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1 **BRANDTODINIUM** GEN. NOV. AND **B. NUTRICULUM** COMB. NOV.
2 **(DINOPHYCEAE), A DINOFLAGELLATE COMMONLY FOUND IN**
3 **SYMBIOSIS WITH POLYCYSTINE RADIOLARIANS¹**

4

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25

1 Abstract

2 Symbiotic interactions between pelagic hosts and microalgae have received little
3 attention, despite the fact that they are widespread in the photic layer of the world
4 ocean where they play a fundamental role in the ecology of the planktonic ecosystem.

5 Polycystine radiolarians (including the orders Spumellaria, Collodaria and
6 Nassellaria) are planktonic heterotrophic protists that are widely distributed and often
7 abundant in the ocean. Many polycystines host symbiotic microalgae within their
8 cytoplasm, mostly thought to be the dinoflagellate *Scrippsiella nutricula*, a species
9 originally described by Karl Brandt in the late nineteenth century as *Zooxanthella*
10 *nutricula*. The free-living stage of this dinoflagellate has never been characterized in
11 terms of morphology and thecal plate tabulation. We examined morphological
12 characters and sequenced conservative ribosomal markers of clonal cultures of the
13 free-living stage of symbiotic dinoflagellates isolated from radiolarian hosts from the
14 three polycystine orders. In addition, we sequenced symbiont genes directly from
15 several polycystine-symbiont holobiont specimens from different oceanic regions.

16 Thecal plate arrangement of the free-living stage does not match that of *Scrippsiella*
17 or related genera, and LSU and SSU rDNA-based molecular phylogenies place these
18 symbionts in a distinct clade within the Peridiniales. Both phylogenetic analyses and
19 the comparison of morphological features of culture strains with those reported for
20 other closely related species support the erection of a new genus that we name
21 *Brandtodinium* gen. nov. and the recombination of *S. nutricula* as *B. nutriculum*
22 comb. nov..

23

24 Key words: dinoflagellate, polycystines, Peridiniales, Radiolaria, *Scrippsiella*,
25 symbiosis, taxonomy, *Zooxanthella*,

1

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1 Introduction

2 Mutualistic associations involving photosynthetic microalgae are common in both
3 benthic and pelagic ecosystems and are essential for establishing and maintaining the
4 structure of marine communities (Caron 2000). Symbiosis between corals and the
5 dinoflagellate genus *Symbiodinium* Freudenthal is fundamental for the survival and
6 ecological success of coral reef ecosystems. Members of the genus *Symbiodinium*
7 have been intensively studied with respect to their morphology and life cycle
8 (Freudenthal 1962; Fitt and Trench 1983; Trench and Blank 1987), and genetic
9 diversity (Coffroth and Santos 2005; Sampayo et al. 2009; LaJeunesse and Thornhill
10 2011; Stat et al. 2011). Studies on this coastal benthic symbiotic relationship
11 significantly increased when the coral-bleaching phenomenon was brought to global
12 attention and associated to increases in sea surface temperature, enhanced light
13 intensity, and ocean acidification (Hoegh-Guldberg et al. 2007).

14 Symbiotic interactions between pelagic hosts and microalgae have received less
15 attention, despite the fact that they are widespread in the photic layer of the world
16 ocean where they play a fundamental role in the ecology of the planktonic ecosystem
17 (Stoecker et al. 2009; Decelle et al. 2012). Recent studies have demonstrated that
18 dinoflagellate symbionts of Foraminifera belong to *Pelagodinium* Siano, Montresor,
19 Probert et de Vargas, a genus that is related to *Symbiodinium* within the order
20 Suessiales (Siano et al. 2010), and that Acantharia typically associate with members
21 of the prymnesiophyte genus *Phaeocystis* Lagerheim (Decelle et al. 2012), although
22 one taxon, *Acanthochiasma* sp., can contain multiple symbiotic partners, including
23 distantly related dinoflagellates (from the genera *Pelagodinium*, *Heterocapsa* Stein,
24 *Azadinium* Elbrächter et Tillmann and *Scrippsiella* Balech ex Loeblich III) as well as
25 a haptophyte (Decelle et al. 2012b).

1 Polycystine radiolarians (including the orders Spumellaria, Collodaria and
2 Nassellaria) are single-celled, heterotrophic, biomineralizing planktonic protists from
3 the Rhizaria lineage that are widely distributed in the ocean and are found throughout
4 the entire water column (Boltovskoy et al. 2010). Many polycystines host microalgae
5 within their cytoplasm (Anderson 1983). Cells containing photosynthetic microalgae
6 have been shown to survive for longer periods in nutrient-poor water than those that
7 do not have microalgal partners and the microalgae are therefore assumed to be
8 symbionts that play a nutritive role for the hosts (Anderson 1983).

9 Polycystines form associations with various dinoflagellate, prymnesiophyte and
10 prasinophyte partners (usually not at the same time), with dinoflagellates being the
11 most common symbiotic partners (Anderson 1976, 1983; Anderson et al. 1983). In the
12 late nineteenth century, Karl Brandt was the first to recognize that the “yellow cells”
13 within polycystines, actinian corals and hydrozoans were microalgae, which he
14 collectively described in the new genus *Zooxanthella* Brandt (Brandt 1881), although
15 they were not immediately recognized as dinoflagellates. Soon afterwards, the species
16 *Z. nutricula* Brandt was proposed for the symbiont of the collodarian polycystine
17 *Collozoum inerme* collected from the western Mediterranean Sea and it was stated in
18 the description that this species was presumably identical to the yellow cells of other
19 polycystines (Brandt 1882). The subsequent taxonomic history of this genus and
20 species have been very confused (see review by Blank & Trench 1986), and the
21 plural noun ‘zooxanthellae’ has persisted as a colloquialism used to describe marine
22 microalgal endosymbionts in general.

23 The symbionts of the ‘by-the-wind sailor’ hydrozoan jellyfish *Velevella velevella* were
24 reported to be similar to those of polycystines initially by Hovasse (1922), who
25 initially described the *in hospite* symbionts of Mediterranean *V. velevella* as *Endodinium*

1 *chattoni* Hovasse (*E. chattonii* under ICBN Art. 73). Taylor (1971) and Hollande and
2 Carré (1974) further characterized the *in hospite* stage of *E. chattonii* and the latter
3 authors proposed the reclassification of the polycystine symbionts (*Z. nutricula*) as *E.*
4 *nutricula* (Brandt) Hollande et Carré (*E. nutricula* under ICBN Art. 73), despite the
5 fact that Hovasse (1924) had in fact previously recombined *E. chattonii* as *Z. chattonii*
6 (Hovasse) Hovasse. Banaszak et al. (1993) isolated a culture of the symbiont of *V.*
7 *velella* from the Pacific, which they considered slightly different from *E. chattonii*
8 (larger cell size and presence of trichocysts *in hospite* and in culture). Based on SEM
9 observations of the morphology and arrangement of thecal plates in the motile stage,
10 Banaszak et al. (1993) classified their organism in the genus *Scrippsiella* as a new
11 species, *S. velellae* Banaszak, Iglesias-Prieto et Trench (a name later validated by
12 Trench 2000). These authors also transferred *E. chattonii* and *E. nutricula* to
13 *Scrippsiella* as *S. chattonii* (Hovasse) Banaszak, Iglesias-Prieto et Trench and *S.*
14 *nutricula* (Brandt) Banaszak, Iglesias-Prieto et Trench, respectively (Banaszak et al.
15 1993), but these names remain technically invalid because reference was not made to
16 the exact page of the basionym.

17 Using molecular methods, Gast and Caron (1996) found that the dinoflagellate
18 symbionts in six different polycystine species from the Sargasso Sea (the collodarians
19 *Collozoum caudatum* and *Thalassicolla nucleata*, three unidentified collodarian
20 species and the spumellarian *Spongostaurus* sp.) had identical SSU rDNA sequences
21 that they assigned to *Scrippsiella nutricula*. These molecular analyses indicate that
22 taxonomically divergent radiolarians can contain the same symbiotic dinoflagellate.
23 Since these analyses were conducted directly on symbionts extracted from the hosts
24 (i.e., not cultured), the morphology of the motile stage of the symbiotic algae assigned
25 to *S. nutricula* was not investigated, and has still never been reported. Gast and Caron

1 (1996) also sequenced the SSU rDNA of the symbiont of *V. veleva* from the Sargasso
2 Sea and found that the sequence was very similar to those of the radiolarian symbionts
3 (4 differences out of 1802 base pairs). They therefore also assigned this *V. veleva*
4 symbiont to *S. nutricula*.

5 Here we examined the morphology and molecular phylogenetic position of clonal
6 cultures of the free-living stage of dinoflagellates isolated from several different
7 polycystine radiolarian hosts, including *Collozoum*, the taxon from which
8 *Zooxanthella nutricula* was originally described. In addition, we sequenced symbiont
9 genes directly from several polycystine-symbiont holobiont specimens (including
10 collodarian, spumellarian and nassellarian hosts) from different oceanic regions.

11 Accurate morpho-molecular characterization and taxonomic designation of symbionts
12 from the genus *Symbiodinium* has been key for studies of the ecology and functioning
13 of coral reef systems and ~~is it~~ is likewise likely to prove important for future studies
14 on the widespread pelagic symbiosis involving polycystine radiolarian hosts.

