J. Eukaryot. Microbiol., 48(3), 2001 pp. 362–367

Molecular Identification of Algal Endosymbionts in Large Miliolid Foraminifera: 1. Chlorophytes

JAN PAWLOWSKI,[a] MARIA HOLZMANN,[a] JOSÉ F. FAHRNI[a] and PAMELA HALLOCK[b]

[a]*Department of Zoology and Animal Biology, University of Geneva, route de Malagnou 154, CH-1224 Chêne-Bougeries/Geneva, Switzerland, and* [b]*College of Marine Science, University of South Florida, St. Petersburg, Florida 33701, USA*

ABSTRACT. Large miliolid foraminifers bear various types of algal endosymbionts including chlorophytes, dinoflagellates, rhodophytes, and diatoms. Symbiosis plays a key role in the adaptation of large foraminifera to survival and growth in oligotrophic seas. The identity and diversity of foraminiferal symbionts, however, remain largely unknown. In the present work we use ribosomal DNA (rDNA) sequences to identify chlorophyte endosymbionts in large miliolid foraminifera of the superfamily Soritacea. Partial 18S and complete Internal Transcribed Spacer (ITS) rDNA sequences were obtained from symbionts of eight species representing all genera of extant chlorophyte-bearing Soritacea. Phylogenetic analysis of the sequences confirms the previous fine structure-based identification of these endosymbionts as belonging to the genus *Chlamydomonas.* All foraminiferal symbionts form a monophyletic group closely related to *Chlamydomonas noctigama.* The group is composed of seven types identified in this study, including one previously morphologically described species, *Chlamydomonas hedleyi.* Each of these types can be considered as a separate species, based on the comparison of genetic differences observed between other established *Chlamydomonas* species. Several foraminiferal species share the same symbiont type, but only one species, *Archaias angulatus,* was found to bear more than one type.

Key Words. *Chlamydomonas,* evolution, ITS, phylogeny, ribosomal RNA, Soritacea, symbiosis, 18S.

SYMBIOSIS plays a key role in the evolution of large benthic calcareous foraminifers. Most of the large shallow-water species live in symbiosis with different types of algae. Algal symbionts provide their foraminiferal hosts with energy from photosynthesis necessary for survival and growth in oligotrophic environments. They also promote calcification by uptake of CO_2 and host metabolites (Hallock 1999).

The large miliolid foraminifera of the superfamily Soritacea are hosts of three groups of algal endosymbionts: chlorophytes, dinoflagellates, and unicellular rhodophytes (Lee and Anderson 1991). Current classification divides Soritacea into two families Peneroplidae and Soritidae, the latter one composed of two subfamilies Archaiasinae and Soritinae (Loeblich and Tappan 1988; Sen Gupta 1999). The subfamilies Archaiasinae and Soritinae bear chlorophytes and dinoflagellates, respectively. The family Peneroplidae includes both ornamented rhodophyte-bearing *Peneroplis* and unornamented chlorophyte-bearing *Laevipeneroplis* (Hallock 1999). Hallock and Peebles (1993) and Gudmundson (1994) argued to include the chlorophyte-bearing *Laevipeneroplis* in the Archaiasinae. Here, we examine the identity of chlorophyte endosymbionts living in several Archaiasinae, as well as those of the genera *Laevipeneroplis* and *Parasorites,* the latter one considered by some authors as a member of the Soritinae (Lehmann 1961; Gudmundson 1994). The diversity of dinoflagellate symbionts of Soritinae is presented in the second article of this series (Pawlowski et al. 2001).

The chlorophyte-bearing foraminifera comprise at least 13 species classified in five genera (Hallock and Peebles 1993; Seiglie, Grove, and Rivera 1977). The majority of these species are endemic to the Western Atlantic region. Only two species, *Parasorites* (*Broeckina*) *orbitolitoides* and *Laevipeneroplis proteus* (also called *Laevipeneroplis malayensis* by Hallock 1999), have been reported from the Indo-Pacific (Crapon de Caprona d'Ersu 1985). Recent molecular phylogenetic studies (MH., unpubl. data) demonstrate, however, that both Indo-Pacific species are not directly related to their Caribbean homonyms. In particular, the Indo-Pacific *Parasorites* (Loeblich and Tappan 1988) forms a separate clade that branches at the base of the Soritinae (MH., unpubl. data). Although the taxonomic status of *Parasorites* is unclear, its chlorophyte endosymbionts are examined herein.

Corresponding Author: J. Pawlowski—Telephone number: + 41 22 349 86 44 ext. 440; FAX number: + 41 22 349 26 47; E-mail: jan.pawlowski@zoo.unige.ch

Earlier studies of chlorophyte symbionts in Archaiasinae led to their identification as belonging to the genus *Chlamydomonas* and to the description of two new species: *Chlamydomonas hedleyi* and *Chlamydomonas provasoli,* isolated respectively from *Archaias angulatus* (Lee et al. 1974; Müller-Merz and Lee 1976) and *Cyclorbiculina compressa* (Lee et al. 1979). Later, *C. hedleyi* was also observed in *Laevipeneroplis proteus* by Leutenegger (1984). These investigations, based on in situ or in toto observations of morphological and ultrastructural features, provided limited information on diversity of symbionts.

