J. Phycol. **41,** 880–886 (2005)

DOI: 10.1111/j.1529-8817.2005.00111.x

SYMBIODINIUM (PYRRHOPHYTA) GENOME SIZES (DNA CONTENT) ARE SMALLEST AMONG DINOFLAGELLATES[1]

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Using flow cytometric analysis of fluorescence, we measured the genome sizes of 18 cultured "free-living" species and 29 *Symbiodinium* spp. isolates cultured from stony corals, gorgonians, anemones, jellyfish, and giant clams. Genome size directly correlated with cell size, as documented previously for most eukaryotic cell lines. Among the smallest of dinoflagellates, *Symbiodinium* spp. (6–15 μm) possessed the lowest DNA content that we measured (1.5–4.8 pg · cell^{-1}). Bloom-forming or potentially harmful species in the genera *Alexandrium*, *Karenia*, *Pfiesteria*, and *Prorocentrum* possessed genomes approximately 2 to 50 times larger in size. A phylogenetic analysis indicated that genome/cell size has apparently increased and decreased repeatedly during the evolution of dinoflagellates. In contrast, genome sizes were relatively consistent across distantly and closely related *Symbiodinium* spp. This may be the product of intracellular host habitats imposing strong selective pressures that have restricted symbiont size.

Key index words: **C-values; dinoflagellates; genome size; *Symbiodinium*; zooxanthellae**

Abbreviation: **DAPI, 4′,6-diamidino-2-phenylindole; ITS, internal transcribed spacer**

Dinoflagellates (Pyrrophyta) are primarily unicellular biflagellates. Collectively, they form one of three main clades comprising the alveolates, an ancient eukaryotic lineage that includes the ciliates and apicomplexan parasites. Dinoflagellates occur in both marine and freshwater habitats and are common constituents of phytoplankton communities. Of the more than 2000 known species, some are responsible for harmful algal blooms, whereas other taxa are appreciated for their impressive bioluminescence. Each species exhibits either phototrophy (photosynthesis), heterotrophy, or mixotrophy (Taylor 1987). Their ecological breadth also extends to parasitic and mutually symbiotic relations involving numerous vertebrate and invertebrate hosts (Cachon 1987, Trench 1993). The widespread symbioses between *Symbiodinium* and coral reef–dwelling invertebrates are especially notable because these mutualistic associations are essential for the existence of coral reef ecosystems (Muscatine and Porter 1977, Muscatine 1990).

Scientific fascination with dinoflagellates extends far beyond their obvious ecological and economic importance. They possess numerous unique cellular, molecular, and biochemical traits not observed in "text book" life forms and model organisms. Such disparities include 1) permanently condensed chromosomes lacking conventional histones; 2) DNA that contains some hydroxymethyluracil in place of thymine, unique among eukaryotes (Rizzo 1987); 3) unique toxic molecules and sterols (Shimizu 1987, Withers 1987); 4) complex organelle structures such as light sensing ocelloids, ejectile nematocyst and trichocysts, and the pusule, an invagination probably involved in osmoregulation and nutrient acquisition (Dodge and Greuet 1987); 5) an elaborate cell covering or thecal system (amphiesma) (Dodge and Greuet 1987); and 6) unu-

[1]Received 16 December 2004. Accepted 30 April 2005.
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sual genes and gene arrangements, such as bacterial type II RUBISCO (Whitney et al. 1995) and minicircular plastid DNA (Zhang et al. 1999). Collectively, these and other unique cytological, ultrastructural, and genetic attributes present intriguing topics of research. In summary, examination of the dinoflagellates underscores the degree of divergence that has occurred in the evolution of eukaryotic life forms.

The economic, ecological, and evolutionary significance of dinoflagellates establishes a persuasive argument for sequencing genomes representative of this group. However, one major obstacle to achieving this goal is that dinoflagellates typically possess genomes many times greater than the $3.0\,pg \cdot cell^{-1}$ (approximately) of the human genome. For example, *Prorocentrum micans* has one of the largest genomes measured for photosynthetic protists (e.g. 230 to 280 $pg \cdot cell^{-1}$; Velduis et al. 1997). Because past surveys of genome size among dinoflagellates were taxonomically limited (Rizzo 1987, Velduis et al. 1997, Parrow and Burkholder 2002), species may yet exist with small genomes more suitable for sequencing.