15

16 Material and Methods

17 *Samples and culture isolation*

18 The radiolarian specimens from which the holobiont sequences or cultures originated
19 were isolated from samples collected in 2010-2012 by net tows (20 to 150 micron
20 mesh size) in the bay of Villefranche-sur-Mer (France), off Sesoko Island, Okinawa
21 (Japan) and in the South Pacific Ocean during the Tara Oceans expedition (Table 1,
22 Supplementary Figs 1 and 2). The polycystines were first sorted from fresh net
23 samples under a binocular microscope, cleaned by successive transfers in sterile
24 seawater in Petri dishes, then left in an illuminated and temperature-regulated
25 incubator for several hours to self-clean. Individual clean specimens were then

1 identified based on their morphology and imaged under an inverted microscope. Some
2 specimens were then transferred to guanidinium isothiocyanate (GITC) buffer for
3 direct DNA extraction from holobionts. The dinoflagellate cultures were obtained by
4 micropipette isolation of single symbiont cells released from live radiolarian
5 specimens that were microdissected under an inverted microscope. The resulting
6 monoclonal cultures were maintained in filter-sterilized seawater with K/2(-Tris, -Si)
7 medium supplements (Keller et al. 1987) at 22°C with an irradiance of 70–80 μmol
8 $\text{photons m}^{-2}\text{s}^{-1}$ in a 12:12 light:dark regime. The cultures have been deposited in the
9 Roscoff Culture Collection (<http://www.sb-roscoff.fr/Phyto/RCC>). [LM images of](#)
10 [radiolarian holobionts from which sequences / cultures were obtained are shown in](#)
11 [Supplementary Figures 1 and 2](#). Detailed information related to each of the samples
12 used in this study can be found in the RENKAN database at [http://abims.sb-](http://abims.sb-roscoff.fr/renkan/)
13 [roscoff.fr/renkan/](http://abims.sb-roscoff.fr/renkan/).

15 *Microscopy preparations and observations*

16 Light micrographs of living cells were taken using a Zeiss Axiophot light microscope
17 equipped with a Zeiss AxioCam digital camera system (Carl Zeiss, Oberkochen,
18 Germany). For scanning electron microscopy (SEM), dinoflagellate cells were fixed
19 in 1% (v:v) formol for 2 hours at room temperature. Samples were then gently filtered
20 onto 3 μm pore-size Nucleopore polycarbonate filters (Pleasanton, CA, USA), washed
21 with distilled water, dehydrated in an ethanol series (25%, 50%, 75%, 95%, 100%),
22 and critical point dried. The filters were mounted on stubs, sputter coated with gold,
23 and examined with a FEI Quanta™ 200 SEM (FEI, Hillsboro, Oregon, USA)

25 *DNA extraction, sequencing and phylogenetic analyses*

1 Genomic DNA was extracted from exponentially growing cultures of the strains using
2 a NucleoSpin Plant II DNA extraction kit (Macherey-Nagel), or from holobionts
3 using the method described in De Vargas et al. (2002).

4 Partial nuclear LSU and SSU rDNA genes were PCR amplified using Phusion high-
5 fidelity DNA polymerase (Finnzymes) in a 25 µl reaction volume and the following
6 thermocycler steps : an initial denaturation step at 98°C for 30 sec, followed by 35
7 cycles at 98°C for 10 sec, 30 sec at the temperature of semi-hybridization chosen for
8 each set of primers, and 30 sec at 72°C, with a final elongation step of 10 min at
9 72°C. The eukaryote primer set 63F (ACGCTT GTCTCAAAGATT) / 1818R
10 (ACGGAAACCTTGTTACGA) (T_m 50°C) (Lepere et al. 2011) was used to amplify
11 the SSU rDNA of the dinoflagellate cultures, whereas the dinoflagellate specific
12 primer set DIN464F (TAACAATACAGGGCATCCAT) / S69
13 (CCGTCADTTTCCTTTRAGDTT) (T_m 53°C) was used to target the dinoflagellates in
14 the holobiont samples. The D1-D2 fragment of the LSU rDNA was amplified using
15 the dinoflagellate specific primers Ldino6 (MCC CGCTGAATTTAAGCATA) /
16 Ldino1 (AACGATTTGCAGGTCAGTACCGC) (T_m 55°C) from both cultures and
17 holobionts. PCR products were then sequenced at the GENOSCOPE (CEA, Evry,
18 France).

19 The sequences generated from the studied strains and holobionts (GenBank accession
20 numbers: ~~XXXX~~ [KF557491 to KF557545](#) ~~to XXXX~~) were aligned with other LSU
21 and SSU rDNA sequences from GenBank (release 194.0, February 2013) attributed to
22 *Scrippsiella* and related Peridinales genera, as well as representatives of the
23 Suessiales as an outgroup. Alignments were generated using MUSCLE implemented
24 in Seaview v.4.0 (Gouy et al. 2010) with subsequent manual verification. [The LSU](#)

1 rDNA data set contained 48 sequences (675 unambiguously aligned positions) and the
2 SSU rDNA data set contained 57 sequences (652 unambiguously aligned positions).

3 Phylogenetic analyses were conducted with Maximum Likelihood (ML) and Bayesian
4 methods. The ML analysis was carried out using MEGA v. 5.1 (Tamura et al. 2011)
5 with the General Time Reversible (GTR) as the best model of nucleotide substitution
6 and considering a gamma distribution with a proportion of invariable sites (I) set at 5
7 by default. Bootstrap supports for the tree were obtained after 1000 replicates. ~~The~~
8 ~~tree was visualized and edited in Fig Tree v 1.3.1 (Rambaut 2010).~~ Bayesian analyses
9 were conducted using Mr Bayes v.3.2.1 (Huelsenbeck and Ronquist 2001) using the
10 same model of evolution. For each gene marker, two Markov Chain Monte Carlo
11 (MCMC) chains were run for 1 million generations, sampling every 500 generations
12 (diagnostic frequency = 5000). The standard deviation of split frequencies between
13 the 2 runs was <0.01 in both LSU and SSU rDNA analyses. ~~For both ML and~~
14 Bayesian analyses, the trees were visualized and edited in Fig Tree v 1.3.1
15 (Rambaut 2010). ~~The~~ In the trees presented herein the posterior probabilities (PP)
16 associated to each node in the Bayesian topologies ~~were are~~ reported on the ML
17 topologies.

19 Results

20 *Microscopy Observations*

21 In our culture conditions, ~~our the~~ clonal strains of polycystine symbionts tended to
22 contain a mixture of motile thecate cells and larger, irregularly-shaped non-motile
23 cells devoid of the typical features of motile cells (theca, cingulum, sulcus), the latter
24 more closely resembling the *in hospite* symbiotic state. The proportion of motile and
25 non-motile cells varied between strains and through growth cycles for each strain. The

1 overall morphology and thecal plate pattern of motile cells was identical for several
2 different strains observed. The following descriptions and illustrations are based on
3 observations of strain [VFR1-1RCC3387](#).
4 Cells are 10.5 to 15 μm in length (average 13.1 μm , $n=30$) and 9.1 to 11.2 μm in
5 width (average 10.4 μm , $n=30$). The epitheca is larger than the hypotheca. Observed
6 under LM, cells have a slightly convex conical epitheca with a well-pronounced
7 apical horn (Fig. 1A, 1B, 1D). The hypotheca is rounded (Fig. 1A, 1D). The nucleus
8 is large and occupies the center of the cells (Fig. 1B, 1D). One or two golden-yellow
9 chloroplasts are present around the cell periphery, sometimes appearing as a single
10 plastid bordering the cell periphery (Fig. 1D). One large circular pyrenoid (sometimes
11 two) is often visible in LM (Fig. 1A-D). No eyespot is visible in light microscopy.
12 Cells swim steadily in a straight line, rotating around the transapical axis. They
13 suddenly stop, change direction at different angles from the original path, often back-
14 tracking.
15 In SEM, the epitheca appears conical (Fig. 2A) to rounded (Fig. 2C), and the smaller
16 hypotheca is symmetrical and rounded in ventral (Fig. 2A) and dorsal (Fig. 2C) view.
17 The plate tabulation is Po, X, 4', 3a, 7", 5C, 4S, 5"', 1'''' (Figs 2A-E, 3A-D). The pore
18 plate (Po) is circular and surrounded by a high collar and is connected to the first
19 apical plate by a long well-defined rectangular canal plate (X) (Figs 2A, 3A, 3C).
20 Three intercalary plates are interposed on the dorsal side of the cell between the apical
21 series and the second epithecal (precingular) series (Figs 2C-D, 3B-C). The first
22 intercalary plate (1a) is five-sided and borders only one of the apical plates (2'),
23 whereas the second and third intercalary plates (2a and 3a) are six-sided and both
24 border two apical plates (Figs 2C-D, 3C). The cingulum is located in the median
25 portion of the cell and descends slightly, displaced by approximately one third of its

1 own width (Figs 2A, 2C, 3A-B). It is very wide and shallow and is constituted by a
2 single series of five rectangular plates, the first being much narrower than the others
3 (Fig. 2A-C, 2E, 3A-B). The sulcus is fairly shallow and narrows towards the antapical
4 end (Fig. 2A-B). The sulcal area comprises four plates (Fig. 2B, 3A). One of these
5 (Sd) forms a conspicuous flange extending over the median area of the sulcus,
6 partially covering the sulcal area (Fig. 2B). There appears to be a single plate (Ss)
7 beneath this flange (Fig. 2B). Flagella were not preserved in our SEM preparations. In
8 the hypotheca, a series of 5 trapezoid plates of similar size borders the cingulum. A
9 single six-sided antapical plate completes the hypothecal tabulation (Fig. 2E, 3D). The
10 cell surface is mostly smooth. We have never observed a peduncle in either LM or
11 SEM preparations.