DNA-based molecular systematic techniques provide tools for identification and assessment of genetic diversity in various groups of protists. Ribosomal DNA (rDNA) sequences were used to examine the molecular phylogeny and genetic diversity in benthic and planktonic foraminifera (Holzmann, Piller, and Pawlowski 1996; Pawlowski et al. 1997; de Vargas et al. 1999). Molecular data were also used to investigate phylogenetic relationships within the genus *Chlamydomonas* and related chlorophytes (Buchheim et al. 1996; Coleman 1999; Coleman and Mai 1997). In the present study, we use rDNA sequences to identify foraminiferal chlorophyte symbionts and to examine their diversity and evolutionary history.

MATERIALS AND METHODS

Collection of specimens. Foraminifera were collected from the western Atlantic (Florida Keys), western Pacific (Okinawa, Guam), and the Great Barrier Reef (Lizard Island). Detailed data concerning collection date, sites, and Genbank accession numbers of new sequences are given in Table 1.

DNA extraction, amplification, and sequencing. Prior to the DNA extraction, the specimens were cleaned with a fine brush in order to remove any debris and associated microorganisms and washed in sterile sea water. DNA of foraminifera and their symbionts was extracted by using either DOC lysis buffer (Holzmann and Pawlowski 1996) or DNeasy Plant Minikit (Qiagen, Basle, Switzerland). All specimens, except very small ones, were broken and only small fragments were taken for DNA extraction. The remaining parts of the tests were preserved for further identification and SEM study. Additionally, DNA was extracted from cultures of *Chlamydomonas hedleyi* and *Chlamydomonas provasoli,* two species of symbionts isolated from Archaiasinae and deposited in the American Type Culture Collection as ATCC # 50216 and ATCC # 50217, respectively. Each DNA extraction received of Foraminifera DNA Collection identification number that appears in Table 1.

One μl of DNA extract was added to each PCR that was

Table **1**. Collection locality and date of foraminiferal hosts, DNA extract number, and GenBak/EMBL accession number of symbiont sequences.

Host species	Collection site	Date	DNA extract[a]	Accession number
Androsina lukasi	Florida Keys, Fl	Jul'98	849	AJ297796
Androsina lukasi	Florida Keys, Fl	Jul'98	850	AJ297795
Archaias angulatus	Florida Keys, Fl	Jul'98	879	AJ297799
Archaias angulatus	Florida Keys, Fl	Jul'98	880	AJ297798
Archaias angulatus	Florida Keys, Fl	Jul'98	881	AJ297801
Broeckina sp.	Conch Reef, Fl	Jul'98	835	AJ297804
Broeckina sp.	Conch Reef, Fl	Jul'98	922	AJ297805
Cyclorbiculina compressa	Florida Keys, Fl	Jul'98	878	AJ297800
Laevipeneroplis bradyi	Florida Keys, Fl	Jul'98	825	AJ297802
Laevipeneroplis proteus	Florida Keys, Fl	Jul'98	876	AJ297803
Laevipeneroplis sp.	Guam, Micronesia	Mar'00	2259	AJ297807
Laevipeneroplis sp.	Guam, Micronesia	Mar'00	2264	AJ297806
Parasorites sp.	Sesoko, Okinawa	Aug'96	277	AJ297811
Parasorites sp.	Sesoko, Okinawa	Aug'96	278	AJ297810
Parasorites sp.	Sesoko, Okinawa	Oct'96	312	AJ297813
Parasorites sp.	Sesoko, Okinawa	Oct'96	313	AJ297812
Parasorites sp.	Sesoko, Okinawa	Nov'96	323	AJ297809
Parasorites sp.	Lizard Island	Aug'97	483	AJ297808
Parasorites sp.	Guam, Micronesia	Jul'99	1634	AJ297814

[a] Foraminiferal DNA Collection identification number (see Material and Methods).

performed in a total volume of 50 μl with an amplification profile consisting of 40 cycles of 30 s at 94 °C, 30 s at 50–55 °C and 120 s at 72 °C, followed by 5 min at 72 °C for final extension. The amplified PCR products were purified using High Pure PCR Purification Kit (Roche, Rotkreuz, Switzerland), sequenced directly with ABI PRISM Big Dye Terminator Cycle Sequencing Kit, and analysed with an ABI 377 DNA sequencer (Perkin-Elmer, Rotkreuz, Switzerland), all according to the instructions of the manufacturers.

