Symbiosis, 14 (1992) 265–281
Balaban, Philadelphia/Rehovot

Endosymbiotic Diatoms from Larger Foraminifera Collected in Pacific Habitats

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Received November 20, 1991; Accepted March 12, 1992

Abstract

The restricted geographic sampling sites and limited number of diatom-bearing hosts available to us in our previous study (Lee et al., 1989) challenged us to include more Indo-Pacific larger foraminifera in our evaluation of this unusual endosymbiotic phenomenon. Almost two hundred specimens from Palau and over four hundred specimens from Kudaka Island, Japan were examined during the course of this study. New hosts examined included, *Baculogypsina sphaerulata*, *Neorotalia calcar*, *Calcarina gaudichaudi*, *C. defrancei*, and *C. spengleri*. Fairly large numbers of some of the species studied in collections from the Red Sea, *Amphistegina lessonii*, *A. lobifera*, and *Heterostegina depressa*, were also available in the new collections to provide another aspect to the comparison. *Nitzschia frustulum* var. *symbiotica* was again the most abundant endosymbiotic species (24% of the total isolations). It was followed in abundance by *Amphora erezii* (20.4%), *Cocconeis andersonii* (10.5%), and *Fragilaria shiloi* (11.5%). In

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general, the new clones were smaller in size, but were the same species as encountered previously. The abundances of free-living diatoms present in the habitat of the foraminifera on Kudaka Island were also studied.

The results of the present study, when coupled to those reported earlier (Lee et al., 1980a,b, 1989) lead to several conclusions: (1) Larger foraminifera do not host the most abundant diatoms in their habitat. (2) They must acquire their endosymbionts by passage during asexual reproduction or possibly from their parents remains if they are gamontogamic, or the habitat if not, after sexual reproduction. (3) There does not seem to be a host/symbiont specific relationship in diatom-bearing larger foraminifera in the same context as has been implied for dinoflagellates and their hosts (Trench, 1987, 1988; Trench and Blank, 1987). (4) Very few species of small (< 10 μm) pennate diatoms are involved in the phenomenon. Ten species were found in 90% of symbionts isolated. This suggests that there must be some aspect of specificity between hosts and their endosymbionts. The most common diatoms (*Nitzschia frustulum* var. *symbiotica*, *N. laevis*, *N. panduriformis*, *Fragilaria shiloi*, *Amphora roettgeri*, *A. erezii*) which are involved in over 75% of all the symbiont systems examined, belong to a variety of taxonomically distantly related genera. *Nitzschia* species accounted for only 55% of all the isolates.

1. Introduction

During the last decade, detailed knowledge of the diatom endosymbionts of larger foraminifera has gradually unfolded (Lee, 1983, 1991; Leutenegger, 1983, 1984; Lee et al., 1991). The identification of the individual endosymbiotic diatoms has been made possible for two reasons: (1) because they remain viable even when their cellular hosts have been mechanically disrupted and their contents transferred to media which support their growth; and (2) because the newly released endosymbionts, which are frustule-less in their hosts, form frustules in culture. This is indeed fortunate, since it is the architecture of the diatom frustule which is used as the basis of the taxonomy of the group at this time.

Although it has been recognized in early TEM observations (Hansen and Buchardt, 1977) that the same host might harbor more than one species of endosymbiotic diatom, it was only after the report of several thousand cultural isolations and identifications that a general picture emerged (Lee et al., 1989). In the latter study, six diatom-bearing species of larger foraminifera, *Amphistegina lessonii*, *A. lobifera*, *Heterostegina depressa*, *Operculina ammonoides*, *Borelis schlumbergeri*, and *Neorotalia calcar* (Hottinger et al., 1991), were harvested mainly (83%) from the Gulf of Elat (Red Sea). The rest of the collections were harvested from the Indo-Pacific ranging from the Great

Barrier Reef (9%), Hawaii (3.6%), and Palau (3.1%) in the Pacific to Mombasa harbor (Kenya) on the Indian Ocean. While over 20 different species (and varieties) of diatoms were collectively isolated from the foraminifera studied, one species, *Nitzschia frustulum* var. *symbiotica*, was the most commonly isolated endosymbiont. Two other species of *Nitzschia*, *N. panduriformis* var. *continua* and *N. laevis*, were also among the top 5 most commonly isolated (72.8%) diatom species. The remaining two species, *Amphora roettgeri*, and *Fragilaria shiloi*, are taxonomically distinct enough from the first three to suggest that the biological basis for entering symbiotic relationships with foraminifera is fairly wide spread among pennate diatoms. Significant numbers of all three species of *Nitzschia* (*N.f. symbiotica*, *N.p. continua*, and *N. laevis*) were isolated from hosts collected at every depth. Other diatom species were isolated more commonly from hosts collected at either very shallow (e.g. *F. shiloi*), or greater than average depths (25 m; e.g. *Achnanthes maceneryae*, *Amphora* sp (J) and *Protokeelia hottingeri*). Although not the rule, since hosts generally harbored different species of diatoms, some host-symbiont bias was observed (e.g. *N.f. symbiotica* was isolated in 92.9% of the total isolations [28 specimens] from *Neorotalia calcar*).

