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Endosymbiotic Diatoms from Previously Unsampled Habitats

JOHN J. LEE[1]* and MARIA CORREIA[2]
[1]*Department of Biology, City College of CUNY, Convent Ave @ 138 St., New York, NY 10031, USA, Tel. +1-212-650-8545, Fax. +1-212-650-8585, E-mail. jlee@ccny.cuny.edu;*
[2]*Ciclo de Estudos Biomédicos, Centro Regional das Beiras, Universidade Católica Portuguesa, Estrada da Circunvalação, 3504-505 Viseu, Portugal*

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Abstract

New endosymbiotic diatom isolations from previously unsampled field sites are reported. *Nitzschia laevis* was the most commonly isolated diatom from populations of *Amphistegina lobifera* collected at both the North Beach in the Gulf of Eilat and at Sifnos, Greece. *Nitzschia frustulum* var. *symbiotica* (as emended by Lee et al., 2000) was the second most abundant species of diatoms found in the *Amphistegina* examined from the North Beach, Gulf of Eilat, (31%) and Sifnos, Greece, (28%) and the most abundant diatom species present in the specimens examined from Coconut Beach, Lizard Island, Great Barrier Reef. New observations are not entirely in consonance with previous observations, but one general conclusion has not changed: a small number of diatom species (~20) are involved in the endosymbiotic phenomenon and certain species are dominant in every population. A rare, yet to be described, nitzschioid diatom was found and figured.

Keywords: Larger foraminifera, endosymbiotic diatoms, *Nitzschia laevis*, *Amphistegina lobifera*, *Nitzschia frustulum* var. *symbiotica*

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*The author to whom correspondence should be sent.

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1. Introduction

Symbiosis with diatoms as the smaller endosymbiotic partner is a rather unusual phenomenon in shallow tropical seas. Yet, four families of very abundant larger foraminifera are hosts to diatoms (reviewed by Lee, 1994, 1995, 2001). The fossil record of probable diatom-bearing hosts is quite impressive (mountains of deposits) and a testimony to the success of this form of symbiosis from the late Mesozoic to the present (Lee, 1995). We are still at the discovery phase of these host-symbiont relationships. Only a few experimental studies of host-symbiont specificity have been attempted (Lee et al., 1983, 1986). Sampling thus far has been opportunistic, and not synoptic at any location with respect to season or over time. Based on the examination of ~3,000 specimens from <20 different habitats over 20 years (Lee et al., 1980, 1989b, 1992, 1995), it is concluded that the relationship between the partners is not finical (Lee, 2001). Although the same host species harbored, at any particular sampling time, any one or two of several dozen taxonomically diverse pennate diatom species, six species, *Nitzschia frustulum* var. *symbiotica* (Lee et al., 2000), *Nitz. laevis*, *Nitz. panduriformis* var. *continua*, *Nanofrustulum* (*Fragillaria*) *shiloi*, Amphora roettgerii and *Amp. erezi* accounted for 75% of all the associations. Common to all of the endosymbiotic diatoms is a 104 kDa antigen, which is recognized by receptors on the host reticulopodia (Chai and Lee, 1999, 2000). Several of the rarer endosymbiotic diatoms (e.g., *Achnanthes maceneryae*, *Navicula muscatini*, *Protokeelia hottingeri*) turned out to be very interesting, hitherto undescribed, species.

Because the relationship between hosts and their symbionts is so imprecise, the question of the diatom symbiont species found in any given population is always open and begs inquiry. In the past several years, we have had the opportunity to sample, or receive samples, from previously unexamined populations. The samples from Sifnos are the first obtained from diatom-bearing foraminifera in the Mediterranean Sea.