For this primary reason, we surveyed genome sizes (C-values, defined as the DNA content per haploid nucleus) for a range of cultured dinoflagellates via flow-cytometric analysis of 4′,6-diamidino-2-phenylindole (DAPI, Sigma Inc., St. Louis, MO, USA) stained cells for a range of cultured dinoflagellates. Our focus was on *Symbiodinium*, but we included representatives from familiar bloom-forming or potentially toxic genera (e.g. *Alexandrium*, *Karenia*, and *Pfiesteria*) as well as species from other genera available in culture. These were compared with measurements on various *Symbiodinium* spp. in the subgeneric clades A, B, C, D, E, and F (reviewed in Baker 2003). Sequences of the highly conserved (Maroteaux et al. 1985) and functionally constrained (Elela and Nazar 1997) 5.8S ribosomal gene (rDNA) provided the basis of a simple phylogenetic reconstruction involving most of the taxa examined in this study. Based on this phylogeny, we trace the evolution of genome/cell size across these lineages. Compared with other lineages, the evolutionarily divergent *Symbiodinium* spp. possess limited genome and cell sizes.

MATERIALS AND METHODS

Culturing and fixation. Cells were harvested while in exponential (log) growth to maintain consistent and accurate comparisons of nuclear DNA content (Allen et al. 1975, Loeblich 1976). After centrifugation (1000 *g* for 4 min), the resulting pellet (100,000–250,000 cells) was fixed with 1 to 10 mL of methanol:acetic acid (3:1 vol/vol) and then placed on ice for 20 min. The cells were recovered by centrifugation and the supernatant removed. The fixation step was repeated two or three times, with the addition of fresh fixative. This process removed essentially all the chloroplast pigments that might potentially interfere with subsequent nuclear fluorescence detection. After a fourth centrifugation and decantation, a 90% methanol wash was used to remove the acetic acid. After 5 min on ice, the cells were centrifuged and a fresh volume of 90% methanol was added. The fixed cells were then immediately shipped at 4° C by overnight courier to the University of Arizona for DNA measurements.

DNA content measurements. Cells were recovered by centrifugation at 1000 *g* for 4 min. They were stained by addition of 4 mL of a solution containing $20\,g \cdot mL^{-1}$ DAPI buffered by 20 M MOPS, pH 7. DNA content was measured using the DNA stain DAPI on a CCA-II Flow Cytometer (Partec, Munster, Germany) that was aligned using AlignFlo fluorescent microspheres (Molecular Probes, Eugene, OR, USA). Flow rates were typically $500\,cells \cdot s^{-1}$, and uniparametric histograms were accumulated to at least 1000 events. Coefficient of variation values for the DNA content peaks were typically less than 10%. DNA content measurements were calibrated using chicken red blood cells and *Arabidopsis thaliana* and *Nicotiana tabacum* nuclei (Johnston et al. 1999). Calculations were performed based on the chicken red blood cell standard of 3.0 pg per nucleus (Johnston et al. 1999), using a conversion factor of 1 pg = 9.65×10^8 bp (Bennett 2000). To define the degree of technical variability in the method of measurement, the same culture of "*Gymnodinium*" *simplex* (CCMP419) was measured seven times on three different occasions (quotation marks designate taxonomic uncertainty [Daugbjerg et al. 2000] or that the taxon lacks a formal description). In addition, replicate samples for other taxa were analyzed whenever possible (see Results).

The asexual vegetative stages of all dinoflagellates (with the possible exception of *Noctiluca*) are thought to be haploid (haplontic; Pfiester and Anderson 1987, Coats 2002). For example, nuclear reconstructions (Blank 1987) and molecular genetic evidence (Santos and Coffroth 2003) indicate that *Symbiodinium* spp. are haploid. For the purposes of this study, we assumed that the cells of each species were haploid.

