We examined a free-living *Symbiodinium* species by light and electron microscopy and nuclear-encoded partial LSU rDNA sequence data. The strain was isolated from a net plankton sample collected in near-shore waters at Tenerife, the Canary Islands. Comparing the thecal plate tabulation of the free-living *Symbiodinium* to that of *S. microadriaticum* Freud., it became clear that a few but significant differences could be noted. The isolate possessed two rather than three antapical plates, six rather than seven to eight postcingular plates, and finally four rather than five apical plates. The electron microscopic study also revealed the presence of an eyespot with brick-shaped contents in the sulcal region and a narrow anterior plate with small knob-like structures. Bayesian analysis revealed the free-living *Symbiodinium* to be a member of the earliest diverging clade A. However, it did not group within subclade A I (=temperate A) or any other subclades within clade A. Rather, it occupied an isolated position, and this was also supported by sequence divergence estimates. On the basis of comparative analysis of the thecal plate tabulation and the inferred phylogeny, we propose that the *Symbiodinium* isolate from Tenerife is a new species (viz. *S. natans*). To elucidate further the species diversity of *Symbiodinium*, particularly those inhabiting coral reefs, we suggest combining morphological features of the thecal plate pattern with gene sequence data. Indeed, future examination of motile stages originating from symbiont isolates will demonstrate if this proves a feasible way to identify and characterize additional species of *Symbiodinium* and thus match ribotypes or clusters of ribotypes to species.

**Key index words:** free-living *Symbiodinium*; LSU rDNA; molecular phylogeny; plate tabulation; ultrastructure

**Abbreviations:** BA, Bayesian analysis; ITS, internal transcribed spacer; LB, longitudinal basal body; LSC, longitudinal striated collar; NJ, neighbor joining; src, striated root connective; TB, transverse basal body; TMRE, transverse microtubular root extension; TSC, transverse striated collar; vc, ventral connective

The term zooxanthellae refers to the golden-brown-colored algae living in mutualistic symbiosis with various invertebrate and protist hosts. Although they may include cryptophytes and diatoms, dinoflagellates constitute by far the most common group (Trench and Blank 1987, Rowan 1998). Earlier studies suggested that the endosymbionts represented a single pandemic dinoflagellate species, *Symbiodinium microadriaticum* (McLaughlin and Zahl 1966), but the advent of ultrastructural, biochemical, and later molecular systematics suggested the presence of different *Symbiodinium* species, and even dinoflagellate species from other genera, for example, *Amphidinium*, *Aureodinium*, *Gloeodinium*, *Gymnodinium*, and *Scripsiella* (Trench 1987, Wakefield et al. 2000). Still, species of *Symbiodinium* are the most widespread types of endosymbionts and have been found, besides cnidarians, in such diverse groups as foraminifers, radiolarians, ciliates, mollusks, and sponges (Baker 2003). The genus comprises four validly described species (*S. microadriaticum*, *S. pilosum* Trench et R. J. Blank ex. Trench, *S. kawaiitii* Trench et R. J. Blank ex. Trench, and *S. goreauii* Trench et R. J. Blank ex. Trench) and seven species without a formal description (*S. bermudense*, *S. californicum*, *S. cariborum*, *S. corculorum*, *S. meandrinae*, *S. muscatinei*, and *S. pulchrorum*) (Banaszak et al. 1993, LaJeunesse et Trench 2000). Additionally, nuclear-encoded LSU gene of *Gymnodinium linucheae* (Trench et Tinh) has ≥98% sequence similarity with *S. microadriaticum* and also belongs to *Symbiodinium* (Wilcox 1998). The correct combination was made by LaJeunesse (2001) as *S. linucheae* (Trench et Tinh) LaJeunesse. *Gymnodinium bei Spero* and the CCMP 1321 strain referred to as *Gymnodinium simplex* (Lohmann) Kofoed et Swezy are also closely related to *Symbiodinium*, and a free-living strain from New Zealand referred to as “*Gymnodinium varians*” Maskell has turned out to be a misidentified *Symbiodinium* species. All three species share a large deletion in the D2 region of the LSU gene

