J. Phycol. **39,** 440–448 (2003)

PHYLOGENETIC EVIDENCE FOR THE CRYPTOPHYTE ORIGIN OF THE PLASTID OF *DINOPHYSIS* (DINOPHYSIALES, DINOPHYCEAE)[1]

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Photosynthetic members of the genus *Dinophysis* Ehrenberg contain a plastid of uncertain origin. Ultrastructure and pigment analyses suggest that the two-membrane-bound plastid of *Dinophysis* spp. has been acquired through endosymbiosis from a cryptophyte. However, these organisms do not survive in culture, raising the possibility that *Dinophysis* spp. have a transient kleptoplast. To test the origin and permanence of the plastid of *Dinophysis*, we sequenced plastid-encoded *psb*A and small subunit rDNA from single-cell isolates of *D. acuminata* Claparède et Lachman, *D. acuta* Ehrenberg, and *D. norvegica* Claparède et Lachman. Phylogenetic analyses confirm the cryptophyte origin of the plastid. Plastid sequences from different populations isolated at different times are monophyletic with robust support and show limited polymorphism. DNA sequencing also revealed plastid sequences of florideophyte origin, indicating that *Dinophysis* may be feeding on red algae.

Key index words: **dinoflagellates; *Dinophysis*; endosymbiosis; kleptoplastidy; mixotrophy; plastid evolution**

Abbreviations: **GTR, general time reversible; LSU, large subunit; ME, minimum evolution; pp, posterior probabilities; pSSU, plastid small subunit**

Understanding the origin of plastids in the evolutionarily diverse algae remains a central issue in molecular and organismal evolution (Bhattacharya and Medlin 1995). The dinoflagellate algae, which are members of the protist supergroup Alveolata (Cavalier-Smith 1998), provide a model for addressing the endosymbiotic origin of plastids. This is because dinoflagellates have an unmatched propensity for plastid loss and replacement. Thus far, five different types of plastids have been identified in the dinoflagellates: those containing peridinin, 19′-hexanoyloxyfucoxanthin of haptophyte origin (Tengs et al. 2000), the diatom-like plastid of *Peridinium foliaceum* Stein and *P. balticum* (Levander) Lemmermann (Chesnick et al. 1996, Inagaki et al. 2000), the prasinophyte-like plastid of *Lepidodinium viride* Watanabe et al. (Watanabe et al. 1987), and the cryptophyte-like plastid of *Dinophysis* spp. (Schnepf and Elbrächter 1988). In addition, many dinoflagellates appear to lack plastids altogether. As many as eight independent plastid losses and three replacements are suggested for the dinoflagellate photosynthetic lineage (Saldarriaga et al. 2001), although this study did not include *Dinophysis* spp. However, little molecular data specifically addresses dinoflagellate plastid replacement. In addition, the existing host trees are often unresolved with regard to relationships among the photosynthetic group and the nature of the ancestral dinoflagellate (Daugbjerg et al. 2000, Tengs et al. 2000, Fast et al. 2001, Saldarriaga et al. 2001). Molecular sequence data have confirmed, however, the diatom origin of the plastid in *P. foliaceum* and *P. balticum* and the haptophyte origin of the plastid in three unarmoured dinoflagellates, *Karenia brevis* (Davis) Hansen et Moestrup, *Gymnodinium aureolum* (Hulbert) Hansen, and *Karlodinium micrum* (Leadbeater et Dodge) Larsen (Chesnick et al. 1996, Tengs et al. 2000). No such molecular phylogenetic evidence exists for the prasinophyte-like plastid of *Lepidodinium viride* and the cryptophyte-like plastid of *Dinophysis* spp., until recently for the latter case (Takishita et al. 2002).

Dinophysis spp. is comprised of photosynthetic and nonphotosynthetic members and is globally distributed in coastal and oceanic waters. Members of this genus have been implicated in diarrhetic shellfish poisoning and have a significant impact on shellfish industries in some parts of the world (Boni et al. 1993, Giacobbe et al. 2000). Nonphotosynthetic species feed by myzocytosis, a process whereby a peduncle (or feeding tube) sucks up the cytoplasm from a prey, leaving behind the plasmalemma (Hansen 1991). Photosynthetic species share this structure, and although they

[1]Received 6 July 2002. Accepted 26 December 2002.

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have never been observed feeding, food vacuoles are often found in their cytoplasm, clearly indicating mixotrophy (Jacobson and Andersen 1994, Koike et al. 2000). Despite many efforts, *Dinophysis* spp. have thus far proven to be unculturable (Sampayo 1993, Jacobson and Andersen 1994, Maestrini 1998). This has led to the suggestion that the plastid of *Dinophysis* might be a kleptoplast, a temporary but functioning plastid captured from a prey (Melkonian 1996). Gaining insights into the biology of *Dinophysis*, particularly with regard to the evolution of its plastid, is important because it may provide clues to its maintenance in culture. This would allow more detailed analyses addressing *Dinophysis* life history and toxin production.

