The ecology and evolution of symbiotic dinoflagellates (*Symbiodinium*, Dinophyta) on tropical coral reefs

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Sessile invertebrates

Symbiotic dinoflagellates
(*Symbiodinium*)
Symbiosis is nutritional in nature

Importance of Coral Reefs

- Biodiversity hotspot
- Economic service
- Environmental service
- Educational service
Coral Bleaching

The history of *Symbiodinium* diversity

- Once thought to be a single pandemic species
- A variety of techniques and experimental approaches have demonstrated that zooxanthellae are a heterogeneous group of many strains and species organized into clades
The current state of 
Symbiodinium diversity

Specificity and Flexibility of 
Symbiodinium and their host

- Scleractinian corals: Clades A, B, C, D, and F.
- Soritid foraminifera: Clades C, F, and G.
- Sea anemones: Clades A, B, C, D, and E.
- Zoanthids: Clades A, B, C, D.
- Milleporina fire corals: Clades A, B, C.
- Tridacnid clams: Clades A, C.
High specificity in invertebrate-\textit{Symbiodinium} associations

Have we developed a biased view of invertebrate-\textit{Symbiodinium} associations?
Symbiodinium and ribosomal genes

Fig. 3. The eukaryotic nuclear rDNA array. NTS: non-transcribed spacer; ETS: external transcribed spacer; ITS: internal transcribed spacer.

Stat et al. (2006) PPEES

Internal transcribed spacer 2 (ITS2) structure in Symbiodinium

Fig. 3. Proposed consensus Symbiodinium internal transcribed spacer (ITS2) secondary structure. Lowercase letters (a–f) denote single-stranded regions within the structure. Roman numerals (I–IV) represent helices. Details for each region within and among the Symbiodinium clades are summarized in Table 1.

Hunter et al. (2007) J Phycol
Conserved processing sites in the ITS2 of *Symbiodinium*

![Diagram](image)

**Fig. 3.** Conserved nucleotide tract identified from *Symbiodinium* internal transcribed spacer (ITS2) sequences. The tract is located 3’ of the apical loop, and approximately at the base of stem 11. Marked positions are as follows: *****100%** nucleotide conservation at a position: **11** of 12 sequences (>90%) share identical nucleotide at a position: <**30%** of the 12 sequences possess one or two specific nucleotides at a position. *Symbiodinium* isolates are named as in Tables 1 and 2.

Hunter et al. (2007) *J Phycol*

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Internal transcribed spacer 2 (ITS2) evolution in *Symbiodinium*

![Diagram](image)

**Table 3.** Mutations in stem 119b of *Symbiodinium* clade G isolates 1582 and 1584 (Pochon et al. 2001).

<table>
<thead>
<tr>
<th>Position in ITS2 base</th>
<th>Isolate 1582</th>
<th>Isolate 1584</th>
<th>Pairs with nucleotide at position</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>U</td>
<td>A</td>
<td>132</td>
</tr>
<tr>
<td>130</td>
<td>C</td>
<td>A</td>
<td>128</td>
</tr>
<tr>
<td>130</td>
<td>U</td>
<td>A</td>
<td>N/A (occurs in loop)</td>
</tr>
<tr>
<td>140</td>
<td>U</td>
<td>C</td>
<td>N/A (occurs in loop)</td>
</tr>
<tr>
<td>142</td>
<td>G</td>
<td>A</td>
<td>150</td>
</tr>
<tr>
<td>150</td>
<td>G</td>
<td>U</td>
<td>112</td>
</tr>
<tr>
<td>150</td>
<td>G</td>
<td>U</td>
<td>111</td>
</tr>
<tr>
<td>150</td>
<td>U</td>
<td>C</td>
<td>108</td>
</tr>
</tbody>
</table>

Compensatory mutations that preserve base pairing in stems are highlighted in bold. See Fig. 2 for location of mutations in the context of the secondary structure for stem 119b.