12

13 *Phylogenetic Analyses*

14 PCR amplifications of DNA extracts from culture strains and uncultured holobionts
15 led to generation of 35 partial SSU rDNA (~650 bp) and 22 partial LSU rDNA (~675
16 bp) sequences of dinoflagellate symbionts from spumellarian, collodarian and
17 nassellarian hosts collected in the Mediterranean Sea and in the North and South
18 Pacific oceans (Table 1). For each gene the vast majority of these sequences were
19 identical (see below) and hence only a subset of 15 SSU rDNA and 10 LSU rDNA
20 sequences, representing a cross-section of host diversity, were included in datasets for
21 phylogenetic reconstructions. Phylogenetic analyses on the SSU and LSU rDNA
22 datasets demonstrated that all of our sequences grouped together in a distinct and
23 highly supported clade (hereafter called clade B) within the dinoflagellate order
24 Peridinales (full ML and Bayesian statistical support; Figs. 4 and 5). In both SSU and
25 LSU rDNA phylogenies, this clade included two distinct sub-clades, B1 and B2, each

1 containing sequences that are 100% identical irrespective of host taxon and oceanic
2 region. In our SSU rDNA phylogenetic tree (Fig. 4), sub-clade B1 included the
3 majority of symbiont sequences recovered in this study (including those from five
4 culture strains isolated from *Collozoum* colonies from the Mediterranean Sea and
5 Pacific Ocean), as well as published sequences that correspond to the symbionts of
6 five collodarians and one spumellarian collected in the Atlantic Ocean (Gast and
7 Caron 1996). Sub-clade B2 contained the sequences generated in the present study of
8 the symbionts of two collodarian holobionts as well as one published sequence
9 (U52357) of the symbiont of the jellyfish *Velevella velevella* (Gast and Caron 1996). In
10 both phylogenetic reconstructions, the monophyletic clade B containing the sequences
11 of polycystine symbionts was phylogenetically distinct from the well-supported clade
12 containing members of the genus *Scrippsiella* (including the holotype species *S.*
13 *sweeneyae* Loeblich III), but overall the phylogenetic relationships between clades
14 within the Peridinales were not clearly resolved in our analyses. When sequences of
15 members of the genus *Bystrum*, which have a plate tabulation pattern similar to
16 *Scrippsiella*-like peridinales (Table 2), were included in phylogenetic analyses, they
17 formed a distinct mono-generic clade which fell on a long branch that altered overall
18 tree topology (Supplementary Figure 3). In the SSU rDNA phylogeny (Figure 4), note
19 that the sequence labeled “uncultured alveolate from Nasselaria” (DQ916409) and the
20 two sequences labeled “Dinophyceae from Collodaria” (DQ116021 and DQ116022)
21 correspond to non-photosynthetic dinoflagellate parasites of Radiolaria (Gast 2006).

22

23 Discussion

24 Dinoflagellates that form symbiotic relationships with metazoan or protistan hosts are
25 characterized by complex life cycles, with an alternation of symbiotic and free-living

1 stages with considerable morphological and physiological differentiation between
2 them. Within the host cells, the symbionts are typically coccoid without flagella, and
3 the cingulum and sulcus are no longer apparent (Trench and Blank 1987). In the free-
4 living stage, cells tend to regain their original morphology (Freudenthal 1962; Spero
5 1987, Siano et al. 2010). Since the taxonomy of dinoflagellates is largely based on
6 comparison of the number, shape and arrangement of the thecal plates (or amphiesmal
7 vesicles in athecate species) that form the periplast of free-living motile cells, the
8 establishment of clonal cultures from symbionts extracted from their hosts is critical
9 for accurate taxonomic assignation.

10 The genus *Zooxanthella* was originally created to collectively describe the symbionts
11 of diverse hosts from the Mediterranean Sea, including polycystines, corals and
12 hydrozoans (Brandt 1881) and *Z. nutricula* was created to describe the symbionts of
13 the collodarian polycystine *Collozoum inerme* (Brandt 1882). The taxonomic history
14 of *Zooxanthella* has subsequently been confusing, with *Z. nutricula* being alternately
15 combined within *Endodinium*, *Amphidinium* Claperède et Lachmann (see review of
16 the nomenclatural history of endosymbiotic dinoflagellates by Blank and Trench,
17 1986) and most recently (albeit technically invalidly) within *Scrippsiella* (Banaszak et
18 al. 1993).

19 Our observations of the plate tabulation pattern of cultured motile cells of the free-
20 living stage of the dinoflagellate isolated from diverse polycystine hosts clearly show
21 that it is a member of the order Peridiniales (bilateral symmetry, cingulum only
22 slightly displaced, presence of Po and X plates, presence of 3 intercalary plates in the
23 epitheca) and that it should not be classified in the genus *Scrippsiella*, nor in the
24 related genera *Calciodinellum*, *Bysmatrum*, *Pentapharsodinium*, or *Ensiculifera*. All
25 of these latter genera are described as possessing 2 antapical plates, whereas the

1 polycystine symbiont reported here possesses a single antapical plate (Table 2, Figs
2 2E and 3D). The presence of a single antapical plate is rare in the order Peridinales,
3 occurring notably in a group of heterotrophic genera (*Podolampas* Stein,
4 *Blepharocysta* Ehrenberg, and *Lissodinium* Matzenauer) characterized by the absence
5 of both a cingulum and a depressed sulcus (Gómez et al. 2010) and a group of
6 heterotrophic taxa (*Diplopsalis* Bergh, *Preperidinium* Mangin, *Boreadinium* Dodge et
7 Hermes) characterized by having large lenticular-shaped cells. The radiolarian
8 symbionts are clearly morphologically and ecologically distinct from these other
9 peridinales with ~~that have~~ a single antapical plate.

10 The polycystine symbionts also differ from *Scrippsiella* and *Bysmatrum* (but not from
11 *Pentapharsodinium* and *Ensiculifera*) in possessing 5 (rather than 6) cingular plates.

12 The wing-like flange that covers the sulcal area has not been described in any of these
13 related genera. This structure resembles the peduncle cover plate (PC) of
14 heterotrophic dinoflagellates in the peridinalean family Pfiestereaceae Steidinger et
15 Burkholder emend. Litaker. Motile forms of members of the Pfiestereaceae feed
16 myzocytotically by means of a peduncle that emerges close to the flagella and that
17 can attach to microalgal prey or epidermal cells of live fish (e.g. Steidinger et al.
18 2006). We have not observed a peduncle in the taxon described here, but should it be
19 present, the Sd plate should rather be termed PC and the plate formula would become:

20 Po, X, 4', 3a, 7'', 5c, 3s, PC, 5''', 1''''.

21 Comparison of morphological characters strongly supports a generic level separation
22 of the polycystine symbiont reported here from other described Peridinales taxa, a
23 conclusion that is corroborated by phylogenetic analyses. In both SSU and LSU
24 phylogenies (Figs 4 and 5), the analyzed polycystine symbionts (including several
25 cultures isolated from *Collozoum* colonies) formed a well-supported clade within the

1 Peridiniales, clearly distinct from *Scrippsiella* and related genera and distant from
2 other dinoflagellate taxa known to form symbiotic relationships such as the
3 suessialeans *Symbiodinium* and *Pelagodinium*.

4 In light of both morphological and genetic differences from existing genera, this taxon
5 should clearly be classified in a distinct genus. Although *S. nutricula* was previously
6 classified within the genus *Endodinium*, this genus was created to describe the
7 symbiont of *Velella velella* from the Mediterranean and there is sufficient doubt as to
8 whether these organisms are actually closely related (see below) to preclude
9 reinstatement of this combination, which in any case should be considered
10 synonymous with *Z. nutricula*. Strict adherence to nomenclatural rules would hence
11 dictate the use of the genus *Zooxanthella* for this species, but we agree with numerous
12 previous authors (e.g. Blank and Trench 1986; Trench and Blank 1987; Banaszak et
13 al. 1993) who have convincingly argued that *Zooxanthella* should be rejected as a
14 confusing name that has been widely applied to divergent taxa. We therefore propose
15 the erection of a new genus, which we name *Brandtodinium* Probert et Siano in
16 reference to Karl Brandt who first described this species (Brandt 1882), and the
17 transfer of *Z. nutricula* to this new genus as *Brandtodinium nutriculum* comb. nov.. In
18 the absence of a holotype, not provided in the original description of the species, we
19 designate Fig. 2, SEM illustrations of plate tabulation of the motile stage of the
20 culture strain ~~VFR1-1~~[RCC3387](#) of this species, as the neotype for the species.

21 Whereas the generic level distinction of *Brandtodinium* from other peridinialeans is
22 obvious, the relationship of this genus to other genera within the Peridiniales is not
23 clear. In terms of overall morphology of the motile stage (e.g. cell size and shape,
24 plate tabulation), *Brandtodinium* has several features in common with members of the
25 Calciodinellaceae Taylor, a family that includes *Scrippsiella*. The Calciodinellaceae,

1 however, are characterized by the production of calcified resting cysts, a feature that
2 we have not observed in *Brandtodinium*. As discussed above, *Brandtodinium* also has
3 certain morphological similarities with members of other groups such as the
4 Pfiestereaceae. An unexpectedly close genetic relationship between *B. nutriculum* (as
5 *Z. nutricula*) and a small group of taxa in which photosynthesis takes place by a
6 tertiary endosymbiont derived from a diatom (Horiguchi and Pienaar 1994), the
7 ‘dinotoms’ (Imanian et al. 2011), was recently reported (Gottschling and McLean
8 2013). These investigators employed a ‘maximal taxon sample’ approach by inferring
9 relationships based on a concatenated SSU, LSU and ITS rDNA sequence alignment
10 irrespective of whether all of these sequences were available for the taxa included (i.e.
11 an alignment with significant gaps). Our individual SSU and LSU phylogenies do not
12 recover this relationship. The present study provides strong evidence from two highly
13 conserved phylogenetic markers (SSU and LSU rDNA) to support the conclusion
14 from our observations of the morphology of free-living cells that *Brandtodinium* is a
15 taxonomically distinct genus within the Peridinales. We chose not to employ an
16 approach comparable to that of Gottschling and McLean (2013) because in-depth
17 assessment of evolutionary and phylogenetic relationships between *Brandtodinium*
18 and other members of the order Peridinales goes beyond the scope of our research.
19 We nevertheless provide evidence that *Brandtodinium* is distinct from the dinotom
20 genera (*Durinskia* Carty et Cox, *Galeidinium* Tamura et Horiguchi, *Kryptoperidinium*
21 Lindemann, and some species currently assigned to *Peridiniopsis* Lemmermann or
22 *Peridinium* Ehrenberg) on the basis of morphological criteria, notably because
23 dinotom genera all have 2 antapical plates whereas *B. nutriculum* possesses a single
24 antapical plate, but also because the characteristic highly visible eyespot of dinotoms
25 is absent in *B. nutriculum*.