Fluorescence emission from DNA stained with DAPI is proportional to the adenine and thymine (AT) content. Major across-species differences in the proportion of these nucleotides relative to cytosine and guanine (GC) would affect our DNA measurements. The AT proportions previously measured for *Symbiodinium* sp. representatives in clades A, C, and F ranged between 50.0% and 53.6% (Blank and Huss 1989). The conservation in AT/GC ratios should not adversely impact conclusions drawn from measurements of *Symbiodinium* spp. C-values. However, for species such as *Heterocapsa pygmea* whose nuclear AT content has been measured as 36%, we expect a systematic underestimation of C-values relative to previous findings (Triplett et al. 1993). The degree of underestimation should not affect conclusions regarding species suitable for genome sequencing because it would at worst represent an impossible factor of 2.

To test the relative precision of our measurements, C-value estimates made for several *Symbiodinium* spp. were compared against previously published estimates of chromosome volumes. In the dinokaryotic nucleus, chromosomes are condensed throughout mitosis and interphase. Therefore, chromosome volume was calculated from three-dimensional reconstructions based on transmission electron micrographs taken of serial sections through the nucleus (Blank and Trench 1985, Trench and Blank 1987, Blank and Huss 1989). Ideally, the use of isolated nuclei would have improved our accuracy because staining whole cells results in some nonspecific background fluorescence.

Cell size measurements. Light microscopy and an eye-piece reticule calibrated with a stage micrometer were used to measure cell sizes. Cell length ranges for the free-living species were obtained from the Center for Culture of Marine Phytoplankton (CCMP) database (Table 1). For *Symbiodinium* spp., coccoid cell sizes can vary considerably within a culture (Domotor and D'Elia 1986). To minimize the variability of this measurement, observations were made along the division plane of dividing cells (doublets) from cultures in log

TABLE 1. The DNA content in cultured dinoflagellates from non-*Symbiodinium* genera.

Taxon (Dinophyceae)	CCMP culture no.	Size in μm (median length)	pg · cell^{-1} (*n*)	Published flow cytometric and (nonflow cytometric) valuations in pg · cell^{-1}	GenBank accession no.
Alexandrium andersonii	2222	20–24 (22)	21.8 (2)		
Alexandrium insuetum	2082	22–28 (25)	30.8 (2)		AB006996
Alexandrium lusitanicum	1888	17–19 (18)	31.0		AJ005050
Alexandrium tamarense	1598	20–30 (25)	103.5		AB006992
Amphidinium carterae	1314	12–18 (15)	5.9 (2)	9.8[a](3.2[b]/2.7[c])	
Gymnodinium simplex	419	8–12 (10)	11.6 (7)		AY686651
Heterocapsa triquetra	449	18–20 (19)	24.1	19.1[a]	AF527816
Heterocapsa pygmaea	1322	9–14 (11.5)	3.8 (2)	(9.10[d])	AB084094
Polarella glacialis	2088	9–14 (11.5)	7.0 (2)		
Karenia brevis	2229	20–28 (24)	57.1	(101.6[c]/112.5[c])	AF352369
Karenia mikimotoi	429	28–30 (29)	100.1		AF318224
Karlodinium galatheanum	1974	10–18 (14)	16.9 (2)		AF172716
Karlodinium galatheanum	2282	8–20 (14)	16.3		AF352366
Katodinium rotundatum	1542	11–16 (13.5)	3.6		
Pfiesteria piscicida	1830	10–12 (11)	5.5 (2)	5.7[e]	AY245693
Pfiesteria shumwayae	2357	10–18 (14)	19.8	14.7[e]	AY245694
Prorocentrum balticum	1260	10–15 (12.5)	8.3 (2)		
Prorocentrum dentatum	1517	14–17 (15.5)	6.6 (2)		
Prorocentrum micans	689	22–38 (30)	115.2; 225.0	217.1[a](42.0[c])	AF370879
Prorocentrum minimum	1329	7–16 (12.5)	6.9 (3; SD = 0.35)		AF208244

Ranges in length dimensions (and median length) are provided. Numbers in parentheses refer to replicate flow cytometer readings. Accession numbers used to reconstruct the 5.8S phylogeny of Fig. 3 are also listed.

[a]Flow cytometric values (Veldhuis et al. 1997) using SYTOX stain.