The plastid of the photosynthetic *Dinophysis* differs from the typical dinoflagellate plastid in that it contains phycoerythrin and alloxanthin but lacks peridinin (Vesk et al. 1996, Hewes et al. 1998, Meyer-Harms and Pollehne 1998). The plastid has paired stacked thylakoids with electron-dense contents, similar to cryptophyte thylakoids (Schnepf and Elbrächter 1988, Lucas and Vesk 1990). Unlike cryptophyte plastids with four membranes, the plastids of *Dinophysis* are surrounded by only two membranes (Schnepf and Elbrächter 1988, 1999, Lucas and Vesk 1990). These ultrastructural and biochemical attributes, consistently observed in phototrophic *Dinophysis* species, suggest that the plastid of *Dinophysis* is a long-established and permanent acquisition from a cryptophyte (Schnepf and Elbrächter 1999). Our primary objective was to test the hypothesis that the plastid of *Dinophysis* originated from a cryptophyte and thereby gain insights into endosymbiotic plastid replacement. We report DNA sequence data from the plastid of *D. acuminata*, *D. acuta*, and *D. norvegica* and present phylogenetic analyses of these data.

MATERIALS AND METHODS

Cell collection and isolation. Populations of *D. acuminata*, *D. acuta*, and *D. norvegica* were collected from 10-μm net tows. Single swimming cells were isolated with a stretched pipet and rinsed three times in 0.22-μm filtered seawater (Table 1). Except for acumGC06 (see Table 1), live isolated cells were shipped overnight to Iowa City for processing. DNA was extracted from all other samples in Rhode Island on the day of isolation.

DNA extraction. For each sample, individually picked cells were pooled and resuspended in approximately 10 μL of 0.22-μm filtered seawater and frozen in liquid nitrogen. Total DNA was extracted from the frozen and thawed cells using the Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA).

PCR amplification, cloning, and sequencing. Amplifications of plastid genes were done in two rounds of PCR using nested primers. The primer sequences are listed in Table 2. In the first round of PCR amplification of *psb*A, we used the nonspecific primers psbAF and psbAR2. After sequencing, the *psb*A coding region from acumGC08, a *Dinophysis*-specific primer, psbAF-dino, was used with psbAR2 in the second round of PCR for all other samples. All reactions were done with an initial denaturation at 94° C for 10 min; followed by 35 cycles of 94° C for 1 min, 50° C for 1 min, and 72° C for 2 min; and concluded with a 10-min extension at 72° C. Products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) or gel purified on a 1% agarose gel, excised, and extracted using the QIAquick gel purification kit (Qiagen). PCR reactions yielding heterogeneous products were cloned using the pGEM-T Vector System (Promega, Madison, WI, USA) following the manufacturer's protocol. Plasmid DNA was isolated and purified using the QIAprep Spin Mini Prep Kit (Qiagen).

Plastid small subunit (pSSU) rDNA fragments were amplified using nested PCR with the external primers SG1 and plas16SR in the first round. This was followed by reactions containing plas130F and plas16SR. The cycle conditions and the purification protocol were as described above. A cryptophyte-like specific primer, 16SdinoINT, was used with SG1 to screen cloned pSSU fragments using PCR. The nucleotide sequences of the amplified regions of *psb*A and pSSU rDNA were determined over both strands using BigDye dye terminator sequencing (Applied Biosystems, Foster City, CA, USA) on an automated DNA sequencer (model ABI 3100 or 3700, Applied Biosystems).

Phylogenetic analyses. Sequences of *D. acuminata*, *D. acuta*, and *D. norvegica psb*A and pSSU rDNA were aligned with previously determined *psb*A and pSSU rDNA sequences from red algae and red algal-derived secondary plastids (Table 3) (Yoon et al. 2002a,b) using ClustalW (Thompson et al. 1994) and optimized manually. The sequences of two distantly related green algae (Chlorophyta) were included in the analyses that were outgroup-rooted with the glaucophyte (*Cyanophora paradoxa* Korshikov). We also included two cryptophyte-like pSSU rDNA sequences isolated by environmental PCR (Rappé et al. 1998), OCS20 (AF001654) and OM283 (U70724), and pSSU rDNA sequences determined by Takishita et al. (2002) (AB073108–AB073116). Identical *Dinophysis* sequences of Takishita et al. (2002) are represented by *D. fortii* (AB073118). Sequence alignments were edited using BioEdit v5.0.9 (Hall 1999). Phylogenetic analysis was done using PAUP* 4.0b8 (Swofford 2002) and the DNA alignments of *psb*A (957nt) and pSSU rDNA (1223nt). These alignments (and inferred trees) are available at the TreeBASE website (http://treebase.bio.buffalo.edu/treebase/) under the accession number SN1296.