1 Banaszak et al. (1993) described the dinoflagellate symbiont of the jellyfish *Veleva*
2 *veleva* from the Pacific as *Scrippsiella velevae* and also (albeit invalidly) transferred
3 *Endodinium (=Zooxanthella) chattonii*, the symbiont of Mediterranean *V. veleva*, to
4 *Scrippsiella*, as *S. chattonii*. These authors gave the thecal plate formula for *S.*
5 *velevae* as pp (=Po, X), 4', 3a, 7'', 5c, 3s, 5''', 2''''', which corresponds neither to
6 that of *Scrippsiella* nor to that of *Brandtodinium* (Table 2). The spine-like
7 protuberance on the first cingular plate illustrated in Figure 11 (page 520) of
8 Banaszak et al. (1993) is a characteristic feature of the genus *Enciculifera*, to which
9 we believe this species should have been assigned. However, the SEM images
10 illustrated in Banaszak et al. (1993) do not permit verification of whether this
11 organism really has 3 sulcal plates (as stated in the description), rather than 5, as
12 diagnostic for members of the genus *Enciculifera*. It could also be inferred that *S.*
13 *chattonii*, the symbiont of Mediterranean *V. veleva*, might also be transferred to
14 *Enciculifera*, but unfortunately no morphological data has ever been provided for the
15 free-living stage of this taxon. It is noteworthy that the only existing sequence (SSU
16 rDNA) of a symbiont of *V. veleva* (from the Sargasso Sea, Atlantic Ocean) produced
17 by Gast and Caron (1996) falls within our *Brandtodinium* clade, in the sub-clade B2
18 composed of three identical sequences, two of which we generated from Pacific
19 polycystine holobionts. This sub-clade is distinct from the sub-clade B1 formed by the
20 group of identical sequences from all of our Pacific (South and North) and
21 Mediterranean culture strains of *B. nutriculum* isolated from polycystines, from
22 several Pacific polycystine holobionts that we sequenced, and from the Sargasso Sea
23 polycystine symbionts sequenced by Gast and Caron (1996). Gast and Caron (1996)
24 did not observe the morphology of the dinoflagellate symbionts of Sargasso Sea *V.*
25 *veleva* that they sequenced, but we predict that they would have plate tabulation

1 consistent with our description of *Brandtodinium*. If this were the case, it would mean
2 that *V. verella* is capable of forming symbiotic associations with different
3 dinoflagellate genera (*Brandtodinium* and *Scrippsiella* (or *Ensiculifera*)), possibly
4 with a biogeographical pattern (*Brandtodinium* in the Atlantic and possibly
5 Mediterranean, *Scrippsiella* (or *Ensiculifera*) in the Pacific). The capacity of hosts to
6 form associations with different symbionts has already [been](#) observed for other
7 pelagic organisms (Siano et al. 2010; Decelle et al. 2012b). A comparison of genetic
8 sequences from morphologically characterized cultured *V. verella* symbionts from the
9 Pacific Ocean, Sargasso Sea and Mediterranean Sea could be helpful in establishing
10 the validity of historical descriptions of these symbionts and their relationship to *B.*
11 *nutriculum*.

12 *Brandtodinium* has been found (in this and previous studies) in association with
13 diverse polycystine radiolarian hosts from the North and South Pacific Ocean,
14 Sargasso Sea, and Mediterranean Sea. In light of the abundance of symbiotic
15 polycystines in the world ocean, *Brandtodinium* likely plays a key ecological role in
16 primary and secondary production at a global scale. Putting aside associations with
17 parasitic alveolates ([Gast 2006](#); Bråte et al. 2012) that can be considered as a form of
18 symbiosis, all Collodaria investigated so far harbor only *Brandtodinium* species as
19 symbionts. At present, *Brandtodinium* is the only symbiont identified for Nassellaria,
20 but information for this radiolarian group remains extremely scarce. *Brandtodinium*
21 has now been found in association with numerous spumellarian hosts, but unlike the
22 other polycystine lineages, other types of (non-dinoflagellate) microalgal and
23 cyanobacterial symbionts have also been reported for this group (Anderson 1983;
24 Gast and Caron 2001; Yuasa et al. 2005). With *Brandtodinium* also probably found in
25 symbiosis with jellyfish, it is clear that *Brandtodinium*, like the suessialean

1 dinoflagellates *Pelagodinium* and *Symbiodinium*, is a generalist symbiont. In this
2 context it is interesting to note that the known genetic diversity (in terms of SSU and
3 LSU rDNA sequences) of *Brandtodinium* and *Pelagodinium*, both of which form
4 symbiotic relationships with planktonic hosts, is relatively low (2 clades described
5 within each of these genera) compared to that of *Symbiodinium* (9 divergent clades
6 and multiple sub-clades, Stat et al. 2008; Pochon and Gates 2010) that is
7 predominately found in association with benthic host organisms. This apparent trend
8 might be explained by the relatively low number of studies on symbiosis in the
9 pelagic realm, but might also be real and reflect inherent differences between life and
10 symbiotic processes in planktonic and benthic ecosystems (Decelle 2013).

11

12 Taxonomic appendix

13 *Brandtodinium* Probert et Siano *gen. nov.*

14 Diagnosis: Photosynthetic dinoflagellate. Motile cells covered by 6 series of thecal
15 plates: 3 in the epitheca, 2 in the hypotheca (including single antapical plate), and 1 in
16 the cingulum. One transverse and one longitudinal flagellum. Large nucleus located in
17 central part of cell. One or two peripheral chloroplasts, golden-yellow in color. One or
18 two large circular pyrenoids.

19 Type species: *Brandtodinium nutriculum* (Brandt) Probert et Siano *comb. nov.*

20 Etymology: the genus name for this dinoflagellate (= *dinos*) derives from Karl Brandt
21 who first described *Zooxanthella* in 1882.

22

23 *Brandtodinium nutriculum* (Brandt) Probert et Siano *comb. nov.*

24 Basionym: *Zooxanthella nutricula* Brandt in Brandt (1882): 140

1 Synonyms: *Endodinium nutricula* (Brandt) Hollande et Carré in Holland and Carré
2 (1974); *Scrippsiella nutricula* (Brandt) Banaszak, Iglesias-Prieto et Trench in
3 Banaszak et al. (1993).
4 Neotype : Fig. 2 in this publication.
5 Diagnosis: Plate tabulation: Po, X, 4', 3a, 7'', 5c, 4s, 5''', 1'''''. Epitheca larger than
6 hypotheca. Epitheca convex conical with well-pronounced apical horn. Hypotheca
7 rounded. Wide and shallow cingulum located in the median portion of the cell,
8 displaced by a small fraction of its own width. Sulcal area with 4 plates, one of which
9 forms a wing-like flange over the median part of the sulcus. Single antapical plate.
10 Cells on average 13.1µm in length by 10.4µm in width. Symbiont of polycystine
11 radiolarians.

12 Type locality: Bay of Villefranche sur Mer (France), Western Mediterranean Sea

13 Authentic culture strain: RCC3387 in the Roscoff Culture Collection.

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- 6

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- 1 Table 1. List of specimens used to obtain symbiont sequences ([images of host cells](#)
 2 [are shown in Supplementary Figures 1 and 2](#)).

Host ID	Host taxonomy	Sampling site	Strain code	18S SSU rDNA GenBank acc # accession number	28S LSU rDNA GenBank acc # accession number
Holobionts					
PAC1	Collodaria (solitary)	South Pacific 21°17.462 S, 105°9.476 W	n.a.	*****KF557503	KF557534***** ***
PAC2	Collodaria (colony)	South Pacific 21°17.462 S, 105°9.476 W	n.a.	KF557504** *****	n.a.
PAC3	Collodaria (solitary)	South Pacific 23°42.949 S, 107°20.141 W	n.a.	KF557505** *****	KF557535***** ***
PAC4	Collodaria (solitary)	South Pacific 23°42.949 S, 107°20.141 W	n.a.	KF557506** *****	KF557536***** ***
PAC6	Collodaria (solitary)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557507** *****	n.a.
PAC7	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557508** *****	n.a.
PAC8	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	n.a.	KF557537***** ***
PAC9	Collodaria (colony)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557509** *****	KF557538***** ***
PAC10	Collodaria (solitary)	South Pacific 24°48.085 S, 110°33.307 W	n.a.	KF557510** *****	n.a.
PAC11	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557511** *****	KF557539***** ***
PAC14	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557512** *****	KF557540***** ***
PAC15	Collodaria (solitary)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557513** *****	*****n.a.
PAC16	Collodaria (colony)	South Pacific 24°23.025 S, 113°58.068 W	n.a.	KF557514** *****	n.a.
PAC17	Collodaria	South Pacific	n.a.	KF557515**	KF557541*****

