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Host specificity and coevolution of Flavobacteriaceae endosymbionts within the siphonous green seaweed *Bryopsis*

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Abstract

The siphonous green seaweed *Bryopsis* harbors complex intracellular bacterial communities. Previous studies demonstrated that certain species form close, obligate associations with Flavobacteriaceae. A predominant imprint of host evolutionary history on the presence of these bacteria suggests a highly specialized association. In this study we elaborate on previous results by expanding the taxon sampling and testing for host-symbiont coevolution. Therefore, we optimized a PCR protocol to directly and specifically amplify Flavobacteriaceae endosymbiont 16S rRNA gene sequences, which allowed us to screen a large number of algal samples without the need for cultivation or surface sterilization. We analyzed 146 *Bryopsis* samples, and 92 additional samples belonging to the Bryopsidales and other orders within the class Ulvophyceae. Results indicate that the Flavobacteriaceae endosymbionts are restricted to *Bryopsis*, and only occur within specific, warm-temperate and tropical clades of the genus. Statistical analyses (AMOVA) demonstrate a significant non-random host-symbiont association. Comparison of bacterial 16S rRNA and *Bryopsis rbcL* phylogenies, however, reveal complex host-symbiont evolutionary associations, whereby closely related hosts predominantly harbor genetically similar endosymbionts. Bacterial genotypes are rarely confined to a single *Bryopsis* species and most *Bryopsis* species harbored several Flavobacteriaceae, obscuring a clear pattern of coevolution.

Keywords: alga; bacteria; coevolution; codivergence; endosymbiosis

1. Introduction

Bacteria living within the body or cells of eukaryotes are extremely abundant and widespread (Dale and Moran, 2006; Ryan et al., 2008; Kikuchi, 2009). These endosymbiotic bacteria often contribute to diverse metabolic host functions, making their presence favorable or even essential (Relman, 2008). Eventually, both the bacterial partner and the host may lose their autonomy and become strictly dependent on each other, resulting in an obligate association (Dale and Moran, 2006; Toft and Andersson, 2010). Obligate endosymbiotic bacteria have been shown to form highly host-specific interactions that are maintained across host generations over long periods of time by vertical transmission (Moran et al., 1993; Sachs et al., 2011). This process might give rise to coevolution or cospeciation, evolutionary processes resulting in congruent host and bacterial phylogenies (Peek et al., 1998, Clark et al., 2000, Legendre et al., 2002, Rosenblueth et al., 2012).

In seaweed-bacterial associations, coevolution has only been suggested between the red alga *Prionitis* and its gall-forming *Roseobacter* symbionts (Ashen and Goff, 2000). In the siphonous green seaweed *Bryopsis* (Chlorophyta: Ulvophyceae), bacteria have been observed by electron microscopy in both vegetative thalli and gametes, suggesting a close, specific association between the algal host and bacterial endophytes (Burr and West, 1970). Recently, molecular results showed that geographically diverse *Bryopsis* samples harbor well-defined and rather stable intracellular bacterial communities consisting of a mix of casually and more closely associated species (Hollants et al., 2011a, 2011b, 2013a). Of these bacteria, Flavobacteriaceae symbionts displayed a putatively obligate endobiotic lifestyle and were never isolated from the *Bryopsis* surface and surrounding seawater (Hollants et al., 2011b). The Flavobacteriaceae is a large family of bacteria with diverse eco-physiological characteristics (Bernardet and Nakagawa, 2006). They are known to decompose polysaccharides such as agar, cellulose and carrageenans, making them key players in biotransformation and nutrient recycling processes in the marine environment (Bernardet and Nakagawa, 2006; Goecke et al., 2010; Hollants et al. 2013b). Because of these traits, species of this family often inhabit seaweed surfaces where they have been shown to fulfill antimicrobial (Penesyan et al., 2009; Wiese et al., 2009), pathogenic (Sunairi et al., 1995; Weinberger et al., 1997; Vairappan et al., 2008), algal morphogenic, and

zoospore settlement inducing (Tatewaki et al., 1983; Nakanishi et al., 1996; Matsuo et al., 2003; Patel et al., 2003; Marshall et al., 2006) roles. Many members of the Flavobacteriaceae, like *Algibacter*, *Fucobacter*, *Maribacter*, and *Ulvibacter* species, have been initially isolated from marine macroalgal surfaces (Goecke et al., 2010, 2013). In addition, several intracellular bacterial symbionts of insects belong to the family Flavobacteriaceae and were shown to affect the reproduction of their hosts (Bernardet and Nakagawa, 2006). In *Bryopsis*, the presence of Flavobacteriaceae was found to be highly congruent with the host phylogeny of two warm-temperate to tropical clades (Hollants et al., 2013a). Testing the hypothesis of non-random host-symbiont association and possibly coevolution, however, requires a rich and geographically diverse sampling.

In this study, we aimed to assess the host-symbiont specificity and possible coevolution of Flavobacteriaceae endosymbionts in *Bryopsis*. Since, the experimental design used previously, i.e. labor-intensive unialgal culturing, surface sterilization, clone libraries, and DGGE analyses (Hollants et al., 2010, 2011a, 2011b, 2013a), was unsuitable for detailed screening of *Bryopsis*-associated Flavobacteriaceae endosymbionts, we developed a PCR protocol to specifically and exclusively amplify Flavobacteriaceae endophytic sequences in non-surface sterilized, natural *Bryopsis* samples. To assess the distribution of these Flavobacteriaceae endosymbionts outside *Bryopsis*, we also screened a large number of samples of other genera of green seaweeds. Phylogenetic and statistical analyses were performed to test for non-random host-symbiont association and possibly coevolution.

2. Material and methods

2.1. Algal material

In total 238 green algal samples were screened for the presence of Flavobacteriaceae endosymbionts, including 146 *Bryopsis* samples covering 23 different species, and 92 additional samples of Bryopsidales (genera *Avrainvillea*, *Boodleopsis*, *Caulerpa*, *Chlorodesmis*, *Codium*, *Derbesia*, *Halimeda*, *Rhipilia*, *Tydemania* and *Udotea*), Dasycladales (*Acetabularia*, *Bornetella* and *Neomeris*), Cladophorales (*Aegagropila*, *Anadyomene*, *Apjohnia*, *Boergesenia*, *Boodlea*, *Chaetomorpha*, *Cladophora*, *Cladophoropsis*, *Dictyosphaeria*, *Ernodesmis*, *Microdictyon*,

Rhizoclonium, *Siphonocladus* and *Valonia*) and Ulvales (*Ulva*) (Table S1). Algal samples were collected during different field expeditions and clean portions of the thalli were preserved in silica-gel.

