both cores. The samples were roasted under vacuum at 400°C for 45 minutes and reacted under vacuum through the action of 100 percent H₃PO₄ at 50°C. The ¹⁸O/¹⁶O ratio is reported as δ^{18} O values (per mil) relative to the Chicago Pee-Dee belemnite (PDB-1) standard:

δ ¹⁸ Ο	(¹⁸ O/ ¹⁶ O)sample	- 1	×	1000	
	(¹⁸ O/ ¹⁶ O)standard				

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 11. The relationship between δ¹⁸O and the salinity of segurater in the socither Indian Occurs is heard.
- seawater in the southern Indian Ocean is based on data collected from different cruise tracks of the French M.S. *Galliéni* between Durban, Crozet Island, Kerguelen Island, New Amsterdam Island, Réunion Island, and Madagascar and the Danish M.S. Thala Dan between Australia and Danish M.S. Thala Dan between Australia and Antarctica [J. C. Duplessy, C. R. Acad. Sci. Paris 271, 1075 (1970)]. For seawater with a salinity of less than 35 per mil, an increase of 0.66 per mil in δ^{18} O corresponds to an increase in salinity of 1 per mil. For higher salinities, the salinity of seawater is constant to within 0.1 per mil (at the 1 σ level, where σ is the standard devia-tion) tion). 12. If the difference between the δ^{18} O values of
- If the difference between the δ^{-0} values of seawater at the two sites has remained zero, the temperature difference in the past at the two sites can be calculated from the δ^{18} O of the foraminifera present at the same isotopic stage in the two cores from the formula:

$$T_2 - T_1 = -4.2 (\delta^{18}O_{\text{foram } 2} - \delta^{18}O_{\text{foram } 1})$$

where, according to the simplified paleotemperature equation.

$$T = 16.9 - 4.2 \left(\delta^{18} O_{\text{foram}} - \delta^{18} O_{\text{sea}} \right)$$

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 According to K. Geitzenauer (personal communication), the nannofossil Pseudoemiliania lacunosa boundary is at about 845 cm in core RC 17-69, which is equivalent to the lower part of stage 12 or about 460,000 years B.P.
 Since the maximum glacial-interglacial δ¹⁸O variation measured in abyssal Atlantic benthic foraminifera is 1.6 per mil, this variation is the highest possible without a change in temperature (J. C.
- infera is 1.6 per mil, this variation is the highest possible without a change in temperature (J. C. Duplessy, unpublished results). The very large δ¹⁸O variation of 2.4 per mil observed between the present and stage 12 in *G. sacculifer* of core RC 17-69 implies a strong temperature difference.
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Ecologic and Paleoclimatic Implications of

Morphologic Variation of Orbulina universa in the Indian Ocean

Abstract. Multivariate cluster analysis of various morphologic indices of Orbulina universa populations from the Indian Ocean indicate the existence of two major groups whose geographic distribution corresponds to the equatorial and central water masses. An abrupt change in shell porosity between populations of this planktonic foraminiferal species in plankton as well as sediment samples occurs within or near the 10°S Hydrochemical Front. Orbulina universa is an excellent indicator of oceanographic conditions in the Indian Ocean today, and may be used as an independent check on shifts in water masses during the last glaciation.

Variation in the abundances and species composition of planktonic foraminifera preserved in deep-sea sediments has been shown to be valuable in reconstructing the paleoclimatic history of the oceans (1). This results from the fact that the geographic distribution of assemblages of living species corresponds to the distribution of major oceanic water masses. Temporal shifts in these water masses can thus be monitored by shifts

in faunal zones as recorded in stratigraphic sequences (2, 3).

It is reasonable to expect that within a major water mass, each planktonic foraminiferal species would be characterized by distinct morphologic characters which differentiate it from neighboring populations in adjacent water masses. The existence of such morphological provinces should be most evident in areas where sharp hydrographic bound-

Table 1. Data on mean shell diameter and shell porosity of O. universa in plankton and sediment samples in the Indian Ocean.