Formatted Table

	(colony)	23°42.289 S, 131°12.744 W		xxxxx	xxx
PAC19	Collodaria	South Pacific	n.a.	KF557516xx	KF557542xxxx
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	xxx
PAC21	Collodaria	South Pacific	n.a.	KF557517xx	KF557543xxxx
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	xxx
PAC22	Collodaria	South Pacific	n.a.	KF557518xx	KF557544xxxx
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	xxx
PAC24	Collodaria	South Pacific	n.a.	KF557519xx	n.a.
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	
PAC26	Collodaria	South Pacific	n.a.	KF557520xx	n.a.
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	
PAC27	Collodaria	South Pacific	n.a.	KF557521xx	n.a.
	(colony)	23°42.289 S, 131°12.744 W		xxxxx	
SES47	Collodaria	Sesoko, Japan	n.a.	KF557502xx	KF557546xxxx
	(colony)	26°37'20 N, 127°52'15 E		xxxxx	xxx
SES19	Spumellaria	Sesoko, Japan	n.a.	KF557501xx	n.a.
		26°37'20 N, 127°52'15 E		xxxxx	
SES28	Nassellaria	Sesoko, Japan	n.a.	n.a.	KF557545xxxx
		26°37'20 N, 127°52'15 E			xxx
Vil 210	Spumellaria?	Villefranche-sur-Mer, France	n.a.	KF557522xx	n.a.
		43°41'10 N, 7°18'50 E		xxxxx	
Vil 217	Spumellaria	Villefranche-sur-Mer, France	n.a.	KF557523xx	n.a.
		43°41'10 N, 7°18'50 E		xxxxx	
Vil 219	Spumellaria	Villefranche-sur-Mer, France	n.a.	KF557524xx	n.a.
		43°41'10 N, 7°18'50 E		xxxxx	
Vil 231	Spumellaria	Villefranche-sur-Mer, France	n.a.	KF557525xx	n.a.
		43°41'10 N, 7°18'50 E		xxxxx	
Culture strains					
SES46	Collodaria	Sesoko, Japan	RCC3378	KF557500xx	KF557526xxxx
	(<i>Collozoum</i> colony)	26°37'20 N, 127°52'15 E		xxxxx	xxx
			RCC3379	KF557499	n.a.
SES46B	Collodaria	Sesoko, Japan	RCC3379	xxxxxxx	n.a.
	(<i>Collozoum</i> colony)	26°37'20 N, 127°52'15 E			
VFPO14-8	Collodaria	Villefranche-sur-Mer, France	RCC3380	KF557494xx	KF557530xxxx
	(<i>Collozoum</i>)	43°41'10 N, 7°18'50 E		xxxxx	xxx

	colony)		RCC3381	KF557495	KF557531
			RCC3382	KF557496	KF557532
VFPO14-13	Collocladia (Collozoum colony)	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3384	xxxxxxx	xxxxxxx
VFPO14-14	Collocladia (Collozoum colony)	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3382	xxxxxxx	xxxxxxx
VFPO2-1	Spumellaria	Villefranche-sur-Mer, France 43°41'10N, 7°18'50E	RCC3383	KF557491 xx xxxxx	KF557527 xxxx xxx
			RCC3384	n.a.	KF557528
VFPO2-2	Spumellaria	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3384	n.a.	xxxxxxx
VFPO5	Spumellaria	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3385	KF557492 xx xxxxx	n.a.
VFPO22-2	Spumellaria	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3386	KF557497xx xxxxx	n.a.
VFR1-1	Spumellaria	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3387	KF557498 xx xxxxx	KF557533 xxxx xxx
VFPO10	Nassellaria	Villefranche-sur-Mer, France 43°41'10 N, 7°18'50 E	RCC3388	KF557493 xx xxxxx	KF557529 xxxx xxx

1

2

1 Table 2. Kofoidian plate tabulation of *Brandtodinium* and related genera

<i>Scrippsiella</i>	Po, X, 4', 3a, 6-7'', 6c, 4-7s, 5''', 2''''
<i>Calciodinellum</i>	Po, X, 4', 3a, 7'', 6c, 5s, 5''', 2''''
<i>Bysmatrum</i>	Po, X, 4', 3a, 7'', 6c, 4-5s, 5''', 2''''
<i>Pentapharsodinium</i>	Po, X, 4', 3a, 7'', 5c, 4s, 5''', 2''''
<i>Ensiculifera</i>	Po, X, 4', 3a, 7'', 5c, 5s, 5''', 2''''
<i>Brandtodinium</i>	Po, X, 4', 3a, 7'', 5c, 4s, 5''', 1''''

2

1 Figure captions

2

3 Figure 1. Light micrographs of *Brandtodinium nutriculum* gen. nov., comb. nov..
4 (arrow indicates large pyrenoid). A, B. Ventral view of the cell showing the large
5 nucleus in the central portion of the cell. C. Lateral (slightly antapical) view of the
6 cell. D. Dorsal view of the cell. Scale bars = 5µm.

7

8 Figure 2. SEM micrographs of *Brandtodinium nutriculum* gen. nov., comb. nov..
9 (enumeration of plates follows the Kofoidian tabulation system). A. Ventral view of a
10 cell (flagella lost during fixation). B. Detail of the sulcal region. C. Dorsal view. D.
11 Apical view. E. Antapical view. Scale bars = 2µm.

12

13 Figure 3. Schematic representation of plate patterns of *Brandtodinium nutriculum* gen.
14 nov., comb. nov. (enumeration of plates follows the Kofoidian tabulation system). A.
15 Ventral view (generalized). B. Dorsal view (generalized). C. Apical view
16 (generalized). D. Antapical view (generalized).

17

18 Figure 4. SSU rDNA phylogenetic tree inferred by Maximum Likelihood (ML)
19 analysis. ~~650-652~~ unambiguously aligned positions were considered from an
20 alignment of 57 sequences, including *Brandtodinium* gen. nov.. Sequences obtained in
21 this study are indicated in bold (followed by the type of host from which the sequence
22 was obtained and the number of holobiont specimens or culture strains in
23 parentheses). The tree was rooted with Suessiales (*Symbiodinium* spp. and
24 *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale
25 bar indicating the number of nucleotide substitutions per site. Numbers on branches

1 are statistical support values for the clusters to the right of them (first: ML bootstrap
2 support values, values under 0.5 are not shown; second: Bayesian posterior
3 probabilities, values under 0.5 are not shown; black dots at nodes represent a
4 statistical support of 1 for both methods).

5

6 Figure 5. LSU rDNA phylogenetic tree inferred by Maximum Likelihood (ML)
7 analysis. 675 unambiguously aligned positions were considered from an alignment of
8 48 sequences, including *Brandtodinium* gen. nov.. Sequences obtained in this study
9 are indicated in bold (followed by the type of host from which the sequence was
10 obtained and the number of holobiont specimens or culture strains in parentheses).
11 The tree was rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinium beii*) as
12 the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the
13 number of nucleotide substitutions per site. Numbers on branches are statistical
14 support values for the clusters to the right of them (first: ML bootstrap support values,
15 values under 0.5 are not shown; second: Bayesian posterior probabilities, values under
16 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both
17 methods).

18

19 [Supplementary Figure 1. LM images of host cells from which uncultured symbiont](#)
20 [\(holobiont\) sequences were retrieved.](#)

21

22 [Supplementary Figure 2. LM images of host cells from which cultures were isolated.](#)

23

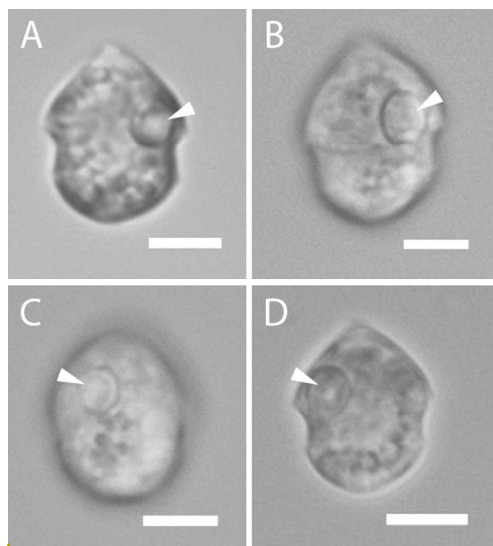
24 [Supplementary Figure 3. SSU rDNA phylogenetic tree inferred by Maximum](#)

25 [Likelihood \(ML\) analysis. 652 unambiguously aligned positions were considered](#)

1 from an alignment of 59 sequences, including *Bysmatrum*. The tree was rooted with
2 Suessiales (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch
3 lengths are drawn to scale, with the scale bar indicating the number of nucleotide
4 substitutions per site. Numbers on branches are statistical support values for the
5 clusters to the right of them (first: ML bootstrap support values, values under 0.5 are
6 not shown; second: Bayesian posterior probabilities, values under 0.5 are not shown;
7 black dots at nodes represent a statistical support of 1 for both methods).
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Figure 1.