The geographic and host bias of our previous study (Lee et al., 1989) challenged us to expand our isolation program to include more Indo-Pacific forms. Only a few calcarinids were found in the previous study and we were anxious to know more about the diatom endosymbionts of these famous Pacific "star sands". There was the possibility that new species of tiny endosymbiont diatoms might lie undiscovered, awaiting the exploration of these previously neglected hosts. Several opportunities for new collections were recently presented to us and it was with great eagerness that we began the studies reported here.

2. Materials and Methods

Collections

The organisms were collected by one of us (R.R.) in March 1987 in shallow back reef habitats in Palau and Kudaka Island (Ryukyu chain, Okinawa Prefecture, Japan). Collections by 2 of us (M.N. and J.J.L.) in the back reef of Kudaka Island were made in August 1987 and July 1989. The foraminifera were washed and resuspended in filtered sea water from the sites. They were then transferred to insulated containers which were carried or mailed to the laboratory at City College of New York where the actual isolations were carried out. At the same time as the foraminifera were collected on Kudaka Island, small samples of the backreef *Aufwuchs* and macrophytes were preserved in 1%

glutaraldehyde to be prepared for later studies of the microphytic communities in which the foraminifera were feeding.

Isolation procedures

The methods we used for isolation, culture, and identification of the endosymbionts were those given previously (Lee and Reimer, 1983, and Lee et al., 1989). Very briefly the technique used was as follows: under a dissection microscope, individual foraminifera were brushed aseptically with #4-0 sable brushes to remove any adhering diatoms. The foraminifera were then aseptically serially washed and transferred with well-to-well in sterilized 9-hole spot plates. After incubation over night at 25°C, foraminifera were re-examined under a Wild dissection microscope (200×) to check that their exterior surfaces were free of contamination. Those with extended reticulopodia were transferred to fresh sterile wells and then cut and crushed with the aid of sterile pairs of fine forcepts (Dumont #5). The contents of each well was aseptically withdrawn by means of a sterilized pasteur pipette and transferred to isolation media (Lee et al., 1980b). The cultures were incubated in front of a bank of fluorescent lights in a temperature controlled room (25°C) for approximately 10 day to 2 weeks. The cultures were then vigorously mixed and aliquots were transferred to test tubes containing 30% H_2O_2. The tubes were placed in a warm water bath until their contents became water clear. The oxidized frustules were then sprayed on the surface of Nucleopore (0.45 μm) filters as the contents of the tubes were pipetted through them.

The filters with the frustules were dried in a vacuum dessicator jar placed in an oven (60°C) over night. They were then mounted on stubs with the aid of double-stick tape and coated with 10 nm of Au/Pd mixture in a Poloron Sputter coater.

Some of the diatoms fixed for community analysis were treated the same way as diatoms from the cultures. The rest of the samples were critical point dried, mounted on stubs with double-stick tape, and sputer coated with 10 nm Au/Pd.

All stubs were examined in a Zeiss digital scanning electron microscope (model DSM 950) at the American Museum of Natural History. Photographs were taken on Kodak 4415 Technical film which was used at an ASA 100 and developed with Kodak HC-110 (dilution B). Prints were made on Kodak Polyprint II (RC).

Statistical analyses

As in our previous study (Lee et al., 1989), the percentages of occurrence of the diatom species were calculated to estimate the probability of encounter of a particular diatom species isolated with respect to location of the collection of the foraminifera, as well as within each host species.

The data were fitted by means of least squares to regression analyses to test the effect of location and host species to the number of encounters of a particular diatom endosymbiont species. Analyses of variance were used to determine whether the estimated regression coefficients departed from zero by more than could be expected by chance. Also, the number of encounters of a particular diatom species was treated as a 2^2 factoral design (SAS Institute, Cary, NC) to test possible interactive effects between the location of the collection and the host species (designated as factors with 2 and 8 levels, respectively).

The data from this study were then pooled with Pacific data from our previous study (Lee et al., 1989) which were collected from the Heron-Wistori Channel, Great Barrier Reef, Australia, and the Makapuu Tide Pool, Hawaii. The above mentioned statistical analyses were repeated for the data with respect to location and season of the collection, and host species.

3. Results and Discussion

Almost two hundred specimens from Palau (193) and over four hundred (434) specimens form Kudaka Island were examined during the course of this study (Table 1). More than one hundred fifty specimens of the most frequently examined hosts, *Calcarina gaudichaudi* (167) and *Baculogypsina sphaerulata*, were examined. One of the authors (R.R.) has been informed that the *Baculogypsina* from Palau may belong to a new, as yet undescribed, species which will be separated from *B. sphaerulata*. Since we do not know the characters which might be used, we did not make the distinction. Significant numbers of other foraminifera, *N. calcar* (77), *C. spengleri* (37), and *C. defrancei* (51), were also studied. Fairly large numbers (31, 62, 32) of the same host species examined in the collections from the Red Sea, *Amphistegina lessonii*, *A. lobifera*, *Heterostegina depressa*, were also available in the new collections for comparison.

