla. siphonifera* and one sequence of *Gln. ruber* branches with *Gln. conglobatus* (Brady). Three groups in the Globigerinidae (i.e., *Gln. sacculifer* + *O. universa*, *Gln. ruber* + *Gln. conglobatus*, and *G. bulloides* + *Turborotalita quinqueloba* (Natland) are supported by high PP, though the BV is relatively low except for the clade of *Gln. ruber* + *Gln. conglobatus*. In addition, *G. falconensis* Blow is sister to the clade of *G. bulloides* + *T. quinqueloba*, and *Glla. siphonifera* is sister to the clade of *O. universa* + *Gln. sacculifer*. However, the relations between these three clades of Globigerinidae are multidivergent.

GENETIC TYPES

Phylogenetic analyses of our data indicates the occurrences of 32 genetic types in 17 of 21 examined morphospecies; *Globorotalia inflata*, *Gl. crassaformis*, *Globigerinita uvula*, and *Globigerina falconensis* are excluded because only a single sequence is present (Figs. 2, 3, and 4). Each genetic type is represented by a robust clade with high support of PP (>0.90) and BV (>85%), and a greater than 2% genetic distance to the other types. Eight out of 17 morphospecies contain multiple genetic types, which so far includes 24 types. Three new genetic types are identified in this study, including two new types in *Globigerinita glutinata* and one new type in *Globorotalia truncatulinoides*. The sub-tree of *Gt. glutinata* identifies two genetic types (Fig. 4a). The comprehensive analyses including all data from the Pacific and Atlantic reveals two genetic types, one of which (Type II in Fig. 4a) occurs only in the Pacific. In the case of *Gl. truncatulinoides*, resolution is insufficient to distinguish the genetic types in the global tree that includes the Globorotaliidae and Candeinidae (Fig. 2a). However, the sub-tree of this morphospecies (Fig. 4b) shows two clades, one of which comprises four previously described types (Type I to IV) and the other is represented by the sequence data from the Northwest Pacific (Fig. 4b). The sequences of the Northwest Pacific clade clearly have a genetic signal corresponding to the genetic type identification in the sequence alignment data, and we identify this clade as the new genetic type V (Fig. 4b).

In the Globorotaliidae, only *Globorotalia truncatulinoides* and *Neogloboquadrina pachyderma* have multiple genetic types (Fig. 2a). Both right- and left-coiled types of *N. pachyderma* have two clades. The sub-tree of *Neogloboquadrina* species strongly indicates the presence of two types in

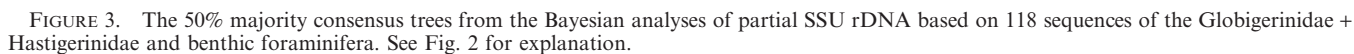
right-coiling *N. pachyderma*: Type I has a bipolar distribution, while Type II is recognized only in the Pacific Ocean (Fig. 2b). In left-coiling *N. pachyderma*, three clades were separated in the sub-tree, though the PP and BV of the clade 3 was low (Fig. 2b). The clades 1 and 3 corresponded to Types I and VII, which were reported only in the Atlantic and Pacific oceans, respectively. The clade 2 included Types II to VI recognized in Darling and others (2007).

All morphospecies in the Globigerinidae and Hastigerinidae, with the exceptions of *Globigerinoides sacculifer*, *Globigerinoides conglobatus* and *Hastigerina pelagica*, are separated into multiple genetic types (Fig. 3). Three types corresponding to three clades are recognized in *Orbulina universa*, while four types are found in *Globigerinella siphonifera* and *Globigerinoides ruber*, of which one in the latter species branches with *Gln. conglobatus* (Fig. 3). The Northwest Pacific specimens of *O. universa* branch with Mediterranean and California specimens in Type I. In *Glla. siphonifera*, only one Type II (named by de Vargas and others, 2001 = Type IIa in Darling and Wade, 2008) was found in the Northwest Pacific in this study. The Northwest Pacific specimens of *Gln. ruber* group into Types II and III (=Types Ia and Ib in Darling and Wade, 2008). Although we have no data from the Northwest Pacific for *Globigerina bulloides* and *Turborotalita quinqueloba*, each morphospecies in our tree forms two clades (Fig. 3). In both morphospecies, clade 1 represents Type I. Types IIa, c, and d of *G. bulloides* and Types IIb and IIc of *T. quinqueloba* are included in clade 2.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

