

SHORT COMMUNICATION

The ‘other’ coral symbiont: *Ostreobium* diversity and distribution

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***Ostreobium* is an endolithic algal genus thought to be an early-diverging lineage of the Bryopsidales (Ulvophyceae, Chlorophyta). *Ostreobium* can live in low-light conditions on calcium carbonate substrata in tropical conditions. It is best known as a symbiont of corals, where it lives deep within the animal skeleton and exchanges nitrogen and carbon, as well as providing nutrients and photoassimilates. In contrast to the relatively well-studied role of the photosynthetic zooxanthellae symbionts in coral (*Symbiodinium*), *Ostreobium* phylogeny, diversity and distribution are all poorly understood. Here, we describe the phylogenetic position and diversity of *Ostreobium* based on plastid 16S ribosomal DNA (rDNA), 18S rDNA and *rbcl* genes from a nuclear genome survey and complete plastid genome, and determined its environmental diversity and distribution by screening the publicly available environmental data for those genes. The results shed light on the phylogeny and the ecology of the ‘other’ coral symbiont.**

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Coral reefs are important biodiversity hotspots, they protect our shores from erosion, mitigate the effect of the sea-level rise and are economically relevant for fisheries and tourism (Bellwood *et al.*, 2004). Accordingly, there have been substantial efforts to preserve these ecosystems and protect the main and most threatened member of the consortia, the corals themselves. Corals are threatened by a number of factors associated with climate change, such as increase in sea temperature, sea-level rise, ocean acidification and the emergence of diseases (Bellwood *et al.*, 2004). Of these, the most direct threat associated with an increase in the sea temperature is coral bleaching (Glynn, 1993). During bleaching events corals expel their zooxanthellae, which are symbionts of the dinoflagellate genus *Symbiodinium* that provide food to the animal in the form of photosynthates (Glynn, 1993). Because of the importance of this symbiotic association, *Symbiodinium* is by far the most-studied microorganism associated with corals, but is not the only coral symbiont. Indeed, the holobiont, the host coral and all its associated symbionts (Rosenberg *et al.*, 2007), is much more complex, and includes a myriad of

bacteria (Rohwer *et al.*, 2002), viruses (Wegley *et al.*, 2007) and a diverse but understudied protist community. Among these protists there are both heterotrophs such as labyrinthulids (Harel *et al.*, 2008; Siboni *et al.*, 2010) or ciliates (Ulstrup *et al.*, 2007; Lobban *et al.*, 2011), a variety of apicomplexan-related lineages, some of which are photosynthetic (for example, *Chromera*) and some of which remain mostly uncharacterized (Janouškovec *et al.*, 2012; Šlapeta and Linares, 2013), and lastly, the clearly photosynthetic genus, *Ostreobium* (Lukas, 1974).

Ostreobium is an endolithic alga that has been found to be associated with calcium carbonate structures in shallow tropical waters, including coral skeletons (Kornmann and Sahling, 1980). *Ostreobium* lives within the skeleton itself, sometimes at concentration so high that its visible as a green ring just below the living cnidarian (Supplementary Figure 1). It has been debated whether *Ostreobium* is a beneficial symbiont or parasite of the coral (Verbruggen and Tribollet, 2011), but the former seems more likely. It absorbs a wavelength of light not used by *Symbiodinium* (Fork and Larkum, 1989) and has been demonstrated to provide photosynthates to the coral (Schlichter *et al.*, 1995). *Ostreobium* has even been proposed to replace zooxanthellae function during bleaching events, perhaps keeping the coral temporarily alive for a possible return of *Symbiodinium* (Fine and Loya, 2002). Despite the potential importance of *Ostreobium* in this system, little is known about the genus (Gutner-Hoch and Fine, 2011).