[b]Diaminobenzoic acid C-value determinations by Holm-Hansen (1969).

[c]Diaminobenzoic acid C-value (Thomas and Farquhar 1978) determinations by Rizzo (1987).

[d]Diaminobenzoic acid C-value determinations by Triplet et al. (1993).

[e]Flow cytometric values (Parrow and Burkholder 2002) using SYTOX stain.

phase (Schoenberg and Trench 1980, Fitt 1985). Size was calculated from an average of 10 to 30 of these doublets. Because isolates possessing the same internal transcribed spacer (ITS) type are similar in size (LaJeunesse 2001), not all isolates were measured. Size measurements on the slightly smaller and rapidly moving motile phase of *Symbiodinium* spp. were not taken (see discussion below).

Phylogenetic analysis. Sequences of the 5.8S ribosomal gene for the cultures used in this study were acquired from GenBank, with the exception of "*Gymnodinium*" *simplex* (Tables 1 and 2). The complete ITS1, 5.8S, and ITS 2 sequence for "*G.*" *simplex* was obtained following the methods described by LaJeunesse (2001). Daugbjerg et al. (2000) showed that CCMP419 was not closely related to the type species of *Gymnodinium*, and therefore it should be reclassified. "*Symbiodinium trenchii*" (unpublished results) corresponds to "type" D1a and occurs in hosts from the Indian, Pacific, and Atlantic oceans. A sequence alignment consisting of 168 characters (including indels) of 18 distinctive sequences was prepared for phylogenetic analysis. Because members of a genus sometimes have the same 5.8S sequence, redundancies were omitted, leaving one sequence in the alignment to stand for more than one species. The proto-alveolate parasite, *Prekinsus marinus* (AF150990), was used as the outgroup (Saldarriaga et al. 2003). Maximum parsimony and maximum likelihood methods for phylogenetic reconstruction were performed using the software package PAUP v 4.10b (Swofford 2000).

RESULTS AND DISCUSSION

Genome size relates to cell size. This study extends the species of dinoflagellates for which genome size measurements are available, most notably by including members of the symbiont genus *Symbiodinium*. The nuclear DNA content for 47 dinoflagellate strains is shown in Tables 1 and 2. The DNA content correlated with cell size (Fig. 1; $r^2 = 0.84$ based on an exponential regression trend line, solid gray) and agreed with initial trends observed for dinoflagellates (Rizzo 1987, Velduis et al. 1997). The mutualistic endosymbionts of coral reef invertebrates, *Symbiodinium* spp., possessed the smallest genomes (Fig. 1). Several clade B "types," as defined by ITS2 sequence identity (LaJeunesse 2002), had genomes less than 2.0 pg · cell^{-1} (Table 2). Therefore, the smallest dinoflagellate genomes were recorded from the smallest *Symbiodinium* spp. (Fig. 1) (LaJeunesse 2001).

The trend illustrated in Figure 1 is similar to most plant and animal cell lineages, with C-values that correlate positively with cell size (Gregory 2001) or carbon content (Holm-Hansen 1969). Numerous explanations for this have been proposed (Gregory 2001), yet it remains unclear whether similar trends exhibited across many diverse and divergent eukaryote lineages are produced by natural selection, historical accident, or simply neutral random processes (Petrov 2001). Because genome size appears to have no relation to genetic and/or organismal complexity ("the DNA C-value paradox," Thomas 1971), it was initially believed that most genomes contained large quantities of secondary "noncoding" DNA representing neutral "junk" (Pagel and Johnstone 1992). Indeed, the DNA content among unicellular dinoflagellates (approximately 1.5 to 170 pg · cell^{-1}, Tables 1 and 2) is greater than most

TABLE 2. The genome sizes of *Symbiodinium* "types" in culture categorized by clade.