---

1Received 22 December 2007. Accepted 21 August 2008.
2Author for correspondence: e-mail gerth@bio.ku.dk.
with *Symbiodinium* (Wilcox 1998). Hence, the genus *Gymnodinium* might be removed from the list of zooxanthellae. The genus *Aureodinium* also seems to be closely related to *Symbiodinium*. They share several characters, for example, the presence of numerous polygonal plates, a stalked pyrenoid, lack of trichocysts, and a coccoid phase in the life history (Dodge 1967).

The molecular phylogenies, based on noncoding (ITS 1 and ITS 2) or coding (5.8S, SSU, LSU rDNA, cp23S rDNA) DNA fragments and also concatenated analyses, have consistently divided the genus into originally three but at present eight clades, designated A to H (Rowan and Powers 1992, Carlos et al. 1999, LaJeunesse 2001, Santos et al. 2002, Baker 2003, Garcia-Cuetos et al. 2005, Pochon et al. 2006). Some of the clades are further subdivided into subclades and numerous molecular “types” representing terminal taxa. About 100 types have been identified so far (Baker 2003), but uncertainty still exists concerning the taxonomic level these types represent (i.e., species, variants, populations, or clonal strains). Often the genetic difference between types is considerably larger than between species or even orders in other dinoflagellate groups (Baker 2003, Coffroth and Santos 2005). Formal descriptions are hampered by the scanty morphological features of the coccoid stage, and details of the motile cells are rarely included (Loeblich and Sherley 1979). Characters used to circumscribe species include size of the coccoid and motile stages, number and volume of chromosomes, number of chloroplasts and pyrenoid stalks, thylakoid stages, number and volume of chromosomes, number of chloroplasts and pyrenoid stalks, thylakoid arrangement, and also isoelectrical characteristics of peridinin–chl α-protein complexes (PCP) (Trench and Blank 1987).

Plate tabulation characteristics are the primary foundation for the taxonomy of armored dinoflagellates (Balech 1980, Fensome et al. 1993), but detailed analyses of the thecal plate pattern of the motile stage of *Symbiodinium* have so far only been performed for *S. microadriaticum* (Loeblich and Sherley 1979). Trench and Blank (1987) observed differences in plate patterns of *S. microadriaticum*, *S. pilosum*, *S. kawagutii*, and *S. goreauii* but did not provide any details. Thus, plate tabulation of *Symbiodinium* spp. could be a potential useful feature as a taxonomic criterion for species identification.

In the present study, we provide detailed plate analyses and include an ultrastructural characterization of a *Symbiodinium* species isolated from the water column in nearshore waters of Tenerife in the northeast Atlantic Ocean. The plate pattern was different from *S. microadriaticum*, and its phylogenetic positions within clade A based on partial LSU rDNA sequences also suggested it as a new species. We propose to name it *Symbiodinium natans* Gert Hansen et Daugbjerg sp. nov.

### Materials and Methods

The clonal culture used in this study was established from a net sample (20 μm mesh size, Aquanet, Kastrup, Denmark) collected from a pier at Callao Salvaje, Tenerife (28°7′36″N, 16°48′56″E), October 2004. The sample was enriched with TL growth medium (Larsen et al. 1994) and incubated at 20°C for about a week. Single cells of potentially interesting small dinoflagellates were isolated using a micropipette and a Labovert inverted microscope (Leitz, Wetzlar, Germany). The *Symbiodinium* culture used in the present study was grown in TL medium, at 20°C and ~40 μmol m⁻² s⁻¹. The culture was maintained for nearly 3 years before a culture cabinet failure unfortunately ended its growth.