For each data set, trees were inferred with the minimum evolution (ME) method using the general time reversible (GTR) model incorporating invariant sites (I) with gamma correction (Γ) (ME-GTR) with 2000 bootstrap replications. The parameters of this model were estimated with PAUP using an ME tree built with HKY85 distances (Hasegawa et al. 1985). MODELTEST (V3.06, Posada and Crandall 1998) identified the GTR + I + Γ model as the best-fit model for both plastid data sets and specified the same parameter estimates as our initial

TABLE 1. Details regarding the origin of the *Dinophysis* samples used in DNA sequencing and phylogenetic analyses.

Dinophysis species	Sampling data	Sampling location	Number of cells per sample	Sample name
D. acuminata	06/04/01	Greenwich Cove, RI (41°39.5′N, 71°27′W)	22	AcumGC06
D. acuminata	08/07/01	Greenwich Cove, RI	10	AcumGC08
D. acuminata	09/26/01	Greenwich Cove, RI	20	AcumGC09
D. acuminata	10/05/01	Watch Hill Cove, RI (41°18.6′N, 71°51.5′W)	20	AcumWH10
D. acuta	09/25/01	Ninigret Pond, RI (41°21′N, 71°39′W)	5	AcutNP09
D. norvegica	04/24/02	Clam Cove, ME (44°08′N, 69°06′W)	20	NorvCC04

TABLE 2. Primers used in this study.

Gene	Primer	Oligonucleotide sequence (5′–3′)
*psb*A	psbAF	ATGACTGCTACTTTAGAAAGACG
	psbAFdino	AGCACTGACAACCGTTTATAC
	psbAR2	TCATGCATWACTTCCATACCT
pSSU rDNA	SG1	GTGCTGCAGAGAGTTYGATCCTGGCTCAGG
	plas16SR	CCCCAGTCACTAGCCCKRCCTTA
	plas130F	ACGTGAGAATYTRCCYYTAGGA
	16SdinoINT	CAGTTACGGCCCAGTAGGGTGCC

analysis. The proportion of invariant sites and gamma parameters for the *psb*A and pSSU rDNA were I = 0.5102, I = 0.4548 and α = 2.4237, α = 0.6208, respectively. Ten heuristic searches with random addition sequence starting trees and tree bisection-reconnection (TBR) branch rearrangements were done to find the optimal ME tree. We also conducted LogDet (ME-LogDet) distance estimates (Lockhart et al. 1994). We studied 2000 bootstrap replicates with LogDet distances. Finally, we did Bayesian analysis of the DNA data (MrBayes V2.0, Huelsenbeck and Ronquist 2001) using the GTR + I + Γ model to infer posterior probabilities (PP) of nodes on the genic trees. A single gamma parameter was used over all codon sites in the *psb*A data set. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) from a random starting tree was initiated three independent times in the Bayesian inference and run for 500,000 generations. A consensus tree was made with the MCMCMC trees after convergence in each analysis.

RESULTS

PCR and sequencing. Cryptophyte-like *psb*A sequences were obtained from all four samples of *D. acuminata*, from *D. acuta*, and from *D. norvegica* (Table 4). All *D. acuminata* and *D. acuta* sequences were identical over the overlapping regions, whereas the *D. norvegica* sequences showed a single polymorphism. Because of poor template quality, amplification and cloning of fragments from acumGC06 yielded only a 545 base pair cryptophyte-like sequence. When PCR was conducted with nonspecific *psb*A primers using acumGC06, acumGC09, acumWH10, and acutNP09 DNA, florideophyte-like *psb*A sequences were obtained. The florideophyte-like sequences from acumGC06 were polymorphic (0.6% difference), whereas those from the other three isolates were identical. No florideophyte-like *psb*A sequences were found from acumGC08 or norvCC04.

PCR amplification of pSSU rDNA from all samples yielded heterogeneous products, which were cloned and sequenced. Cryptophyte-like pSSU rDNA sequences were obtained from all collections except the acumGC06. Heterogeneous products were cloned from acumGC09, acumWH10, and acutNP09; only cryptophyte-like clones were found. Two polymorphic cryptophyte-like clones (see Fig. 2, 3B and 3D) were sequenced from acumWH10. Attempts to isolate florideophyte-like pSSU clones from these three isolates were unsuccessful. Florideophyte-like sequences were obtained from only the acumGC06 and acumGC08 samples. All cryptophyte-like pSSU rDNA sequences showed little polymorphism, ranging from 0.45% to 1.26% difference.