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Since even its phylogenetic position is uncertain, we first confirmed the relationship between *Ostreobium* and other ulvophytes based on three different markers derived from a nuclear genome survey and complete plastid genome (Supplementary Figure 2): plastid 16S rRNA gene, *rbcL* and nuclear 18S rRNA gene. The plastid 16S rRNA gene was the best represented in public databases and best resolved tree (Figure 1a), but the same result was found for *rbcL* and 18S rRNA gene (Supplementary Figure 3 and 4 and Supplementary Tables 2 and 3). The affiliation of *Ostreobium* within the Bryopsidales

(Verbruggen *et al.*, 2009) was confirmed by all the three phylogenetic gene trees, but only statistically supported by 16S rRNA gene (with 100% bootstrap support). Because plastids are sampled along with bacteria in microbial diversity surveys, the 16S rRNA gene tree also included a significant number of environmental *Ostreobium* sequences (ten times more than *rbcL* or 18S rRNA gene: Supplementary Table 1). In the plastid 16S tree, these sequences formed a strongly supported monophyletic group, but were subdivided into three distinct clades (1, 2 and 3), all with 100% support

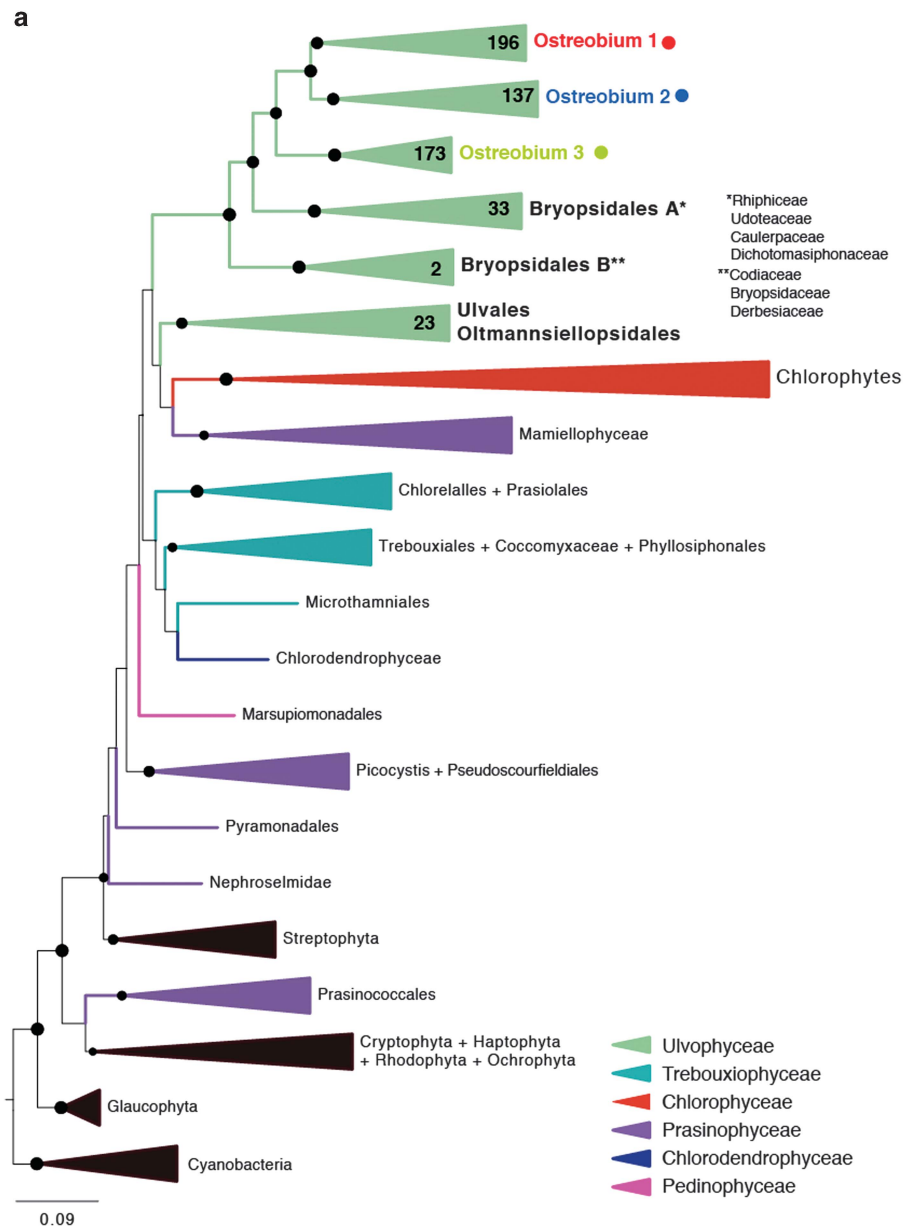


Figure 1 (a) Maximum likelihood phylogenetic tree inferred from the plastid 16S rRNA gene. Taxa includes clustered sequences (97%) from a curated database of ulvophytes ($n=89$, representing 590), a selection of other green algae ($n=41$) and an outgroup of non-chlorophytes ($n=27$). The three *Ostreobium* clades are labeled at the top, and color-coded consistently throughout the figure. (b) Biogeographical distribution of *Ostreobium* clades based on the 16S rRNA gene metadata. (c) Host distribution of *Ostreobium* clades based on the 16S rRNA gene sequence metadata and the host geographical origin. Clade 1 is widespread but dominates in Acroporidae, clade 3 is absent from complex corals and only clade 2 was found in octocorals.