This study analyzed planktic foraminiferal phylogenies separately because the extreme heterogeneity of the SSU rDNA substitution rates makes the phylogenetic relationships between planktic foraminifera difficult to establish. The deep phylogenetic relationships are unclear (Figs. 2a and 3). Indeed, the Globigerinidae evolved fifty to hundred times faster than did some benthic species (Pawlowski and others, 1997), even though comprehensive phylogenetic analysis of many benthic and planktic foraminifera reveals that planktic foraminifera belong in the benthic rotaliid clade (Flakowski and others, 2005; Bowser and others, 2006). The previous SSU rDNA-based phylogenies of planktic foraminifera show the Globigerinidae, Globorotaliidae, and Candeinidae have a polyphyletic origin (Darling and others, 1997, 1999; de Vargas and others, 1997; Stewart and others, 2001) instead of the monophyletic origin inferred from paleontology (e.g., Caron and Homewood, 1983; Tappan and Loeblich, 1988). Most of the previous genetic studies used distance methods to build phylogenetic trees and they included few benthic taxa. Even though trees were analyzed by the Bayesian and maximum likelihood methods using many available sequences from rotaliids, they do not reveal phylogenetic relationships between ancestral lineages of the three planktic families (Ujiié and others, 2008). Moreover, highly divergent rates of rRNA genes were also observed within the Globorotaliidae (de Vargas and Pawlowski, 1998). A limited number of



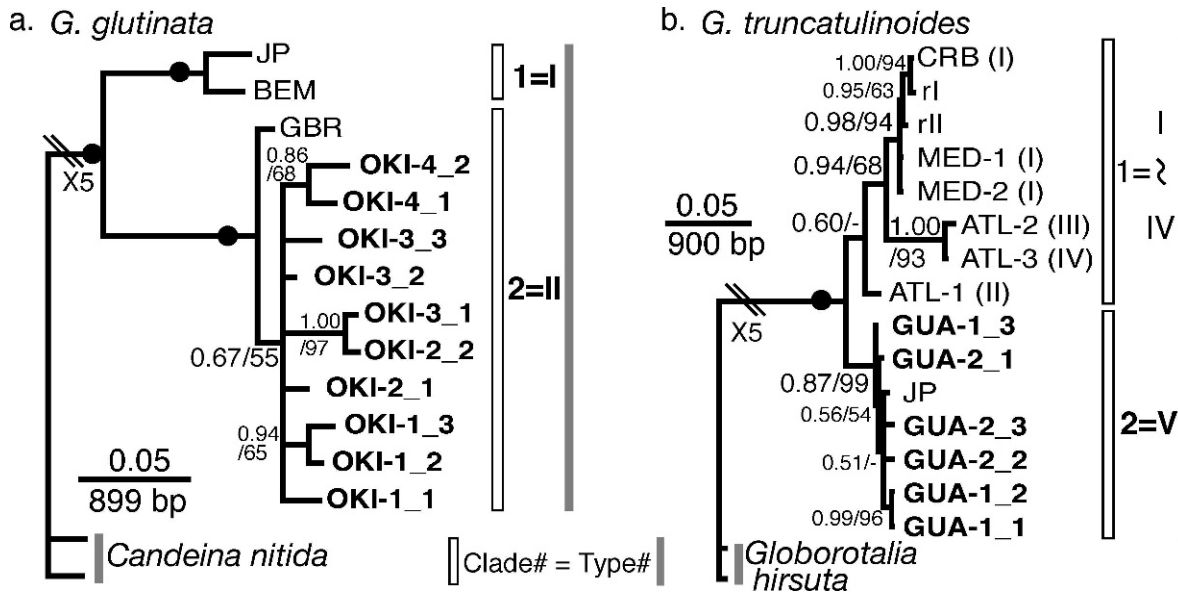


FIGURE 4. The 50% majority consensus trees from the Bayesian analyses of partial SSU rDNA based on (a) 17 sequences of the *Globigerinita glutinata* with *Candeina nitida* as an outgroup, (b) 17 sequences of the *Globorotalia truncatulinoides* with *Glr. hirsuta* as an outgroup.

nucleotide sites around 600 bp is not sufficient to solve the inter-species relationships within the Candeinidae and some of the Globorotaliidae (Fig. 2a).