Phylogenetic analyses. The cryptophyte-like *psb*A sequences obtained from all samples of *D. acuminata*, *D. acuta*, and *D. norvegica* grouped within the cryptophytes in the ME-GTR + I + Γ tree (Fig. 1) with strong bootstrap support from all analyses (ME-LogDet = 100%, ME-GTR = 95%, PP = 100%). The Bayesian posterior probabilities in the consensus trees from the three independent MCMCMC runs were consistent with each other. Identical sequences are represented once in the *psb*A tree as *D. acuminata/ acuta*. Florideophyte-like *psb*A sequences obtained from the acumGC06 collection formed a monophyletic group within the Florideophycidae, and the identical sequences from acumGC09, acumWH10, and acutNP09 were positioned with *Palmaria palmata* (Linnaeus) Kuntze.

pSSU rDNA sequences behaved in a similar manner (Fig. 2). The polymorphic pSSU rDNA sequences of *Dinophysis* spp. formed a monophyletic group (ME-LogDet = 100%, ME-GTR = 98%, PP = 100%) within the cryptophytes. Relationships within the *Dinophysis* clade were not resolved, although there is weak support for the monophyly of the two sequences from the same isolate, acumWH10 clones 3B and 3D (ME-LogDet = 78%, ME-GTR = 57%, PP = 100%). *Dinophysis fortii* is positioned outside our sequences, at the base of the *Dinophysis* clade. The two cryptophyte sequences determined through environmental PCR by Rappé et al. (1998) diverge within the cryptophytes. OCS20 groups within the *Dinophysis* clade, whereas OM283 falls outside these taxa. The florideophyte-like pSSU rDNA sequence obtained from the acumGC08 collection is positioned with *P. palmata*, whereas the florideophyte-like pSSU rDNA from acumGC06 is found in a different position within the Florideophycidae. Unlike Takishita et al. (2002), we found polymorphisms among our pSSU rDNA sequences. If these differences were due to PCR error, we would expect the transition-to-transversion ratio to be approximately equal. However, the observed transition-to-transversion ratio at polymorphic sites in our sequences is 25:6 (4.17:1), making it unlikely that these differences are due solely to PCR error.

DISCUSSION

The genus *Dinophysis* represents a fascinating case study in plastid evolution, because it contains both

TABLE 3. GenBank accession numbers of previously published *psb*A and pSSU rDNA sequences used in the phylogenetic analyses.

Taxa	GenBank accession no.	
	pSSU rDNA	*psb*A
Rhodophyta		
Bangia atropurpurea (Roth) Agardh	AF545616	None
Bangia fuscopurpurea (Dillwyn) Lyngbye	AF170716	AY119735
Porphyra purpurea (Roth) J. Agardh	U38804	U38804
Compsopogon coeruleus (Balbis) Montagne	AF170713	AY119739
Erythrotrichia carnea (Dillwyn) J. Agardh	AF545619	AY119739
Bangiopsis subsimplex (Montagne) Schmitz	AF545620	AY119736
Dixoniella grisea (Geitler) Scott et al.	AF545621	None
Flintiella sanguinaria Ott in Bourelly	AF170719	None
Porphyridium aerugineum Geitler	X17597	None
Rhodella violacea (Kornmann) Wehrmeyer	AF545622	None
Rhodosorus marinus Geitler	AF170719	AY119744
Stylonema alsidii (Zanardini) Drew	AF170714	AY119745
Glaucosphaera vacuolata Korsh	X81903	None
Rhodochaete parvula Thuret	AF545623	AY119743
Chondrus crispus Stackhouse	Z29521	AY119746
Palmaria palmata (L.) Kuntze	Z18289	U28165
Thorea violacea Bory de St. Vincent	AF170721	AY119747
Antithamnion sp.	X54299	X55364
Nemalionopsis tortuosa Yoneda & Yagi	AF170720	None
Cryptophyta		
Chilomonas paramecium Ehrenberg SAG 977.2a	AF545624	AY119748
Chilomonas paramecium Ehrenberg NIES 715	AB073108	None
Chroomonas sp.	AF545625	AY119749
Chroomonas placoidea Butcher ex G. Novarino & I. A. N. Lucas	AB073110	None
Guillardia theta Hill et Wetherbee	AF041468	AF041468
Pyrenomonas helgolandii Santore	AF545626	AY119750
Rhodomonas abbreviata Butcher ex Hill et Wetherbee	AF545627	AT119751
Geminigera cryophila (D. L. Taylor & C. C. Lee) D. R. A. Hill	AB073111	None
Proteomonas sulcata D. R. A. Hill & R. Wetherbee	AB073113	None
Hemiselmis virescens Droop	AB073112	None
Cryptomonas ovata Ehrenberg	AB073109	None
Haptophyta		
Emiliania huxleyi (Lohmann) Hay et Mohler	X82156	AY119752
Isochrysis sp.	X75518	AT119753
Pavlova gyrans Butcher	AF172715	AY119754
Pavlova lutherii (Droop) Green	AF545628	AY119755
Stramenopiles		
Heterosigma akashiwo Carter	M34370	AY119759
Odontella sinensis Greville	Z67753	Z67753
Pylaiella littoralis (L.) Kjellman	X14803	AY119760
Skeletonema costatum (Greville) Cleve	X82154	AY119761
Dinophyta		
Dinophysis fortii Pavillard	AB073115	None
Chlorophyta		
Mesostigma viride Lauterborn	AF166114	AF166114
Nephroselmis olivacea Stein	AF137379	AF137379
Glaucophyta		
Cyanophora paradoxa Korshikov	U30821	U30821

nonphotosynthetic and photosynthetic members, with phycobilin- instead of the "typical" peridinin-containing plastids and a mixotrophic nutritional mode (Jacobson and Andersen 1994, Schnepf and Elbrächter 1999). Many of these species display morphological variability and "small cell" formation, and because of the unavailability of long-term cultures, the life history of *Dinophysis* spp. remains only partially known (Caroppo 2001, Reguera and González-Gil 2001).