Clade (subgenus)	ITS "type"	Culture I.D. (CCMP no.)	DNA (pg · cell^{-1})	Cell size (μm)	Host origin	Geographic origin
A	A1	61 (2464)	2.2	8–11 (6–7)	*Cassiopeia xamachana* (Rhizostomeae)	Caribbean (Florida, USA)
	A1	370 (2467)	2.3		*Stylophora pistillata* (Scleractinaria)	Red Sea (Gulf of Aqaba)
	A1.1	80 (2469)	2.3	8–12 (6–7)	*Condylactis gigantea* (Actiniaria)	Caribbean (Jamaica)
	A2	185 (2461)	3.4	9–13 (7–8)	*Zoanthus sociatus*	Caribbean (Jamaica)
	A3	168 (2457)	3.2		*Tridacna crocea* (Bivalvia)	South West Pacific (Australia)
	A3a	PHMS TDle	3.4		*Tridacna* (Bivalvia)	West Pacific (Phillipines)
	A4	379 (2456)	2.7		*Plexaura homamalla* (Gorgonacea)	Caribbean (Bahamas)
	A12	Culture X	2.2		Unknown	Aquarium reef tank
	A13	PTA1	2.4		*Porites asteroides* (Scleractinaria)	Caribbean (Florida, USA)
	A14	m. miribalis	2.5		*Madracis miribalis* (Scleractinaria)	Caribbean (Florida, USA)
B	B1	2 (2460)	2.1		*Aiptasia pallida* (Actiniaria)	Caribbean (Florida, USA)
	B1	74	1.9	6–9	*Aiptasia pallida* (Actiniaria)	Caribbean (Jamaica)
	B1	147 (2470)	1.9	6–8	*Pseudoterogorgia bipinnata* (Gorgonacea)	Caribbean (Jamaica)
	B1	Pe	1.9		*Pseudoterogorgia elisabethae* (Gorgonacea)	Caribbean (Bahamas)
	B2	PPf	2.8		*Plexaura flexuosa* (Gorgonacea)	Caribbean (Panama)
	B2	10.14b.02	1.5	7.5–11	*Montastraea faveolata* (Scleractinia)	Caribbean (Florida, USA)
	B2.1	141 ($n = 2$)	2.5		*Oculina diffusa* (Scleractinaria)	Western Atlantic (Bermuda)
	B3	385 (2462)	2.4		*Dichotomia* sp. (Coronatae)	Caribbean
	B19	579	2.6		*Briareum* sp. newly settled polyp (Gorgonacea)	Caribbean (Florida, USA)
	B25	571	2.4		*Briareum* sp. newly settled polyp (Gorgonacea)	Caribbean (Florida, USA)
	B26	702	2.9		*Plexaura kuna* newly settled polyp (Gorgonacea)	Caribbean (Panama)
C	C1	152 (2466)	4.8	9–13 (6–8)	*Rhodactis*(*Heteractis*) *lucida* (Corallimorph.)	Caribbean (Jamaica)
	C2	203	2.9		*Hippopus hippopus* (Bivalvia)	West Pacific (Palau)
	C2	HHC1B	3.0		*Hippopus hippopus* (Bivalvia)	West Pacific (Phillipines)
D	D1a	A001	4.1		*Acropora* sp. (Scleractinia)	North west Pacific (Okinawa)
	D1a	2.2b (2556)	3.5	7.5–10	*Montastrea faveolata* (Scleractinia)	Caribbean (Florida, USA)
E	E1	383	3.3		*Anthopleura sola* (= *elegantisima*) (Actiniaria)	East Pacific (California, USA)
F	F1 (FR5)	Mv/135 (2468)	3.0	6–11 (4–6)	*Montipora verrucosa* (Scleractinaria)	Central Pacific (Hawaii, USA)
	F2 (FR5)	133 (2455)	2.6		*Meandrina meandrites* (Scleractinaria)	Caribbean (Jamaica)

The alphanumeric of each ITS "type" refers to the clade (uppercase letter) and ITS sequence identifier (numeral) and, if present, the designation of a co-dominant intragenomic variant (lowercase letter). Example cell size ranges are provided for coccoid and motile phases (in parentheses). The conversion factor for pg to base pair is 1 pg per $.965 \times 10^9$ bp (Bennett 2000). Center for Culture of Marine Phytoplankton accession numbers are given in parentheses in the Culture I.D. column.

other protist lineages (Velduis et al. 1997) and metazoa (Gregory 2001). However, evidence suggests that DNA content within a nucleus possesses important structural and/or volume-related functions (Cavalier-Smith 1978, Beaton and Cavalier-Smith 1999, Gregory 2001).