2. Materials and Methods

During the workshop on the Biology of Living Foraminifera (Lee and Hallock, 2000) held on Lizard Island, Great Barrier Reef, the authors had the opportunity to sample (0.5–20 m depth at low tide) Coconut Beach Cove, a location not far from the Lizard Island Marine Station. There, the diatom-bearing "star sands" (Calcarinidae) are exceptionally abundant. Several small samples of macrophyte green algae with clinging stars were carefully placed in small (250 ml) plastic bags and brought back to the Marine Station for identification and processing.

Two samples were collected by Professor Jonathan Erez and his students (Hebrew University of Jerusalem). Their first sample came from 3 m depth near the Island of Sifnos, Mediterranean Sea in September 2002. The second sample was obtained in January 2003, by SCUBA at ~20 m, from the *Halophila* meadow near the North Beach in the Gulf of Eilat. The samples from Professor Erez were sent to our laboratory in New York for processing. The methods for isolating, culturing and identifying the endosymbiotic diatoms followed those used by us on previous occasions (e.g. Lee et al., 1992).

3. Results and Discussion

Nitzschia laevis was the most commonly isolated diatom from populations of *Amphistegina lobifera* collected at both the North Beach in the Gulf of Eilat and at Sifnos, being found in 78% of the former and 40% of the latter (Table 1).

However, it was found in only 10% of the foraminifera examined from Coconut Beach. That overall consolidated finding may be misleading because *Nitz. laevis* was found in 30% of the *Calcarina hispida* form *spinosus* and 36% of the *Operculina ammonoides* examined. It was not found in *Alveolinella quoyi*, *Amphistegina lobifera*, *Baculogypsina spinosus*, *Heterostegina depressa* or *Parasorites orbitolitoides* examined (Table 2). *Nitzschia laevis* was first identified as an endosymbiont in *Amphistigina lessonii* (9.3%) from Hawaii (Lee et al., 1980). While it was present in only 3.8% of the symbionts collected near the Steinitz Marine Laboratory in the Gulf of Eilat it was more abundant in some samples taken further south in the Gulf (Taba, 12%; Ras Burka, 33.3%) and absent in samples from other populations of foraminifera to the south (Coral Island and El Habik) (Lee et al., 1985). It was found in 10% of diatom isolations from *Amphistegina gibbosa* from the Florida Keys (Lee et al., 1995). It was rare in previous samples from other Pacific habitats (Heron-Wistori Channel, GBR, 6.3%; Mombassa Harbor, Kenya, 1.9%; Palau, 0%).

Nitzschia frustulum var. *symbiotica* (as emended by Lee et al., 2000) was the second most abundant species of diatom found in the *Amphistegina lobifera* examined from the North Beach (31%) and Sifnos (28%) and the most abundant diatom species present in the specimens examined from Coconut Beach (47%, Table 1). This diatom was exceptionally abundant in *Alv. quoyi* (60%), *B. spinosus* (72%), *C. hispida* form *spinosus* (72%), *H. depressa* (50%) and *O. ammonoides* (50%) examined (Table 2). These abundances are not out of line with previous findings since it was abundant in previous samples from Taba (32.1%) and the Steinetz Laboratory (40%) on the Red Sea, Mombassa Harbor (58.5 %) and the Makapuu Tide Pool, Hawaii (42.9%) (Lee et al., 1985). It was not a common symbiont (4%) in *Amphistegina gibbosa* from the Florida Keys (Lee et al., 1995).

Navicula hanseniana was particularly abundant in the population of *Amphistegina lobifera* from the North Beach (26%) and *Alveolinella quoyi* from Coconut Beach (20%), but rare in other hosts (Tables 1 and 2). It was never as abundant in any previous populations examined (Lee et al., 1985, 1992, 1995).

Species of *Amphora* were not found as symbionts among the foraminifera we examined from the North Beach or Sifnos assemblages, but they were widespread in the specimens from Coconut Beach Cove (Table 1). *Amphora tenerrima* was found in 60% of *Alv. quoyi*, and 9% of *C. hispida* form *spinosus* had *A. roettgerii* (Table 2). This pattern of variable abundance has been noted in all of our previous studies including those from the Gulf of Eilat (Lee et al., 1985, 1992, 1995).