*LM.* LM of live cells was made using an Axiovert AX 70 (Olympus, Tokyo, Japan) with a 60x dry lens, NA. 0.90. Microphotography was made using a Axiocam HR digital camera (Zeiss, Jena, Germany).

*SEM.* Cells were fixed in 2% OsO₄ for either 3 min or 40 min and placed on polyl-L-lysine coated circular coverslips or polycarbonate filters of 5 μm pore size (Isopore, Millipore Corp., Bedford, MA, USA). After washing in dH₂O for 1 h, samples were dehydrated in an ethanol series: 30, 50, 70, 96, and 99.9% for 10 min in each change, and finally in two changes of 100% ethanol, 30 min in each change. Critical-point drying was in a BAL-TEC CPD-050 (Balzers, Liechtenstein). The filters or coverslips were mounted on stubs and coated with palladium-platinum and examined in a JEOL JSM-6335F field emission scanning electron microscope (JEOL, Tokyo, Japan).

*TEM.* One vol. culture was added to one vol. fixation cocktail consisting of 2% glutaraldehyde made up in 0.2 M Na-cacodylate buffer with 0.3 M sucrose. The culture was pelleted by centrifugation after 1 h fixation and washed in four changes of buffer with decreasing sucrose concentration: 0.3 M, 0.15 M, 0.075 M, and 0.1 M buffer, 20 min in each change.

Postfixation was 1 h in 2% OsO₄ made up in 0.1 M Na-cacodylate buffer, and the material was dehydrated in an ethanol series and embedded in Spurr’s resin via propylene oxide. The material was sectioned on a Reichert Ultracut E ultramicrotome (Leica, Wetzlar, Germany) using a diamond knife. The sections were collected on slot grids and placed on formvar film. After staining in uranyl acetate and lead citrate, sections were examined in a JEOL JEM-1010 electron microscope operated at 80 kV. Micrographs were taken using a GATAN 792 digital camera (GATAN, Pleasanton, CA, USA).

Several, more or less successful, fixation schedules were applied. The schedule outlined above provided acceptable preservation of the internal parts of the cell but also pronounced osmotic artifacts of the amphiasma (i.e., thecal plates could not be recognized, and both flagella were lost).

**DNA extraction, PCR amplification, and LSU rDNA sequencing.** Ten mL of a clonal culture of *S. natans* was harvested by centrifugation at 3,000 g for 10 min. The resulting pellet was transferred to a 1.5 mL Eppendorf tube and placed at −20°C for ~2 weeks. Following thawing of the cell pellet, total genomic DNA was extracted using the CTAB (hexadecyltrimethyl-ammonium bromide) protocol as previously outlined (Daugbjerg et al. 1994). Amplification of partial nuclear-encoded LSU rDNA by the use of PCR was performed in a 50 μL reaction containing 5 μL 10X Taq buffer (67 mM Tris-HCl, pH 8.3, 2 mM MgCl₂, 16.6 mM (NH₄)₂SO₄, and 10 mM β-mercaptoethanol), 20 μL 0.5 μM dNTP mix, 5 μL 10 μM of each primer, 5 μL 100 mM tetramethylammonium chloride (TMA), and 1 U Taq polymerase (Ampliqon, Herlev, Denmark). The amplification primers were D1R-F (forward primer) combined with 28-1483 (reverse primer). Primer sequences are given in Scholin et al. (1994) and Daugbjerg et al. (2000), respectively. The PCR temperature profile was
Symbiodinium natans sp. nov. Gert Hansen et Daugbjerg.

**Description.** Cellulæ mobiles circiter 10 μm longæ (9.5–11.5 μm) et 8 μm latæ (7.4–9 μm). Epitheca leviter latior quam hypotheca. Apicalis theca angusta elongataque plurimis cum tuberibus in apice sita. Cingulum cinguli unius latitudine dispositum. Ex duabus lineis thecarum pentagonalium constat. Thecae formula x, EAV, 4′, 5a, 8′′, ıs, ıc, 6′, 2′′′. Praesentia unius pyrenoidis duplici calamo in media cellula siti. Nucleus in epitheca situs. Stigma ex plurimis vesiculis latericiis forma in sulco. Pedunculus unus praesens. GenBank accession numerus EU315917.