2.2. DNA extraction and PCR amplification

Algal samples were subjected to total DNA-extraction following a CTAB protocol modified from Doyle and Doyle (1987). To create a *Bryopsis* host phylogeny, chloroplast-encoded *rbcl* genes were amplified as described by Hollants et al. (2011a). For the specific amplification of Flavobacteriaceae endosymbiont 16S rRNA genes, we designed species-specific primers in Kodon v3.5 (Applied Maths, Belgium) with as target group full length Flavobacteriaceae 16S sequences (JF521600-JF521604, HE648933, HE648935, HE648940, and HE648943) obtained in our previous studies (Hollants et al., 2011a, 2013a). Due to the large non-target group (i.e. all other bacterial 16S sequences) only one suitable region (position 690 to 720) for specific primer annealing was found. Consequently, we designed one species-specific primer which we used in both the forward (F695: 5'-GGCAGGTTGCTAAGCCTTAA-3') as well as reverse (R695: 5'-TTAAGGCTTAGCAACCTGCC-3') direction together with the 16S rRNA gene universal primers 1492R and 27F (Lane, 1991), respectively. *Bryopsis* DNA extracts from previous studies (Hollants et al., 2011a, 2013a), in which Flavobacteriaceae endosymbiont DNA was known to be present or absent, were used as templates for the initial PCR optimization experiments. Thermocycling conditions were investigated using gradient-PCR with the following reaction mix: 1× AmpliTaq Gold reaction buffer (Applied Biosystems), 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each primer and 1.25 U/μL AmpliTaq Gold DNA polymerase (Applied Biosystems). Optimized thermocycling conditions were as follows: one cycle of 95°C for 5 min; 25 cycles of 95°C for 1 min, 59°C for 1 min, 72°C for 1 min; one final extension cycle at 72°C for 10 min. PCR amplicons were purified using a Nucleofast 96 PCR clean up membrane system (Machery-Nagel, Germany) according to the manufacturer's instructions and sequenced as described by Hollants et al. (2011a). Flavobacteriaceae endosymbiont 16S sequences were assembled using the BioNumerics 5.1 software (Applied Maths, Belgium), compared with nucleotide databases via BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and chimera-checked using Bellerophon (Huber

et al., 2004). Bacterial and algal sequences were submitted to EMBL under accession numbers HE775438-HE775517.

2.3. Phylogenetic analyses of host and symbiont

Two alignments were created for phylogenetic analyses. The *Bryopsis* alignment consisted of 146 *rbcl* sequences and was 1363 bp long, including 100 variable and 85 parsimony informative positions. The 80 Flavobacteriaceae 16S rRNA gene sequences obtained from *Bryopsis* samples were aligned with 15 Flavobacteriaceae type strains and closest BLAST hits using MUSCLE (Edgar, 2004). The resulting alignment was 1470 bp long, including a small number of gaps, and 500 variable and 398 parsimony informative positions. Models of nucleotide substitution were selected using the Akaike information criterion with JModelTest v0.1.1 (Posada, 2008). Phylogenetic trees were reconstructed by maximum likelihood (ML) using PhyML v3.0 (Guindon and Gascuel, 2003), via the University of Oslo Bioportal website (Kumar et al., 2009). The *Bryopsis rbcl* and bacterial 16S rRNA gene alignment were analyzed under a GTR + G model. Trees were visualized in Mega 4.0 (Tamura et al., 2007) and annotated with Adobe® Illustrator® CS5. Based on the resulting *Bryopsis* phylogram, 23 species were identified as clades of closely related sequences that are preceded by relatively long, well supported branches (Hudson and Coyne, 2002; Leliaert et al., 2009). Phylogenetic analysis of the Flavobacteriaceae 16S dataset resulted in a tree with three well supported clades (Fig. 1B: clades A, B1 and B2). Because the internal branches of clade B2 were largely unresolved, the genetic variation within this clade could be represented more appropriately by a network (Posada and Crandall, 2001). Statistical parsimony networks (Templeton et al., 1992) were constructed with TCS 1.21 (Clement et al., 2000), with calculated maximum connection steps at 95% and alignment gaps treated as missing data. Sequence similarity between the 16S rRNA gene sequences was determined in BioNumerics v5.1 (Applied Maths, Belgium).

2.4. Analysis of host-symbiont coevolution and biogeography

We used different statistical techniques to assess coevolution between Flavobacteriaceae endosymbionts of clade B and the *Bryopsis* host, and to investigate to which degree the

bacterial genetic variation was geographically structured. Analysis of molecular variance (AMOVA) of Flavobacteriaceae 16S sequences was used to investigate the percentage of variation within and between populations, which were predefined as the different host species (*Bryopsis* spp. 20, 21, 22, 23, 24, 28 and *B. myosuroides*) or geographical regions (Atlantic-Mediterranean, East Pacific, Indian Ocean and West Pacific). Because of small sample sizes, *Bryopsis* spp. 25 and 26 were excluded from the analyses. Patterns of genetic structuring among *Bryopsis* species and between geographical regions were estimated using Arlequin v3.5.1.3 (Excoffier and Lischer, 2010). Population pairwise Φ_{ST} values, a measure of population differentiation or genetic distance, were calculated using Tamura–Nei distances.

3. Results and discussion

3.1. Restricted phylogenetic distribution of Flavobacteriaceae endosymbionts

The newly designed PCR protocol was successful in amplifying Flavobacteriaceae sequences directly from algal DNA extracts. Sequencing resulted in unambiguous electropherograms, indicating the primer designed (F/R695) is highly specific for the targeted endosymbionts, and suggesting the exclusive presence of one flavobacterial genotype per host plant. This allowed for screening of a large number of algal samples without the need for culturing, surface sterilization, or molecular cloning. Of the 146 *Bryopsis* samples examined, 80 displayed an amplicon on agarose gel. The 16S rRNA gene sequences were most similar (99% BLAST similarity) to Flavobacteriaceae endosymbiont sequences previously obtained from *Bryopsis* (Hollants et al., 2011a, 2013a). None of the other Bryopsidales or Ulvophyceae algal samples yielded positive amplifications (Table S1), indicating a strong host specificity and an intimate association of the Flavobacteriaceae endosymbionts with *Bryopsis*.

Mapping of the positive amplifications on the *Bryopsis* host phylogram revealed that the presence of Flavobacteriaceae endosymbionts was restricted to two clades (green branches, Fig. 1A): a large clade B containing *Bryopsis* species from tropical and warm-temperate regions and a smaller clade A including *B. vestita* and *B. foliosa* samples from New Zealand and southern Australia, respectively. The non-monophyly of the *Bryopsis* species containing Flavobacteriaceae (although not strongly supported) either indicates that host-endosymbiont associations evolved

twice independently, or that the association has been lost in one or more *Bryopsis* clades (Fig. S1).

Although our data suggest a preference of Flavobacteriaceae endosymbionts for high temperatures, it is difficult to distinguish whether this results from an actual temperature preference of the bacteria or ecological preferences of the host. Host ecological preferences likely play an important role as seaweed species distributions are known to be predominantly determined by seawater temperature regimes (Breeman, 1988). For *Bryopsis*, variation partitioning analysis showed that the presence or absence of Flavobacteriaceae endosymbionts could be largely explained by host phylogenetic factors, which are inevitably interrelated with environmental factors as a result of phylogenetic niche conservatism (Losos, 2008, Hollants et al. 2013a). These results are in agreement with specific host-symbiont associations (Hollants et al., 2013a). Niche conservatism of hosts resulting in temperature-dependent variation of endosymbionts has also been described in other eukaryotes, including sponges, squids and insects (Taylor et al., 2005; Erwin and Thacker, 2008; Toju and Fukatsu, 2011; Zamborsky and Nishiguchi, 2011).

