Palaeogeography, Palaeoclimatology, Palaeoecology 391 (2013) 2–12

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo

Global scale same-specimen morpho-genetic analysis of *Truncorotalia truncatulinoides*: A perspective on the morphological species concept in planktonic foraminifera

Frédéric Quillévéré [a,*], Raphaël Morard [a,b], Gilles Escarguel [a], Christophe J. Douady [c,d], Yurika Ujiié [e], Thibault de Garidel-Thoron [f], Colomban de Vargas [b]

[a] Laboratoire de Géologie de Lyon: Terre, Planètes, Environnement, UMR CNRS 5276, Université Lyon 1, 27-43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France
[b] UPMC Univ Paris 06, UMR CNRS 7144, EPPO: Evolution du Plancton et PaléoOcéans, Station Biologique, BP 74, 29682 Roscoff, France
[c] UMR CNRS 5023 Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, 27-43 Boulevard 11 Novembre 1918, 69622 Villeurbanne Cedex, France
[d] Institut Universitaire de France, France
[e] Shinshu University, Department of Biology, Faculty of Science, Asahi 3-1-1, Matsumoto City, Nagano 390-8621, Japan
[f] Université Aix-Marseille, CNRS/INSU, CdF, IRD, CEREGE, Europôle Méditerranéen de l'Arbois, BP 80, 13545 Aix-en-Provence Cedex 4, France

ARTICLE INFO

Article history:
Received 23 September 2010
Received in revised form 28 February 2011
Accepted 13 March 2011
Available online 21 March 2011

Keywords:
Planktonic foraminifera
Biogeography
Ribosomal DNA
Morphometrics
Species concept

ABSTRACT

Genetic analyses of planktonic foraminifera have unveiled significant levels of cryptic diversity, thus calling into question the usefulness of the morphological species concept for paleoceanographic reconstructions. Here, we present single-specimen combined genetic and morphological analyses performed on living *Truncorotalia truncatulinoides* collected across the world oceans. A combined morphogenetic analysis allows us to (1) detect five different genetic types (Types I to V) within the morphospecies *T. truncatulinoides*, (2) statistically analyze shape variations among these genotypes, and (3) assess the biogeographic patterns and the links between surface ocean properties and the distribution of morphological and genetic diversity within *T. truncatulinoides*. Of the five genetic types, Type I appears to inhabit the warm (sub)tropical waters of the South Hemisphere, Types II and V are found in the warm (sub)tropical waters of the Atlantic and NW Pacific, respectively, and Types III and IV appear to be restricted to the productive subtropical and the cold subpolar frontal zones of the Southern Ocean, respectively. Same-specimen morphogenetic comparisons reveal significant differences in test morphology between the warm (sub)tropical cluster of genotypes (Types I, II, and V) and the colder subpolar cluster of genotypes (Types III and IV). These results indicate that changes in shell conicalness, observed across the subtropical fronts in the Southern Ocean and for a long time interpreted as ecophenotypic variation, reflect genetic differentiation, with large, highly conical left (Indian Ocean) or right-coiled (Pacific Ocean) specimens north of the North Subtropical Front representing genetic Type I, and small, axially compressed and biconvex left-coiled specimens south of this front representing genetic Types III and IV. Our morphogenetic data are consistent with the scenario of a late Pleistocene invasion of the Southern Ocean by newly evolved *T. truncatulinoides* genotypes, specifically adapted to cold water masses. Finally, we build a model based upon test outline analyses, which correctly assigns up to 75% of the specimens to their corresponding cluster of genotypes. Application of this model to sediment samples may contribute to the reconstruction of migrations of the Subtropical Front during the late Pleistocene.

1. Introduction

Calcareous shells (tests) of planktonic foraminifera are commonly used in paleoceanographic studies to reconstruct sea surface conditions and upper ocean structure (e.g., Mulitza et al., 1997). These studies assume that each morphospecies has its own ecological preferences that can be used for reconstruction of past water mass properties (e.g., Kennett, 1968; Malmgren et al., 2001). Therefore, the use of species-specific paleoproxies requires a high degree of taxonomic consistency. Since the CLIMAP global reconstruction of glacial oceanic conditions (CLIMAP, 1976), the morphological definition of planktonic foraminiferal species has been set very broadly, and morphological variation has been classically regarded as intra-specific variability or ecophenotypy (e.g., Hecht et al., 1976; Kennett, 1976; Healy-Williams and Williams, 1981; Healy-Williams et al., 1985).