The amplified fragment includes a part of the 18S gene, the whole ITS region (ITS 1 + 5.8 S + ITS2) and a short fragment of the 28S gene (Fig. 1). The length of the amplification product ranged from 1133–1372 nucleotides (nt). The Chlorophytes-specific primer S15ch (5′-CTTAGTTGGTGGGTTGCC) situated about 600 nt upstream from the 3′-end of the 18S rDNA and the Plants-specific primer L5pl (5′-TTC (AG)CTCGCCGTTACT) situated at the 5′-end of the 28S rDNA were used for PCR amplification. An additional primer S21ch (5′-TACCGATTGGGTGTGCTG) was used for direct sequencing of the whole fragment (Fig. 1). The 5′ region of the amplified fragment was sequenced twice using S15ch primer, while both strands were sequenced for S21ch—L5pl fragment.

Sequence analysis. Sequences were aligned using Clustal X (Thompson, Higgins, and Gibson 1994) and further improved manually by using GDE 2.2 software (Maidak et al. 1994). Two methods were used for sequence analysis: the neighbor-joining (NJ) method (Saitou and Nei 1987), applied to distances corrected for multiple hits, and for unequal transition and transversion rates, using Kimura's two-parameter model (Kimura 1980); and the maximum likelihood (ML) method as implemented in the fast DNAm1 program (Olsen et al. 1994). The reliability of internal branches in the NJ and ML trees was assessed, respectively by 1,000 and 100 bootstrap replicates (Felsenstein 1988). The PHYLO_WIN program (Galtier and Gouy 1996) was used for distance computations, tree building, and bootstrapping.

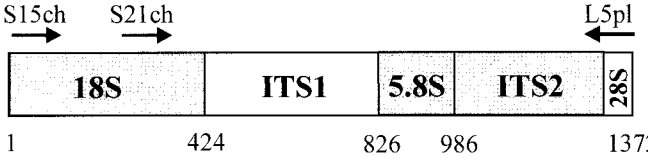

Fig. **1**. Diagram of the amplified and sequenced fragments of the rDNA, indicating the relative length of each region and the position of amplification primers. The numbers below correspond to the length of each region in the sequence of *Androsina lukasi*_850. The 18S and ITS regions used for phylogenetic analyses are shaded.

The sequences obtained in this study were compared to 24 18S rDNA and 13 ITS rDNA sequences of various species of *Chlamydomonas* available in the GenBank. Accession numbers of 18S rDNA sequences are as follows: CNU70782 (*Chlamydomonas noctigama*_a), CNU70787 (*Chlamydomonas noctigama*_b), AF008239 (*Chlamydomonas noctigama*_c), AF008242 (*Chlamydomonas noctigama*_d), CMU70786 (*Chlamydomonas moewusii*), CPU70789 (*Chlamydomonas pitschmanii*), CDU13985 (*Chlamydomonas dysosmos*), AB001037 (*Chlamydomonas pulsatilla*_c), AB001038 (*Chlamydomonas pulsatilla*_a), AB001039 (*Chlamydomonas pulsatilla*_b), CBU70781 (*Chlamydomonas baca*), CZU70792 (*Chlamydomonas zebra*), AF008240 (*Chlamydomonas debaryana*), CRU70790 (*Chlamydomonas rapa*), CAU70788 (*Chlamydomonas asymetrica*), CMU57695 (*Chlamydomonas mutabilis*), CBU70783 (*Chlamydomonas bipapillata*), CMU70785 (*Chlamydomonas macrostellata*), CNU57696 (*Chlamydomonas nivalis*), CRU57697 (*Chlamydomonas radiata*), CFU70784 (*Chlamydomonas fimbriata*), AB007370 (*Chlamydomonas tetragama*), AB001374 (*Chlamydomonas* sp.), CMU57694 (*Chlamydomonas monadina*).

Accession numbers of ITS rDNA sequences are: AF033282 (*Chlamydomonas noctigama*_a), AF033283 (*Chlamydomonas noctigama*_b), AF033284 (*Chlamydomonas allensworthii*_a), AF033285 (*Chlamydomonas allenworthii*_b), AF033292 (*Chlamydomonas smithii*_a), AF033293 (*Chlamydomonas smithii*_b), AF033294 (*Chlamydomonas zebra*), AF033281 (*Chlamydomonas cribrum*), AF033277 (*Chlamydomonas* sp.), AF033295 (*Chlamydomonas* sp.), AF033288 (*Chlamydomonas reinhardtii*), AF033287 (*Chlamydomonas reinhardtii*), AF033290 (*Chlamydomonas reinhardtii*).

Accession numbers of new sequences presented in this study are listed in Table 1.