The results of our molecular phylogenetic study clearly confirm the cryptophyte origin of the *Dinophysis* plastid, which was previously proposed based on plastid pigmentation and ultrastructure (Schnepf and Elbrächter 1999 and references therein) and are consistent with the recent findings of Takishita et al. (2002). Both *psb*A and pSSU rDNA trees firmly place the *Dinophysis* spp. plastid within the cryptophytes as a monophyletic group. Presently, the dearth of available plastid sequences from cryptophytes makes it difficult to identify the potential donor of the *Dinophysis* plastid, although *Geminigera cryophila* (D. L. Taylor & C. C. Lee) D.R.A. Hill groups most closely to the *Dinophysis* clade. In this regard, the presence of the environmental sample OCS20 within the *Dinophysis* clade is intriguing. This sequence shows only two nucleotide differences with our *D. acuta* sequence, so it is unclear if the sequence is from a cryptophyte or from a life history stage of a *Dinophysis*, especially from a "small cell"

TABLE 4. GenBank accession numbers and length (bp) of *Dinophysis* sequences determined in this study.

	*psb*A		pSSU rDNA	
	Cryptophyte-like	Florideophyte-like	Cryptophyte-like	Florideophyte-like
D. acuminata (acumGC06)	AF530391 545 bp[a]	AF530397–AF530399 957 bp each	None	AF530390 1051 bp
D. acuminata (acumGC08)	AF530392 865 bp[a]	None	AF530383 1135 bp	AF530389 571 bp
D. acuminata (acumGC09)	AF530393 899 bp[a]	AF530400 957 bp[b]	AF530384 1200 bp	None
D. acuminata (acumWH10)	AF530394 909 bp[a]	Identical to AF530400 957 bp[b]	AF530385, AF530386 1200, 1191 bp	None
D. acuta (acutNP09)	AF530395 890 bp[a]	Identical to AF530400 957 bp[b]	AF530387 1121 bp	None
D. norvegica (norvCC04)	AF530396 927 bp	None	AF530388 1222 bp	None

[a] These cryptophyte-like *psb*A sequences were identical to each other.
[b] These florideophyte-like *psb*A sequences were identical to each other.

given the mesh size used in the environmental PCR study (Rappé et al 1998). The vegetative cells of *Dinophysis* are too large to pass through the filter used in the environmental PCR study.

Several attributes of the *Dinophysis* plastid support the idea of a permanent plastid. The consistency of plastid morphology across species and populations of *Dinophysis* from around the world argues for a permanent replacement. Furthermore, several characteristics expected to be present in cryptophyte kleptoplasts are absent in *Dinophysis*. For example, a digested plastid has never been observed in this genus, as is common in dinoflagellates with kleptoplasts (Schnepf and Elbrächter 1999). Kleptoplasts remain photosynthetically active for variable periods of time, from approximately 2 days for *Gymnodinium gracilentum* Campbell, to 1 week for *Pfiesteria piscicida* Steidinger et Burkholder, and to 13–14 days for *Gymnodinium acidotum* Nygaard, before being digested or eliminated through cell division, leaving colorless dinoflagellate hosts (Fields and Rhodes 1991, Skovgaard 1998, Lewitus et al. 1999). In short-term life cycle studies in which *Dinophysis* cells were maintained in culture for several cell divisions, daughter cells were never found to lose their plastids (Sampayo 1993, Subba Rao 1995, Reguera and González-Gil 2001), although Takishita et al. (2002) reported plastid loss in culture. In addition, no other cryptophyte organelles or structures besides the plastid have been found inside photosynthetic *Dinophysis*, nor have plastids been reported within food vacuoles (Lucas and Vesk 1990). Together, these observations argue for a permanent plastid replacement in *Dinophysis*.

Our molecular analyses are fully consistent with these findings, showing resounding support for the monophyly of the plastid sequences from *Dinophysis* species collected at different times of the year and from different locations and are also monophyletic with sequences from Japan. However, our data do not rule out the alternative explanation that *Dinophysis* has a kleptoplast acquired from closely related cryptophytes present both in Japan and New England. The discovery of polymorphic pSSU rDNA sequences in our collections is surprising when compared with the identical sequences found by Takishita et al. (2002). These differences would support the idea of kleptoplastidy along the interpretation that *Dinophysis* acquired its plastid from a more diverse group of cryptophytes in New England than in Japan. However, in our opinion the morphological and molecular data do not support convincingly either hypothesis.