In the Candeinidae, the phylogenetic position of *Globigerinita uvula* is unclear and incongruent with the paleontological hypothesis in which it is considered closely related to *Gt. glutinata* (Kennett and Srinivasan, 1983). Although *Gt. uvula* branches as sister to *Candeina nitida*, the whole sequence of *Gt. uvula* is clearly different from the sequences of *Gt. glutinata* and *C. nitida*, especially within variable regions (data available from YU upon request).

In the Globorotaliidae, the monophyletic clade of *Globorotalia menardii*, *Glr. truncatulinoides*, and *Glr. hirsuta* (Fig. 2a) is robust and agrees with previous findings of de Vargas and others (1997). The relationship between *Glr. menardii* and *Glr. truncatulinoides* differs from the paleontological hypothesis that considers *Glr. menardii* as one of the earliest lineages in the genus *Globorotalia* (e.g., see Kennett and Srinivasan, 1983). The grouping between these two rapidly-evolving species results from long-branch attraction (LBA). The same phenomenon also influences the phylogenetic position of *Glr. menardii* in the tree of Darling and others (1999), where this species branches with the Globigerinidae and *Neogloboquadrina dutertrei* is used as the outgroup. Another incongruence between molecular and fossil data is the phylogenetic position of *Glr. inflata*, which is an outgroup of the Globorotaliidae (Fig. 2a) as shown by the tree of de Vargas and others (1997); however, it has been considered a member of another *Globorotalia* lineage descended from the common ancestor of other *Globorotalia* species (Kennett and Srinivasan, 1983). Moreover, *Glr. crassaformis*, which is considered an ancestor of *Glr. truncatulinoides* in the paleontological hypothesis, branches with *Glr. inflata*. When we increased the number of sites within the *Neogloboquadrina* clade to ~800 bp (Fig. 2b), the sister group relationships of *Pulleniatina obliquiloculata* + *N. dutertrei* and *N. pachyderma*

are the same as those determined by Darling and others (2003, 2004); this finding supports the paleontological hypothesis.

In the Globigerinidae, inter-species relationships within clades of *Globigerinoides sacculifer* + *Orbulina universa*, *Gln. ruber* + *Gln. conglobatus*, and *Globigerina falconensis* + *G. bulloides* + *Turborotalita quinqueloba* agree with previous work (Darling and others, 1997, 1999, 2006; de Vargas and others, 1997; Stewart and others, 2001). However, phylogenetic relationships between the three clades are unresolved (Fig. 3). The phylogenetic relationships in our data differ from those in the previously published trees. The *Gln. ruber* + *Gln. conglobatus* clade is an outgroup to the others in all previous trees except for those of Darling and others (1997), who did not obtain high statistical values in each tree. The phylogenetic position of *G. bulloides* differs between de Vargas and others (1997) and Darling and others (1999) because the species has a fast evolutionary rate and these two studies had different taxonomic samplings; de Vargas and others (1997) included another fast-evolving species, *Hastigerina pelagica*. Even after removing *T. quinqueloba*, which shows the longest branch in the tree, the tree topology did not change (data available from YU upon request). On the other hand, *Hastigerina pelagica* is the outgroup for the *Globigerinoides* and *Globigerinella siphonifera* clades (Fig. 3) in the same position as in the NJ and MP trees of de Vargas and others (1997). This phylogenetic position contradicts the paleontological hypothesis that *H. pelagica* diverged from *Glla. siphonifera* and might indicate a missing fossil record for *H. pelagica*, whose thin and fragile shell might be prone to dissolution in the sediment (Hemleben and others, 1979; Caron and others, 1990).