Nanofrustulum (Fragilaria) shiloi was one of the first endosymbiotic diatoms to be identified (Lee et al., 1979, 1980). It was found in 6% of the *Amphistegina lobifera* from Sifnos, but it was more common in *A. lobifera* collected from 6 m (17%), *C. hispida* form *spinosus* (21.2%) and *O. ammonoides* (10%) from Coconut Beach (Table 2).

Nitzschia pandiformis was numerous only in populations of *Amphistegina lobifera* collected from 20 m, and in populations of *C. hispida* form *spinosus* (30.3%) and *H. depressa* (30%) from Coconut Beach.

Table 1. Endosymbiotic diatoms recently isolated.

Endosymbiont	North Beach Gulf of Eilat-Red Sea *Amphistegina lobifera*	Sifnos -Med. Sea *Amphistegina lobifera*	Coconut Beach Cove Lizard Island-GBR Australia (all 7 host species)
Nitzschia frustulum symbiotica	31	28	47
Nitz. laevis	78	40	10
Nitz. frustulum	–	24	15
Nitz. panduriformis	2	–	–
Navicula hanseniana	26	2	<1
Undescribed genus and species (Fig. 1)	–	7	4
Amphora spp.	–	–	22
Nanofrustulum (Fragilaria) shiloi	–	6	7
Navicula spp.		2	
Total specimens	50	50	154

Data reported as % of hosts which harbored a particular diatom species. Some hosts harbored more than 2 species of diatoms, a few harbored 3 species.

Table 2. Diatoms isolated from 7 hosts collected on Coconut Beach cove, Lizard Island, GBR, Australia.

Symbionts	Hosts							
	Alveolinella 3m	*Amphistegina* 6 m	*Amphistegina* 20 m	*Baculogypsina* 3m	*Calcarina* 3m	*Heterostegina* 3m	*Operculina* 3m	*Parasorites* 3m
Amphora bigibba		8.3						
Amphora erezi			6.7	5.6	3			
Amphora roettgerii					9.1			
Amphora sp.		16.7	3.3		3		10	8.3
Amphora tenerrima	60.0		3.3	5.6	24.2			32.3
Undescribed nitzschoid genus and species (Fig. 1)			3.3		9.1			
Cocconeis andersonii	40		10	17	3	83	34	8
Cymbella sp.		8.3						
Nanofrustulum (Fragilaria) shiloi		17			21		10	
Navicula hanseniana	20	8	7		3			
Navicula muscatini					3			
Navicula sp.	20			3	11			33
Nitzschia frustulum						33		
Nitzschia frustulum symbioticum	60	17	40	72	73	50	50	8
Nitzschia laevis					36		30	
Nitzschia panduriformis			10	3	30	33.3		
Nitzschia panduriformis (variety)		42			6			17
Nitzschia sp.			7					25
Number of hosts	14	25	25	30	30	10	10	10

Data reported as % of hosts which harbored a particular diatom species. Some hosts harbored more than 2 species of diatoms, a few harbored 3 species.