RESULTS

Sequence data. Complete endosymbiont sequences were obtained from one specimen of each chlorophyte-bearing fora-

miniferal species and from the cultured strain of *Chlamydomonas hedleyi* (Table 1). A second cultured species, *Chlamydomonas provasoli,* was not included because its PCR amplification gave no positive result. Additionally, 10 shorter sequences, spanning the 18S + ITS1 region, were obtained (using primers S21ch and L5pl) to examine the genetic diversity of symbionts within the same host species. In total, 19 sequences were obtained, including at least two sequences for each foraminiferal species, except *Cyclorbiculina compressa, Laevipeneroplis bradyi,* and *Laevipeneroplis proteus* (Table 1). Symbiont sequences obtained from the same host species and the same locality were identical, except in the case of *Archaias angulatus* for which two individuals from the same location gave two different types of sequences. All PCR products were sequenced directly, an indication that symbiont populations within single foraminiferal cells should be homogeneous.

18S rDNA analysis. The 18S fragment of 20 foraminiferal symbionts, including *C. hedleyi,* was compared to 24 *Chlamydomonas* sequences available from the Genbank database representing 19 different species. The neighbor joining (NJ) analysis of these sequences, based on 377 unambiguously aligned sites, showed foraminiferal symbionts branching together, as a sister group to four sequences of *C. noctigama* (Fig. 2). The clade of foraminiferal symbionts is supported by 78% bootstrap, but reaches 98% when the sequences of rapidly evolving *C. hedleyi* and *A. lukasi* symbionts are removed (data not shown). The foraminiferal symbionts and *C. noctigama* form a robust clade supported by 94% bootstrap value. The close relationships between *C. noctigama* and foraminiferal symbionts are confirmed by maximum likelihood (ML) analysis (data not shown). General topologies of the NJ and ML trees do not differ significantly. In both analyses, the clade containing *C. zebra* was chosen as an outgroup, following Buchheim et al. (1996).

Relationships within the clade of foraminiferal symbionts distinguish six different ''types'' (C1-C6). Two of them (C3, C4) have been found in more than one foraminiferal species: C3 in *L. bradyi, L. proteus,* and *Broeckina* sp. and C4 in *A. angulatus*_881, *A. angulatus*_879, and *C. compressa*. *Archaias angulatus* bears also another closely related C5 type, as well as *C. hedleyi,* which was originally isolated from this species (Lee et al. 1974). Among other foraminiferal species, small differences were also observed between symbiont sequences obtained from *Parasorites* specimens collected in different localities in the central Indo-Pacific (Guam, Japan, Lizard Island), but as these sequences are very closely related (sequence divergence less than 1.0 %), they are considered here as a single type (C1). Interestingly, the symbionts isolated from another Indo-Pacific species, *Laevipeneroplis* sp., do not cluster with those of *Parasorites,* but branch together with the symbionts of the Caribbean *Laevipeneroplis* and *Broeckina* sp. (Fig. 2). The large number of nucleotide substitutions observed in *C. hedleyi* and in C6 symbionts isolated from *A. lukasi* suggests an accelerated rate of evolution in both species, which may artifactually influence their phylogenetic position.

ITS rDNA analysis. Nine ITS sequences of foraminiferal symbionts, including *C. hedleyi*, were compared to 13 ITS sequences available from the Genbank. The alignment of all 20 sequences was only possible for short fragments of 287 sites situated in the 5.8S gene and ITS 2 region, using a secondary structure model for these regions (Coleman and Mai 1997). Phylogenetic relationships of foraminiferal symbionts inferred from ITS sequences (Fig. 3) are similar to those revealed by 18S analysis. All foraminiferal symbionts form a monophyletic group, but its monophyly is well supported ($> 90\%$) only if the rapidly evolving sequences of the type C6 and *C. hedleyi* are removed. The ITS data confirm the close relationship between foraminiferal symbionts and *C. noctigama*: these sister clade are supported by 100% bootstrap and are clearly separated from all other *Chlamydomonas.* The topologies of both NJ and ML trees are identical, and the bootstrap support for particular clades is similar. The branching order within the clade of foraminiferal symbionts is identical to that obtained in the analysis of 18S rDNA (Fig. 2, 3).

DISCUSSION

Symbionts' identity. Molecular data show that the chlorophyte symbionts of large miliolid foraminifera belong to the genus *Chlamydomonas.* This confirms a previous identification based on ultrastructure features of algae isolated or observed in situ in three species of Archaiasinae (Lee et al. 1974, 1979; Leutenegger 1984). Although we have not examined all chlorophyte-bearing species, the fact that the symbionts of all Caribbean and Indo-Pacific genera are closely related points to a single origin of symbiosis between chlorophytes and Soritacea.