GENETIC TYPES

SSU rDNA sequences identify 24 of 32 genetic types in eight morphospecies (Figs. 2a and 3), though we find fewer

genetic types in the morphospecies *Globorotalia truncatulinoides*, *Neogloboquadrina pachyderma*, *Globigerina bulloides*, and *Turborotalita quinqueloba* than previous works (Darling and others, 2000, 2006, 2007; de Vargas and others, 2001). Except for *G. truncatulinoides*, the genetic types have identical mutations in the variable region of the sequence that is mostly omitted in the phylogenetic analyses. The original discrimination of genetic types in *N. pachyderma*, *G. bulloides*, and *T. quinqueloba* corresponded to other information characteristic of mutation in the variable region and to their ecological preferences inferred from their biogeographic distributions. Therefore, most genetic types cannot be described by a robust (high PP-high BV) clade with >2% genetic distance in our trees (Figs. 2b, 3), but we lack new data for these species. In clade 2 of *G. bulloides* and *T. quinqueloba*, two subclades probably are separated into Type IIa + c + d and Type IIb, and Type IIb + c and Type IIa, respectively. *Neogloboquadrina pachyderma* has two genetically distinct lineages between right- and left-coiling forms (Fig. 2a) as shown in Darling and others (2006). Here the right-coiling *N. pachyderma* is renamed as *Neogloboquadrina incompta*, in accordance with the morphological description of Cifelli (1961). However, other data on the Northwest Pacific specimens (Kimoto and Tsuchiya, unpublished) show an incongruence between those coiling directions and phylogenetic positions – two left-coiling sequences (JP-L-3, -4) belong to the clade of right-coiling forms, whereas one right-coiling sequence (JP-R-3) is in the left-coiling clades (Fig. 2b). Whereas some specimens from temperate areas might be aberrant forms (Bauch and others, 2003), a more precise morpho-molecular description of *N. pachyderma* is needed.

This study identifies three new genetic types. Two genetic types of *Globigerinita glutinata* clearly formed monophyletic groups in the resultant tree and sub-tree (Figs. 2a, 4a). These two types differ by ~4% of genetic distance in the sub-tree, although insufficient data is available to determine the morphological differences between them. In general, the *Gt. glutinata* morphospecies is widespread in the world ocean (Bradshaw, 1959). Perhaps these two types correspond to different water masses in the Pacific, as Type I (JP) is from temperate latitudes and Type II (OKI, GBR) is from subtropical latitudes (Appendix 2).

In *Globorotalia truncatulinoides*, genetic Type V revealed by this study is the sister group to the other types (Fig. 4b). Types I, II, III, and IV of *Glr. truncatulinoides* as reported by de Vargas and others (2001) are based on SSU rDNA and ribosomal ITS (Internal Transcribed Spacer) sequences. As shown in Fig. 2a, the SSU rDNA is too conservative to separate these genetic types, in particular Types III and IV. In the alignment data of SSU rDNA sequences, few mutations occur between Types III and IV. On the other hand, sequences of our Pacific specimens categorized into Type V have more numerous single mutations than other types. In the sub-tree (Fig. 4b), the sequence divergence of the four known types (Types I to IV) ranges from 1.2 to 6.7% (de Vargas and others, 2001). Type V, however, is much different, with divergences ranging between 3.9% (Type II) and 8.0% (Type I). Type V is represented only by sequences from the Northwest Pacific, including a sequence

taken from northern Japan (Kimoto and Tsuchiya, unpublished). This type was collected from oligotrophic subtropical areas in the Central Water of the Northwest Pacific that is similar to the hydrographic preference of Type II (de Vargas and others, 2001). In the global distribution of *Glr. truncatulinoides*, Type II is found only in the Atlantic Ocean, whereas the other genetic Types I, III, and IV occur in the Atlantic, Indian, and Southeast Pacific oceans (de Vargas and others, 2004) (Appendix 2). The morphological shape of Type V is similar to Types I and II, but all specimens in this study are right-coiling forms. The right-coiling form is recognized only in Type II (de Vargas and others, 2001).