3.2. *Flavobacteriaceae* genetic diversity

The 80 *Bryopsis*-associated Flavobacteriaceae 16S rRNA gene sequences formed a distinct and well supported clade that included two other sequences from sponge- and coral-associated uncultured bacteria (Thiel et al., 2007; Sunagawa et al., 2009) (Fig. S1). The clade was distantly related to cultured Flavobacteriaceae type strains (85-87% 16S rRNA gene similarity), confirming our previous observation that the Flavobacteriaceae endosymbionts likely represent a new genus (Hollants et al., 2011a). The *Bryopsis*-associated Flavobacteriaceae fell into two smaller clades (Fig. 1B, Fig. S1). Clade A consisted of endosymbionts from *Bryopsis vestita* and *B. foliosa*; clade B included the endosymbionts from the other nine *Bryopsis* species (*Bryopsis myosuroides* and *Bryopsis* spp. 20, 21, 22, 23, 24, 25, 26 and 28). Clade B consisted of two subclades: a small clade B1 and a large clade B2 with unresolved internal branches, which can be better represented as a phylogenetic network. Statistical parsimony analysis resulted in two unconnected networks, corresponding to clade A (three 16S genotypes) and B (26 genotypes).

The unresolved relationships within clade B were reflected in a highly interconnected network (Fig. 1C), which may result from homoplasies or recombination (Posada and Crandall, 2001) (see section 3.3). Pairwise sequence similarity of the 16S rRNA gene sequences (1445 bp) was 99.3-99.9% within clade A, 99.1-100% within clade B, and a maximum of 96.1% between clades A and B (Fig. S1).

3.3. Host-symbiont coevolution and biogeography

We applied different methods for examining the association between Flavobacteriaceae endosymbionts and *Bryopsis* hosts. A possible correlation between endosymbiont and host genetic variation was visually explored by comparing host and symbiont trees and by mapping the Flavobacteriaceae genotypes on the host phylogeny (Fig. 1) or vice versa (Fig. 2A). Strict topological congruence was observed between *Bryopsis vestita* and *B. foliosa* (clade A) and their associated endosymbionts. However, within clade B, correlation between the phylogenies of Flavobacteriaceae and *Bryopsis* was more complex for three reasons. First, several bacterial genotypes were present in different *Bryopsis* hosts. For example, genotype 1 was found in four *Bryopsis* species (spp. 22, 23, 24 and 26), genotype 11 was present in three species (spp. 20, 21 and 28), and genotype 7 in two species (spp. 21, 28). Secondly, most *Bryopsis* species contained multiple Flavobacteriaceae genotypes, with *Bryopsis* sp. 28 possessing as much as 14 different genotypes. Thirdly, relationships among Flavobacteriaceae genotypes were largely unresolved, hampering the reconstruction of reconciled trees.

Because of these complicating factors, we applied statistical approaches that do not require a well-resolved host and symbiont phylogeny for assessing coevolution. AMOVA revealed that 57% of the genetic variation in endosymbiont 16S rRNA gene sequences was attributable to the host species clade divisions and subsequent permutation tests pointed out that this difference was significant ($p < 0.0001$, Table 1), indicating genetic differentiation of endosymbionts between *Bryopsis* species. Pairwise ϕ_{ST} -values between the species are highest between more distantly related species, while genetic differentiation was found to be insignificant between some closely related species (Table 1). Our data also indicated that genetic diversity of endosymbionts was to a large extent geographically structured, with most 16S genotypes being restricted to

one geographical region (Fig. 1D, Fig. 2B). This was supported by AMOVA and pairwise ϕ_{ST} -values that showed significant genetic differentiation between the East Pacific, Atlantic-Mediterranean and Indo-Pacific (Table 2). However, this geographical signal may in part be due to dispersal limitation of the host, which results in confined geographical ranges for most host species. Several observations favor the hypothesis that endosymbiont genetic diversity is primarily structured by host phylogeny. As described above, Flavobacteriaceae endosymbionts were restricted to two *Bryopsis* clades (clade A and B), irrespective of host biogeography. For example, of the five *Bryopsis* species from the Mediterranean Sea, only the two species from clade A harbored Flavobacteriaceae endosymbionts (Fig. 1A). A similar strict phylogenetic distribution of endosymbionts was observed for the different *Bryopsis* species from Pacific Mexico, Pacific Nicaragua, South Africa and the Seychelles. A phylogenetic rather than geographic effect on endosymbiont genetic differentiation was also apparent when examining specific Flavobacteriaceae genotypes within *Bryopsis* clade B. For example, genotype 1 is widely distributed in the Atlantic, Mediterranean and Indo-Pacific, but clearly restricted to a single clade including *Bryopsis* spp. 22, 23, 24 and 26.

There are several potential and non-exclusive explanations for this complex host-symbiont evolutionary association, invoking uptake of bacteria from the environment and/or vertical transmission of bacterial endosymbionts (Burr & West, 1970; Hollants et al., 2011b). First, related *Bryopsis* species may have evolved similar traits that select for the uptake of specific Flavobacteriaceae from the environment. This uptake may be selective and specific to a certain extent only, depending on habitat-specific physiological requirements, *Bryopsis* plants and availability of certain Flavobacteriaceae genotypes in the seawater. Second, the occurrence of specific Flavobacteriaceae genotypes in different *Bryopsis* species may also be explained by lateral transfer of endosymbionts between host species (host-switching). Sea slugs, which are known to graze on siphonous green algae, could act as effective carriers of bacteria between different *Bryopsis* species (Händeler et al., 2010). The observation that *Bryopsis* endosymbionts are related to bacteria encountered in sponge and coral hosts (Fig. S1) might be indicative for host-switching among distantly related eukaryotes (Weinert et al., 2009). Third, the presence of Flavobacteriaceae in related hosts may be explained through vertical inheritance of bacteria

either during sexual reproduction or asexual proliferation by fragmentation or extruded protoplasts that regenerate into new *Bryopsis* plants. The diversity of Flavobacteriaceae genotypes within a single *Bryopsis* species could then be explained by recent and ongoing divergence of endosymbionts. The observation that some endosymbiont genotypes (genotypes 1, 7 and 11, Fig. 1) are found in different *Bryopsis* species may be the result of persistence of ancestral Flavobacteriaceae genotypes in different host lineages. Finally, incongruent host-symbiont coevolution patterns might be biased by ambiguous algal host and endosymbiont species delimitation. For example, the low level of 16S sequence variability proves that this molecular marker offers limited phylogenetic resolution at lower taxonomic levels (Erwin and Thacker, 2008). Faster evolving markers would provide more polymorphic sites and suitable information to assess coevolution patterns.