Motile cells are ~10 μm long (range 9.5–11.5 μm) and 8 μm wide (range 7.4–9 μm). The episphere is slightly larger than the hyposphere. An apical narrow elongated amphibial vesicle (EAV) with numerous knobs is located at the apex. The cingulum is displaced one cingular width and consists of two rows of pentagonal plates. Plate formula: x, EAV, 4′, 5a, 8′′, ıs, ıc, 6′, 2′′′. One two-stalked pyrenoid is situated in median part of the cell. The nucleus is located in the episphere. An eyespot, consisting of numerous brick-containing vesicles, is present in the sulcus. A peduncle is present. GenBank accession number: EU315917.

**Etymology.** natans referring to the free-swimming cells of this species.

**Holotype:** A SEM stub of the clonal culture used in this study has been deposited at the Botanical Museum, University of Copenhagen, accession number CAT2993. Figure 3, A–C, has been chosen to represent the type in accordance to fulfill article 39.1 of the International Code of Botanical Nomenclature (ICBN).

**Type locality:** Callao Salvaje, Tenerife.

**LM.** The typical motile cell had a slightly longer epi- than hyposphere (Fig. 1A), although some variations were noted from very pronounced “mushroom-shaped” (not shown) to an almost equally sized epi- and hyposphere (Fig. 1C). Brownish chloroplast(s) were situated along the cell periphery, and a distinct pyrenoid surrounded by a starch layer was located just below the nucleus (Fig. 1C). A darker brownish body, assumed to be the eyespot, was present in the sulcal area (Fig. 1, A and B). A majority of cells in the log phase were in the motile phase and had a very characteristic “spinning” movement, staying at the same place as if attached to the bottom of the culture flask, only occasionally swimming for a short distance.

The immotile cells measured ~13 μm in diameter and were often packed with storage products, particularly in old cultures (Fig. 1E). Scattered two-celled division stages were occasionally observed on the bottom of the culture flask, but four-celled stages were never recognized (Fig. 1D).
SEM. SEM revealed the transverse and longitudinal flagella, the length of the latter being more than 1.5 times the cell length (Fig. 2A). The cingular displacement was about one cingular width, and a peduncle emerged between the exit points of the two flagella (Fig. 2B).

The thecal plates were evident in many cells (Fig. 2, B–I) allowing us to reconstruct the thecal plate pattern (Fig. 3, A–C). On the episome, a narrow elongated plate or vesicle, measuring ~2 μm in length and 0.2 μm in width was situated at the apex of cells (Fig. 2, F–H). We designate this plate the elongated amphiesmal vesicle (EAV) in accordance with the terminology used by Moestrup and Daugbjerg (2007). About 12 tiny knob-like structures were protruding from the EAV (Fig. 2H). A small plate (x) was associated with the ventral part of the EAV (Figs. 2, F and G; 3, A and B). Four plates, apart from the x-plate bordered the EAV. According to Kofoidian terminology, we consider these as apical plates. Plate 1' was relatively large and rhomboid in shape, while plates 2' and 4' were narrow and somewhat rectangular (Fig. 2G). Eight precingular plates were present (Figs. 2, D–F; 3B), although a specimen with an extra precingular plate caused by division of plate 4' was observed (not shown). Five plates were located between the apical and precingular series, the so-called anterior intercalary plates (Figs. 2, F and G; 3B). The hyposomal plates comprised six postcingular and two antapical plates (Figs. 2I; 3C). The cingulum was made up of numerous pentagonal plates (Figs. 2, C–E; 3A). The exact number was not determined, but judging from the SEM micrographs of cells seen at different angles, the number was estimated to be ~20. The cingular plates were arranged in two rows. The SEM fixation did not allow for a detailed analysis of the sulcal plates, but a large posterior and a narrow left sulcal plate were evident (Fig. 2C). Tiny accessory sulcal plates surrounded the flagellar pores, but their definite numbers and shapes were not evident. Two small plates, a triangular plate situated next to the 8’-plate and the plate located left anteriorly to the transverse flagellar pore, might also be interpreted as either sulcal plates or alternatively as a precingular and a cingular plate, respectively. For the time being, we have designated these as s? (Figs. 2, B and C; 3A).