The diatom species isolated from the foraminifera in this study included *Fragilaria shiloi* (Lee, Reimer and McEnery, 1980b), *Achnanthes maceneryae* (Reimer and Lee (1988), *Cocconeis andersonii* (Reimer and Lee, 1988), *Achnanthes maceneryae* (Lee and Reimer, 1983), *A. erezii* (Reimer and Lee

Table 1. Percentage of occurrence of the diatom species isolated from collections from Palau and Kudaka Island, and from different hosts. The host isolations data are combined for both locations. Each value is a percentage of the number of isolates for each column.

			Location		Host species							
	Total No.	Total %	PAL	KIJ	*A. lessonii*	*A. lobifera*	*H. depressa*	*N. calcar*	*C. spengleri*	*C. defrancei*	*C. gaudi-chaudi*	*B. sphaer-ulata*
Fragilaria shiloi	72	11.5	12.4	11.1	48.4	41.9		2.6		9.8	10.2	4.1
Achnanthes maceneryae	11	1.8	1.6	1.8		4.8		10.4				
Cocconeis andersonii	66	10.5	20.2	6.2		8.1	50.0	5.2	5.4	9.8	12.0	8.2
Amphora roettgeri	106	16.9	7.3	21.2			43.8	35.0		39.2	12.0	14.7
Amphora erezii	128	20.4	2.1	28.6		6.5		3.9			37.7	35.9
Navicula hanseniana	6	1.0	2.1	0.5					5.4	5.9	0.6	
Navicula sp. (W)	26	4.1	9.3	1.8	3.2	12.9			29.7	2.0	1.2	
Nitzschia frustulum v. *symbiotica*	155	24.7	34.7	20.3	48.4	19.4		41.6	54.1	29.4	16.8	19.4
Nitzschia laevis	32	5.1	4.7	5.3					5.4	3.9	7.8	8.8
Nitzschia panduriformis v. *continua*	25	4.0	5.7	3.2		6.5	6.3	1.2			1.8	8.8
Number of isolates	627		193	434	31	62	32	77	37	51	167	170