In conclusion, our results provide strong evidence for a non-random association between *Bryopsis* and its Flavobacteriaceae endosymbionts, whereby more closely related host species predominantly harbor genetically similar endosymbionts, suggestive of coevolution. The physiological ground for this alliance remains unknown from both the host and endosymbiont perspective. It is possible that Flavobacteriaceae endosymbionts offer the algal host an adaptive advantage. Future studies focusing on functional diversity of the endosymbionts should bring additional insights in these little studied algal-bacterial associations.

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Figure and table legends

Figure 1. Flavobacteriaceae endosymbiont data (B and C) plotted on the *Bryopsis* host phylogram (A) and geographical distribution of Flavobacteriaceae 16S rRNA types (D). Green colored branches denote positive amplification of Flavobacteriaceae endosymbiont 16S rRNA genes within the respective algal samples. The TCS parsimony network (C) visualizes phylogenetic relations among the different Flavobacteriaceae 16S rRNA gene types (numbers 1-29) and each black node represents 1 nucleotide mutation separating genotypes. Colored circles (numbers on these circles refer to sequence types) on pictures B and C indicate endosymbiont genotypes and are in picture C proportionally sized to the number of sequences (i.e. Flavobacteriaceae strains) they represent. These distributions are also represented in the pie charts (B and D) in which the numbers again correspond to the endosymbiont 16S rRNA gene types. ML bootstrap values are indicated at the branch nodes (A and B). The scale bar indicates 0.02 (A) and 0.001 (B) nucleotide changes per nucleotide position.

Figure 2. TCS parsimony network of 16S rRNA gene sequences of Flavobacteriaceae endosymbionts. Circles depict endosymbiont genotypes and are proportionally sized to the number of sequences (i.e. Flavobacteriaceae strains) they represent. Colors within the network correspond to (A) *Bryopsis* species as depicted in the host phylogram on the left and (B) geographical location of the host samples as depicted in the map on the right. Each black node represents 1 nucleotide mutation separating genotypes.

Appendix A. Supplementary data

Table S1. Algal samples (taxonomic affiliation, voucher number and geographic location) that were screened for the presence of Flavobacteriaceae endosymbionts.

Figure S1. Maximum likelihood tree showing the phylogenetic position of *Bryopsis* Flavobacteriaceae endosymbionts. The phylogeny was inferred from 16S rRNA gene sequences

determined in this study (**bold**), BLAST hits and Flavobacteriaceae type strains. Bootstrap values and sequence similarity values are indicated at the branch nodes in black and grey, respectively.

Table 1. Pairwise Φ_{ST} values of Flavobacteriaceae endosymbionts between *Bryopsis* host species (clade B).

	<i>B. sp. 22</i>	<i>B. sp. 23</i>	<i>B. sp. 24</i>	<i>B. myosuroides</i>	<i>B. sp. 21</i>	<i>B. sp. 20</i>
<i>B. sp. 22</i>						
<i>B. sp. 23</i>	0.10					
<i>B. sp. 24</i>	0.51	0.41				
<i>B. myosuroides</i>	0.94	0.91	0.27			
<i>B. sp. 21</i>	0.96	0.94	0.45	0.59		
<i>B. sp. 20</i>	0.92	0.88	0.27	0.32	0.03	
<i>B. sp. 28</i>	0.74	0.72	0.36	0.19	0.04	0.02

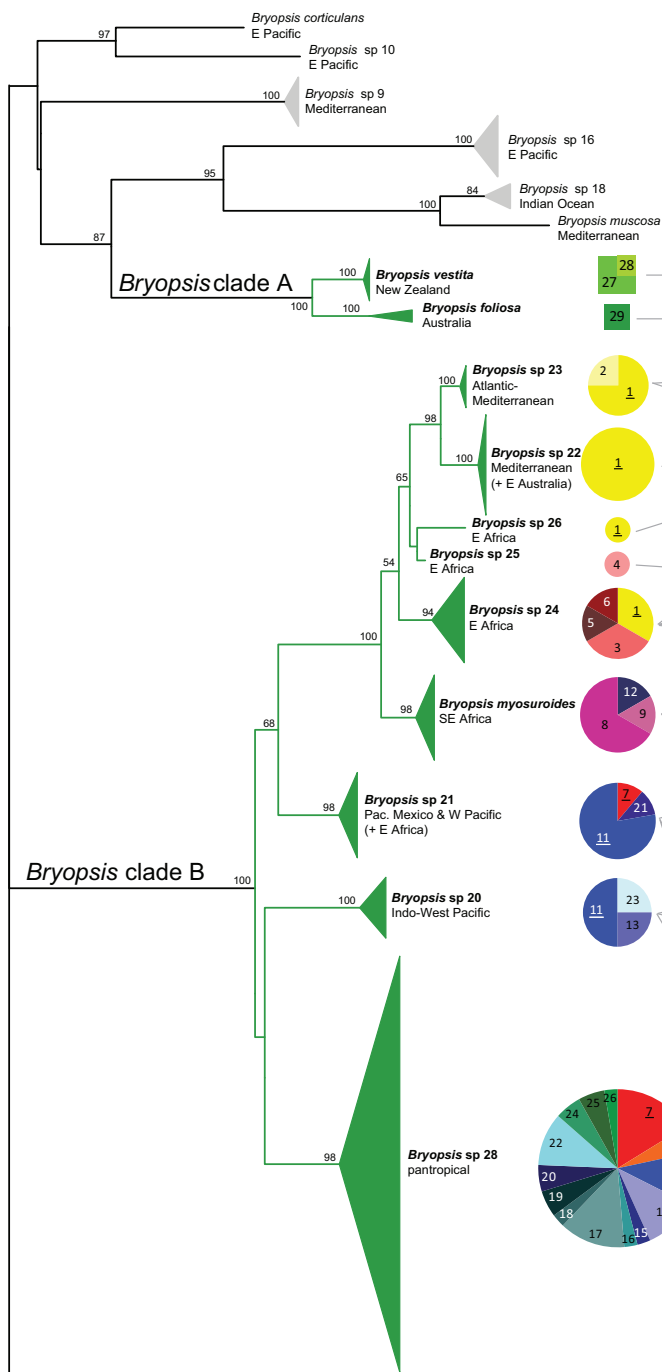
Values in bold are significantly different from zero after Bonferroni correction

Table 2. Pairwise Φ_{ST} values of Flavobacteriaceae endosymbionts between four geographical regions.

