

1 **Supplementary Methods and Materials**

2 **Coral 18S rRNA gene cloning, sequencing and phylogeny analysis**

3 Briefly, the 18S rRNA gene was PCR amplified with the primers 18S1
4 (5'-AACCTGGTTGATCCTGCCA-3') and 18S2
5 (5'-TGCAGGTTACCTACAGAA-3'), as described by Borchiellini et al. (1). The
6 PCR products (~ 1.8k bp) were then purified and ligated into a pCR2.1 TOPO vector
7 (Invitrogen, USA) according to the manufacturer's protocol. Sequences for both
8 directions of the insert were obtained using an ABI sequencing machine (ABI3730).
9 Multi-alignment between the sequences and references was conducted with the
10 ClustalW program, and a bootstrap phylogenetic tree (Fig. S1) was then generated
11 with the MEGA software using the neighbor-joining method (2). The 18S rRNA
12 sequences were deposited into GenBank with the accession number
13 HM067604-HM067613.

14 **TRFLP analysis of the microbial communities**

15 The primers 26F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1055R
16 (5'-CACGAGCTGACGACAGCCAT-3'), corresponding to *Escherichia coli* 16S
17 rRNA gene positions 26-45 and 1055-1074, respectively, were used for the PCR
18 amplification. Amplification was conducted with a Bio-Rad thermal cycler using the
19 following cycling conditions: initial denaturation at 94°C for 5 min; 26 cycles of 94°C

20 for 45 sec, 55°C for 45 sec, and 72°C for 1 min; and final extension for 6 min at 72°C.

21 About 500 ng of PCR products were digested with 10 U of the restriction enzyme

22 *Msp* I at 37°C for 6 h, ethanol was precipitated and re-dissolved in 10 µl of double

23 distilled water (ddH₂O). Ten microliters of purified products were mixed with 0.5 µl

24 of an internal size standard (ET-550R; Amersham Biosciences, NJ, USA), denatured

25 at 95°C for 2 min, snap cooled on ice, and subjected to a MegaBACE genetic analyzer

26 (Amersham Biosciences, NJ, USA) operating in the genotyping mode to generate the

27 chromatography. After electrophoresis, the sizes of the fluorescently labeled terminal

28 restriction fragments (TRFs) were determined by comparison with the size standard

29 using a Genetic Profiler (Amersham Biosciences, NJ, USA). TRFs that were greater

30 than 35 and less than 900 bp were excluded from the statistical analysis to screen out

31 background noise and inaccurate size determination.

32 **References**

- 33 1. **Borchiellini C, Manuel M, Alivon E, Boury-Esnault N, Vacelet V, Le Parco Y.**
- 34 2001. Sponge paraphyly and the origin of Metazoa. *J. Evol. Biol.* **14(1)**:171-179.
- 35 2. **Kumar S, Dudley J, Nei M, Tamura K.** 2008. MEGA: a biologist-centric
- 36 software for evolutionary analysis of DNA and protein sequences. *Briefings in*
- 37 *Bioinformatics* **9**:299-306.