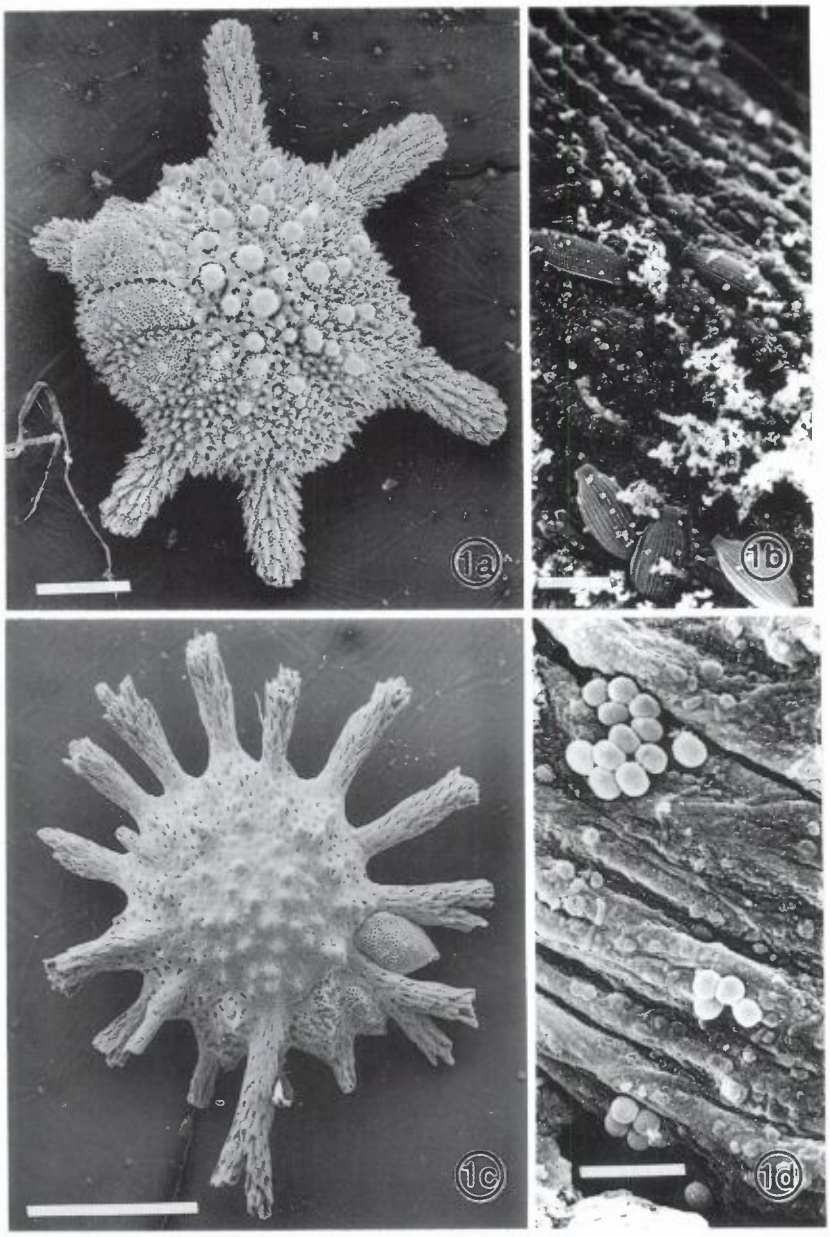

Figure 1. Scanning electron micrographs: (a) *Calcarina spengleri*, one of the "star sand" foraminifera examined in this study; (b) macrophyte on which the foraminifera were grazing showing epiphytic *Amphora* spp. available as potential food; (c) *Calcarina defrancei*, another of the "star sand" hosts; (d) macrophyte on which the foraminifera were grazing showing the abundance of bacteria available as potential food. Bars represent: (a) 0.4 mm; (b) 10 μm; (c) 0.5 mm; (d) 5 μm.

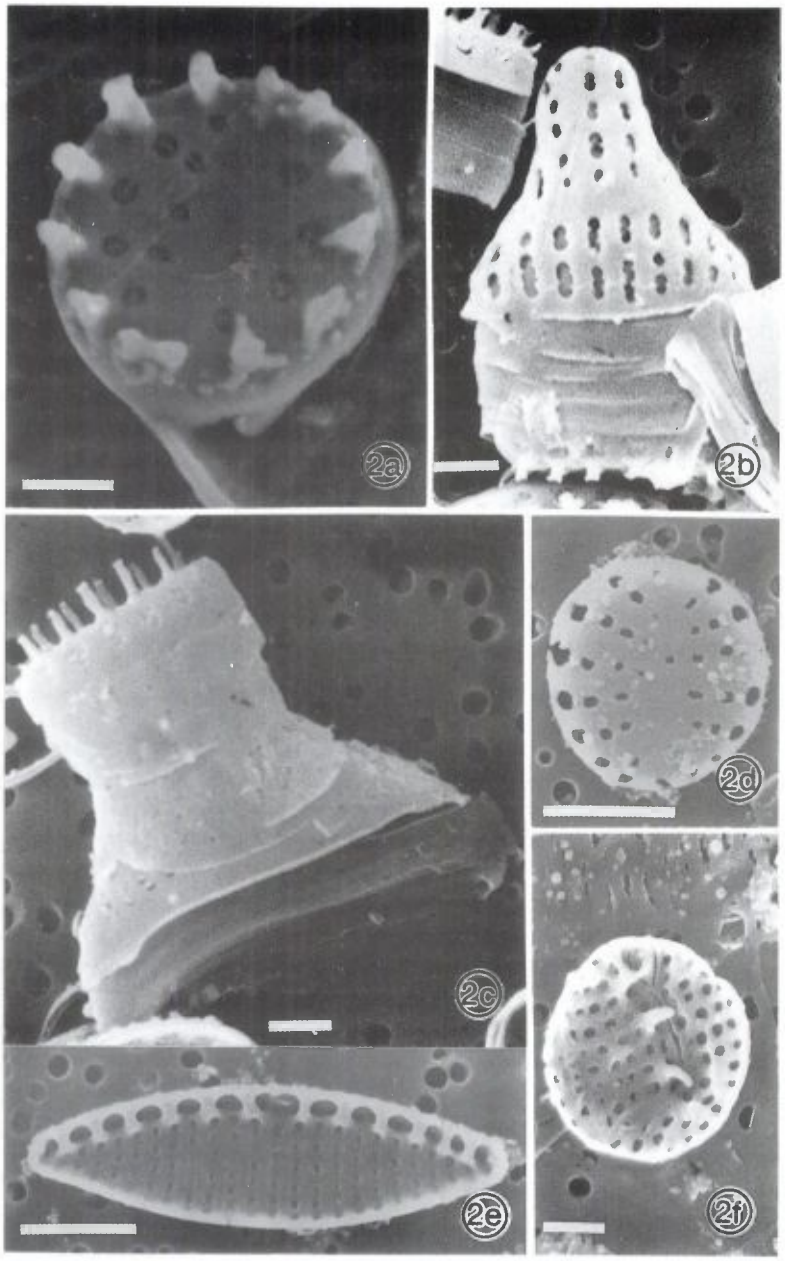

Figure 2. Scanning electron micrographs of endosymbiotic diatoms: (a) ***Fragilaria shiloi***, similar to type; (b&c) *F. shiloi*, showing unusual valve jackets a here-to-fore unknown type of size rejuvination; (d) *F. shiloi*, from an extremely small clone; (e) *Nitzschia frustulum* var. *symbiotica*, from an extremely small spherical clone; (f) *N.f. symbiotica*, from a clone which rejuvinated species as large as this one. Bars represent 1 μm except (e) which represents 2 μm.