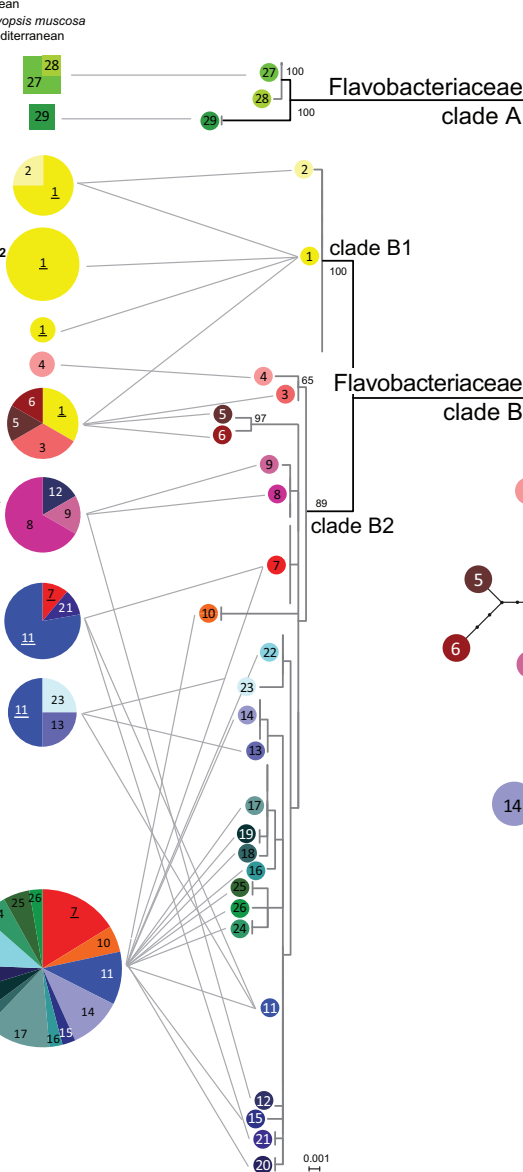
	Atlantic-Mediterranean	East Pacific	Indian Ocean
Atlantic-Mediterranean			
East Pacific	0.66		
Indian Ocean	0.45	0.26	
West Pacific	0.44	0.18	0.05

Values in bold are significantly different from zero after Bonferroni correction

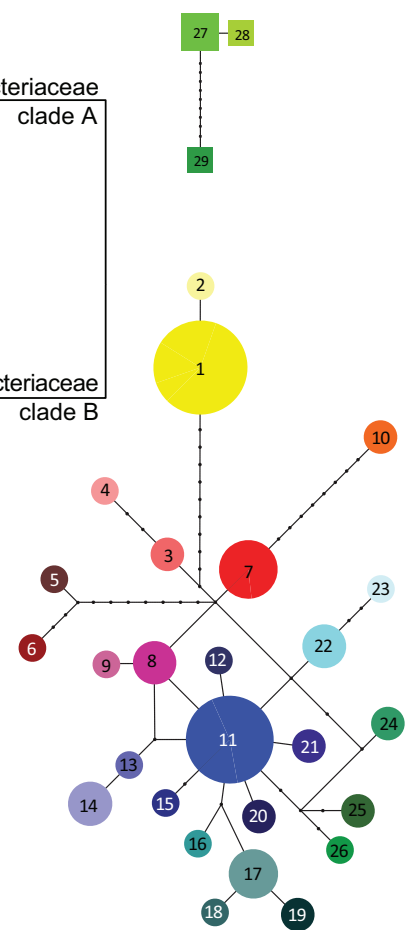
A. *Bryopsis* *rbcL* phylogeny and distribution of Flavobacteriaceae 16S rRNA types in host species