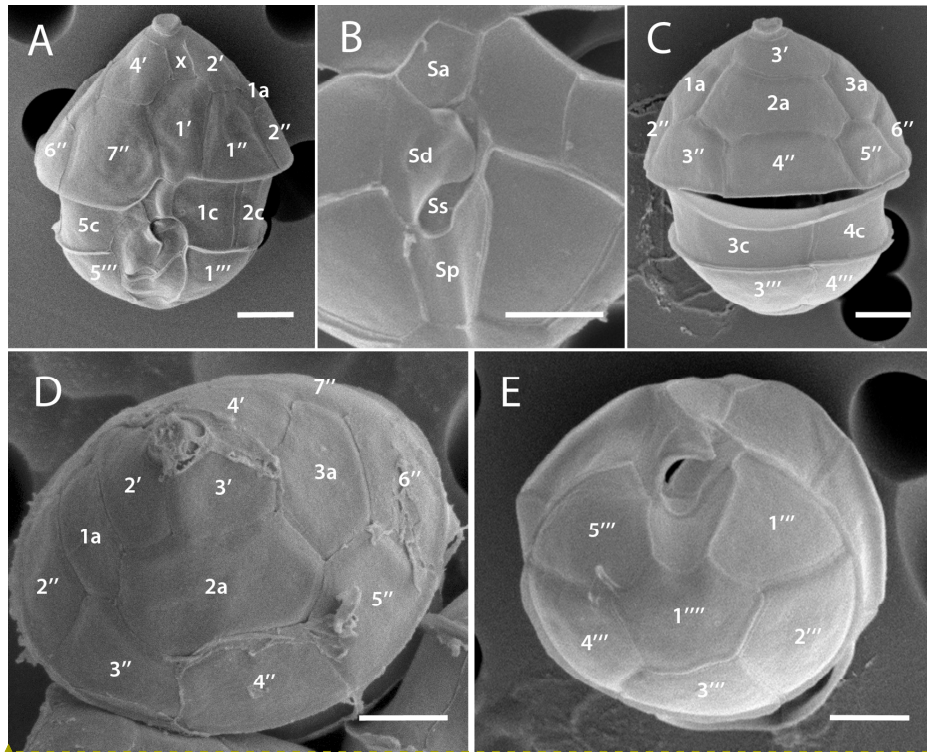


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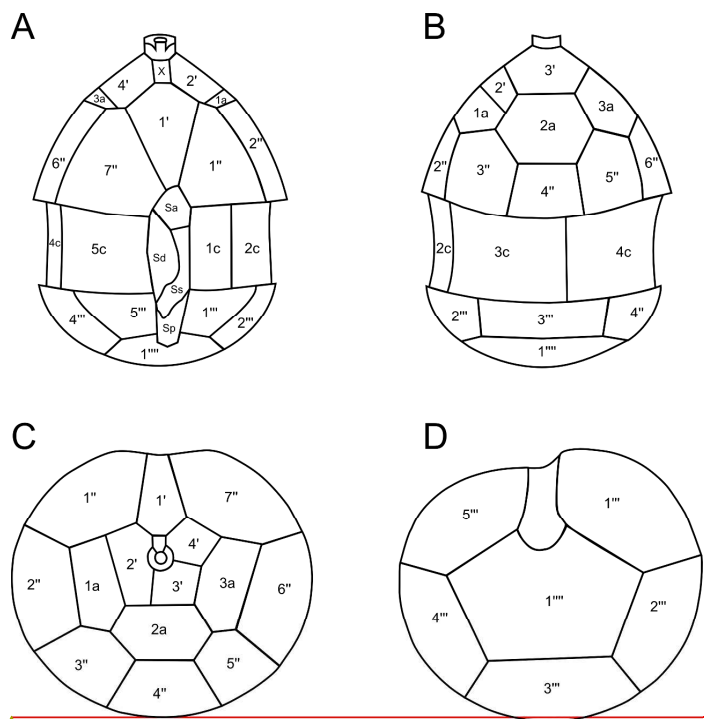
Figure 2.

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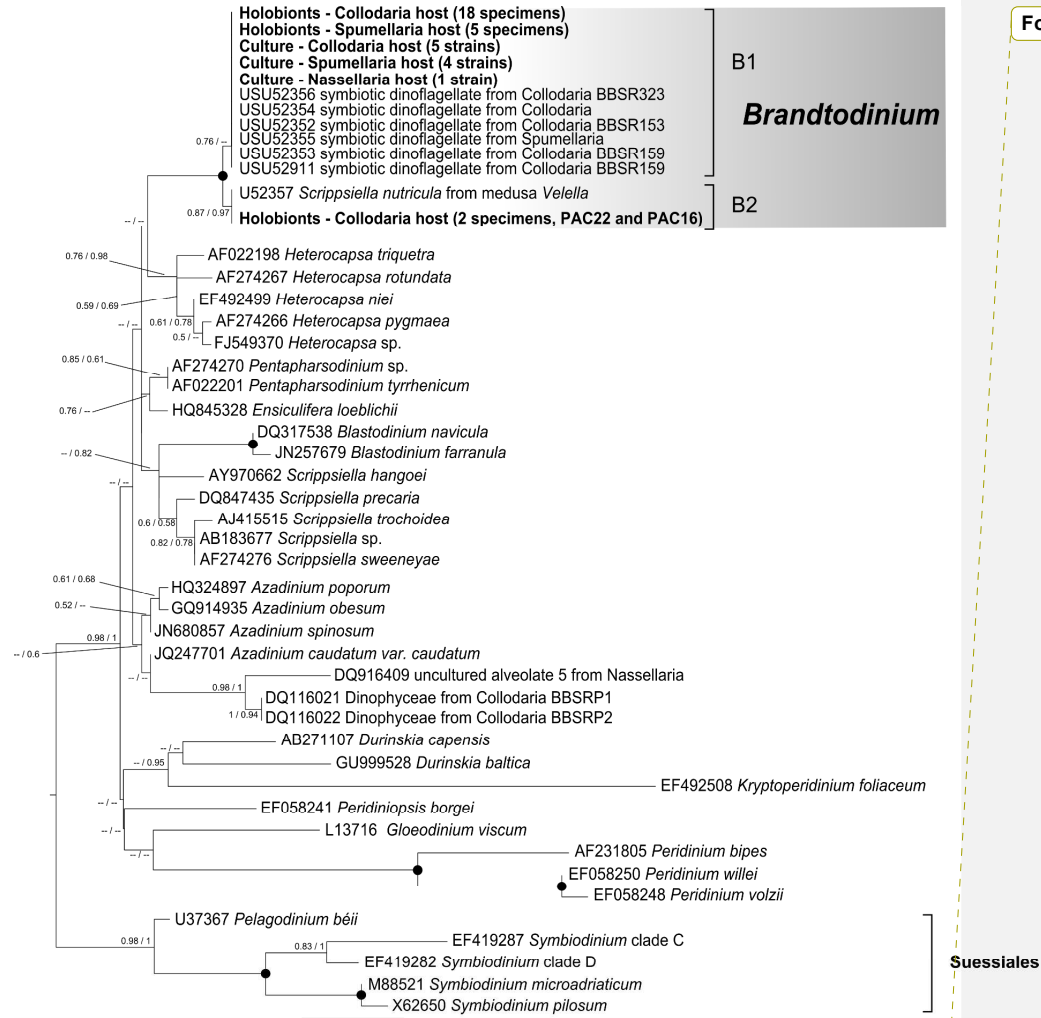
Figure 3.



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Figure 4.



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Figure 5.

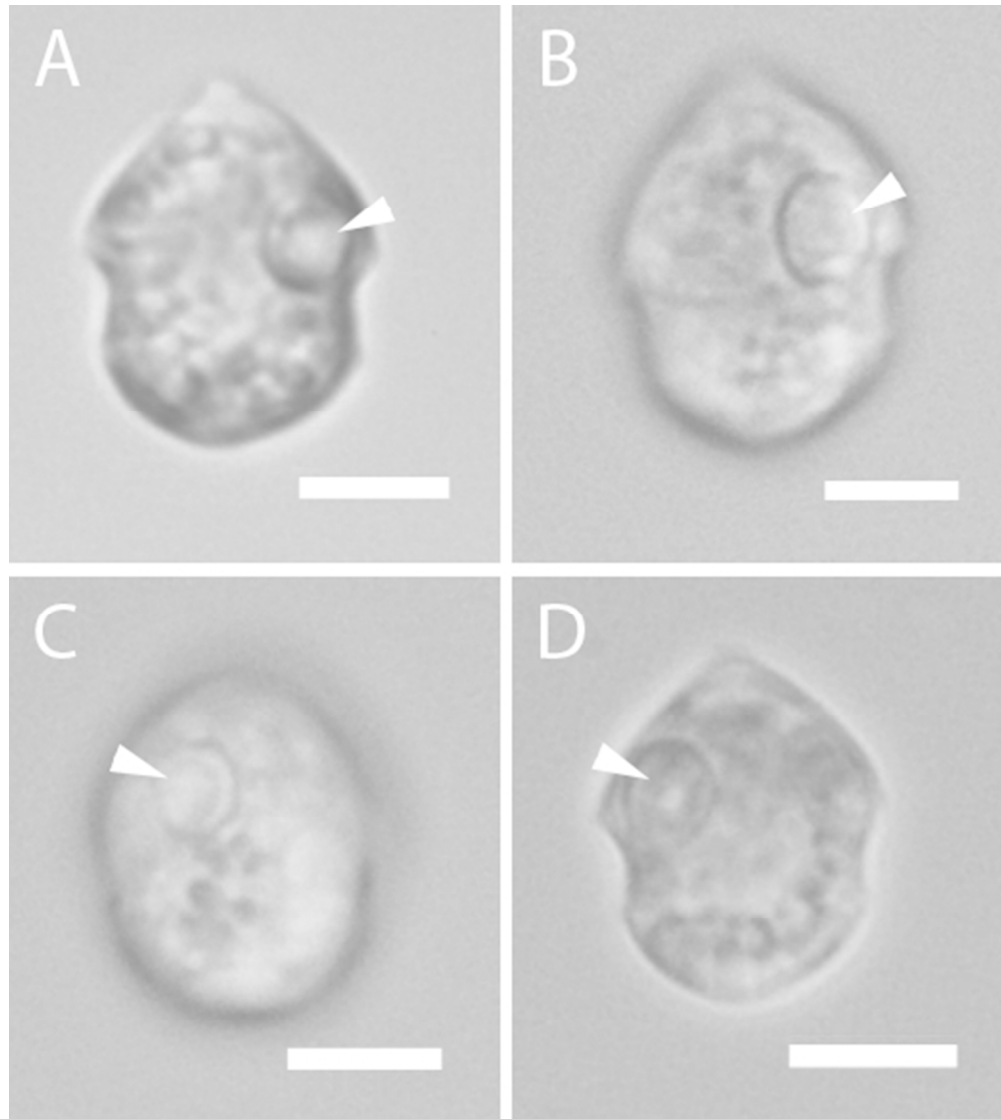


Figure 1. Light micrographs of *Brandtodinium nutriculum* gen. nov., comb. nov.. (arrow indicates large pyrenoid). A, B. Ventral view of the cell showing the large nucleus in the central portion of the cell. C. Lateral (slightly antapical) view of the cell. D. Dorsal view of the cell. Scale bars = 5 μ m. 44x49mm (300 x 300 DPI)