A growing body of molecular studies has revealed that the classical, morphological definition of species in planktonic foraminifera hides higher levels of genetic and ecological differentiation (de

* Corresponding author. Tel.: +33 4 72 44 83 81; fax: +33 4 72 44 83 82.
E-mail address: frederic.quillevere@univ-lyon1.fr (F. Quillévéré).

0031-0182/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.palaeo.2011.03.013

Vargas et al., 1997; Huber et al., 1997; Darling et al., 1999; de Vargas et al., 1999; Darling et al., 2000; de Vargas et al., 2001; Stewart et al., 2001; de Vargas et al., 2002; Bauch et al., 2003; Darling et al., 2004, 2006, 2007; Kuroyanagi et al., 2008; Aurahs et al., 2009; Ujiié and Lipps, 2009; see Darling and Wade, 2008 for a review). Based on the molecular data currently available, phylogenetic analyses of the SSU (Small Subunit) or ITS (Internal Transcribed Spacers) regions of ribosomal DNA (rDNA) on both spatially and environmentally well-sampled extant morphospecies, allowed the recognition of two to seven distinct genotypes per morphospecies (Darling and Wade, 2008). The large genetic distances between many of these genotypes, the high degree of genetic similarity within each genotype, the high age of their divergence times and their environmental and geographical separations strongly suggest that they correspond to cryptic biological species. Paleoceanographic reconstructions using a broad morphospecies concept are consequently based on aggregates of biogeographically and ecologically distinct species; they contain significant noise and the usual assumption of homogeneity made in these reconstructions is called into question (Kucera and Darling, 2002; Kucera et al., 2005a).

It has been shown that subtle morphological differences within certain morphospecies, classically treated as ecophenotypic variants, may represent characters distinguishing among cryptic species adapted to highly different water masses (Huber et al., 1997; Morard et al., 2009). If this discovery could be generalized, it would become possible to transfer the biological information acquired through genetic analysis to paleoceanography. To this end, it would be necessary to detect which characters of the test may permit significant distinction of the planktonic foraminiferal cryptic species. In order to identify such characters, direct morphogenetic comparisons should ideally be conducted on a large number of single individuals collected throughout the geographic ranges of the studied species in order to distinguish morphological variation induced by environmental triggers from that due to genetic isolation. Such comparisons are now feasible with the development of methods that make the DNA extraction step non-destructive for the foraminiferal calcareous test. In this paper, the tests of ~600 genetically characterized *Truncorotalia truncatulinoides* specimens collected in different water masses of the world oceans are morphometrically analyzed.

Truncorotalia truncatulinoides is a non-spinose, non-symbiotic trochospiral planktonic foraminifera that originated 2.8 myrs ago in the subtropical southwest Pacific (Lazarus et al., 1995), later spreading into the Indian and Atlantic Oceans ~2.0 myrs ago (Spencer-Cervato and Thierstein, 1997; but see Sexton and Norris, 2008). Today, *T. truncatulinoides* inhabits a wide depth range, from the surface mixed layer to depths well below the thermocline (Hemleben et al., 1985; Mulitza et al., 1997; Cléroux et al., 2007). Restricted to tropical and subtropical waters in the Northern Hemisphere, it exhibits a wider temperature tolerance in the Southern Hemisphere, where its distribution extends to subpolar waters (e.g., Kennett, 1968; Malmgren, 1983). Morphological changes (large and highly conical to small and axially compressed tests), then considered as ecophenotypic, were correlated to sea surface properties from analyses of subtropical to subpolar surface sediments in the Southern Hemisphere (Kennett, 1968; Healy-Williams and Williams, 1981; Lohmann and Malmgren, 1983; Healy-Williams et al., 1985; Pharr and Williams, 1987; Lohmann, 1992).