Four genetic types are identified in *Globigerinella siphonifera*. This is the morphospecies in which cryptic speciation was first discovered among planktic foraminifera (Huber and others, 1997; Darling and others, 1997). The initial two genetic types (Types I and II) are characterized by different metabolic rates and depth habitats (Huber and others, 1997). Their geographic distribution is different as well, with Type I present in oligotrophic water while Type II is cosmopolitan (de Vargas and others, 2002) (Appendix 2). Type II, a deeper dweller with a high metabolic rate, was recognized as four types by Darling and others (1999) and de Vargas and others (2002), who gave them different sets of names. In our tree, the node between Type IV and Types II and III is supported by relatively low PP and BV, but the genetic distance between them is from 4.0 to 6.0%. Here we confirm the occurrence of Type II in the Northwest Pacific (Fig. 3). All specimens were collected from the Kuroshio Current where the mixed layer is well developed. *Globigerinella calida* nested in Type II of *Glla. siphonifera*. The *Glla. calida* morphospecies sequence was later identified as Type IV (Stewart, 2000).

Multiple genetic types have also been reported in *Orbulina universa* and *Globigerinoides ruber*. The former was considered to consist of three types inferred by using the most variable region of the SSU rDNA (ca. 425/950 bp; de Vargas and others, 1999). The Northwest Pacific specimens of *O. universa* branch with the Mediterranean type identified as Type I in our tree (Fig. 3). This genetic type appears to be characteristic of tropical to subtropical waters with relatively high chlorophyll concentration as well as upwelling areas in the Atlantic Ocean (de Vargas and others, 1999).

In the case of *Globigerinoides ruber*, three genetic types and two non-pigmented phenotypes (Types II and III) occur in the Northwest Pacific Ocean. Another genetic type (Type I) with a pink-pigmented phenotype occurs only in the Atlantic Ocean. The same types were previously reported from other areas (de Vargas and others, 1997; Darling and others, 1999). In addition, another genetic type of *Gln. ruber* with a non-pigmented phenotype (Type IV) collected off California (Type IIa in Darling and others, 1999) branches as a sister group to *Gln. conglobatus*. The two lineages of *Gln. ruber* might have evolved from *Gln. subquadratus* Brönnimann or *Glr. obliquus* Bolli (Darling and others, 1999). Both lineages might have diverged from a common ancestor, either *Gln. primordius* or *Gln. altiapertura*, based on the similarities between juvenile tests of *Gln. subquadratus* and *Gln. obliquus* (Cordey, 1967). The

morphological classification of *Gln. obliquus* remains uncertain. Here, we consider *Gln. ruber* as a paraphyletic lineage from which *Gln. conglobatus* descended (Fig. 3). These two major lineages of *Gln. ruber* reflect morphological and ecological differences based on the molecular analysis of central Pacific specimens (Kuroyanagi and others, 2008). Types I to III are identified as *Gln. ruber* s.s., which is dominant at shallow depths, whereas Type II is *Gln. ruber* s.l., which is dominant in deeper water.

GEOGRAPHIC DISTRIBUTION OF GENETIC TYPES

Most genetic types have broad, transoceanic distributions unrestricted by physiographic barriers. The exceptions are six genetic types from four morphospecies: Type II of *Globigerinita glutinata*, Type II of right-coiling *Neogloboquadrina pachyderma*, Type VII of left-coiling *N. pachyderma*, and Type V of *Globorotalia truncatulinoides*, all of which are restricted to the Pacific Ocean (Appendix 2; Figs. 2a and 3). On the other hand, Type II of *Glr. truncatulinoides* and Type I of *Globigerinoides ruber* occur only in the Atlantic Ocean. Types I and II (=II–VI in Darling and Wade, 2008) are distributed separately between the Northern and Southern hemispheres. The restricted geographic distributions of *Nq. pachyderma*, *Glr. truncatulinoides*, and *Gln. ruber* might reflect the adaptation of each genetic type to a water mass with specific hydrographic conditions (e.g., temperature, chlorophyll). In the case of *Gt. glutinata*, future research might discover other types with broad geographic distributions because relatively few studies have been carried out in the world ocean. With increased geographic sampling, novel types might also be discovered in the other morphospecies that are currently considered monotypic.