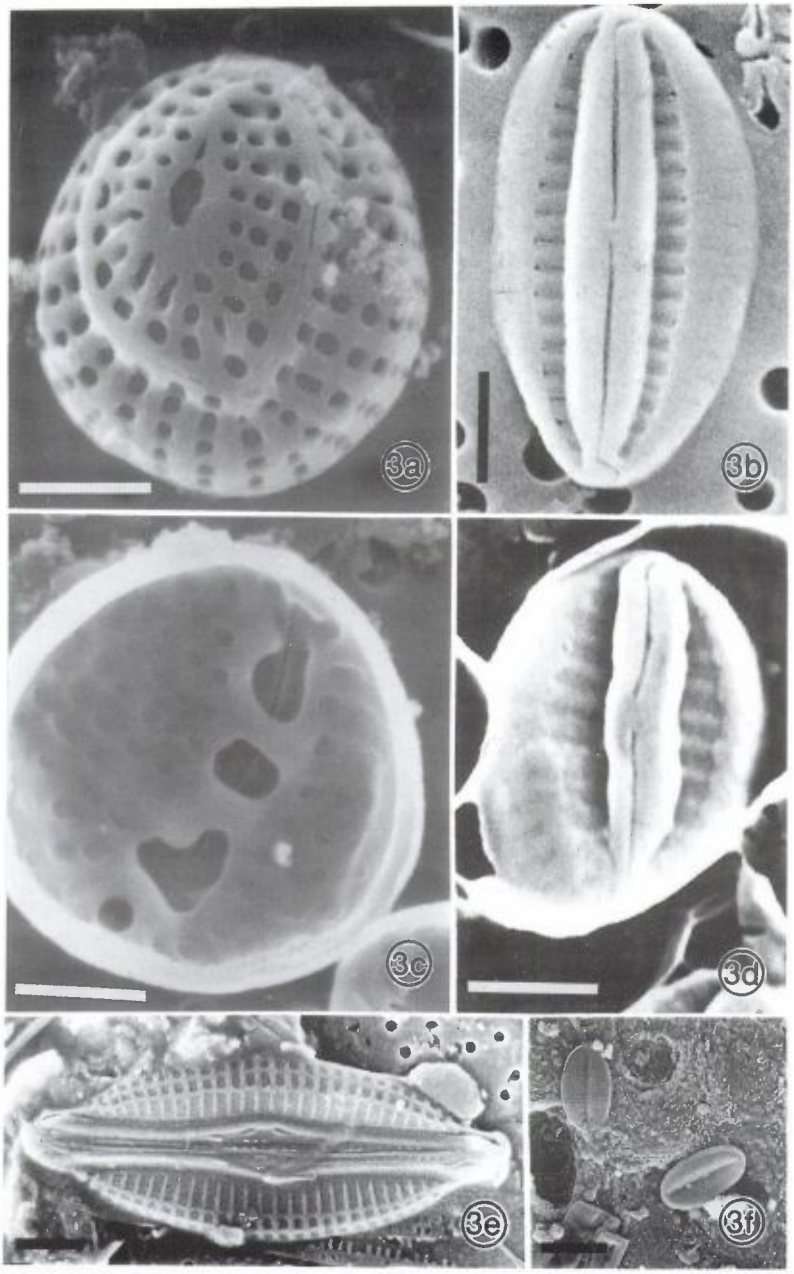

Figure 3. Scanning electron micrographs of endosymbiotic diatoms (a–d) and 2 free-living forms (e&f): (a&c) *Nitzschia frustulum* var. *symbiotica*, spherical clones, (a) raphe curved in an arc, (c) with irregular keel fibulae, (b&c) unidentified species of *Navicula* (W), from a very large clone, (c) from a very small clone; (e&f) common species of diatoms found in the habitat of the foraminifera, (e) *Amphora* sp., (f) *Cocconeis placentula*. Bars represent: (a–d) 1 μm; (e) 2 μm; (f) 5 μm.