Our data clearly indicate the filiation between foraminiferal symbionts and *C. noctigama,* suggesting that the latter species may be the symbionts' ancestor. The phylogenetic position of *C. noctigama* (considered as a synonym of *C. geitleri*) was analysed by Buchheim et al. (1996). Based on nuclear and chloroplast sequence data, they identified seven main lineages within the genus *Chlamydomonas.* Three of these lineages: ''*Euchlamydomonas*'' lineage, ''*C. eugametos*'' lineage, and ''*C. radiata*'' lineage, whose sequences are available in the Genbank, can be distinguished in our analyses. The relationships between these lineages revealed by our data are in general agreement with those presented by Buchheim et al. (1996). According to these authors, *C. geitleri/C. noctigama* and *C. pitschmanii* belong to the ''*C. eugametos*'' lineage. Given the close relationships between *C. noctigama* and foraminiferal symbionts, the latter can also be included to this lineage. Although the 71% bootstrap support for the ''*C. eugametos*'' lineage in our 18S analyses is lower than in Buchheim's nuclear and chloroplast analyses (96–100%), its definition is confirmed by the position of *C. monadina* as a sister group to this lineage in our and Buchheim's analyses. *Chlamydomonas noctigama* is also associated with *C. pitschmannii* and *C. eugametos* in the analysis of ITS rDNA, although this association is much less robust than in the 18S data (Coleman and Mai 1997).

Genetic diversity. The 6 types of *Chlamydomonas* symbionts identified here are characterized by very low sequence divergence within each type and relatively high divergence between them. Sequences of the same type isolated from different foraminiferal species are identical or almost identical ($< 1.0\%$). Similar low sequence variations are observed within different strains of cultured species of *Chlamydomonas,* such as *C. noctigama* ($< 0.8\%$), *C. allensworthii* (0%), and *C. reinhardtii* ($< 0.4\%$). On the other hand, sequence variations between different types of foraminiferal symbionts range from 1.8 to 6.2%, with a mean value of 5%. These values correspond to the sequence divergences observed between some well-defined species, for example, between *C. pitschmannii* and *C. moewusii* (18S rDNA) and between *C. allensworthii* and *C. smithii* (ITS rDNA). If we consider these values as a species distinction criterion, then each type of foraminiferal symbiont should be considered as a separate species.

Our data are in agreement with previous ultrastructure-based studies of foraminiferal symbionts that led to the description of two new species of *Chlamydomonas*: *C. hedleyi* and *C. provasoli* (Lee et al. 1974, 1979). Both species were distinguished by their pyrenoid structure. *Chlamydomonas provasoli* bears pyrenoids that are surrounded by a higher number of starch grains and penetrated by more double-thylakoids disposed in