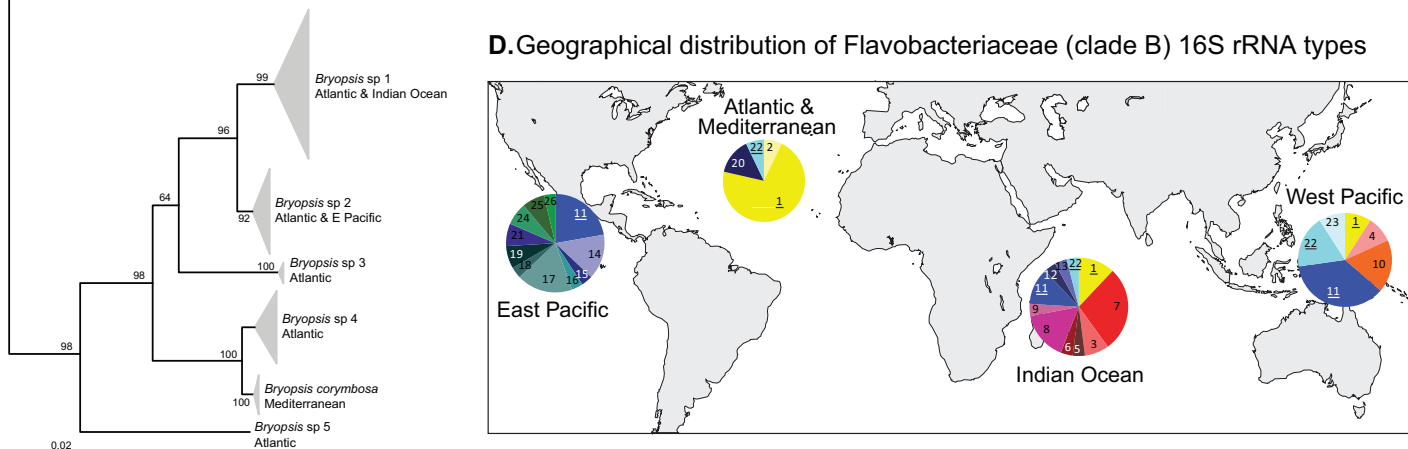
B. Flavobacteriaceae 16S rRNA phylogeny



C. Flavobacteriaceae 16S rRNA networks



D. Geographical distribution of Flavobacteriaceae (clade B) 16S rRNA types



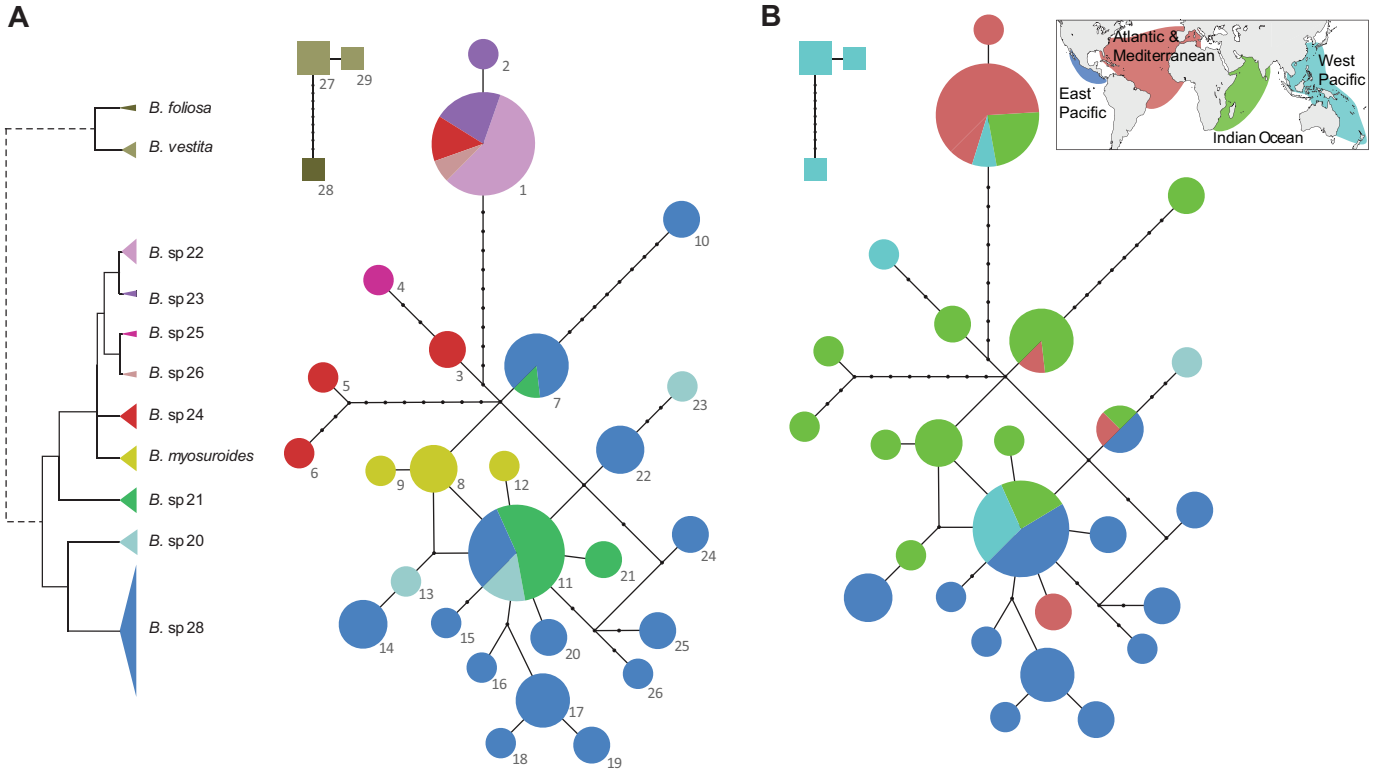


Table S1. Algal samples (taxonomic affiliation, voucher number and geographic location) that were screened for the presence of Flavobacteriaceae endosymbionts.

Order	Genus/species	Voucher number	Country	Ocean/Sea
Bryopsidales	<i>Avrainvillea asarifolia</i>	LL0044	Belize	Atlantic Ocean
Bryopsidales	<i>Avrainvillea nigricans</i>	LL0005	Belize	Atlantic Ocean
Bryopsidales	<i>Avrainvillea silvana</i>	LL0045	Belize	Atlantic Ocean
Bryopsidales	<i>Boodleopsis pusilla</i>	LL0046	Belize	Atlantic Ocean
Bryopsidales	<i>Bryopsis corticulans</i>	HV1535	USA	Pacific Ocean
Bryopsidales	<i>Bryopsis corymbosa</i>	HEC4772	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis corymbosa</i>	HV1237	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis corymbosa</i>	ODCMZ1	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis corymbosa</i>	ODCMZ2	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis foliosa</i>	F0001	Australia	Indian Ocean
Bryopsidales	<i>Bryopsis foliosa</i>	F0002	Australia	Indian Ocean
Bryopsidales	<i>Bryopsis muscosa</i>	HV1238	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis myosuroides</i>	F.0172	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	F.0174	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	F.0175	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	KZN0156	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	KZN2318	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	ODC1185	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	ODC1186	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis myosuroides</i>	ODC1187a	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis vestita</i>	F0082b	New Zealand	Pacific Ocean
Bryopsidales	<i>Bryopsis vestita</i>	Joe1	New Zealand	Pacific Ocean
Bryopsidales	<i>Bryopsis vestita</i>	Joe2	New Zealand	Pacific Ocean
Bryopsidales	<i>Bryopsis vestita</i>	Joe3	New Zealand	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	F.0173	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	F0006	Argentina	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	FL62	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	HEC10851	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	HEC10881	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	JH001	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	JH002	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	JH003	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	KZN0920	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	KZN931	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	SEY382	Seychelles	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	SEY477	Seychelles	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	Sn10839	Indonesia	Pacific Ocean

Bryopsidales	<i>Bryopsis</i> sp. 1	TS133	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	TS172	South Africa	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 1	West4583	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	EE4	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	HVGoes	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	WB3	USA	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	WB4	USA	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	WE2	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	WE3	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	YB1	USA	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 2	YB2	USA	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 3	HV880	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 3	ODC1380	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4b	BR	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	BY	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	HV1340	Spain	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	HV1341	Spain	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	HV1370	Spain	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	WE1	Netherlands	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 4c	West4718	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 5	HV1388	France	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 9	HEC1637	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 9	JH021	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 9	JH022	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 9	JH023	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 9	JH025	France	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 10	MX0359	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	F.0112	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	HV1559	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	HV1757	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	HV1779	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	HV1780	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 16	MX0254	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 18	HEC15265	Madagascar	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 18	KZN0800	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 18	ODC1187b	South Africa	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 20	F.0176	Malaysia	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 20	HEC14151	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 20	HEC14796	Mauritius	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 20	HEC16048	Sri Lanka	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 20	HEC8671	Kenya	Indian Ocean

Bryopsidales	<i>Bryopsis</i> sp. 20	KE1	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	FL1173	Philippines	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	HV1682	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	HV1686	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	MX0036	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	MX0156	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	MX0253	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21	MX19	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 21b	TZ170	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 22	HV1227	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	HV1228	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	HV1229	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	HV1240	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	HV1241	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	HV2122	Australia	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 22	MZ4	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	ODCMZ3	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 22	ODCMZA	Spain	Mediterranean Sea
Bryopsidales	<i>Bryopsis</i> sp. 23	HV967	USA	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 23	HV968	USA	Atlantic Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC10690	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC11314	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC12192	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC14026	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC14932	Madagascar	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	HEC9474	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	ODC679	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 24	TZ583	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 25	HV1983	Japan	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 26	HEC14026	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 26	HEC9417	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0096	New Caledonia	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0097	New Caledonia	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0104	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0105	Costa Rica	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0107	Costa Rica	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0108	Costa Rica	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0109	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0110	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0111	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0113	Nicaragua	Pacific Ocean

