

COMMENT

PHYLOGENETIC ANALYSIS OF A FREE-LIVING STRAIN OF *SYMBIODINIUM* ISOLATED FROM JIAOZHOU BAY, P.R. CHINA¹

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Recently, the isolation of a free-living strain of symbiotic dinoflagellate belonging to the genus *Symbiodinium* was reported. Although the specimen procured and characterization from Jiaozhou Bay, P.R. China is a *Symbiodinium* spp., the manner in which this isolate was classified is inconsistent with the currently used and accepted *Symbiodinium* cladal nomenclature. To avert unnecessary confusion in the field, I place this important scientific contribution into the proper context and state of *Symbiodinium* research.

Key index words: cnidarian; phylogeny; *Symbiodinium*; symbiosis; zooxanthellae

I was very pleased to see the report of Gou et al. (2003) on the isolation and characterization of a free-living strain of symbiotic dinoflagellate belonging to the genus *Symbiodinium*. Although healthy *Symbiodinium* cells are continuously expelled into the environment by a variety of invertebrates (Steele 1977, Stimson and Kinzie 1991, Maruyama and Heslinga 1997) and host predators (Augustine and Muller-Parker 1998, Seavy and Muller-Parker 2002), few have been observed or cultured from field-collected water samples (Loeblich and Sherley 1979, Chang 1983, Carlos et al. 1999). Clearly, the specimen procured from Jiaozhou Bay, P.R. China by Gou et al. (2003) is a *Symbiodinium* spp. On the other hand, I do believe that there were serious omissions and interpretation problems in this study. These issues concern *Symbiodinium* classification and need to be corrected to avoid undue confusion.

The systematics of *Symbiodinium*, like other dinoflagellates, has historically relied on morphological characters (Trench and Blank 1987). However, the fact that phylogenies based on such characters do not necessarily delineate related assemblages (Wilcox 1998) demonstrates their shortcomings. Rowan and Powers (1991), using sequence data of nuclear small subunit rDNAs, provided the first evolutionary classification of *Symbiodinium*. They subdivided divergent lineages into several major groups or clades, designat-

ated A through C. Initially, field-collected populations were rapidly analyzed and classified to a particular clade via RFLPs of small subunit rDNAs. This system was enthusiastically adopted by the scientific community and subsequently refined to include the sequencing and fragment analyses of other rDNA genes and molecules (Baker 2003). Currently, algal populations and cultures are typically placed into one of seven clades (i.e. *Symbiodinium* clades A, B, C [Rowan and Powers 1991], D [Carlos et al. 1999], E [*Symbiodinium californicum*; LaJeunesse and Trench 2000, LaJeunesse 2001], F [LaJeunesse 2001], and G [Pawlowski et al. 2001, Pochon et al. 2001]) (Fig. 1), with each clade probably comprised of many species (Rowan 1998). Gou et al. (2003) applied this framework to classify the sequences used in their phylogenetic reconstruction (Gou et al. 2003, fig. 1); from this analysis, the authors state that their free-living strain is a member of a “novel” clade. Unfortunately, this conclusion is founded on an inaccurate portrayal of the current *Symbiodinium* cladal nomenclature and, in fact, disregards some of the most relevant published literature.

The crux of the problem resides in the manner that Gou et al. (2003) uses the *Symbiodinium* cladal designations and how particular strains are assigned to these groups. For example, in figure 1 in Gou et al. (2003), three sequences (accession numbers AF238261–AF238263; please note that the host names given in their figure 1 for these accession numbers are incorrect and should read *Montastrea faveolata*, *M. franksi*, and *Siderastrea siderea*, respectively) are said to belong to *Symbiodinium* clade E. These sequences, originally posted to GenBank by Toller et al. (2001) as members of *Symbiodinium* clade E, were subsequently recognized as members of *Symbiodinium* clade D by a number of independent laboratories and studies since 2001 (Baker 2003). Thus, sequences AF238261–AF238263 should be grouped with the unusual symbiont from the sponge *Haliclona koremella* (AB016578; please note that this host name is incorrectly assigned to accession number AJ271766 within *Symbiodinium* clade C; the host name for AJ271766 should be *Fungia scutaria* [Darius et al. 2000]) under the banner *Symbiodinium* clade D.