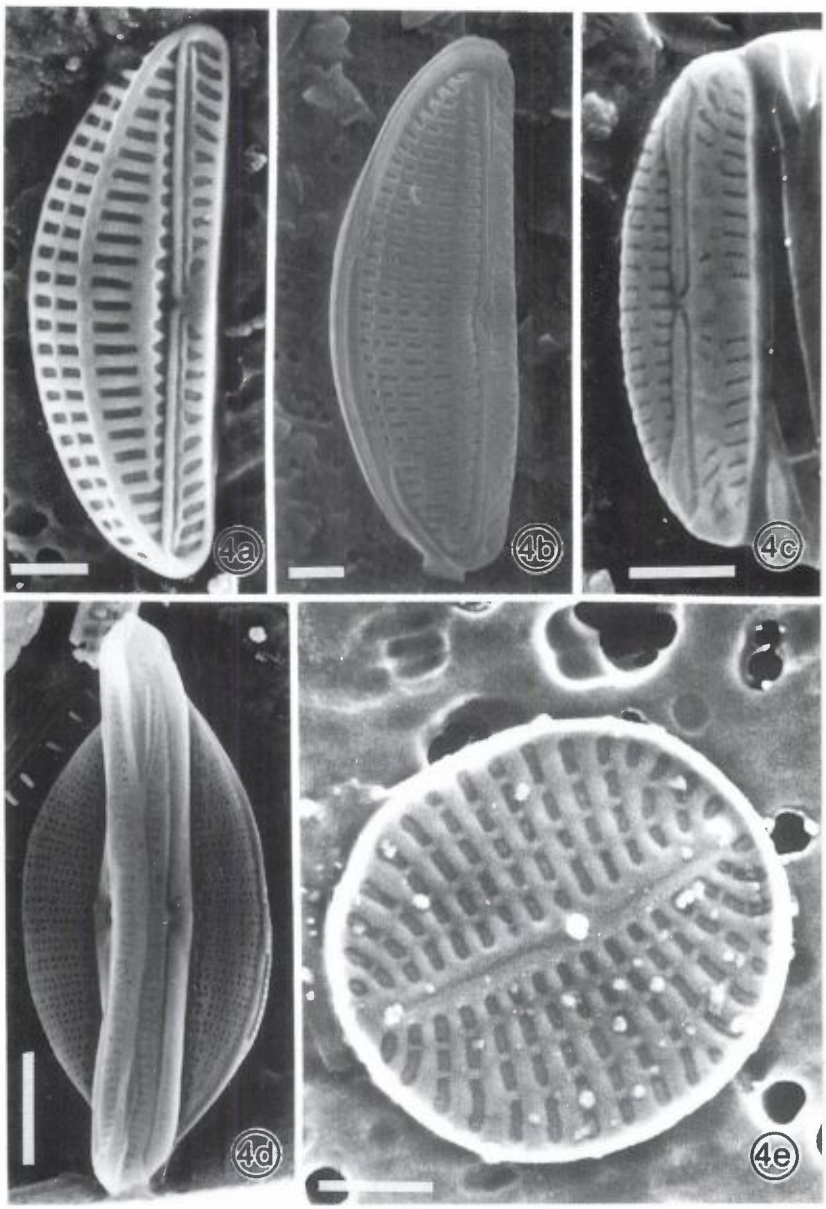

Figure 4. Scanning electron micrographs of free-living diatoms from the habitat of the foraminifera (a–d) and one symbiotic form; (a) *Amphora* sp.; (b) *Amphora* sp.; (c) *Amphora* sp.; (d) *Amphora* sp.; (e) *Cocconeis andersonii*. Scale bars represent: (a&e) 1 μm; (b–d) 2 μm.

(1988), *Navicula hanseniana* (Lee and Reimer (1983), *Nitzschia frustulum* var. *symbiotica* (Lee and Reimer, 1983), *N. laevis* and *N. panduriformis* var. *continua* Grun. (Lee et al., 1979a,b). There were very few surprises in the new isolations. Only one here-to-fore undescribed new species of *Navicula* was found (Figs. 3b&d). As in the previous study (Lee et al., 1989), *Nitzschia frustulum* var. *symbiotica* was the most frequently isolated (24.7%) species (Table 1). Although many of the new isolates resembled the type, many of the new isolates were much smaller and almost spherical (Figs. 2e, 3a&c). Generation of larger-sized specimens was not observed in the third serial cultures transferred from the original isolates of tiny clones (< 2.5 μm), so we must conclude that they are probably genetically locked into this unusually small size range. Except for *Heterostegina depressa* significant numbers of *Nitzschia frustulum* var. *symbiotica* were isolated from all the hosts examined. Two species of *Amphora*, *A. erezii* and *A. roettgeri* were the next most frequently (20.4% and 16.9% respectively) isolated endosymbionts. *A. erezii* was not found in *C. spengleri*, *C. defrancei*, *A. lobifera*, or *H. depressa* but was abundant in *C. gaudichaudi* and *Baculogypsina sphaerulata*. On the other hand, *A. roettgeri* was frequently isolated from *N. calcar*, *C. defrancei* and *H. depressa*. Many of the specimens of the other two frequently isolated species, *Fragilaria shiloi* (11.5%) and *Cocconeis andersonii* (10.5%) were also tiny (< 3 μm) and subspherical (Fig. 2d). The pattern of size rejuvination in some clones of *F. shiloi* was exceptional for diatoms (Figs. 2b&c) and will be the subject of a separate report (Reimer and Lee, unpublished).

Three species of diatoms, *N. panduriformis* var. *continua*, *N. laevis* and a new species of *Navicula* sp(W) were also frequently (4–5%) isolated. The new species (Figs. 3f, 4a-d) will be described separately (Reimer and Lee, in prep. *op. cit.*). While different specimens of most of the host species examined harbored a variety (5–8) of different species, two hosts in the collections, *A. lobifera* and *H. depressa*, were more restrictive (Table 1). The endosymbionts in the latter 2 foraminifera were almost equally divided between 2 different algal species: *Fragilaria shiloi* and *N.f. symbiotica* in the case of *A. lobifera* and *Cocconeis andersonii* and *Amphora roettgeri* in the case of *H. depressa*. Taken as a whole, we were not able to correlate isolations of particular diatom species with particular hosts or particular locations where they were collected (Table 2). Perhaps a more accurate picture of the diatom species distribution in Pacific foraminiferal hosts is obtained when we combine the data obtained from all the Pacific diatom-bearing hosts (Lee et al., 1989, Hawaii [MTP], Great Barrier Reef [GBR], with those of the paper, Palau [PAL] and Kudaka Island [KIJ]; Table 3). The total isolate data base increased to nearly nine hundred. *Nitzschia frustulum* var. *symbiotica* remained the most abundant of

Table 2. ANOVA F-values for the diatom species isolated as endosymbionts from collections at Palau and Kudaka Island, and with different host species. All values were not significant ($P \geq 0.05$).