Many other dinoflagellate species grow to sizes larger than those we measured. Our survey was limited to smaller isolates for technical reasons: Large and/or nonspherical cells, as they pass through the flow cytometer, can orient in such a way as not to pass through the focus of the illuminations light source and/or of the optics that are used to collect the fluorescence emission. This has the effect of broadening the fluorescence intensity distribution of these types of cells, thereby imparting unacceptable levels of variation on the DNA content measurements. For this reason, species or isolates whose cells were greater than 40 μm in length were not examined.

Has culturing substantially affected genome size? Extended time in culture has been suggested to reduce the C-value and/or increase ploidy in a dinoflagellate strain (Holt and Pfiester 1982, Parrow and Burkholder 2002). No indication was found that time in culture had substantially altered the genome sizes of cultured *Symbiodinium* investigated for this study. The C-values measured among different isolates of a particular ITS type were remarkably consistent (Table 2). For, example "A1" cultures #61 originating from *Cassiopea xamachana* in the Caribbean and #370 from *Stylophora pistillata* Esper in the Red Sea differed by 0.1 pg · cell^{-1} (Table 2). These readings are well within the SE (for technical replications) of our method. Similarly, the values of seven independent measurements of "*G.*" *simplex* ranged from 9.1 to 12.4 pg · cell^{-1} with an average of 11.6, variance of 1.53, and SD of 1.24. The "B1" *Symbiodinium* listed in

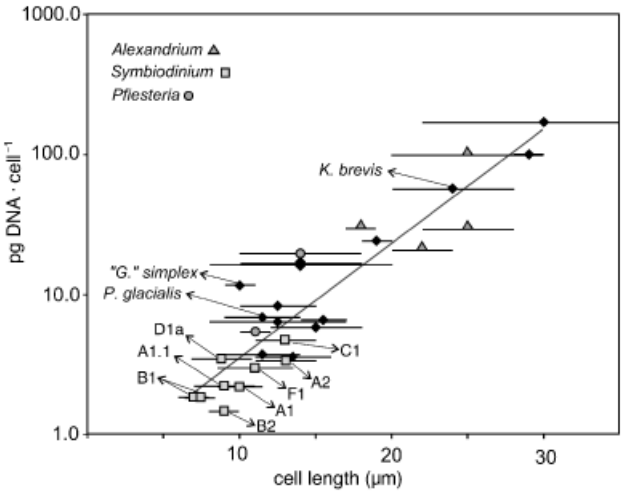

FIG. 1. Flow cytometric analyses of fluorescence by genomic DNA (pg) stained with DAPI as it relates to cell length (given as the median and range). Increased genome size correlates with increased cell size, a trend observed for most other eukaryotes (Petrov 2001). *Symbiodinium* spp. are among the smallest dinoflagellates, and their genomes are correspondingly small. The representative harmful algal bloom genera *Alexandrium*, *Karena*, and *Pfiesteria* have 2- to 50-fold larger genomes of *Symbiodinium* spp. Other species listed in Table 1 are represented by black diamonds. An exponential trend line is included ($R^2 = 0.84$). Measurements were made on logarithmically growing cells (Allen et al. 1975).

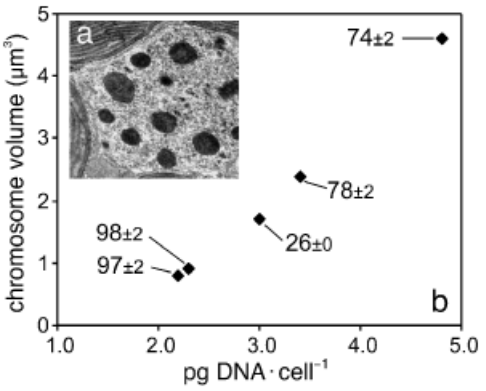

FIG. 2. Genome size (DNA content) for described *Symbiodinium* spp. in relation to chromosome volume and number. (a) An electron micrograph through the dinokaryotic nucleus of a *Symbiodinium* sp. showing condensed chromosomes (or "condensed DNA regions," Udy et al. 1993). (b) From left to right, plotted values correspond to the species *S. microadriaticum* subspecies *microadriaticum* (Blank and Huss 1989), *S. microadriaticum* subspecies *condylactis* (Blank and Huss 1989), *S. kawagutii* (Trench and Blank 1987), *S. pilosum* (Trench and Blank 1987), and *S. goreauii* (Trench and Blank 1987). Chromosome volume estimates (μm^3) were based on TEM three-dimensional reconstructions (Trench and Blank 1987, Blank and Huss 1989). Chromosome number and variance are indicated for each taxon.