The designation *Symbiodinium* clade E has been used by LaJeunesse (2001) to describe the symbionts found

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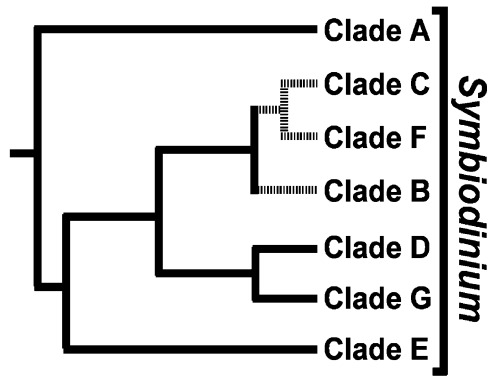


FIG. 1. Phylogeny of the seven recognized clades of symbiotic dinoflagellates in the genus *Symbiodinium*. The phylogeny is presented as a consensus cladogram synthesized from studies using nuclear (small subunit, 5.8S, large subunit) and chloroplast (cp23S) rDNAs to infer relationships among the clades. The relative positions of and relationships between *Symbiodinium* clades B, C, and F are variable depending on the molecule analyzed and the phylogenetic method used; the dashed lines comprising the branches leading to these clades represent this uncertainty. Phylogenies inferred from protein-encoding genes of the *Symbiodinium* chloroplast (*psbA*, Takishita et al. 2003) and mitochondrial (*cox1*, Takabayashi et al. 2004) genomes are congruent with the relationships recovered from the various rDNAs and provide further confidence in the phylogenetic inferences and molecular taxonomy of the clades. A comprehensive review of the history and current state of *Symbiodinium* phylogenetics and systematics is presented in Baker (2003).

in some populations of the temperate anemone *Anthopleura elegantissima* and a *Symbiodinium* culture (informally described as *S. californium* by Banaszak et al. 1993) isolated from these same cnidarians (LaJeunesse and Trench 2000). In addition, dinoflagellate culture CCMP 421, which can be obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), was thought to be an isolate of *Gymnodinium varians* when it was originally cultured from a water sample taken in Wellington Harbor, New Zealand (Chang 1983). However, phylogenetic studies based on various rDNAs have placed this isolate in the genus *Symbiodinium* (Rowan and Powers 1992, Wilcox 1998, Saldarriaga et al. 2001) and, more specifically, within *Symbiodinium* clade E (LaJeunesse 2001, Pochon et al. 2001, Santos et al. 2002). As depicted in figure 1 in Gou et al. (2003), their free-living strain (AY1-60124), along with the two *Symbiodinium* clade E isolates mentioned above, form a monophyletic lineage with 100% bootstrap support. Given this, there is no doubt that the Gou et al. (2003) free-living strain belongs to *Symbiodinium* clade E and is not a candidate for a “novel” *Symbiodinium* clade F.

In their report, Gou et al. (2003) proposed *Symbiodinium* clade F as a “novel” clade. Because the Gou et al. (2003) clade F is actually comprised of members of the previously established *Symbiodinium* clade E, the proposal made by these authors is invalid. Along with this, *Symbiodinium* clade F is not novel as stated by Gou et al. (2003); this cladal designation has seen extensive usage in categorizing the *Symbiodinium* cultures isolated

from some cnidarian hosts (LaJeunesse 2001, Santos et al. 2002), the *Symbiodinium* populations of the scleractinian *Alveopora japonica* (Rodriguez-Lanetty et al. 2003), and the symbionts of foraminiferans (Pawlowski et al. 2001, Pochon et al. 2001). In fact, cultures from *Montipora verrucosa*, such as CS-156 (AB016594), have been characterized as members of *Symbiodinium* clade F (LaJeunesse 2001, Santos et al. 2002) and should have been indicated as such in figure 1 in Gou et al. (2003).

In conclusion, the isolation and characterization of a free-living strain of *Symbiodinium* by Gou et al. (2003) is an important scientific contribution. To maximize the impact of this work, however, it should be placed into the proper context and state of *Symbiodinium* research. I hope this comment has accomplished that and averted unnecessary confusion in the field of *Symbiodinium* nomenclature.

I thank A. C. Baker, M. A. Coffroth, R. A. Kinzie III, T. C. LaJeunesse, X. Pochon, and M. Rodriguez-Lanetty for their support in preparing this comment.

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