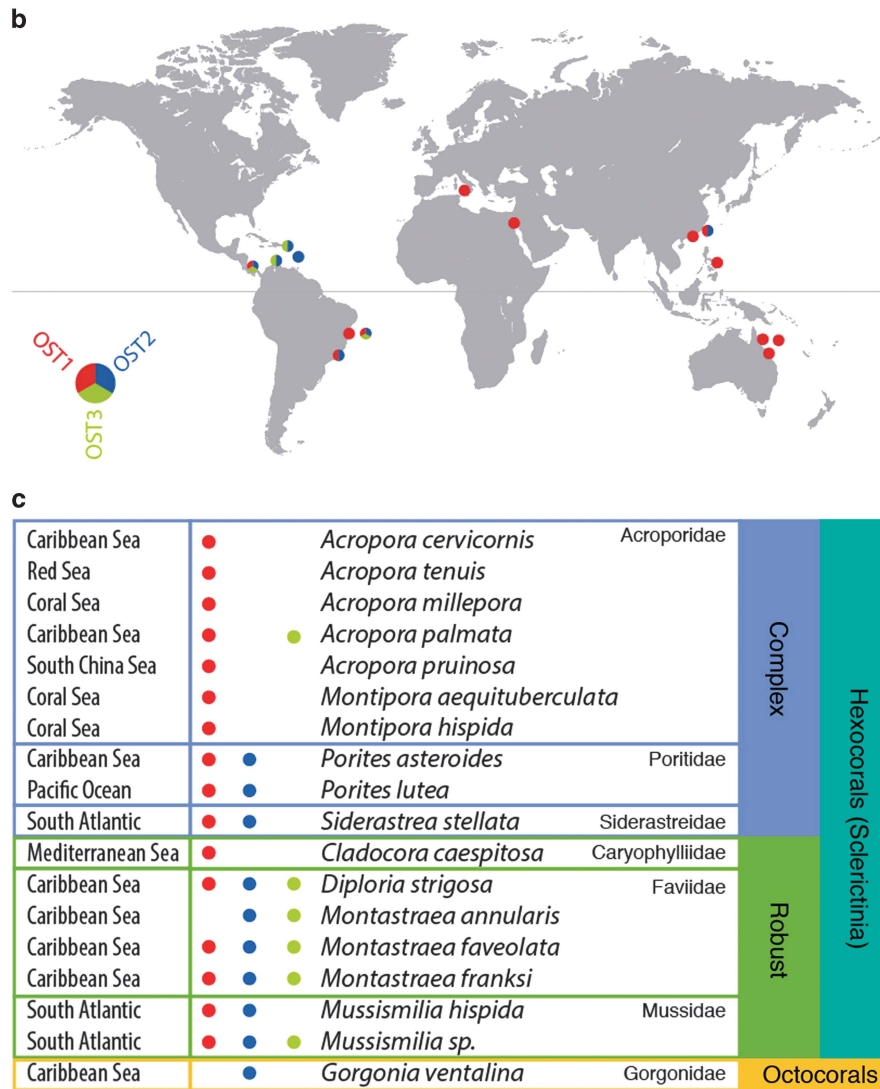


Figure 1 Continued.

(Figure 1a). The sequence level diversity within these groups is similar to the rest of the Bryopsidales combined, and the divergence between any two subgroups is typically greater than that between bryopdisalean genera, altogether suggesting that a substantial amount of diversity exists within what we call ‘*Ostreobium*’.

The environmental 16S rRNA gene data also presents the first opportunity to study the geographical distribution of *Ostreobium* (and indeed is currently the only molecular marker with sufficient data to address such questions: only a few *rbcL* genes are known and all are from strains that fall into the same clade as our genomic isolate, and the genomic isolate provided the first and only nuclear 18S from this genus). We obtained environmental metadata from all the publicly available Ulvophyte environmental sequences in order to link these sequences to their geographical distribution, environment and, eventually, host (Supplementary Table 1). This immediately revealed that *Ostreobium* is almost exclusively

associated with coral reefs: we found no association with shells as it has previously been described (Kornmann and Sahling, 1980), and the two sequences that are not associated to corals were obtained from reef-dwelling sponges. Even more interesting, however, is that the three well-supported *Ostreobium* clades show a complex biogeographical distributed across different reefs (Figure 1b). *Ostreobium* clade 1 is most common globally, but is relatively rare in the Caribbean. In contrast, clades 2 and 3 are more common in the Caribbean, present in the South Atlantic, and rare elsewhere. Interestingly, the distribution of *Ostreobium* clade 1 overlaps with *Symbiodinium* C-dominated regions, whereas *Ostreobium* clade 2 and 3 overlap with *Symbiodinium* A and B-dominated regions. Of course, these distribution patterns represent dominance and not simple presence and absence, and other local aspects of distribution (such as depth, latitude, and host-associations) make the patterns more complex for *Symbiodinium* (Baker, 2003) and probably also *Ostreobium*. But the apparent degree