Interestingly, *Dinophysis* nuclear genes also show very little sequence divergence (Guillou et al. 2002). The sequence variation observed in the plastid genes is consistent with variation seen in the nuclear large subunit (LSU) rDNA of *Dinophysis* spp. Relationships among photosynthetic *Dinophysis* are poorly resolved, which is perhaps an indication that the cryptophyte plastid acquisition and/or the radiation of the photosynthetic lineage are relatively recent events. The low divergence observed in both *psb*A and the pSSU rDNA sequences supports this hypothesis. This could reflect a shared evolutionary history between nuclear and plastid genes in *Dinophysis*, indicating a permanent replacement. However, this similarity may only be a coincidence. Analysis of more variable nuclear and plastid markers (e.g. nuclear rDNA internal transcribed spacer and *rbc*L spacer) will be necessary to determine if plastid gene trees are congruent with nuclear gene trees.

There are several possibilities for the origin and evolution of the cryptophyte plastid in *Dinophysis* spp. It is presently unclear whether the common ancestor of the genus contained a peridinin plastid or whether the photosynthetic species arose from a heterotrophic ancestor. Phylogenetic analyses of nuclear loci have thus far failed to resolve the position of *Dinophysis* in host trees (Saunders et al. 1997, Daugbjerg et al. 2000). The plastid could have been acquired in the common ancestor of all species of *Dinophysis*, which