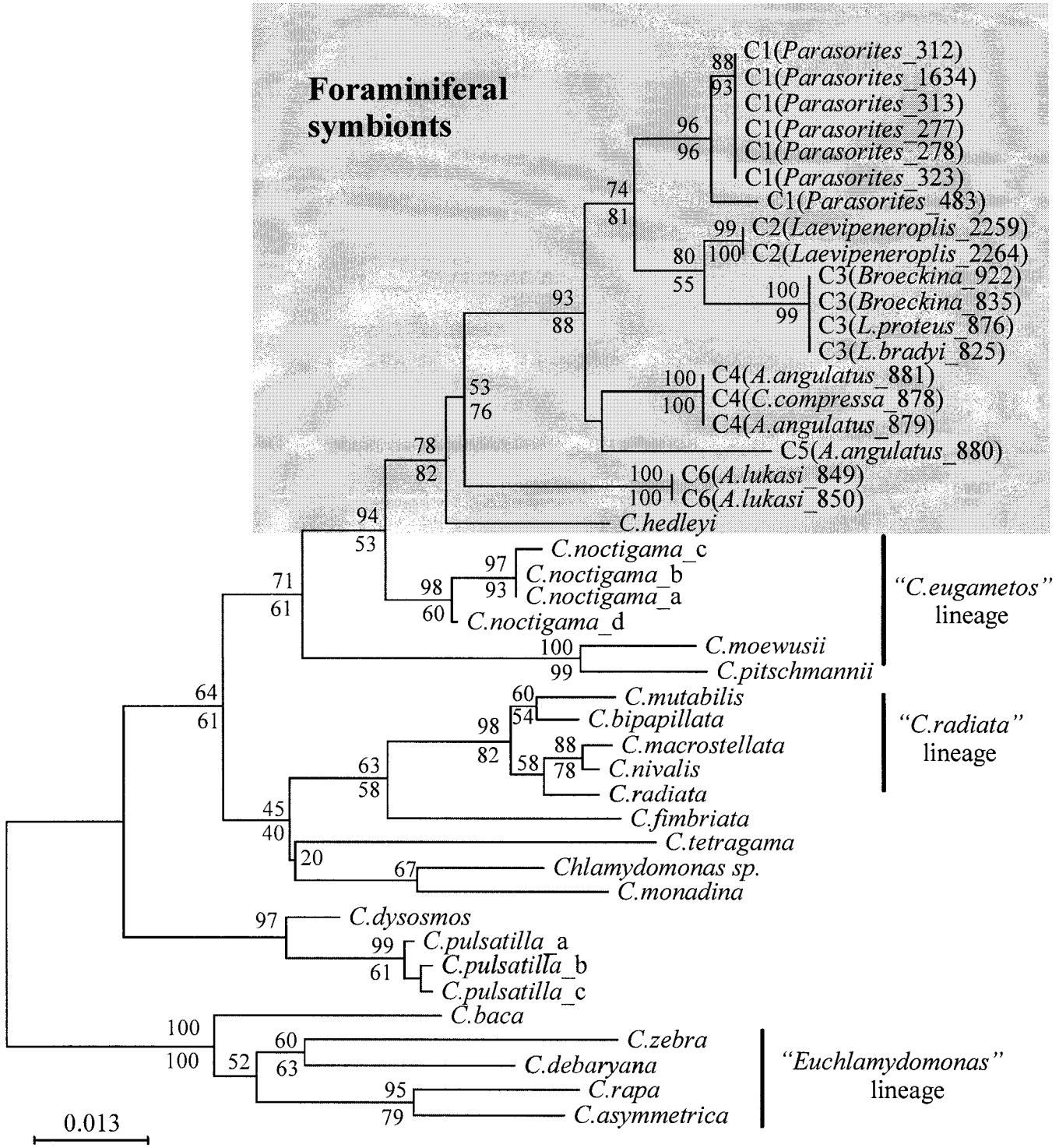

Fig. 2. The 18S rRNA tree of *Chlamydomonas* species including the symbionts of foraminifera, inferred using neighbor joining method. The denomination of foraminiferal symbiont sequences corresponds to the type of symbiont (C1 to C6), name of foraminiferal host, and number of DNA extract (see Materials and Methods). The numbers above and below the branches represent percentage bootstrap support in NJ and ML analyses, respectively.

several planes than in *C. hedleyi* (Leutenegger 1984). We attempted to obtain rDNA sequences of both species, deposited in the ATCC, to determine their genetic differences. PCR amplification of *C. provasoli,* however, proved to be unsuccessful. Further joint molecular and morphological studies will be necessary to characterize the morphological features of the different types identified in this study.

Evolutionary history. A single origin of chlorophyte symbiosis as suggested by symbiont DNA sequences is in congruence with the monophyletic origin of chlorophyte-bearing Sor-