**Fig. 4.** Variation in the secondary structure of *Symbiodinium* internal transcribed spacer (ITS2) and its relationship with the clade phylogeny for the genus. Hatched branches leading to *Symbiodinium* clades B, C, E, and I indicate these clades as representing the transition model of ITS2 secondary structure. The phylogenetic relationships between the major clades of *Symbiodinium* are represented as a consensus cladogram from various studies and molecules (see Colwell and Hartman 2003 for additional details).

Hunter et al. (2007) *J Phycol*
**Symbiodinium** and ribosomal genes

Fig. 3. The eukaryotic nuclear rDNA array. NTS: non-transcribed spacer; ETS: external transcribed spacer; ITS: internal transcribed spacer.

Stat et al. (2006) PPEES

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Measuring ITS2 intragenomic diversity in *Symbiodinium*

Table 1. Summary of bacterially cloned *Symbiodinium* internal transcribed spacer (ITS) sequences derived from five clonal isolates in clades A to E. Unconnected genetic distances (a) are presented for each region of the ITS.

<table>
<thead>
<tr>
<th>ITS2 Type</th>
<th>Culture</th>
<th>No. of clones</th>
<th>No. of unique classes</th>
<th>% of clones identical to direct sequencing of PCR product</th>
<th>ITS1 p</th>
<th>ITS1-rDNA p</th>
<th>ITS2 p</th>
<th>Total p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>77</td>
<td>22</td>
<td>7</td>
<td>72.7%</td>
<td>0.0093</td>
<td>0.0156</td>
<td>0.0306</td>
<td>0.0099</td>
</tr>
<tr>
<td>B1</td>
<td>13</td>
<td>22</td>
<td>13</td>
<td>40.5%</td>
<td>0.0099</td>
<td>0.0126</td>
<td>0.0121</td>
<td>0.0056</td>
</tr>
<tr>
<td>C1</td>
<td>152</td>
<td>22</td>
<td>18</td>
<td>18.2%</td>
<td>0.0124</td>
<td>0.0189</td>
<td>0.0206</td>
<td>0.0112</td>
</tr>
<tr>
<td>D1</td>
<td>A951</td>
<td>15</td>
<td>13</td>
<td>22.2%</td>
<td>0.0090</td>
<td>0.0063</td>
<td>0.0166</td>
<td>0.0052</td>
</tr>
<tr>
<td>E2</td>
<td>CCMP 421</td>
<td>24</td>
<td>18</td>
<td>29.2%</td>
<td>0.0301</td>
<td>0.0126</td>
<td>0.0126</td>
<td>0.0066</td>
</tr>
</tbody>
</table>

Thornhill et al. (2007) Mol Ecol
Identification and exclusion of potential ITS2 pseudogenes in *Symbiodinium*

C15

Reexamination of ITS2 diversity from *Symbiodinium* populations *in hospite*
Intragenomic variation, pseudogenes and PCR artifacts can confound biodiversity estimates in eukaryotic microbial systems.

Table 2. Summary of secondary structural disruptions and PCR-chimeras identified from bacterial internal transcribed spacer (ITS) sequences. Sequences were derived from either five cloned isolates or from populations of Hawaiian coral in the genus Porites.

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>ITS2 type determined by DGGE</th>
<th>Total no. of clones</th>
<th>No. of unique clones with ITS2 disruptions</th>
<th>No. of unique clones with ITS2 disruptions</th>
<th>No. of unique PCR-chimeras</th>
<th>Total p between clones without identifiable disruptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 77</td>
<td>A0</td>
<td>22</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Culture 13</td>
<td>B1</td>
<td>22</td>
<td>13</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Culture 152</td>
<td>C1</td>
<td>22</td>
<td>18</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Culture 103</td>
<td>D1a</td>
<td>18</td>
<td>13</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Culture CCMP 421</td>
<td>E2</td>
<td>24</td>
<td>18</td>
<td>3</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Porites spp.</td>
<td>C15</td>
<td>66</td>
<td>241</td>
<td>20</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

*Apprill & Gates (2007); Lahannese et al. (2004a). Includes 23 novel ITS2 clones reported by Apprill & Gates (2007) and 'type' C15, previously reported by Lahannese et al. (2004a).