	Host species	Location
Fragilaria shiloi	3.02	0.33
Achnanthes maceneryae	1.05	0.36
Cocconeis andersonii	0.07	0.98
Amphora roettgeri	0.52	3.31
Amphora erezii	1.71	1.63
Navicula hanseniana	0.48	0.67
Navicula sp. (W)	1.25	1.61
Nitzschia frustulum v. *symbiotica*	0.28	0.02
Nitzschia laevis	4.29	0.51
Nitzschia panduriformis v. *continua*	0.97	<0.00
Degrees of Freedom	1.16	1.16

all the isolates, it being found in 25.9% of all the associates. While their absolute percentage dropped, *Amphora roettgeri* (14.6%) and *A. erezii* (15.4%) remained the second and third most abundant species. *Fragilaria shiloi* remained fourth most abundant (12%). It was followed by *Cocconeis andersonii* (7.6%), *Nitzschia laevis* (5.9%), *N. panduriformis* var. *continua* (4.8%), and *N. valdestriata* (3.6%) and 8 other less abundant (< 3%) species. It was significant to note that when the Pacific species data were combined, a greater variety of diatom species was isolated from both *Amphistegina lessonii* (11) and *Heterostegina depressa* (5). Though abundant in most hosts, *Fragilaria shiloi* has not yet been isolated from a single specimen of *H. depressa* (Lee et al., 1989 or present data). Although *N.f. symbiotica* was common (20%) in *H. depressa* from the Red Sea, it was not recovered from any specimens of the same host collected at Pacific stations. *Achnanthes maceneryae* which was abundant in two host species, *H. depressa* (17.7%) and *Operculina ammonoides* (29.8%), collected from the Red Sea (Lee et al., 1989) was rarely encountered in Pacific hosts. The exception was *Neorotalia calcar.*

We were fortunate to collect many bits of algae and coral rubble with foraminifera on them at Kudaka Island. As before, we expended a great deal of time and energy to search them for species of diatoms which were the same as those we found as endosymbionts. Since we were searching for very small species, all of our studies were done with the aid of the SEM. Small species of *Amphora, Cocconeis*, *Navicula*, and *Nitzschia* were quite abundant (Figs. 3–4). In fact the potential for foraminifera to recruit small-sized diatoms was greater on Kudaka Island than in any other site we have studied. Small size

Table 3. Percentage of occurrence of the isolated diatom endosymbiont species of this study combined with the data of Lee et al. (1989) from Pacific collections. The location of collection data are combined for all host species, and similarly the host isolations data are combined for all locations. Each value is a percentage of the number of isolates for each column.

			Location				Host species								
	Total no.	Total %	MTP	GBR	PAL	KU	*A. lessonii*	*A. lobifera*	*H. depressa*	*B. schlumbergeri*	*N. calcar*	*C. spengleri*	*C. deformis rancei*	*C. gaudichaudi*	*B. sphaerulata*
Fragilaria shiloi	108	12.0	9.6	31.0	11.0	11.1	17.4	28.1		30.0	2.6		9.8	10.2	4.1
Fragilaria sp. (K)	11	1.2			5.0			6.2							
Achnanthes maceneryae	11	1.2			1.4	1.8		1.7			10.4				
Achnanthes sp. (L)	5	0.6	2.7					2.8							
Cocconeis andersonii	68	7.6	1.1		17.8	6.2	1.4	2.8	22.9		5.2	5.4	9.8	12.0	8.2
Amphora roettgeri	131	14.6	6.4	22.4	6.4	21.2	8.7		37.1	10.0	35.1		39.2	12.0	14.7
Amphora tenerrima	17	1.9	2.7	1.7	5.0		0.7	9.0							
Amphora erezii	138	15.4	1.1	13.8	1.8	28.6	1.4	2.2	4.3	50.0				37.7	35.9
Navicula hanseniana	10	1.1	2.1		1.8	0.5		2.2					5.4	5.9	0.6
Navicula reissii & *N. muscatinei*	11	1.2		19.0			7.2			10.0					
Navicula sp. (W)	26	2.9			8.2	1.8	0.7	4.5			3.9	27.9	2.0	1.2	
Nitzschia frustulum v. *symbiotica*	233	25.9	41.7		30.6	20.3	39.9	28.1			41.6	54.1	29.4	16.8	19.4
Nitzschia frustulum variety	1	0.1	0.5					0.6							
Nitzschia laevis	53	5.9	9.1	6.9	4.1	5.3	11.6	2.8				5.4	3.9	7.8	8.8
Nitzschia panduriformis v. *continua*	43	4.8	8.0	5.2	5.0	3.2	5.1	6.7	7.1		1.3			1.8	8.8
Nitzschia valdestriata	32	3.6	15.0		1.8		5.8	2.2	28.6						
Number of isolates	898		187	58	219	434	138	178	70	10	77	37	51	167	170

Table 4. Percentage of occurrence of the isolated diatom endosymbiont species of this study combined with the data of Lee et al. (1989) for all collections. Each value of the number of isolates for each column.