TEM. General ultrastructure: Thin sectioning revealed the presence of numerous chloroplast profiles located along the cell periphery (Fig. 4, A and B). It is possible that these profiles represented a single chloroplast. Numerous parallel thylakoid bands arranged in groups of three were present, and three membranes surrounded the chloroplast(s) (Fig. 6G). A large pyrenoid surrounded by a distinct polysaccharide cap was situated in the central part of the cell. The pyrenoid was two-stalked, that is, attached to two of the chloroplast profiles (Fig. 4A). A typical dinoflagellate cell nucleus with condensed chromosomes, the dinokaryon, was situated in the anterior part of the cell (Fig. 4A). Mitochondrial profiles with tubular cristae, and also lipid globules were scattered throughout the cell (Fig. 4A). Pusules were associated with both the transverse and longitudinal flagellar canal (Figs. 5B; 6, A and B). They were not studied in detail but appeared to consist of a convoluted tube. Trichocysts were never observed in the literally hundreds of cell profiles examined.

The eyespot was located in the sulcus and consisted of numerous flattened vesicles or cisternae containing electron translucent brick-shaped structures. A microtubular strand, probably the r1 flagellar root, was situated between the eyespot and the inner amphiesmal membrane (Fig. 4C). Unfortunately, the amphiesma could not be studied due to osmotic artifacts (Fig. 4A); for example, the thecal plates appeared to have been lost. However, plates inside the amphiesmal vesicles have previously been demonstrated in S. microadriaticum (Loeblich and Sherley 1979).

Flagellar apparatus, striated collars, and peduncle: The two slightly overlapping flagellar basal bodies were inserted at an angle of ~100° with respect to each other (Fig. 5, F and G). A typical complement of flagellar roots was associated with the basal bodies. Thus, a microtubular root (r1) consisting of 16 microtubules was associated with the left side of the longitudinal basal body (LB) (Figs. 5, E–G; 6, A–E), and the r2 root comprising one microtubule embedded in a dense fiber was situated on the right ventral side of the LB (Figs. 5, E–G; 6, D and E). Two roots, r3 and r4, were attached to the right...
dorsal and left side of the transverse basal body, respectively. The former consisted of a single microtubule that nucleated numerous microtubules (TMRE) (Fig. 5, C–G), the latter was a compound root consisting of a striated fiber (Figs. 5, E–G; 6, H and I) and a single microtubule (not shown). Several fibers interconnected the various components of the flagellar apparatus. The two basal bodies were connected by a small striated connective, the bbc (Fig. 5, E–G). The r1 root was attached to the LB by two small fibers, the C1LB/r1 and C2LB/r1 (Fig. 6E), and to the TB by a small striated fiber,
the $r_1$/TBc (Figs. 5, E–G; 6G). The $r_1$ and $r_4$ roots were interlinked by a striated fiber, the src (Fig. 5, F and G). A dense fiber was situated on the right dorsal side of the $r_1$ root (Figs. 6, B–E).

Two dense collars or sphincters encircled each of the flagellar canals, the longitudinal (LSC) and transverse striated collar (TSC), although the striation was not apparent (Figs. 5, A, B, and E–G; 6, A–C; G–I). The two collars were interlinked by a dense fiber, probably representing the ventral ridge fiber (Figs. 5, B and C; 6, B–F). A ventral connective (vc) originating from the $r_1$ flagellar root attached to the ventral ridge and the LSC (Figs. 5F; 6, C and D).