The transoceanic distribution of the majority of genetic types, in particular the warm-water species, implies that gene flow between the Pacific and Atlantic oceans is a recent phenomenon. Although cold (<8°C) surface waters effectively block warm-water species from migrating around South America, they might have migrated around the Cape of Good Hope because part of the Intermediate Water formed in the Indian Ocean joins the Agulhas Current and then the Atlantic Benguela Current (Schmitz, 1995). The water temperature is warm enough (>14°C) in the upper 200 m layer near the Cape (Read and Pollard, 1993) to support the transport of some tropical and subtropical species through these current systems, especially during warm interglacial periods.

CONCLUSIONS

This study provides the first description of planktic foraminiferal cryptic diversity in the Northwest Pacific. Bayesian phylogenetic analysis of specimens from the Northwest Pacific and other oceans creates a phylogenetic tree of 192 sequences from 21 morphospecies with 36 genetic types. Multiple genetic types are recognized in eight morphospecies: *Globigerinita glutinata*, *Globorotalia truncatulinoides*, *Neogloboquadrina pachyderma*, *Orbulina universa*, *Globigerinella siphonifera*, *Globigerinoides ruber*, *Globigerina bulloides*, and *Turborotalita quinqueloba*. Thirty

of 36 genetic types have modern transoceanic distributions, probably due to recent or current gene flow between the Pacific and Atlantic oceans by means of the global oceanic circulation system.

ACKNOWLEDGMENTS

We are much indebted to M. Nishida of University of Tokyo and J. Inoue of University College London, for their assistance throughout this study. We thank K. Kimoto of JAMSTEC and S. M-Lynch of University of California, Berkeley, for their kind suggestions and assistance in sampling and laboratory work. H. Ujiie gave us valuable advice concerning the fossil record and morphological identification of planktic foraminifera. We thank S. Fay of the University of California, Berkeley, and J. Pawlowski of University of Geneva for their constructive reviews of the manuscript, and two other anonymous reviewers for additional comments. YU acknowledges KT02-15 and KH04-2 cruise members and the crews of the R/V *Tansei-maru* and R/V *Hakuho-maru* for their assistance on board their ships. YU was supported by the Japan Society for the Promotion of Science. YU did most of this study at University of California, Berkeley, with support in part by a grant from the University of California Museum of Paleontology (UCMP). This paper is UCMP contribution no. 1915.

REFERENCES

- BAUCH, D., DARLING, K., SIMSTICH, J., BAUCH, H. A., ERLKENKEUSER, H., and KROON, D., 2003, Paleocceanographic implications of genetic variation in living North Atlantic *Neogloboquadrina pachyderma*: *Nature*, v. 424, p. 299–302.
- BÉ, A. W. H., 1977, An ecological, zoogeographical and taxonomic review of Recent planktonic foraminifera, in Ramsay, A. T. S. (ed.), *Oceanic Micropaleontology*: Academic Press, New York, p. 1–100.
- BOWSER, S. S., HABURA, A., and PAWLOWSKI, J., 2006, Molecular evolution of foraminifera, in Katz, L. A., and Bhattacharya, D. (eds.), *Genomics and Evolution of Microbial Eukaryotes*: Oxford University Press, USA, p. 78–93.
- BRADSHAW, J. S., 1959, Ecology of living planktonic foraminifera in the north and equatorial Pacific Ocean: Contributions from the Cushman Foundation for Foraminiferal Research, v. 10, p. 25–64.
- CARON, D. A., ANDERSON, O. R., LINDSEY, J. L., FABER JR., W. W., and LIM, E. L., 1990, Effects of gametogenesis on test structure and dissolution of some spinose planktonic foraminifera and implications for test preservation: *Marine Micropaleontology*, v. 16, p. 93–116.
- , and HOMEWOOD, P., 1983, Evolution of early planktic foraminifera: *Marine Micropaleontology*, v. 7, p. 435–462.
- CIFELLI, R., 1961, *Globigerina incompta*, a new species of pelagic foraminifera from the North Atlantic: Cushman Foundation Foraminiferal Research Contribution, v. 12, p. 83–86.
- CORDEY, W. G., 1967, The development of *Globigerinoides ruber* (d'Orbigny 1893) from the Miocene to Recent: *Palaeontology*, v. 10, p. 647–659.
- DARLING, K. F., and WADE, C. M., 2008, The genetic diversity of planktic foraminifera and the global distribution of ribosomal RNS genotypes: *Marine Micropaleontology*, v. 67, p. 216–238.
- , —, KROON, D., and LEIGH BROWN, A. J., 1997, Planktonic foraminiferal molecular evolution and their polyphyletic origins from benthic taxa: *Marine Micropaleontology*, v. 30, p. 251–266.
- , —, —, and BIJMA, J., 1999, The diversity and distribution of modern planktonic foraminiferal small subunit ribosomal RNA genotypes and their potential as tracers of present and past ocean circulations: *Paleoceanography*, v. 14, p. 3–12.