Table 2 have similar C-values despite being isolated between 5 and 30 years ago from different hosts and geographic regions. Therefore, the close similarity in genome size among various isolates, cultured for various lengths of time, and sharing identical ITS sequences suggests that their genomes are stable in culture (Table 2).

Chromosome volume is proportional to estimated DNA content. The picogram values derived using our DAPI nuclear staining methods are similar to those found using flow-cytometric analysis of florescence using SYTOX green (Table 1) (Velduis et al. 1997, Parrow and Burkholder 2002). Both sets of values were proportional to DNA content but typically higher than DNA contents calculated using fluorometric measurements on extracted nucleic acids (Table 1) (Thomas and Farquhar 1978). Although these results are complementary, a second source of evidence supports the accuracy and relative precision of our measurements.

Dinoflagellates and euglenoids are among the few eukaryotic cell types that possess permanently condensed chromosomal bodies (Fig. 2a). The number and size of these is conserved in various species (Holt and Pfiester 1982), indicating genetic control. The condensed state therefore allows direct volumetric measurements for each species. Previous calculations made from three-dimensional nuclear reconstructions from various *Symbiodinium* spp. correspond linearly with DAPI fluorescence (Fig. 2b).

The accuracy and precision of DNA measurements may be directly attributed to the close similarity in genome size between *Symbiodinium* spp. and the chicken red blood cell standard (Johnston et al. 1999). We observed unusual variability between measurements on the largest species of our survey, *Prorocentrum micans* (225.0 and 115.2 $pg \cdot cell^{-1}$ respectively). The wide range in cell size of *P. micans* may explain this variation in DNA concentration (Table 1). Alternatively, because each measurement on this species was taken at separate times, the 2-fold differences in DNA content between dinoflagellate cells in log versus stationary phase (Allen et al. 1975) may better explain this discrepancy.

Endosymbiosis: constraints in the evolution of cell and genome size. Phylogenetic analysis of the dinoflagellates in our survey indicates that genome/cell size has increased and decreased repeatedly over the evolutionary history of this group (Fig. 3). For *Symbiodinium* spp., the ranges in genome and cell sizes are conserved within a narrow range relative to "free-living" dinoflagellate genera (Figs. 1 and 3). The numerous physical, ecological, and evolutionary constraints placed on an intracellular existence may largely explain the conservation of small cell and genome sizes across distantly related *Symbiodinium* spp.

Are *Symbiodinium* genomes smaller than those that would be predicted based on cell size alone? Many pathogenic and mutualistic intracellular bacterial symbionts have smaller genomes than their free-living relatives, often as a result of gene loss and increased