Bryopsidales	<i>Bryopsis</i> sp. 28	F.0114	Nicaragua	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0115	Panama	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0116	Panama	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0117	Panama	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0119	Panama	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	F.0120	Panama	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC10527	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC10657	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC11198	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC12942	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC14609b	Mauritius	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC6728	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC9490	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HEC9510	Kenya	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HOD-RUN98-33	Reunion	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HOD-RUN98-34	Reunion	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HV1609	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HV1614	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HV566	Philippines	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	HV679	Philippines	Pacific Ocean
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Bryopsidales	<i>Bryopsis</i> sp. 28	MX0314	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	MX164	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	MX344	Mexico	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	ODC1747	Philippines	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	PH167	Philippines	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	PH222	Philippines	Pacific Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	SEY323	Seychelles	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	SEY357	Seychelles	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	TZ0053	Tanzania	Indian Ocean
Bryopsidales	<i>Bryopsis</i> sp. 28	TZ0088	Tanzania	Indian Ocean
Bryopsidales	<i>Caulerpa cupressoides</i>	MX0382	Mexico	Pacific Ocean
Bryopsidales	<i>Caulerpa mexicana</i>	LL0104	Bahamas	Atlantic Ocean
Bryopsidales	<i>Caulerpa peltata</i>	HV2030	Japan	Pacific Ocean
Bryopsidales	<i>Caulerpa prolifera</i>	LL0113	Belize	Atlantic Ocean
Bryopsidales	<i>Caulerpa racemosa</i>	LL0118	Martinique	Atlantic Ocean
Bryopsidales	<i>Caulerpa racemosa</i>	MX0174	Mexico	Pacific Ocean
Bryopsidales	<i>Caulerpa serrulata</i>	LL0010	Fiji	Pacific Ocean
Bryopsidales	<i>Caulerpa sertularioides</i>	MX0316	Mexico	Pacific Ocean
Bryopsidales	<i>Caulerpa taxifolia</i>	LL0131	Fiji	Pacific Ocean
Bryopsidales	<i>Chlorodesmis</i> sp.	HV1774	Sri Lanka	Indian Ocean

Bryopsidales	<i>Chlorodesmis</i> sp.	MX0081	Mexico	Pacific Ocean
Bryopsidales	<i>Codium arabicum</i>	TZ0517	Tanzania	Indian Ocean
Bryopsidales	<i>Codium decorticatum</i>	G.371	Brazil	Atlantic Ocean
Bryopsidales	<i>Codium duthiae</i>	HEC10919	South Africa	Atlantic Ocean
Bryopsidales	<i>Codium dwarkense</i>	TZ0818	Tanzania	Indian Ocean
Bryopsidales	<i>Codium fragile</i>	HEC1554	South Africa	Atlantic Ocean
Bryopsidales	<i>Codium fragile</i>	HV1099	USA	Atlantic Ocean
Bryopsidales	<i>Codium fragile</i>	HV1392	France	Atlantic Ocean
Bryopsidales	<i>Codium fragile</i>	HV1786	Mexico	Pacific Ocean
Bryopsidales	<i>Codium geppiorum</i>	TZ0370	Tanzania	Indian Ocean
Bryopsidales	<i>Codium isabellae</i>	G.083	Panama	Pacific Ocean
Bryopsidales	<i>Codium spongiosum</i>	HV2489	Australia	Indian Ocean
Bryopsidales	<i>Derbesia</i> sp.	HV1079	Netherlands	Atlantic Ocean
Bryopsidales	<i>Derbesia</i> sp.	HV1448	Netherlands	Atlantic Ocean
Bryopsidales	<i>Derbesia</i> sp.	HV1600	Mexico	Pacific Ocean
Bryopsidales	<i>Derbesia</i> sp.	MX0021	Mexico	Pacific Ocean
Bryopsidales	<i>Derbesia</i> sp.	TZ0612	Tanzania	Indian Ocean
Bryopsidales	<i>Halimeda borneensis</i>	W0168	Micronesia	Pacific Ocean
Bryopsidales	<i>Halimeda copiosa</i>	LL0417	Belize	Atlantic Ocean
Bryopsidales	<i>Halimeda cuneata</i>	G.905	South Africa	Indian Ocean
Bryopsidales	<i>Halimeda discoidea</i>	LL0020	Panama	Pacific Ocean
Bryopsidales	<i>Halimeda distorta</i>	H.0097	British IO Territory	Indian Ocean
Bryopsidales	<i>Halimeda gigas</i>	HA0238	Australia	Pacific Ocean
Bryopsidales	<i>Halimeda gigas</i>	W0162	Micronesia	Pacific Ocean
Bryopsidales	<i>Halimeda macroloba</i>	LPT0034	Thailand	Pacific Ocean
Bryopsidales	<i>Halimeda opuntia</i>	HA0373	Australia	Pacific Ocean
Bryopsidales	<i>Halimeda opuntia</i>	LL0459	Panama	Atlantic Ocean
Bryopsidales	<i>Halimeda opuntia</i>	LPT0030	Thailand	Indian Ocean
Bryopsidales	<i>Halimeda tuna</i>	H.0086	British IO Territory	Indian Ocean
Bryopsidales	<i>Halimeda tuna</i>	HV889	Spain	Mediterranean Sea
Bryopsidales	<i>Rhipilia orientalis</i>	HEC10402	Papua New Guinea	Indian Ocean
Bryopsidales	<i>Tydemanina expeditionis</i>	HV873	Philippines	Pacific Ocean
Bryopsidales	<i>Udotea unistratea</i>	LL0051	Belize	Atlantic Ocean
Cladophorales	<i>Aegagropila</i> sp.	Aeg 1	Ukraine	Freshwater
Cladophorales	<i>Anadyomene</i> sp.	FL1113	Philippines	Pacific Ocean
Cladophorales	<i>Anadyomene</i> sp.	TZ0177	Tanzania	Indian Ocean
Cladophorales	<i>Apjohnia laetevirens</i>	HV2291	Australia	Pacific Ocean
Cladophorales	<i>Apjohnia laetevirens</i>	HV2342	Australia	Pacific Ocean
Cladophorales	<i>Boergesenia forbesii</i>	Boerg1	Seychelles	Indian Ocean
Cladophorales	<i>Boergesenia forbesii</i>	FL1114	Philippines	Pacific Ocean
Cladophorales	<i>Boergesenia</i> sp.	JAP073	Japan	Pacific Ocean