- , and STEWART, I. A., KROON, D., DINGLEM, R., and LEIGH BROWN, A. J., 2000, Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers: *Nature*, v. 405, p. 43–47.
- , KUCERA, M., WADE, C. M., VON LANGEN, P., and PAK, D., 2003, Seasonal distribution of genetic types of planktonic foraminifer morphospecies in the Santa Barbara Channel and its paleoceanographic implications: *Paleoceanography*, v. 18, 10.1029/2001PA000723.
- , PUDSEY, C. J., and WADE, C. M., 2004, Molecular evidence links cryptic diversification in polar planktonic protists to Quaternary climatic dynamics: *Proceedings of the National Academy of Sciences USA*, v. 101, p. 7657–7662.
- , KROON, D., and WADE, C. M., 2006, A resolution for the coiling direction paradox in *Neoglobobulimina pachyderma*: *Paleoceanography*, v. 21, 10.1029/2005PA001189.
- , and WADE, C. M., 2007, Global molecular phylogeography reveals persistent Arctic circumpolar isolation in a marine planktonic protist: *Proceedings of the National Academy of Sciences USA*, v. 104, p. 5002–5007.
- DE VARGAS, C., and PAWLOWSKI, J., 1998, Molecular versus taxonomic rates of evolution in planktic foraminifera: *Molecular Phylogenetics and Evolution*, v. 9, p. 463–469.
- , ZANINETTI, L., and PAWLOWSKI, J., 1997, Phylogeny and rates of molecular evolution of planktonic foraminifera: SSUrDNA sequences compared to the fossil record: *Journal of Molecular Evolution*, v. 45, p. 285–294.
- , NORRIS, R., ZANINETTI, L., GIBB, S. W., and PAWLOWSKI, J., 1999, Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to ocean province: *Proceedings of the National Academy of Sciences USA*, v. 96, p. 2864–2868.
- , RNEAUD, S., HILBECHT, H., and PAWLOWSKI, J., 2001, Pleistocene adaptive radiation in *Globorotalia truncatulinoides*: Genetic, morphologic, and environmental evidence: *Paleobiology*, v. 27, p. 104–125.
- , BONZON, M., REES, N. W., PAWLOWSKI, J., and ZANINETTI, L., 2002, A molecular approach to biodiversity and biogeography in the planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny): *Marine Micropaleontology*, v. 45, p. 101–116.
- , SÁEZ, A. G., MEDLIN, L. K., and THIERSTEIN, H. R., 2004, Super-species in the calcareous plankton, in Thierstein, H. R., and Young, J. R. (eds.), *Coccolithophores – from Molecular Processes to Global Impact*: Springer, Berlin, p. 271–298.
- FLAKOWSKI, J., BOLIVAR, I., FAHRI, J., and PAWLOWSKI, J., 2005, Actin phylogeny of foraminifera: *Journal of Foraminiferal Research*, v. 35, p. 93–102.
- HALL, T. A., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT: *Nucleic Acids Symposium Series*, v. 41, p. 95–98.
- HEMLEBEN, C., BÉ, A. W. H., SPINDLER, M., and ANDERSON, O. R., 1979, “Dissolution” effects induced by shell resorption during gametogenesis in *Hastigerina pelagica* (d'Orbigny): *Journal of Foraminiferal Research*, v. 9, p. 118–124.
- HUBER, B. T., BIJMA, J., and DARLING, K., 1997, Cryptic speciation in the living planktonic foraminifer *Globigerinella siphonifera* (d'Orbigny): *Paleobiology*, v. 23, p. 33–62.
- JOBB, G. A., VON HAESELER, A., and STRIMMER, K., 2004, TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology*, v. 4, 10.1186/1471-2148-4-18.
- KENNETT, J. P., and SRINIVASAN, S., 1983, *Neogene Planktonic Foraminifera*: Hutchinson Ross Pub. Co., New York, 265 p.