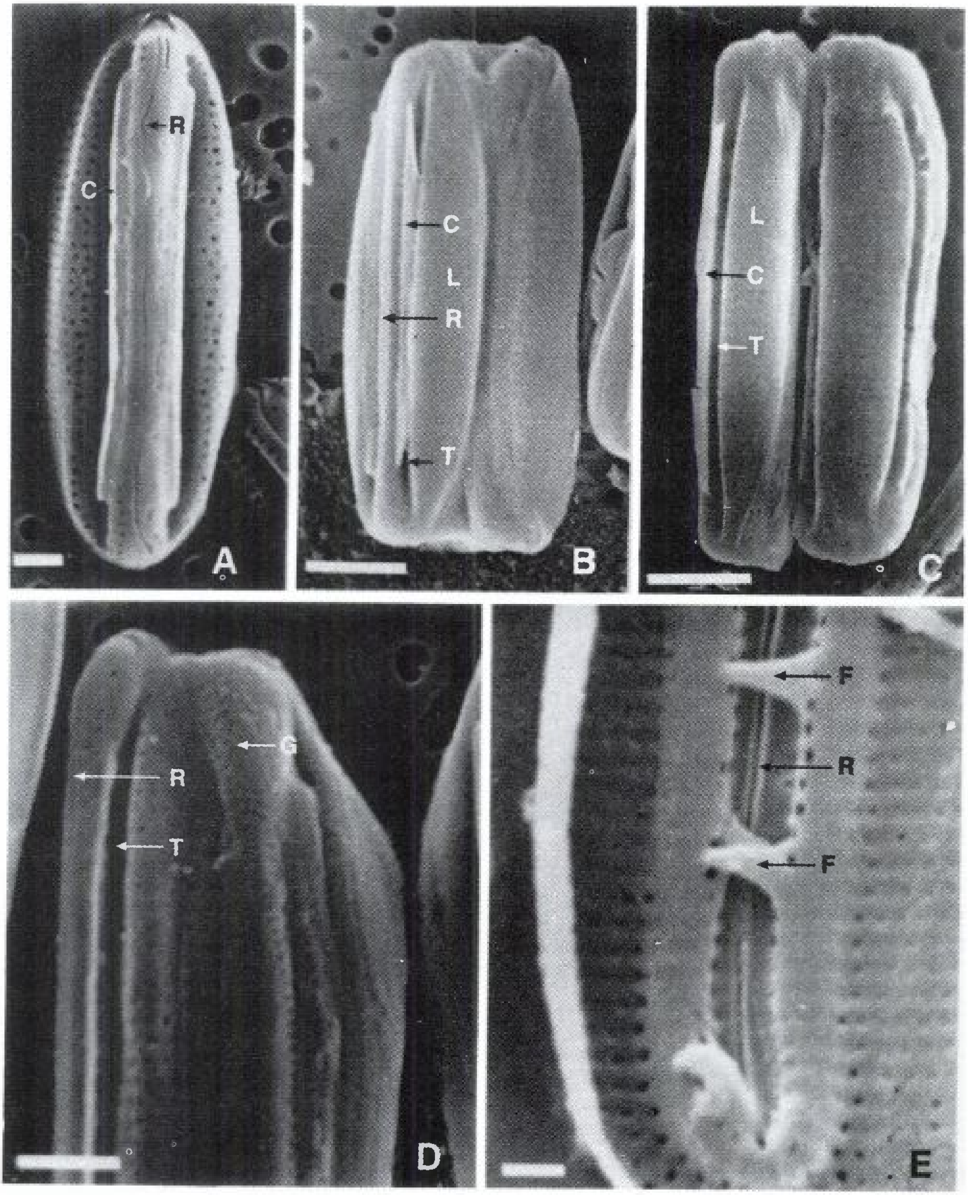

Figure 1. SEMs of a new (genus and species) diatom endosymbiont found in hosts examined from Coconut Beach, Lizard Island, Great Barrier Reef, Australia. C - Conopeum; F - Fibula; G - Girdle band; L - Lateral chamber; R - Raphesternum; T - Tubular space.
See continuation of legend on next page.

These observations differ somewhat from overall observations in previously published data from other sites (Lee et al., 1995). Most of the species of larger foraminifera at Coconut Beach had more (40–72%) *Nitzschia frustulum* var. *symbiotica* than the average of assemblages of foraminifera from other sites (30.6%). *Nitzschia laevis* was much more abundant in populations from North Beach and Sifnos than in hosts at any of the previous sites sampled. The average abundance of *N. shiloi* at most sites was ~8.6% in previous collections but in those sampled for this paper it was relatively rare except in the 6 m collections of *Amphistegina lobifera* and *C. hispida* form *spinosus* at Coconut Beach. While *N. panduriformis* var. *continua* was fairly abundant in all our previous collections (11%) it was only abundant in the 20 m *Amphistegina lobifera* and *C. hispida* form *spinosus* and *H. depressa* from Coconut Beach. A previously unrecognized variety of *Nitzschia panduriformis* was found in *A. lobifera* and *Parasorites* from Coconut beach.