Our choice to study *Truncorotalia truncatulinoides* was motivated by previous molecular analyses along a latitudinal gradient in the southwest Atlantic, which revealed the existence of four distinct genotypes based on their specific ITS rDNA sequences (de Vargas et al., 2001). Recently, a novel genetic type (Type V) was described based on SSU rDNA variations (Ujiié and Lipps, 2009). Morphometric and genetic analyses of plankton samples linked the morphological latitudinal cline observed from surface sediment samples in the Southern Hemisphere to a zonal distribution pattern of four different genetic types of this species (de Vargas et al., 2001). However, most of these genetic and morphometric analyses were not performed on the same specimens, failing to *unequivocally* demonstrate that the cryptic species of *T. truncatulinoides* indeed exhibit distinct morphologies. Here, same-specimen morphogenetic analyses on a larger number of individuals from the world oceans allow us to directly investigate the significance of the relationships between genetic and morphological differentiation within this morphospecies.

2. Material

Specimens were collected using plankton tows from the subtropical to the subpolar oceans (Fig. 1). In the Indian and Pacific Oceans, they were collected onboard the *Marion Dufresne* during the cruise OISO-4 (January–February 2000; Metzl, 2000; 177 specimens) and onboard the *R/V Roger Revelle* during the cruise REVELLE (January–February 2004; 281 specimens), respectively. For these two cruises, genetic data by de Vargas et al. (2004) were supplemented by doubling the number of genotyped specimens. Additional specimens were collected in the Atlantic Ocean onboard the *R/V Ronald H. Brown* as part of the CMarZ project (April 2006; 242 specimens), in the Mediterranean Sea offshore Marseille onboard the *R/V Antédon II* (February 2008; 96 specimens), and in the northwest Pacific during the cruise KT-06-11 onboard the *R/V Tansei-maru* (June 2006; 17 specimens). At OISO-4, REVELLE and KT-06 stations, plankton samples were collected using vertical ring net tows (100 μm mesh size) from ~200 m depth to the sea surface. At CMarZ and Marseille stations, 1/4-m^2 Multiple Opening/Closing Nets and Environmental Sensing Systems (MOCNESS; Wiebe et al., 1985) and 1-m^2 MULTINETs with 100 μm mesh sizes were used, respectively, to sample specific layers of the water-column.

In summary, our study is based on the analysis of 813 living specimens of *Truncorotalia truncatulinoides* from 36 stations (Table 1). Each of these specimens was isolated on the day of collection into the GITC* buffer, and stored at −20 °C. Other specimens collected at 28 of the sampling stations were directly dried after filtration.

Hydrographic data are used to characterize surface water mass features and boundaries. At OISO-4 stations, Conductivity Temperature and Depth (CTD) casts provided water temperature and chlorophyll-a fluorescence profiles of the 250 m upper water column. At all other sampled stations, temperature (SST) measurements were also recorded by the shipboard loggers between 7 and 10 m depth.

3. Methods

3.1. DNA extraction, PCR amplification and RFLP analysis

We used the GITC* buffer procedure to extract DNA and retain calcareous tests of the specimens (de Vargas et al., 2004; Morard et al., 2009). Following de Vargas et al. (2001), for each individual, the ITS-2 region was amplified using the foraminiferal specific primers 5.8S12f and L2TAigta. All positive PCR products were digested using the endonuclease *Sau 96 I* (New England Biolabs). Distinct RFLP patterns were UV-detected after slow migration of the digested PCR-products on 3% agarose gel, and ethidium bromide staining, then allowing distinction between Types I + V, II, III and IV as described by de Vargas et al. (2001). Since the band patterns of Type V (Ujiié and Lipps, 2009) were identical to those of Type I, we developed an additional RFLP procedure to discriminate between these two genotypes, using the enzyme *BbVI* (New England Biolabs), which cuts the sequence 5′…GCAGC$(N)_8$…3′. 5 μl of the PCR products was mixed with a solution containing 3.5 μl of distilled water, 1 μl of NEB buffer 2 solution (New England Biolabs) and 0.5 μl of the *BbVI* enzyme, and then incubated at 37 °C during 2 h. In this method, Type I of *Truncorotalia truncatulinoides* displayed a two band pattern at 400 and 200 bp, while Type V displayed no cut.