- KUROYANAGI, A., TSUCHIYA, M., KAWAHATA, H., and KITAZATO, H., 2008, The occurrence of two genotypes of the planktonic foraminifer *Globigerinoides ruber* (white) and paleo-environmental implications: *Marine Micropaleontology*, v. 68, p. 236–243.
- NYLANDER, J. A. A., 2004, MrModeltest v2. Program distributed by the author: Evolutionary Biology Centre, Uppsala University.
- PAWLOWSKI, J., BOLIVAR, I., FAHRI, J., DE VARGAS, C., GOUY, M., and ZANINETTI, L., 1997, Extreme differences in rates of molecular evolution of foraminifera revealed by comparison of ribosomal DNA sequences and the fossil record: *Molecular Biology and Evolution*, v. 14, p. 498–505.
- READ, J. F., and POLLARD, R. T., 1993, Structure and transport of the Antarctic Circumpolar Current and Agulhas Return Current at 40°E: *Journal of Geophysical Research*, v. 98, C7, p. 12281–12296.
- RONQUIST, F., and HUELSENBECK, J. P., 2003, MrBayes 3: Bayesian phylogenetic inference under mixed models: *Bioinformatics*, v. 19, p. 1572–1574.
- SCHMITZ, W. J., JR., 1995, On the interbasin-scale thermohaline circulation: *Review of Geophysics*, v. 33, p. 151–173.
- STEWART, I. A., 2000, The molecular evolution of planktonic foraminifera and its implications for the fossil record: Unpublished Ph.D. Dissertation, University of Edinburgh.
- , DARLING, K. F., KROON, D., WADE, C. M., and TROELSTRA, S. R., 2001, Genotypic variability in subarctic Atlantic planktic foraminifera: *Marine Micropaleontology*, v. 43, p. 143–153.
- SWOFFORD, D. L., 2002, PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4.0b10: Sinauer Associates, MA.
- TAPPAN, H., and LOEBLICH, A. R., JR., 1988, Foraminiferal evolution, diversification, and extinction: *Journal of Paleontology*, v. 62, p. 695–714.
- UJIIÉ, Y., and UJIIÉ, H., 2000, Distribution and oceanographic relationships of modern planktonic foraminifera in the Ryukyu Arc region, northwestern Pacific Ocean: *Journal of Foraminiferal Research*, v. 30, p. 336–360.
- , KIMOTO, K., and PAWLOWSKI, J., 2008, Molecular evidence for an independent origin of modern triserial planktonic foraminifera from benthic ancestors: *Marine Micropaleontology*, v. 69, p. 334–340.
- YANG, Z., 1994a, Estimating the pattern of nucleotide substitution: *Journal of Molecular Evolution*, v. 39, p. 105–111.
- , 1994b, Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods: *Journal of Molecular Evolution*, v. 39, p. 306–314.
- XU, X. D., YAMASAKI, M., ODA, M., and HONDA, M. C., 2005, Comparison of seasonal flux variations of planktonic foraminifera in sediment traps on both sides of the Ryukyu Islands, Japan: *Marine Micropaleontology*, v. 58, p. 45–55.

Received 29 October 2008

Accepted 23 January 2009

APPENDIX 1

List of 206 sequence data used for the reconstruction of the phylogenetic tree. Bold letters indicate new data obtained from the northwestern Pacific Ocean in this study. This table can be found on the Cushman Foundation website in the JFR Article Data Repository (<http://www.cushmanfoundation.org/jfr/index.html>) as item number JFR_DR200902.

APPENDIX 2

All of genetic types recognized in 16 morphospecies in this study and their geographical distribution. Previous works were compiled by Darling and Wade (2008). This table can be found on the Cushman Foundation website in the JFR Article Data Repository (<http://www.cushmanfoundation.org/jfr/index.html>) as item number JFR_DR200902.