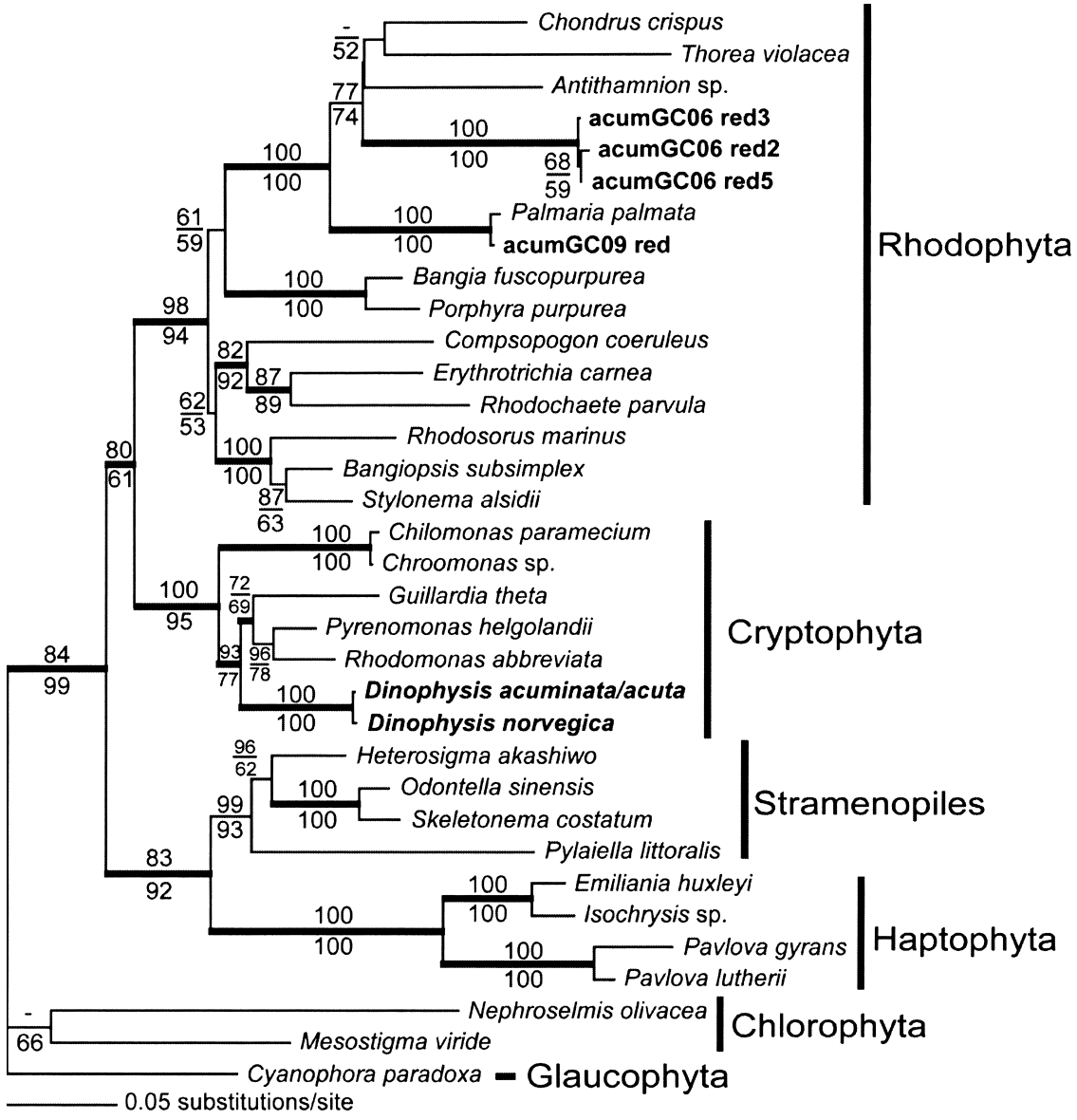

FIG. 1. ME-GTR + I + Γ tree of *psb*A sequences including *Dinophysis*, red algae, and red algal-derived plastid sequences. Bold letters indicate sequences determined in this study. *Dinophysis acuminata/D. acuta* represents identical *psb*A sequences from acumGC06, acumGC08, acumGC09, acumWH10, and acutNP09. AcumGC09 red represents florideophyte-like sequences from acutNP09, acumGC09, and acumWH10 that are identical. A total of 957 nucleotide positions was used in this analysis. LogDet bootstrap values greater than 50% are shown above the branches, and ME-GTR + I + Γ bootstrap values greater than 50% are shown below the branches. Thick branches indicate greater than 95% posterior probability for groups to the right from a Bayesian inference determined from a consensus of 4701 trees. The tree is rooted on the branch leading to the glaucophyte *Cyanophora paradoxa*.

would imply losses in multiple taxa (i.e. in *D. rotundata* Claparède et Lachman, *D. rapa* Stein, *D. schuettii* Murray et Whitting, etc.). A more parsimonious explanation would be that all photosynthetic species are monophyletic. Phylogenetic analysis of nuclear LSU rDNA sequences supports this view because *D. rotundata*, a nonphotosynthetic species, is positioned as a sister to all photosynthetic species (Guillou et al. 2002). These limited data imply that nonphotosynthetic *Dinophysis* species ancestrally lacked a cryptophyte plastid. Given that the nuclear LSU rDNA analysis included only one nonphotosynthetic species, increased taxon sampling from both photosynthetic and nonphotosynthetic species is required to unravel plastid evolution in *Dinophysis*.

In extant cryptophytes, the nucleomorph lies in the periplastidial compartment between the two pairs of membranes that surround the plastid (Douglas et al. 1991). The nucleomorph is the remnant of the red algal nuclear genome that was reduced after secondary endosymbiosis in the ancestor of cryptophyte and other