Although new observations are not entirely in consonance with previous observations one general conclusion has not changed: a small number of diatom species (~20) are involved in the endosymbiotic phenomenon and certain species are quite dominant in every population. With the exception of *N. frustulum* var. *symbiotica,* the dominant diatoms are not necessarily the same ones in any given population.

The fact that many hosts harbor more than one symbiotic species at the same time raises the question of the adaptive value of different symbionts in changing environmental conditions. This has barely been probed. One study examined the photoadaption of four endosymbiotic diatoms isolated in axenic culture from their foraminiferan hosts (Lee et al., 1982). Their data suggested that the diatoms are very well adapted to function as endosymbionts in a wide range of the euphotic zone. All 4 diatoms had photocompensation points near 2% of incident light at the sea surface. In the Gulf of Eilat, depending upon weather and season, this would be reached between 40–50 m. There were differences in the optimal irradiance among the 4 different diatoms studied.

Figure 1. A) Valve face view. Conopeum detached from valve face (scale = 1 µm). B) Valve face some-what lateral view showing conopeum attached to valve face enclosing a tubular space. Lateral chamber of each valve clearly delineated in this view (scale = 2 µm). C) Girdle view showing lateral chambers and tubular space beneath each conopeum (detached from valve face of chambers in this specimen so that the tubular space is more visible (scale = 2 µm). D) Higher magnification of the apical end of the specimen figured in C (above) (scale = 0.5 µm). E) Interior view of raphe-sternum showing fibulae (scale = 0.5 µm).

Nanofrustulum (*Fragilaria*) *shiloi* and *Nitzschia laevis*, both isolated as endosymbionts of *Amphistegina lessonii*, grew fastest in a light regime of 312 μW cm^{-2}, the highest level tested. The two endosymbiotic diatoms isolated from *Heterostegina depressa* (*Nitzschia valdestriata* and *N. panduriformis*) grew best at lower light levels (19 μW cm^{-2}) and were photoinhibited at higher light levels.

Results from Eilat and elsewhere seem in consonance with the distribution of *Heterostegina depressa* on the shaded side of the Makapuu Tide Pool in Hawaii (Röttger, 1976). Experiments with *Amphistegina lessonii* and *A. lobifera* indicated in general that light intensities substantially affected their optimum growth rates (Röttger et al., 1980; Hallock, 1981).

Many avenues of future research offer promise for insight into questions of symbiont diversity in diatom-bearing hosts. One approach would be to examine the same population throughout the year. This might be aided by using immunolabeling technique to quantitatively analyze populations of symbionts released from crushed diatom-bearing hosts (Lee et al., 1989a). Another approach would be to examine the symbionts in the same host species over environmental gradients of temperatures or light. Additional laboratory experiments of isolated endosymbiotic diatoms could also yield some insight.

We encountered again a rare, as yet to be described nitzschioid diatom (Figs. 1A–D). It has a raphae-sternum system which has some characteristics suggestive of the genera *Gomphotheca*, *Nitzschia*, and *Denticula*, but it is really not close to any of these genera. The characters which separate it from other genera are: 1) a raphe-sternum running from pole to pole which divides the valve into two lateral chambers, 2) a conopeum extending from the raphe-sternum to the surface of each lateral chamber enclosing a tubular space, 3) a copular canal, running almost pole to pole ventrolateral to each lateral chamber and enclosed by copulae, 4) it has two sets of 6–8 fibulae on each side of the raphe-sternum system. This interesting rare diatom begs further study and eventual formal description.

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