The peduncle consisted of a microtubular strand terminating in electron dense fibrous material that seemed to represent part of the ventral ridge. It is likely that this material represented a closed sphincter similar to the LSC and TSC. Numerous electron dense bodies were situated in the vicinity of the microtubular strand (Fig. 5, A–B).

Phylogeny. Figure 7 illustrates the phylogeny deduced from BA, including 106 *Symbiodinium* LSU rDNA sequences and two outgroup taxa (i.e., *Gymnodinium beii* and *G. simplex*). The tree topology for clade A to H was consistent with Garcia-Cuetos et al. (2005), and similar to this study, our analyses did not reveal details of the relationship between clades G, D, and the cluster that comprised clades B, F, H, and C, formed a trichotomy. Clade A branched at the base of the tree forming a sister clade to the remaining *Symbiodinium* clades (B to H). The individual clades designated A–E and G–H were highly supported in terms of posterior probabilities and bootstrap values from NJ analyses (1.0 = 100% in BA and ≥91% in NJ; Fig 7). Clade F received little bootstrap support from NJ analysis (54%) but high posterior probability (94%) from BA. *S. natans* clustered with 14 *Symbiodinium* LSU rDNA sequences assigned to clade A. Clade A was originally described more than 16 years ago (Rowan and Powers 1991). For reasons of comparison, we have divided clade A into four subclades, viz. $A_I$ (=temperate A, sensu Visram et al. 2006 but including a ribotype from eastern Australia, DQ060760), $A_{II}$ (=*Symbiodinium* pilosum and an unnamed Jamaican isolate, AF427456), $A_{III}$ (=*S. natans*), and $A_{IV}$ (=*Symbiodinium* from a diverse assemblage of Cnidaria host species sampled in tropical waters worldwide). Within clade A, subclade $A_I$ diverged first followed by subclade $A_{II}$. *S. natans* formed its own lineage (subclade $A_{III}$) and thus took a somewhat
isolated position. Subclade A_III formed a sister taxon to subclade A_IV, which contained two isolates identified as *S. microadriaticum* in addition to many unidentified taxa. BA provided high posterior probabilities for the major branches of subclades in clade A (>92%), whereas NJ bootstrap values only provided high support for A_I and A_II (100% and 96%, respectively; Fig. 7). In NJ bootstrap analysis, the position of subclade A_II and A_III was switched compared to the topology shown in Figure 7. Otherwise, the resulting tree topologies were almost identical.

**Divergence estimates of subclades in clade A.** Table 1 shows divergence estimates for all pair-wise comparisons within subclades (intracladal divergence) and among subclades (intercladal divergence) of clade A comprising *S. natans*. The divergence estimates are based on 520 base pairs including the highly divergent domain D2 of the LSU rDNA gene. The intracladal divergence suggested by Bayesian phylogeny (Fig. 7) ranged from a few substitutions (≤6 base pair differences within subclades A_I and A_II) up to 11 substitutions for subclade A_IV (range 1–11). Thus, subclade A_IV is the most diverse, and future morphological studies of motile cells are likely to recognize more species than the currently identified *S. microadriaticum*. The intercladal comparison revealed that the sequence of *S. natans* is most divergent to subclades A_I and A_II (43–46 substitutions (=8.9%–9.5%) and 21–23 substitutions (=4.2%–4.6%), respectively). Interestingly, the intercladal sequence divergence estimates between subclades A_III (=S. natans) and A_IV is only slightly higher than the intracladal differences of subclade A_IV (1.8%–2.8% and 0.2%–2.2%, respectively), indicating that these *Symbiodinium* taxa are more closely related and probably diverged more recently.