Code	Sample	Latitude	Longi- tude	Mean shell diameter (µm)	Mean shell porosity (%)	Ocean depth (m)	Surface temper- ature (°C)	Surface density (g/liter)
			Centi	ral group				
Sedim	ents							
Α	V 14-81	28°26′S	43°47′E	608	5.17	3634	20.8	24.5
С	RC 11-124	36°6′S	90°13′E	417	6.19*	3775	14.0	26.5
D	V 18-222	38°34′ S	140°37′E	646	5.73	1904	13.0	26.5
J	V 18-207	25°38′S	87°7′E	662*	5.17	3434	19.0	25.0
K	V 16-93	30°12′S	91°43′E	511	5.44	2769	16.0	26.0
L	Monsoon 45	18°41′S	87°51′E	624	5.71	1735	23.5	23.5
0	RC 11-123	37°59′S	86°39′E	421	5.66	3766	12.0	26.5
P	RC 11-120	43°31′S	79°52′E	413	5.10	3193	9.0	26.5
Ō	V 20-170	21°48′S	69°14′E	596	4.81	2479	20.7	23.5
Ŕ	RC 8-61	46°32′S	125°34′E	385†	4.50†	4254	11.0	26.5
			Means	528.3	5.35			
Plankt	ton							
1	V 16-131	29°52′S	62°36′E	536	3.48		23.9	
2	RC 9-111	28°27′S	101°57′E	525	5.00		23.9	
3	V 16-110	42°40′ S	45°40′E	443†	3.31		14.9	
4	V 20-194	22°18′S	68°0′E	576	4.22		21.0	
5	V 19-148	12°44′ S	82°1′E	681	6.79*		24.8	
7	V 14-142	13°35′S	64°39′E	697	6.08		28.0	
10	RC 8-29	35°49′S	109°57′E	723*	3.19†		18.9	
			Means	597.3	4.58			
			Equato	orial group				
Sedim	ents		•					
В	CH 99-64	13°18′S	46°59′E	694	7.72†	3243	24.8	23.5
E	Dodo 204	3°9′N	94°6′E	765	12.07	2150	28.4	23.5
F	RC 9-155	7°24′ N	72°48′E	728	12.22	1765	28.0	23.5
G	Dodo 117	18°21′S	62°4′E	657†	9.44	3394	22.8	23.5
Н	V 19-183	8°7′N	62°47′E	819*	17.35*	4451	27.3	22.5
I	V 19-200	4°13′S	41°33′E	690	9.23	2692	25.0	23.5
Μ	Dodo 193	2°12′S	69°15′E	724	9.98	3494	27.9	22.5
Ν	V 14-105	14°18′N	51°0′E	664	11.52	2120	24.8	23.0
			Means	717.6	11.19			
Planki	ton							
6	V 19-154	0°27′N	80°37′E	714†	9.71†		28.8	
8	V 19-168	0°29′S	53°41′E	787	10.96		27.4	
9	V 19-160	7°26′N	61°4′E	822	10.68		28.0	
- 11	V 20-180	17°10′S	99°12′E Means	860* 795.7	11.18* 10.63		24.2	

*Maximum. †Minimum.



Fig. 1. (a) Dendrogram based on group average cluster analysis for *O. universa* populations from sediment (letters A to R) and plankton-tow (numbers 1 to 11) samples. Similarity between samples is based on a distance coefficient scaled between 0 (most similar) to 100 (least similar). The clustering procedure is described by Pritchard and Anderson (6). Sample H in the sediment data set is anomalous in the sense that it is most similarly related to the product of the central water and equatorial clusters. This results from its large shell size and shell porosity. On the basis of its shell morphology and geographic location, we assume that this sample has a greater affinity with the equatorial than the central water cluster. (b) The boundaries between the equatorial and central water mass populations in the plankton and sediment correspond approximately with the position of the 10°S Hydrochemical Front (8). Sample locations 1, 3, O, P, and R occur slightly south of the central water mass in the region of the Subtropical Convergence.

aries prevent complete mixing of a species population. Under these conditions, major discontinuities in morphologic gradients may occur which would allow each water mass to be uniquely defined. If such is the case, then changes in the distribution of morphotypes would provide independent confirmation for shifts in major water masses during the geological past (2, 3).

We investigated the existence of such morphologic provinces in *Orbulina universa* populations from plankton tows and sediment core tops in the Indian Ocean north of 50°S. We conclude that for this species, two morphologic provinces exist on the basis of a major discontinuity in the gradient of shell porosity between populations in the equatorial and central water masses of the Indian Ocean.

The morphologic variables used in this study are those previously determined by Bé et al. (4). Populations of O. universa are morphologically characterized by a spherical shell which is perforated by pores of two sizes. Variations in the number and diameter of these pores give rise to variations in the pore concentration (number of pores per 10,000 μ m²), and shell porosity (percentage of pore space per unit area). In total, eight morphologic parameters (shell diameter, shell thickness, and the concentration and porosity of small, large, and total pores) from 18 core-top and 11 plankton-tow samples were analyzed by group average cluster analyses (5, 6). Cluster analysis is a multivariate statistical procedure widely used in biological and paleontological



Fig. 2. Plots of (a) shell porosity against shell diameter, (b) shell porosity against water density, and (c) shell diameter against water density for *O. universa* in the sediment cluster group given in Fig. 1a.

studies for comparing a large number of samples on the basis of many variables (Q-mode analysis) and organizing groups (7). The cluster analyses for populations of O. universa in plankton and sediment samples are shown as dendrograms in Fig. 1a. The input data for the cluster analyses include no information on the location of each sample. Yet, Fig. 1b shows discrete geographic distributions of two morphologic groups for both the plankton and the sediment samples. This boundary generally corresponds to the 10°S Hydrochemical Front as defined by Wyrtki (8). While the exact location of the boundary is limited by the number of samples, there is general agreement in the boundary position for both sediment and plankton data.