of overlap in biogeographical distributions between these two unrelated symbionts suggests a possible correlation, which would require detailed analysis based on additional data specifically testing the detailed distribution of *Ostreobium* in nature. Finally, we investigated the distribution of *Ostreobium* clades among coral host species (Figure 1c), and observed *Ostreobium* clade 1 to dominate in Acroporidae, and *Ostreobium* clade 3 to be virtually absent from complex corals. Only *Ostreobium* clade 2 was found in octocorals, but from a single data set. The number of analyzed host in the different areas is limited so there is the possibility that this distribution might be affected by the general biogeographic patterns.

Our observations regarding the environmental diversity and distribution of *Ostreobium* indicate first of all that *Ostreobium* is closely associated to corals and not widespread in other environment that has been sampled. Second, the distribution of *Ostreobium* suggests that it shares similar biogeographical patterns as *Symbiodinium*, and that distinct subclasses have host species preference. We hypothesize that *Ostreobium* might have co-evolved with both coral and *Symbiodinium*, being a stable member of the holobiont through the ages. Further analyses are needed to improve our knowledge about the biogeographical and host distribution of *Ostreobium* if we are to test this, and to determine the nature of the relationship of its relationship with *Symbiodinium*.

Conflict of Interest

The authors declare no conflict of interest.

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