**Discussion**

*Identity of the species.* Previously, the plate tabulation had only been analyzed in two strains of *S. microadriaticum*, one isolated from decaying *Chondrus crispus* Stackh. and one isolated from *Cassiopea* sp. The free-living and in hospite strain had essentially a similar plate arrangement (Loeblich and Sherley 1979), although the variations in tabulation given for the free-living and the symbiotic strain were 1 pr, 5′, 5a–6a, 9–10″, ca. 20c, 8–9s, 7–8‴, 3‴‴ and 1 pr, 5′, 4a–6a, 10–11″, ca. 20c, 8–9s, 7–8‴, 3‴‴, respectively. The plate arrangement of *S. natans* is very similar to *S. microadriaticum*, but with some significant differences. For example, *S. natans* has two rather than three antapical plates and only six
postcingular plates compared to the seven to eight postcingular plates in *S. microadriaticum*. Furthermore, the number of apical plates is only four in *S. natans* but five in *S. microadriaticum*. Particularly, the differences in the hyposomal plate pattern are noteworthy. Apart from the cingular and sulcal plates, the hyposomal plates are considered the most stable and conservative (Balech 1980). These differences alone justify *S. natans* as different from *S. microadriaticum* sensu Loeblich and Sherley.
Additional differences are the episomal nucleus and displaced cingulum in *S. natans* compared to the hyposomal nucleus and nondisplaced cingulum in *S. microadriaticum* (Freudenthal 1962). Our phylogenetic analyses (Fig. 7) and sequence divergence estimates (Table 1) based on partial LSU sequences confirmed *S. natans* as separate from *S. microadriaticum*, but also from *S. pilosum* and *S. linucheae*, all belonging to clade A. The two other validly described species, *S. goreauii* and *S. kawagutii*, are not included in the present analysis due to lack of sequence data available (<25 base pairs) but belong to clade C and F, respectively (Baker 2003). Thus, molecular data also confirmed *S. natans* as a new species.

The rather different hyposomal plate pattern of *S. natans* and *S. microadriaticum*, irrespective of the fact that both species belong to clade A is somewhat
Table 1. Comparison in absolute number of differences and divergence estimates based on the Kimura-2-Parameter model using PAUP* (ver. 4b10) based on 520 unambiguously aligned base pairs of LSU rDNA sequences. See Figure 7 for definition of subclades A1 to AIV.

<table>
<thead>
<tr>
<th>Subclades A_i to A_V</th>
<th>Range of absolute number of differences</th>
<th>Range of divergence estimates based on Kimura-2-P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1–A_2</td>
<td>1−9</td>
<td>0.2−1.2</td>
</tr>
<tr>
<td>A_2–A_3</td>
<td>52−57</td>
<td>11.0−12.1</td>
</tr>
<tr>
<td>A_3–A_4</td>
<td>43−46</td>
<td>8.9−9.9</td>
</tr>
<tr>
<td>A_4–A_5</td>
<td>47−56</td>
<td>9.6−11.9</td>
</tr>
<tr>
<td>A_5–A_6</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>A_6–A_7</td>
<td>21–23</td>
<td>4.2−4.6</td>
</tr>
<tr>
<td>A_7–A_8</td>
<td>24–29</td>
<td>4.8−5.9</td>
</tr>
<tr>
<td>A_8–A_9</td>
<td>9–14</td>
<td>1.8−2.8</td>
</tr>
<tr>
<td>A_9–A_10</td>
<td>1–11</td>
<td>0.2−2.2</td>
</tr>
</tbody>
</table>

puzzling. The plate pattern of species within other clades is unknown, but such differences would be expected to reflect inter- rather than intracladal differences.