The morphologic characteristics of plankton and sediment samples in each cluster group are compared in Table 1. For both sediment and plankton data, populations in the equatorial water mass are larger in shell diameter and thicker in shell walls than populations in the central waters. The plankton populations are, on the average, somewhat larger in shell size and thinner in shell walls than those in the sediment.

Table 1 also shows that while the mean values for all samples in each cluster group are different, the ranges of measured variables in both clusters overlap for all but one morphologic index. For example, the mean shell diameters of O. universa in sediment samples beneath the equatorial and central water masses are 718 and 528 μ m, respectively. However, in the size range between 657 and 662 μ m, the two cluster groups overlap, and therefore size alone could not be used to distinguish the two water masses. This is of considerable importance in paleoclimatic studies since a decrease in shell size in fossil populations over the size range of overlap between the two cluster groups would not necessarily mean a major shift in water mass boundaries in subtropical latitudes. Only in the case of shell porosity does a discontinuity exist in the morphologic gradient between the two cluster groups. This is evident from Fig. 2a, where shell porosity is plotted against shell diameter for the sediment samples. These data show that all samples in the central water mass fall along a line with a different slope from the line representing those in the equatorial waters. Thus, the gradient in one of these morphologic parameters must be discontinuous between the two water masses; in Fig. 2, b and c, shell porosity and shell diameter are plotted against water density. Variations in shell diameter are clearly gradational between the two water masses, but variations in shell porosity are discontinuous (9). All equatorial samples are drawn from waters of similar density, but show a wide range of variation in shell porosity. Central water mass samples, on the other hand, show a narrow range of porosity variation over wide changes in water density (10). Thus, shell porosity is a critical morphologic index whose geographic variation probably reflects fundamental ecologic differences between O. universa populations in the equatorial and central water masses.

The discontinuity in morphologic gradient in populations of O. universa provides an important paleoclimatic index. Its value may be in providing an independent check on proposed paleoclimatic reconstructions. McIntyre et al. (3) recently compared August temperatures in the Indian Ocean today and 18,000 years before present (B.P.) as derived from faunal paleotemperature estimates. The morphologic boundary between the equatorial and central water masses today parallels the August 24°C isotherm. This isotherm, computed for the time of the last major ice age (18,000 years B.P.), is essentially in the same position as today, indicating that no major displacement in water masses had occurred in this region. Independent verification of this paleoclimatic reconstruction may be derived from analysis of morphologic gradients in populations of O. universa from this region.



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Activating Factor for the Iron Protein of Nitrogenase from

Rhodospirillum rubrum

Abstract. As isolated from Rhodospirillum rubrum, the iron protein of nitrogenase has little or no activity. It can be activated by incubating it with a trypsin-sensitive, oxygen-labile component (activating factor) plus adenosine triphosphate and a divalent metal ion. After activation, the iron protein retains its nitrogenase activity when the activating factor is removed.

Although the molybdenum-iron protein of Rhodospirillum rubrum nitrogenase has been partially purified (1), little success in purifying the iron protein has been reported. However, several observations have indicated that the R.



rubrum nitrogenase system differs from nitrogenase in other bacteria; chromatophores (membrane fragments containing the photosynthetic apparatus) strongly inhibit nitrogenase activity in crude extracts, and a high level of $MgCl_2$ (25 mM) is optimal for the assay of nitrogenase in the presence of only 5 mM adenosine triphosphate (ATP) (2). The time course of reductions catalyzed by R. rubrum nitrogenase is nonlinear, and a lag phase of 10 to 15 minutes is common (3). Our discovery of an activating factor (AF) for

Fig. 1. Assays were performed in 21-ml vaccine bottles at 30°C. Total volume of the reaction mixture was 1 ml; it contained 5 mM ATP, 30 mM creatine phosphate, 0.05 mg of creatine phosphokinase, 0.4 ml of crude extract which had a protein concentration of 8.4 mg/ml, 10 mM $Na_2S_2O_4$, and 40 mM triethanolamine-acetate buffer. The gas phase was 90 percent H₂ and 10 percent C₂H₂. C₂H₄ was measured by gas chromatography. O-25 mM Mg²⁺ and 0.5 mM Mn²⁺; \square --0. -10 mM Mg²⁺ and 0.5 mM Mn²⁺; \triangle -mM Mg²⁺; \triangle - \Diamond , 10 mM Mg²⁺. $-\triangle$, 25

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