Cladophorales	<i>Boodlea</i> sp.	FL1110	Philippines	Pacific Ocean
Cladophorales	<i>Boodlea</i> sp.	Huisman nov06	Australia	Indian Ocean
Cladophorales	<i>Boodlea</i> sp.	TZ0147	Tanzania	Indian Ocean
Cladophorales	<i>Chaetomorpha antennina</i>	MX275	Mexico	Pacific Ocean
Cladophorales	<i>Chaetomorpha crassa</i>	FL1132	Philippines	Pacific Ocean
Cladophorales	<i>Chaetomorpha crassa</i>	ODC1640	Kenya	Indian Ocean
Cladophorales	<i>Chaetomorpha</i> sp.	FL1092	Philippines	Pacific Ocean
Cladophorales	<i>Chaetomorpha</i> sp.	TZ0877	Tanzania	Indian Ocean
Cladophorales	<i>Cladophora vagabunda</i>	Bernecker 73493	Costa Rica	Pacific Ocean
Cladophorales	<i>Cladophora vagabunda</i>	HEC15734	Madeira	Atlantic Ocean
Cladophorales	<i>Cladophora vagabunda</i>	TZ0203	Tanzania	Indian Ocean
Cladophorales	<i>Cladophoropsis vaucheriiformis</i>	HEC10097	Japan	Pacific Ocean
Cladophorales	<i>Cladophoropsis vaucheriiformis</i>	HEC7547	Papua New Guinea	Pacific Ocean
Cladophorales	<i>Cladophoropsis vaucheriiformis</i>	TZ0826	Tanzania	Indian Ocean
Cladophorales	<i>Dictyosphaeria cavernosa</i>	FL1091	Philippines	Pacific Ocean
Cladophorales	<i>Dictyosphaeria cavernosa</i>	TZ0197	Tanzania	Indian Ocean
Cladophorales	<i>Dictyosphaeria sericea</i>	HV2275	Australia	Pacific Ocean
Cladophorales	<i>Dictyosphaeria</i> sp.	Bernecker86	Costa Rica	Pacific Ocean
Cladophorales	<i>Dictyosphaeria versluysii</i>	TS253	Hawaii	Pacific Ocean
Cladophorales	<i>Dictyosphaeria versluysii</i>	TZ0156	Tanzania	Indian Ocean
Cladophorales	<i>Ernodesmis verticillata</i>	Bernecker 73483	Costa Rica	Pacific Ocean
Cladophorales	<i>Microdictyon boergesenii</i>	BW00392	Panama	Indian Ocean
Cladophorales	<i>Microdictyon tenuius</i>	HEC16007	Sri Lanka	Indian Ocean
Cladophorales	<i>Rhizoclonium africanum</i>	TZ0781	Tanzania	Indian Ocean
Cladophorales	<i>Rhizoclonium</i> sp.	FL1164	Philippines	Pacific Ocean
Cladophorales	<i>Siphonocladus tropicus</i>	Siph3	Canary Islands	Atlantic Ocean
Cladophorales	<i>Valonia macrophysa</i>	BW00825	Panama	Pacific Ocean
Cladophorales	<i>Valonia</i> sp.	FL1120	Philippines	Pacific Ocean
Cladophorales	<i>Valonia</i> sp.	IT028B	Hawaii	Pacific Ocean
Cladophorales	<i>Valonia</i> sp.	TZ0148	Tanzania	Indian Ocean
Dasycladales	<i>Acetabularia dentata</i>	HEC12349	Philippines	Pacific Ocean
Dasycladales	<i>Acetabularia ryukyuensis</i>	HEC12329	Philippines	Pacific Ocean
Dasycladales	<i>Bornetella oligospora</i>	FL1108	Philippines	Pacific Ocean
Dasycladales	<i>Neomeris annulata</i>	HEC12327	Philippines	Pacific Ocean
Dasycladales	<i>Neomeris vanbosseae</i>	TZ0198	Tanzania	Indian Ocean
Ulvaes	<i>Ulva</i> sp.	JH3epi	France	Atlantic Ocean
Ulvaes	<i>Ulva</i> sp.	Qingdao1	China	Pacific Ocean

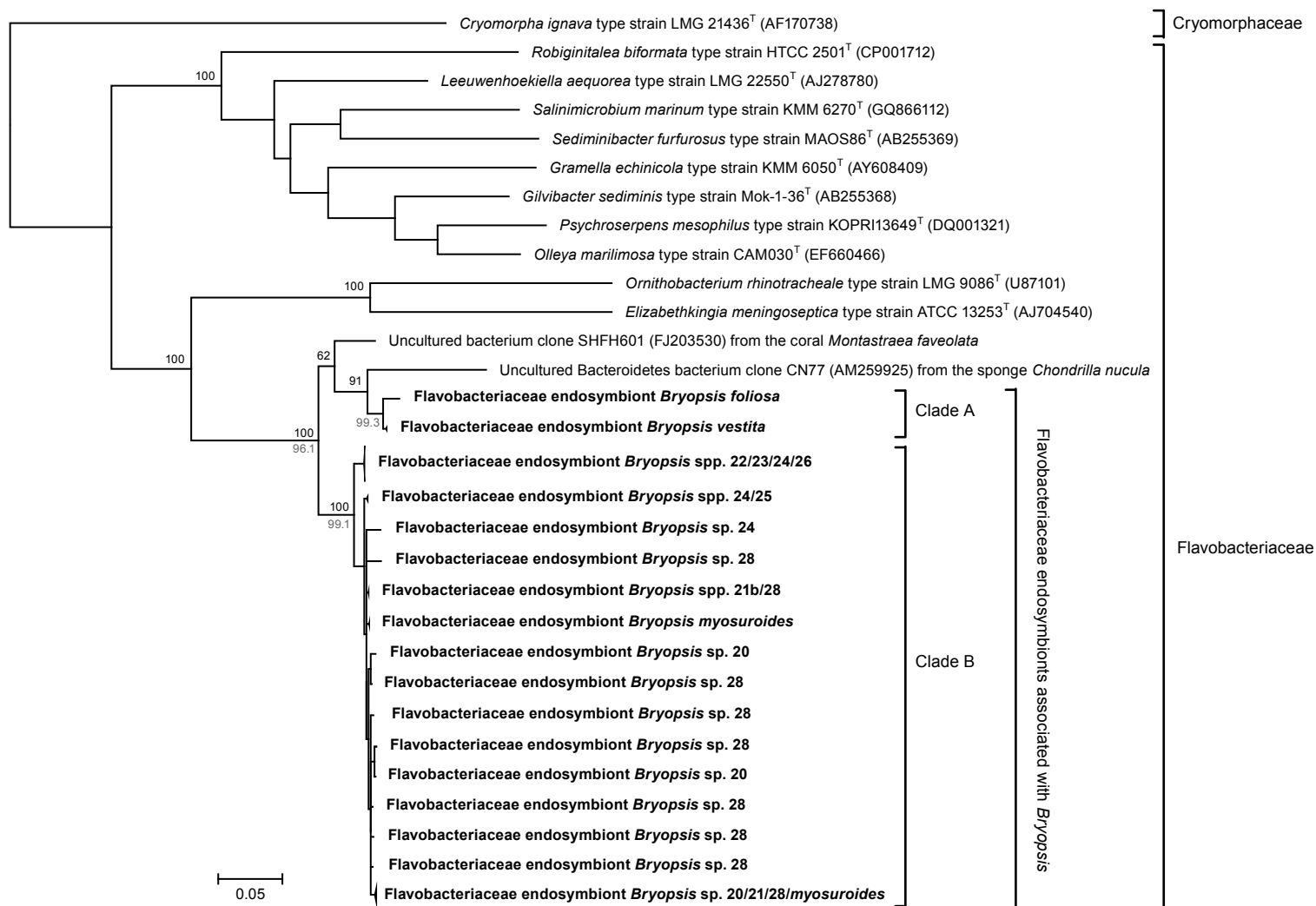


Figure S1. Maximum likelihood tree showing the phylogenetic position of *Bryopsis* Flavobacteriaceae endosymbionts. The phylogeny was inferred from 16S rRNA gene sequences determined in this study (bold), BLAST hits and Flavobacteriaceae type strains. Bootstrap values and sequence similarity values are indicated at the branch nodes in black and grey, respectively.