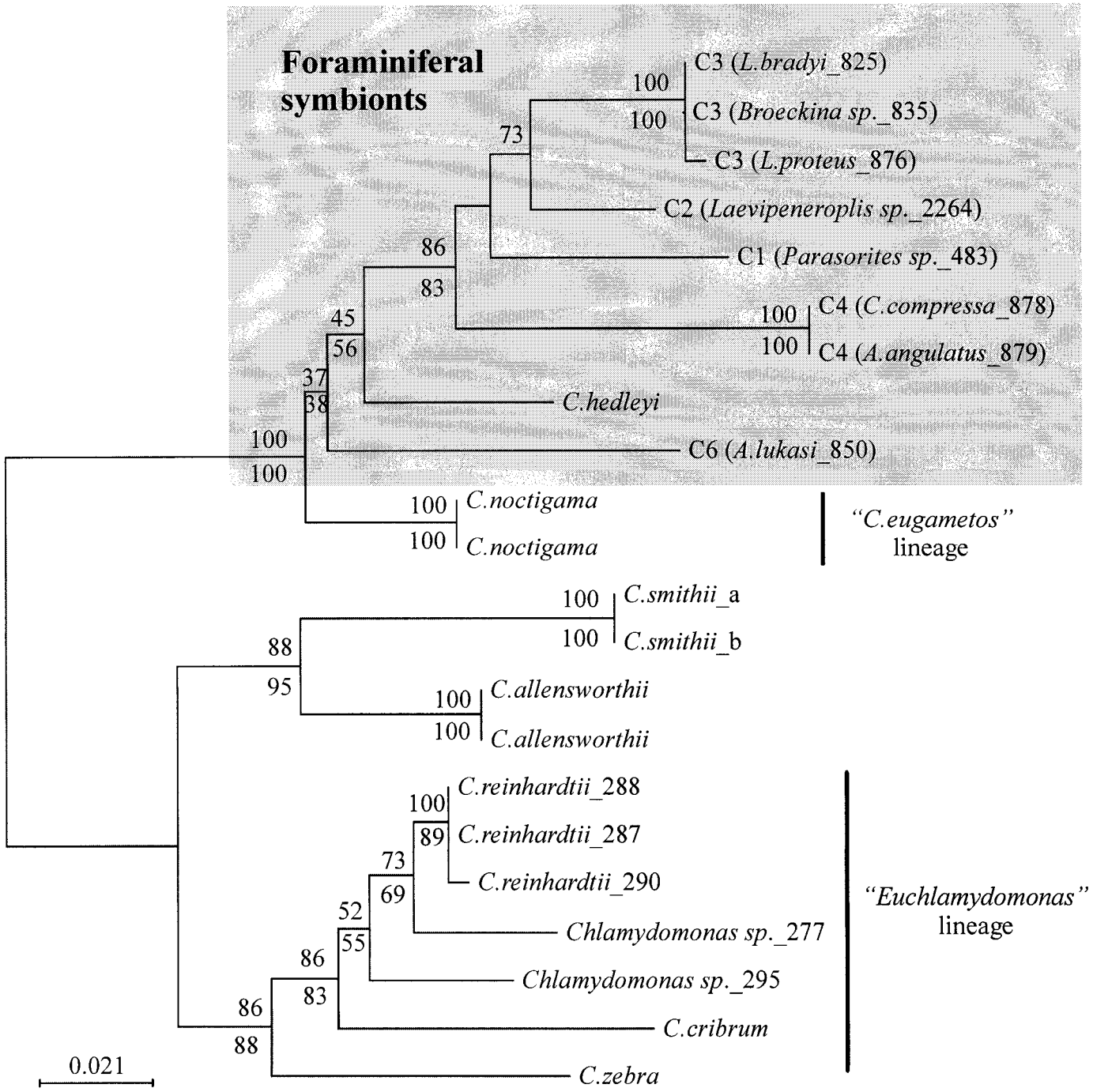

Fig. **3**. The ITS tree of the rRNA gene region of *Chlamydomonas* including the symbionts of foraminifera, inferred using neighbor joining method. The denomination of foraminiferal symbiont sequences corresponds to the type of symbiont (C1 to C6), name of foraminiferal host, and number of DNA extract (see Materials and Methods). The numbers above and below the branches represent percentage bootstrap support in NJ and ML analyses, respectively.

itacea inferred from phylogenetic analysis of foraminiferal rDNA sequences (MH., unpubl. data), and supports proposals by Hallock and Peebles (1993), Gudmundson (1994), and others to include members of the genus *Laevipeneroplis* in the Archaiasinae. According to these data, the divergence of Archaiasinae from rhodophyte-bearing Peneroplidae, about 45 mya (Haynes 1981), was most probably driven by a change of endosymbionts. Similarly, the change of symbionts from chlorophytes to dinoflagellates led to the radiation of Soritinae, some 25 mya ago (Haynes 1981). Molecular phylogeny of Soritacea suggests that Caribbean Archaiasinae evolved from a lineage represented today by Indo-Pacific *Laevipeneroplis* sp., while Soritinae diverged from the Indo-Pacific genus *Parasorites* (MH., unpubl. data). The ancestral character of Indo-Pacific lineages is in agreement with fossil data suggesting a widespread circumtropical distribution of Archaiasinae in the past

(Reiss and Hottinger 1984). It contrasts, however, with the present distribution of this family, represented in majority by western Atlantic species (Hallock and Peebles 1993).

Interestingly, our data show some congruence between trees of symbionts and their hosts. The branching order of the symbionts of *Parasorites* sp., *Laevipeneroplis* spp., and *Broeckina* sp. (types C1, C2, C3) is in accordance with the phylogeny of corresponding host foraminiferal species (MH., unpubl. data). Both data sets agree that the Indo-Pacific *Parasorites* sp. and *Laevipeneroplis* sp. branch before the radiation of three western Atlantic archaiasinids (*L. proteus, L. bradyi,* and *Broeckina* sp.). Both trees differ, however, in the position of three other western Atlantic species (*A. lukasi, A. angulatus,* and *C. compressa*). The symbionts of these species (i.e. types C4, C5, C6, and *C. hedleyi*) branch at the base of the clade (Fig. 2), while their hosts form a monophyletic group with other Caribbean foraminiferal species. This discrepancy could be explained by higher nucleotide substitution rates, particularly demonstrated by the C6 type and *C. hedleyi.* To further test the hypothesis of co-evolution of chlorophytes and their foraminiferal hosts, additional sampling of chlorophyte-bearing foraminifera would be necessary.

ACKNOWLEDGMENTS

The authors thank Johann Hohenegger, Dana E. Williams, Colomban de Vargas, Rob Rowan, and Xavier Pochon for help in collecting foraminifera. This study was supported by the Swiss National Science Foundation grant 3100–49513.96 (J. P.). Collection of material by P. Hallock was funded by NOAA's National Undersea Research Center at the University of North Carolina at Wilmington's Florida Keys Program (Contract No. 9703–66) and by the U.S. Environmental Protection Agency NCERQA grant No. GAD#R825869.

LITERATURE CITED

Buchheim, M. A., Lemieux, C., Otis, C., Gutell, R. R., Chapman, R. L. & Turmel, M. 1996. Phylogeny of the Chlamydomonadales (Chlorophyceae): a comparison of ribosomal RNA gene sequences from the nucleus and the chloroplast. *Mol. Phyl. Evol.,* **5**:391–402.

Coleman, A. W. 1999. Phylogenetic analysis of ''Volvocacae'' for comparative genetic studies. *Proc. Natl. Acad. Sci. USA,* **96**:13892–13897.