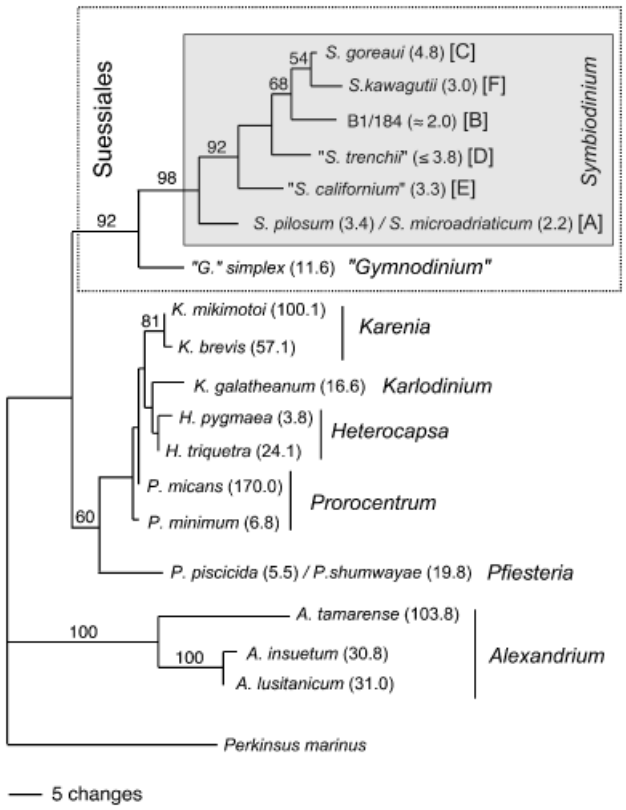

FIG. 3. Maximum likelihood reconstruction based on the 5.8S rRNA gene from genera whose DNA content (in parentheses) are presented in Figure 1. *Symbiodinium* and *Alexandrium* contain taxa with high sequence divergence relative to other genera. A small cell size (6–14 μm) and hence genome size (DNA content) are conserved across various *Symbiodinium* lineages (clade designations are in brackets). This phylogeny, while based on a small number of nucleotides (168 bases), corresponds to previously published reconstructions (Saldarriaga et al. 2001). Numbers associated with internal branches are bootstrap values (>50%) based on 500 replicates. *Prekinsus marinus* was used as the out group taxon (Saldarriaga et al. 2003).

physiological dependence on the host (Moran and Wernegreen 2000). The sister taxa to *Symbiodinium* spp., "*G.*" *simplex* and *Pollarella glacialis* (Montresor et al. 2003), have similar cell lengths (10–12 and 9–14 μm, respectively), yet their genomes are approximately 2.4 to 7 times greater (Fig. 3, Tables 1 and 2). Indeed, values for most *Symbiodinium* spp. fell slightly below the exponential trend line seen in Figure 1. This observation may be an artifact of the size calculations presented for *Symbiodinium* spp. These measurements were based on their coccoid life stage, which tends to be 20%–30% larger than the motile phase (Blank and Huss 1989) (Table 2). Adjustments for this discrepancy would bring *Symbiodinium* spp. C-values more into line with the general trends observed for other dinoflagellates. Although further comparative genomic analyses are needed, the small C-values among *Symbiodinium* spp. may be nothing more than the simple scaling of genome sizes relative to cell size.

Candidates for genome sequencing. It is only a matter of time before sequencing of the first dinoflagellate genome commences. The taxon to be selected should depend on a number of factors, including 1) relative economic and ecological importance, 2) a tractable genome size for sequencing, 3) suitability for genetic manipulation (Lohuis and Miller 1998), and 4) a genome that is representative of the group as a whole. Although *Symbiodinium* clearly meet criteria 1 through 3, unfounded concerns that their associations with invertebrates may have substantially modified their genomes remain. Indeed, they lack many of the complex organelles found in other taxa (Dodge and Greuet 1987). Nonetheless, isolates analyzed in this study are widely distributed host generalists (LaJeunesse 2005) that persist as viable cells in the external environment (Van Oppen et al. 2001, LaJeunesse et al. 2004). They are easily isolated from the host and maintained "happily" in culture as independent free-living dinoflagellates (host "specialists," however, have yet to be cultured, personal observation). Therefore, these are typical dinoflagellates not lacking important housekeeping genes. Given all these factors and considerations, we believe *Symbiodinium* spp. represent leading candidates for genome sequencing.

We thank Robert Moore for pointing out to us the need for additional genome measurements including the various *Symbiodinium* spp. R. K. Trench, D. Poland, N. Kirk, and J. Holmberg contributed comments on the manuscript. Funding for TCL was provided by an NSF grant (OCE-0137007) and FIU start-up. M. A. C.'s contributions were supported by NSF grants OCE-99-07319 and NSF OCE 03-13708 and R. A. A.'s contributions were supported by NSF grant DBI-9910676. C. Lewis provided technical assistance, and the Niagara Falls Aquarium, Niagara Falls, New York supplied seawater for maintaining algal cultures at the SUNY Buffalo laboratory.

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