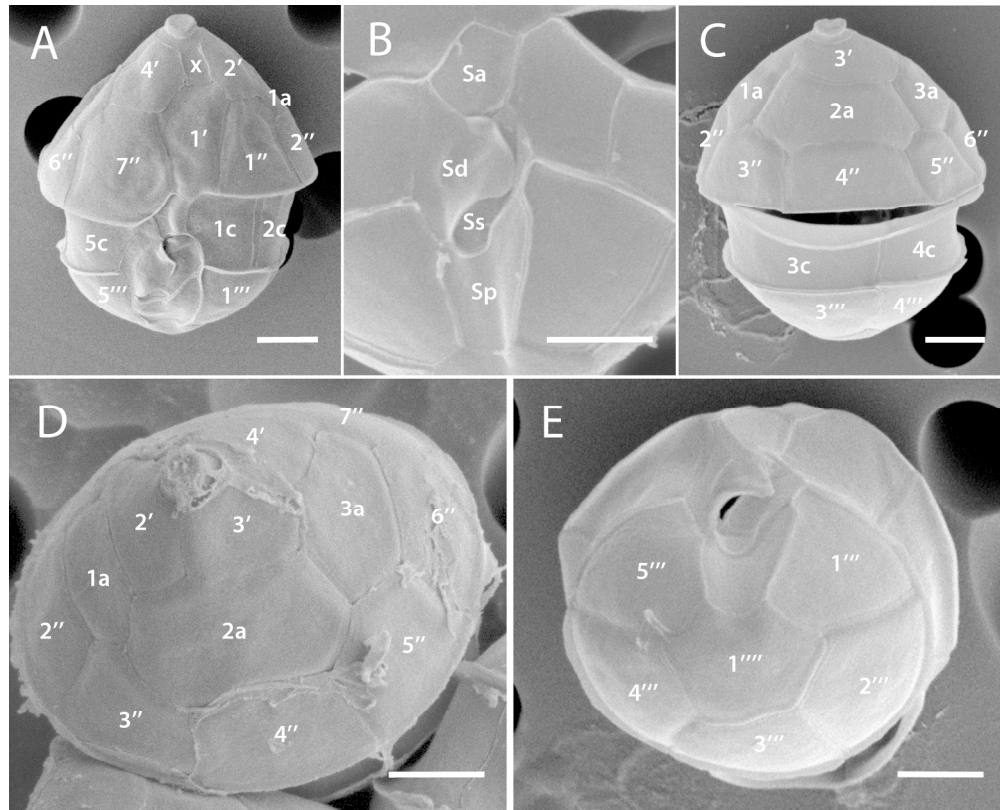


Figure 2. SEM micrographs of *Brandtodinium nutriculum* gen. nov., comb. nov.. (enumeration of plates follows the Kofoidian tabulation system). A. Ventral view of a cell (flagella lost during fixation). B. Detail of the sulcal region. C. Dorsal view. D. Apical view. E. Antapical view. Scale bars = 2 μ m. 175x140mm (300 x 300 DPI)

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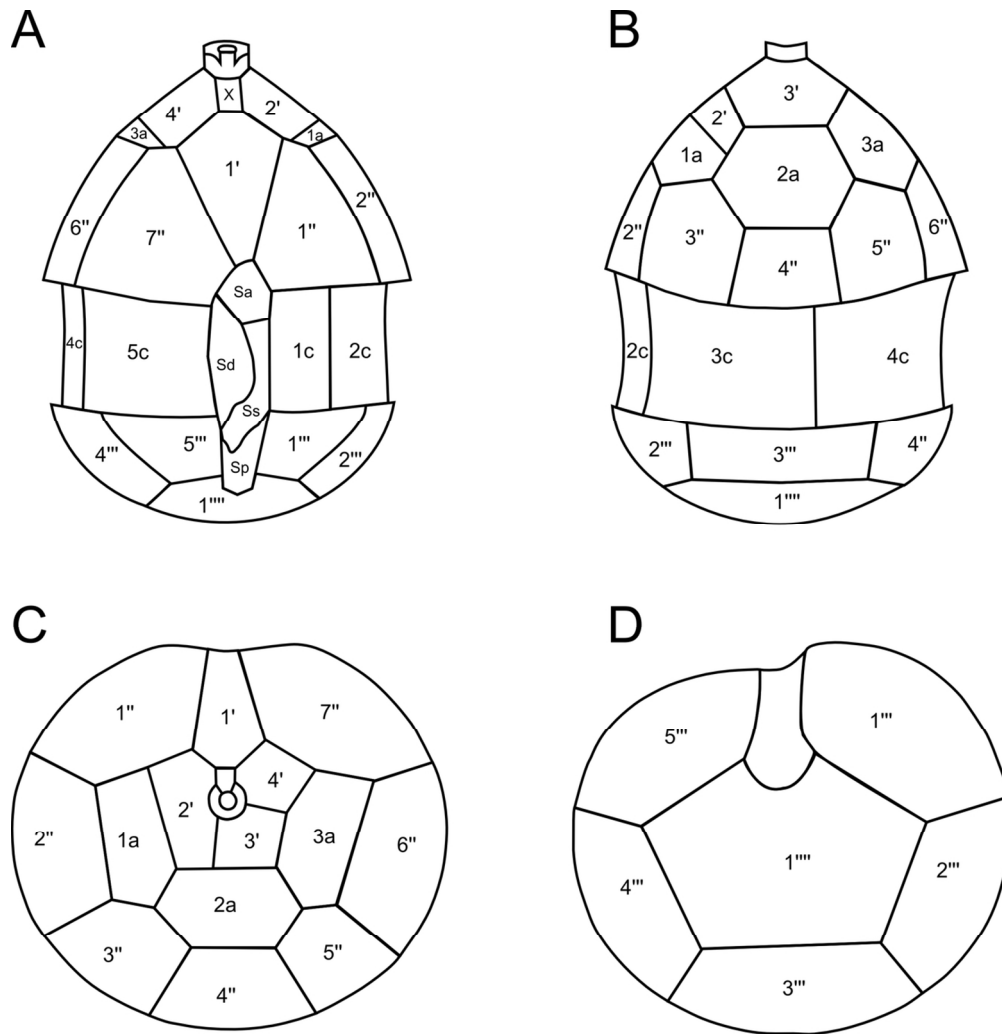


Figure 3. Schematic representation of plate patterns of *Brandtodinium nutriculum* gen. nov., comb. nov. (enumeration of plates follows the Kofoidian tabulation system). A. Ventral view (generalized). B. Dorsal view (generalized). C. Apical view (generalized). D. Antapical view (generalized).
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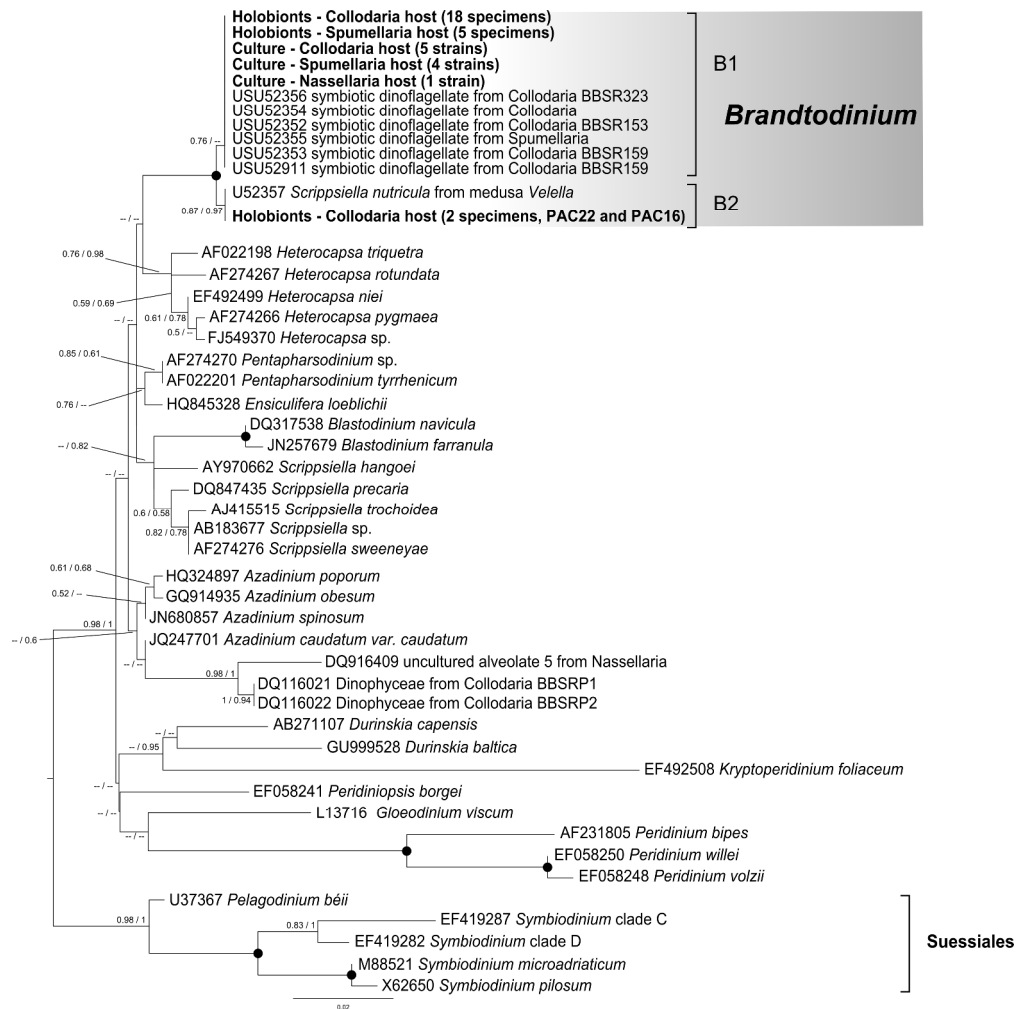


Figure 4. SSU rDNA phylogenetic tree inferred by Maximum Likelihood (ML) analysis. 652 unambiguously aligned positions were considered from an alignment of 57 sequences, including *Brandtodium* gen. nov..