Coleman, A. W. & Mai, J. C. 1997. Ribosomal DNA ITS-1 and ITS-2 sequence comparisons as a tool for predicting genetic relatedness. *J. Mol. Evol.,* **45**:168–177.

Crapon de Caprona d'Ersu, A. C. 1985. Contribution à l'étude de Soritidae actuels (Formainifères) 3. Sous-familles des Archaiasinae, Meandropsininae et Soritinae et conclusions générales. *Rev. Paléobiologie,* **4**:347–390.

de Vargas, C., Norris, R., Zaninetti, L., Gibb, S. W. & Pawlowski, J. 1999. Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proc. Natl. Acad. Sci. USA,* **96**:2864–2868.

Felsenstein, J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annu. Rev. Genet.,* **22**:521–565.

Galtier, N. & Gouy, M. 1996. SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci.,* **12**:543–548.

Gudmundson, G. 1994. Phylogeny, ontogeny, and systematics of Recent Soritacea Ehrenberg, 1839 (Foraminiferida). *Micropaleontology,* **40**: 101–155.

Hallock, P. 1999. Symbiont-bearing foraminifera. *In*: Sen Gupta, B. K. (ed.), Modern Foraminifera. Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 123–139.

Hallock, P. & Peebles, M. W. 1993. Foraminifera with chlorophyte endosymbionts: habitats of six species in the Florida Keys. *Mar. Micropaleontol.,* **20**:277–292.

Haynes, J. R. 1981: Foraminifera. MacMillan Publishers, London.

Holzmann, M. & Pawlowski, J. 1996. Preservation of foraminifera for DNA extraction and PCR amplification. *J. Foraminif. Res.,* **26**:264–267.

Holzmann, M., Piller, W. & Pawlowski, J. 1996. Sequence variations in large-subunit ribosomal RNA gene of *Ammonia* (Foraminifera, Protozoa) and their evolutionary implications. *J. Mol. Evol.,* **43**:145–151.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.,* **16**:111–120.

Lee, J. J. & Anderson, O. R. 1991. Symbiosis in foraminifera. *In*: Lee, J. J. & Anderson, O. R. (ed.), Biology of Foraminifera. Academic Press, London, p. 157–220.

Lee, J. J., Crockett, L. J., Hagen, J. & Stone, R. J. 1974. The taxonomic identity and physiological ecology of *Chlamydomonas hedleyi* sp. nov., algal flagellate symbiont from the formainifer *Archaias angulatus. Brit. Phycol. J.,* **9**:407–422.

Lee, J. J., McEnery, M. E., Kahn, E. & Schuster, F. 1979. Symbiosis and the evolution of larger foraminifera. *Micropaleontology,* **25**:118–140.

Lehmann, R. 1961. Strukturanalyse einiger Gattungen der Subfamilie Orbitolitinae. *Ecol. Geol. Helv.,* **54**:597–667.

Leutenegger, S. 1984. Symbiosis in benthic foraminifera: specificity and host adaptation. *J. Foraminif. Res.,* **14**:16–35.

Loeblich, A. R. Jr. & Tappan, H. 1988. Foraminiferal genera and their classification. Van Nostrand Reinhold, New York.

Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. 1994. The Ribosomal Database project. *Nucl. Acids Res.,* **22**:3485–3487.

Müller-Merz, E. & Lee, J. J. 1976. Symbiosis in the larger foraminiferan *Sorites marginalis* (with notes on *Archaias* spp.). *J. Protozool.,* **23**: 390–396.

Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. 1994. FastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood: *Comp. Appl. Biosci.,* **10**:41–48.

Pawlowski, J., Holzmann, M., Fahrni, J. F., Pochon, X. & Lee, J. J. 2001. Molecular identification of algal endosymbionts in large miliolid foraminifera: 2. Dinoflagellates. *J. Eukaryot. Microbiol.,* **48**: 367–373.

Pawlowski, J., Bolivar, I., Fahrni, J., De Vargas, C., Gouy, M. & Zaninetti, L. 1997. Extreme differences in rates of molecular evolution of foraminifera revealed by comparison of ribosomal DNA sequences and the fossil record. *Mol. Biol. Evol.,* **14**:498–505.

Reiss, Z. & Hottinger, L. 1984. The Gulf of Aqaba (Elat). Springer, Berlin.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.,* **4**:406–425.

Seiglie, G. A., Grove, K. & Rivera, J. A. 1977. Revision of some Caribbean Archaiasinae, new genera, species, and subspecies. *Ecol. Geol. Helv.,* **70**:855–883.

Sen Gupta, B. K. 1999. Systematics of modern foraminifera. *In*: Sen Gupta, B. K. (ed.), Modern Foraminifera. Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 7–36.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.,* **22**:4673–4680.

Received 11/09/00, 02/26/01; accepted 02/26/01