Ultrastructure and phylogeny of Symbiodinium. Recent studies have shown that Symbiodinium together with Polarella glacialis Montresor, Proacaccini et Stoecker, Wolosynskia halophila (Biecheler) Elbr. et Kremp, and W. pseudopalustris (J. Schiller) Kisselev constitute a well-supported clade (Kremp et al. 2005, Moestrup et al. 2008). The ultrastructural feature unifying these groups is the peculiar eyespot consisting of cisternae with brick-shaped contents, type E eyespot uniting these groups is the peculiar eyespot sensu Moestrup and Daugbjerg, with respect to the various connectives interlinking the flagellar roots and the basal bodies, with one notable difference, the presence of a “hub-spoke” structure around the basal bodies in B. dodgei (Moestrup et al. 2008). A structure previously only observed in fading agglutinata Moestrup, K. Lindbl. et Daugbjerg (Robert et al. 1995, as W. limnetica Bursa) and Wolosynskia pascheri (Such.) Stoch sensu Wilcox (1989). The flagellar apparatus of B. anauniensis differs from Symbiodinium and Borghiella by the presence of a ventral fiber, an incomplete longitudinal striated collar, and a peculiar lamellar body surrounding the basal bodies (Hansen et al. 2007). Loeblisch and Sherley (1979) observed a projection between emergence points of the two flagella in Symbiodinium microadriaticum and considered it to be a peduncle. Thin section TEM of S. natans confirms that Symbiodinium has a peduncle similar to that observed in a many dinoflagellates. It is not the microtubular basket type present in, for example, Gyrodinium lebouriae Herdmann (Lee 1977) and Paulsenella sp. (Schnepf et al. 1985), but the more common type consisting of a single microtubular strand. Its function is puzzling as it is hardly used for food uptake in an organism living as an endosymbiont presumably for most of its life history. Loeblisch and Sherley (1979) suggested it to be used for attachment, due to the rapid spinning behavior of the motile cell indicating attachment to the substrate. However, if S. natans is truly free living, the peduncle might be used for food uptake. For example, B. anauniensis also has a peduncle, and
this species is mixotrophic feeding on, for example, small cryptophytes (Hansen et al. 2007). Interestingly, *B. dodgei* lacks a peduncle (Moestrup et al. 2008).

It is clear that several features separate the clade comprising *Polarolla*, *Symbiodinium*, *Wołoszynskia halophila*, and *W. pseudopalustris* from the clade comprising *Baldinia* and *Borghiella*. However, it is less obvious what morphological characters link these groups into a major clade, apart from the fact that all species lack trichocysts and possess two rows of cingular plates (Montresor et al. 1999, Kremp et al. 2005, Moestrup et al. 2008).

An interesting question is whether the free-living stage of *S. natans* is permanent or merely a temporary motile stage awaiting the right host. Its phylogenetic position within clade A does not suggest any obvious hosts. However, clade A in the present analysis primarily consists of *Symbiodinium* spp. within cnidarians, but a non-cnidarian host cannot be excluded. Studies based on free-living strains of *Symbiodinium* are rare, and their infection potential, if any, remains largely unknown (Loeblich and Sherley 1979, Carlos et al. 1999, Gou et al. 2003). However, a recent study based on several strains of free-living *Symbiodinium* from Florida Keys, showed these were able to infect cnidian recruits, although some strains belonging to clade A appeared to be noninfectious (Coffroth et al. 2006). Due to the unfortunate breakdown of our culture cabinet resulting in the death of *S. natans*, studying of its infection potential is currently not possible.

In conclusion, the present study has indicated the potential application of plate tabulation for the taxonomy of *Symbiodinium*. Details of the plate arrangement have only been made on two species, and therefore more species from different clades (e.g., B to H) need to be analyzed before an evident evaluation of inter- and intracladal plate variation can be undertaken. However, in an attempt to improve our understanding of species distribution of *Symbiodinium* on a local and regional scale, we propose to combine information from molecular methods with observations of plate tabulation characteristics.

This study was financed by the Carlsberg Foundation to G. H. and N. D. (equipment grant), and Danish Science Research Council to N. D. We thank Lydia Garcia-Cuetos for the initial alignment comprising *Symbiodinium* LSU rDNA sequences. We thank Charlotte Hansen for technical help in running the ABI 377 sequencer.