Sequences obtained in this study are indicated in bold (followed by the type of host from which the sequence was obtained and the number of holobiont specimens or culture strains in parentheses). The tree was rooted with *Suessiales* (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. Numbers on branches are statistical support values for the clusters to the right of them (first: ML bootstrap support values, values under 0.5 are not shown; second: Bayesian posterior probabilities, values under 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both methods).

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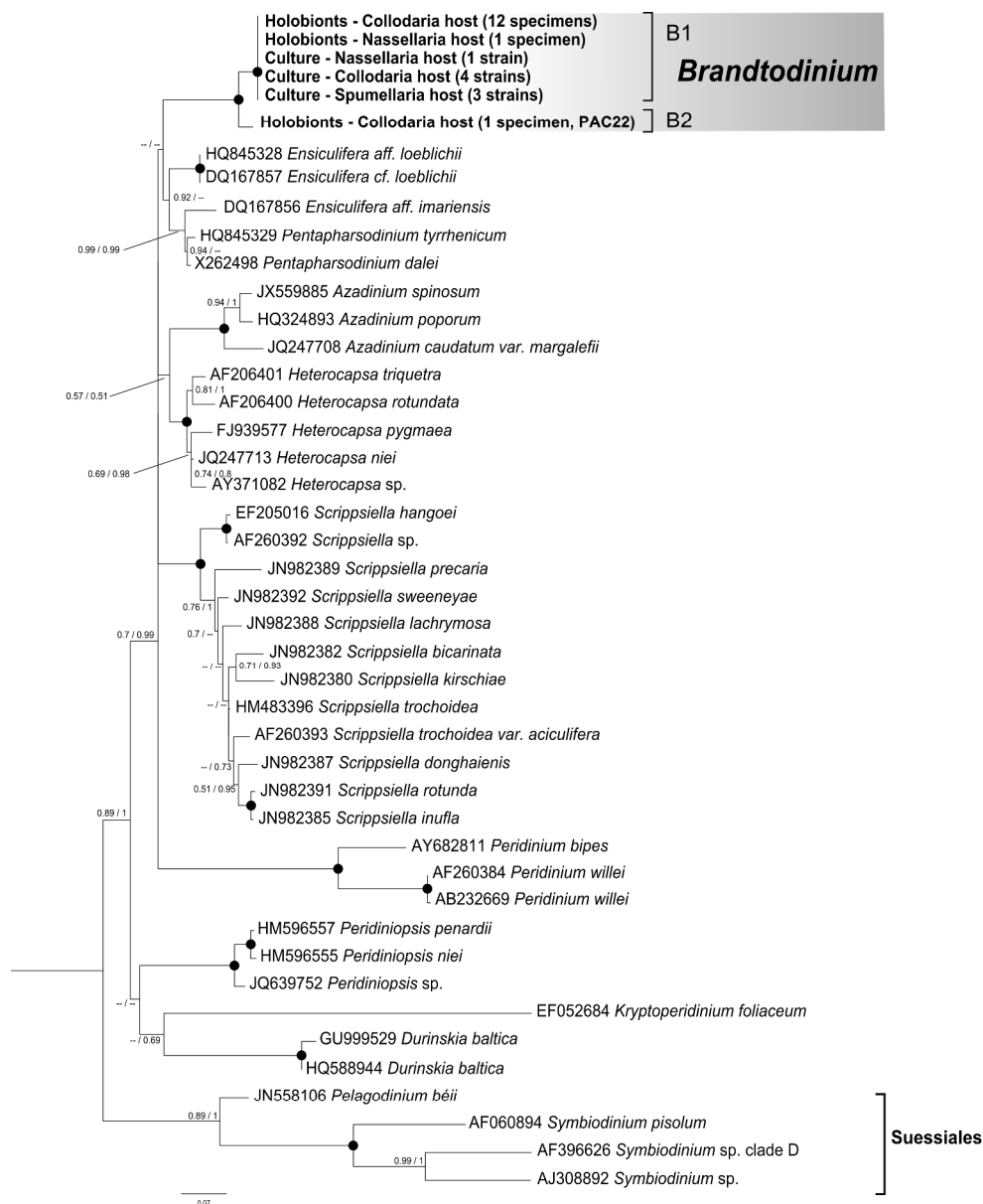


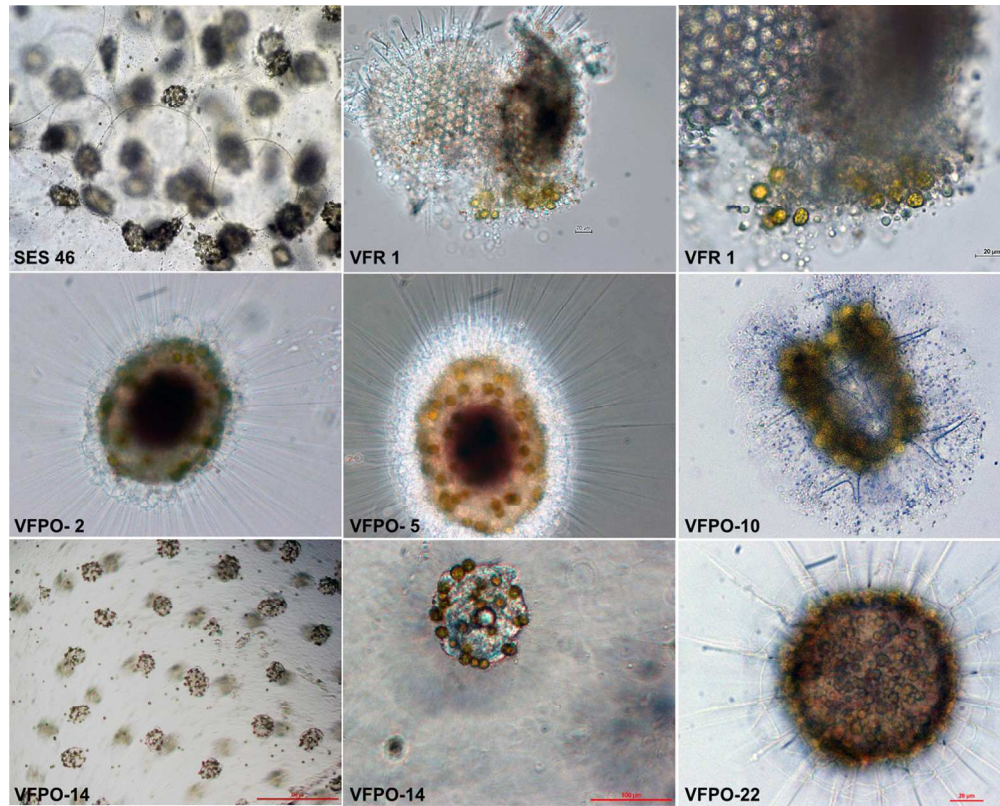
Figure 5. LSU rDNA phylogenetic tree inferred by Maximum Likelihood (ML) analysis. 675 unambiguously aligned positions were considered from an alignment of 48 sequences, including *Brandtodiinium* gen. nov..

Sequences obtained in this study are indicated in bold (followed by the type of host from which the sequence was obtained and the number of holobiont specimens or culture strains in parentheses). The tree is rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinidium beii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site. Numbers on branches are statistical support values for the clusters to the right of them (first: ML bootstrap support values, values under 0.5 are not shown; second: Bayesian posterior probabilities, values under 0.5 are not shown; black dots at nodes represent a statistical support of 1 for both methods).

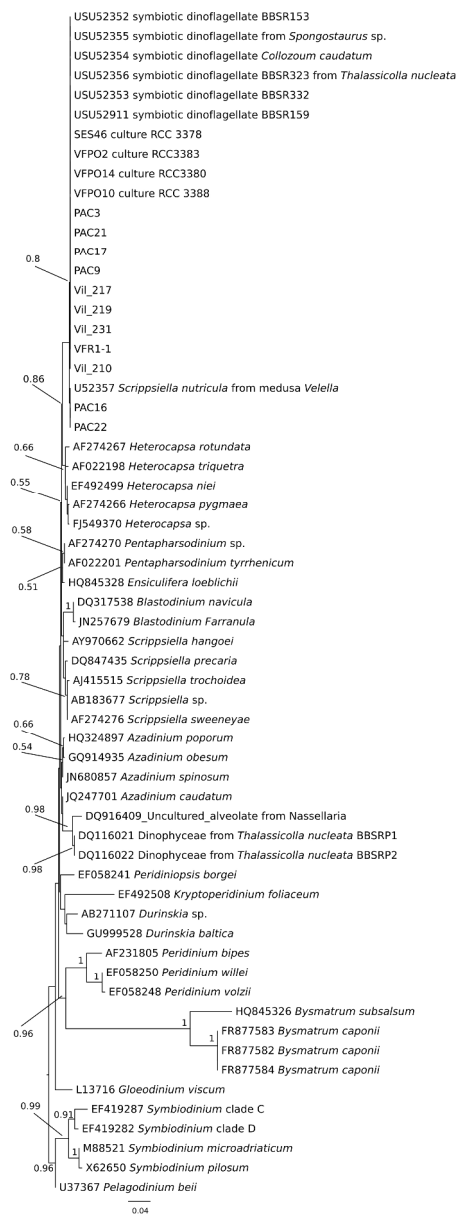
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Supplementary Figure 1. LM images of host cells from which uncultured symbiont (holobiont) sequences were retrieved.
165x291mm (300 x 300 DPI)



Supplementary Figure 2. LM images of host cells from which cultures were isolated.
170x136mm (300 x 300 DPI)



Supplementary Figure 3. SSU rDNA phylogenetic tree inferred by Maximum Likelihood (ML) analysis. 652 unambiguously aligned positions were considered from an alignment of 59 sequences, including *Bysmatrum*. The tree was rooted with Suessiales (*Symbiodinium* spp. and *Pelagodinium béii*) as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of nucleotide substitutions per site.

Numbers on branches are ML bootstrap support values (values under 0.5 are not shown).
 163x440mm (299 x 299 DPI)