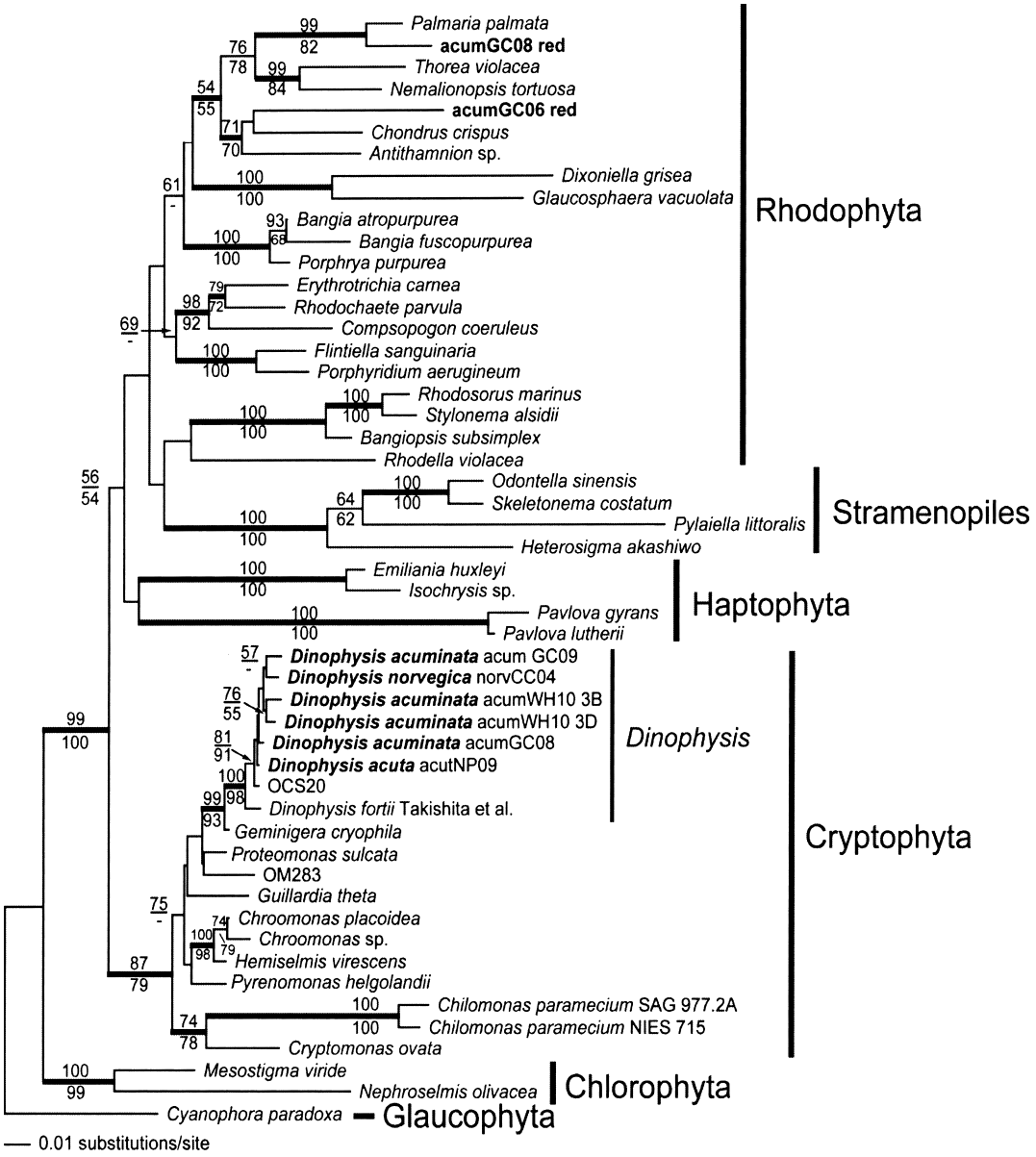

FIG. 2. ME-GTR + I + Γ tree of pSSU rDNA sequences including *Dinophysis*, red algae, and red algal-derived plastid sequences. Bold letters indicate sequences determined in this study. A total of 1285 nucleotide positions was considered in this analysis. LogDet bootstrap values greater than 50% are shown above the branches, and ME-GTR + I + Γ bootstrap values greater than 50% are shown below the branches. Thick branches indicate greater than 95% posterior probability for groups to the right from a Bayesian inference determined from a consensus of 4471 trees. The tree is rooted on the branch leading to the glaucophyte *Cyanophora paradoxa*.

chromist algae (i.e. haptophytes and stramenopiles) (Yoon et al. 2002b). It contains 30 plastid-targeted proteins and hundreds of other genes needed to express these coding regions (Douglas et al. 2001). Among the plastid proteins are several known to be essential for plastid function (FtsZ, rubredoxin, Hlip, Tic22). In Dinophysiaceae, only one pair of membranes surrounds the plastid, and there is no evidence of the presence of a nucleomorph (Lucas and Vesk 1990). If the nucleomorph was not acquired along with the plastid, and if the essential nucleomorph plastid-targeted genes were not transferred to or already present in the nucleus of *Dinophysis*, then it is unlikely that this organism would be able to maintain the plastid with its own gene complement. How *Dinophysis* spp. have circumvented the need for the nucleomorph encoded proteins is unknown. If *Dinophysis* once had a peridinin-containing plastid, it may already have these genes in the nucleus. A search for nucleomorph genes in *Dinophysis* may address this issue. Absence of these essential gene products would support the model of kleptoplastidy.

The feeding behavior of *Dinophysis* might explain our detection of florideophyte DNA in the single-cell

isolates. One possibility is that florideophyte DNA was present in the food vacuoles of these cells. We believe this to be a likely explanation because mixotrophy has been reported in several photosynthetic species of *Dinophysis* (Jacobson and Andersen 1994, Koike et al. 2000). *Dinophysis* species feeding on red algal cells has, however, never been reported. Heterotrophic *Dinophysis* species have only been observed feeding on ciliates (Elbrächter 1991, Hansen 1991). Identification of food vacuole contents of mixotrophic species is inconclusive, although ciliates are a possible prey (Jacobson and Andersen 1994, Koike et al. 2000). Our study suggests that the use of molecular methods may prove useful in determining the identity of *Dinophysis* prey. Another explanation for the presence of florideophyte DNA in our samples may be through contamination with these algal cells during the isolation process or with their DNA during PCR amplification. However, all isolated cells were rinsed three times and resuspended in 0.22-μm filtered seawater, making it unlikely that red algal cells or spores could have remained in our samples. In addition, the detected florideophyte sequences are evolutionarily distant from all species of red algae currently studied in our laboratory. Interestingly, we were able to amplify either florideophyte-like *psb*A or pSSU rDNA from all samples collected in Rhode Island; however, no red algal sequences were found in *D. norvegica* isolates from Maine. This may indicate that feeding on red algal cells is a local, species-specific, or seasonal behavior. Future studies detailing food vacuole contents and culturing *Dinophysis* spp. with florideophyte cells will be necessary to fully explain these results.

J. D. H was supported by an Institutional NRSA (T 32 GM98629) from the National Institutes of Health. H. S. Y. was supported by the Post Doctoral Fellowship Program of the Korean Science and Engineering Foundation. Dr. P. E. Hargraves kindly shared laboratory facilities at the University of Rhode Island. This work was supported by grants from the National Science Foundation awarded to D. B. (DEB 01-0774, MCB 01-10252). We thank two anonymous reviewers for critically reading this manuscript.

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