			Host species									
	Total no.	Total %	*A. lessonii*	*A. lobifera*	*H. depressa*	*B. schlumbergeri*	*O. ammonoides*	*N. calcar*	*C. spengleri*	*C. defrancei*	*C. gaudichaudi*	*B. sphaerulata*
Fragilaria shiloi	272	10.3	81	141		15	4	2		5	17	7
Fragilaria sp. (K)	15	0.6		15								
Achnanthes maceneryae	90	3.4	13	3	50	2	14	8				
Achnanthes sp. (L.)	24	0.9		24								
Cocconeis andersonii	94	3.6	13	17	16		3	4	2	5	20	14
Amphora roettgerii	227	8.6	35	46	48	1	5	27		20	20	25
Amphora tenerrima	29	1.1	5	18	6							
Amphora erezii	184	7.0	9	28	18	5					63	61
Amphora sp. (J)	31	1.2				1	30					
Entomoneis paludosa v. *densistriata*	9	0.3		9								
Navicula hanseniana	77	2.9	24	44	2	1			2	3	1	
Navicula reissi & *N. muscatinei*	40	1.5	31	4		5						
Navicula sp. (W)	71	2.7	16	32		5		4	11	1	2	
Protokeelia hottingeri	26	1.0	5	14	7							
Nitzschia frustulum v. *symbiotica*	834	31.6	242	368	57	7	6	58	20	15	28	33
Nitzschia frustulum variety	44	1.7	15	27	2							
Nitzschia laevis	207	7.8	65	71	14	13	12		2	2	13	15
Nitzschia panduriformis v. *continua*	318	12.0	118	98	73	6	3	2			3	15
Nitzschia valdestriata	49	1.9	9	16	20	4						
Number of isolates	2641		681	975	313	65	77	105	37	51	167	170

is apparently not enough to qualify a diatom to be a potential endosymbiont. Species we have isolated as endosymbionts were extremely rare ($\ll 1\%$). We were able to identify the characteristics of more than 1×10^5 specimens in our SEM studies. We found 18 specimens of *Fragilaria shiloi*, 14 *N. panduriformis* var. *continua*, 9 *N. laevis*, 4 *N.f. symbiotica* and 2 *Navicula reissii* in our entire search.

The results of the present study, when coupled to those reported earlier (Lee et al., 1980a,b, 1989) led to several conclusions: (1) Larger foraminifera do not host the most abundant diatoms in their habitat. (2) They must acquire their endosymbionts by passage during asexual reproduction or from their parents remains, or the habitat after sexual reproduction. (3) There does not seem to be a host/symbiont specific relationship in diatom-bearing larger foraminifera in the same context as has been implied for dinoflagellates belonging to the *Symbiodinium*-complex and their hosts (Trench 1987, 1988; Trench and Blank, 1987). (4) Very few species of small (< 10 μm) pennate diatoms are involved in the phenomenon. Ten species were found in 90% of symbionts isolated. This suggests that there must be some aspect of specificity between hosts and their endosymbionts. The most common diatoms (*Nitzschia frustulum* var. *symbiotica*, *N. laevis*, *N. panduriformis*, *Fragilaria shiloi*, *Amphora roettgeri*, *A. erezii*) which are involved in over 75% of all the symbiont systems examined, belong to a variety of taxonomically distantly related genera. *Nitzschia* species accounted for only 55% of all the isolates.

Recent studies in our lab have shown that foraminiferal digestion often begins outside of the organism's shell in the extended rhizopodia (Lee et al., 1991; Faber and Lee, 1992). It is reasoned that those algal species which escape initial external digestion by the foraminifera have the potential to establish themselves as endosymbionts. How is this accomplished? Do the diatoms have some sort of recognition signals on their surfaces which can be identified by the pseudopods of the foraminifera? Do the diatoms produce something which interferes with the digestive process? What is the mechanism? While the present study has laid to rest some old questions on recruitment of symbionts, and host specificity, it has given rise to new ones. It is with eager anticipation that we will take steps in new directions.

Acknowledgements

This reseach was aided by PSC/CUNY grant 6-69182 (JJL) and a grant from the Deutsche Forschungsgemeinschaft (RR). The scanning electron microscope at City College CUNY used in this study was purchased with funds provided by a NSF grant (DIR-8804964). Dr. Charles Reimer, Diatom Herbarium,

Philadelpha Academy of Natural Sciences, was very helpful to us in the identification of the diatoms. Mr. Joji Nakama, University of the Ryukyus, assisted us in the field study at Kudaka Island.

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