PCR conditions, cloning and sequencing

***Calkinsia aureus* epibionts.** PCR amplification for the 8F/1492R primer pair and the Arch25F/1492R pair was: 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, 45°C for 1 minute, 72°C for 1.5 minutes, and a final cycle of 72°C for 7 minutes. For the 341F/1492R primer pair amplification was: 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 50°C for 1 minute, and 72°C for 2.5 minutes, and a final cycle of 72°C for 10 minutes. Amplified DNA was checked for quality by agarose gel electrophoresis, bands were gel purified using the Qiaquick Gel Extraction Kit (Qiagen), and cloned into the vector pCR4-TOPO using the TOPO TA Cloning Kit (Invitrogen) following the manufacturer's instructions. Plasmid DNA from 20 clones was prepared using a MWG Biotech RoboPrep2500, and inserts were sequenced bi-directionally using the universal M13 primers and an Applied Biosystems 3730XL capillary sequencer at the Keck Facility at the Josephine Bay Paul Center at the Marine Biological Laboratory (MBL), Woods Hole, MA.

***Bihospites bacati* epibionts.** PCR amplifications consisted of an initial denaturing period (95 °C for 3 minutes), 35 cycles of denaturing (93 °C for 45 seconds), annealing (5 cycles at 45°C and 30 cycles at 55 °C, for 45 seconds), extension (72 °C for 2 minutes), and a final extension period (72 °C for 5 minutes). The amplified DNA fragments were purified from agarose gels using UltraClean 15 DNA Purification Kit (MO Bio, CA, USA), and subsequently cloned into the TOPO TA Cloning Kit (Invitrogen, CA, USA).

CARD-FISH protocol

Catalyzed Reporter Deposition FISH (CARD-FISH) was performed by hand picking and rinsing individual cells in sterile seawater and fixing them in 2% (final concentration) paraformaldehyde for one hour, then rinsing them 3 times with 5 ml sterile phosphate buffered saline (PBS) by filtration onto a 0.2μm pore size, 25mm Isopore GTTP filter (Millipore, USA). After air-drying, the filters were overlaid with 37°C 0.2% (w/v) Metaphor agarose and filters were dried at 50°C. To inactivate endogenous peroxidases, filter sections were incubated in 10ml of 0.01 M HCl for 10 minutes at room temperature. Filters were washed in 50ml 1X PBS, then in 50 ml of distilled, deionized water (ddH_20). The epibiont cells were permeabilized by incubating the individual filter pieces in 2.0 ml Eppendorf microfuge tubes for 60 minutes at 37°C in a lysozyme solution (0.05 M EDTA, pH 8.0; 0.1 M Tris HCL, pH 8.0; 10 mg/ml lysozyme). The filters were washed in 50 ml ddH_20 for 2 minutes, followed by 50 ml of absolute ethanol (96%) and air-dried. Hybridization buffer and probe were mixed 300:1 in 2.0 ml Eppendorf tubes (probe at 50ng/microliter). For 50 ml of hybridization buffer we mixed 3.6 ml 5 M NaCl, 0.4 ml 1 M Tris HCl and ddH_20 depending on formamide concentration for each probe used (see Table 1). Two grams of dextran sulfate were added and the mixture heated (40-60°C) and shaken until the dextran sulfate was dissolved. After cooling, formamide was added (% formamide noted for each probe used in Table 1), 2.0 ml Blocking Reagent were added (50 ml of 100mM maleic acid in ddH_20 combined with 50 ml of 150mM NaCl and pH adjusted to 7.5 with NaOH, plus 10 g Roche Blocking Reagent (Roche Diagnostics GMbH, Germany)), and volume adjusted to 20 ml with ddH_20. Hybridization was performed at 46°C for 2 hours. Filters were washed for 5 minutes by placing them in 50ml tubes of wash buffer (0.5ml 0.5M EDTA, 1.0 ml 1M Tris HCl plus volume of 5M

NaCl depending on probe used (see Table 1) and ddH_20 to make 50 ml). After washing, filters were transferred to 50 ml 1X PBS (pH 7.6) for 15 minutes at room temperature. 1000 microliters of amplification buffer (4 ml 10X PBS, 16 ml 5M NaCl and sterile ddH_20 were mixed to a volume of 35 ml, then 4 g dextran sulfate (Sigma-Aldrich, USA) were added and mixture was heated to 40-60°C until dextran sulfate was dissolved. After cooling, 0.4 ml Blocking Reagent (see above) was added and water to a final volume of 40 ml, and the solution was filtered through a 0.2µm filter unit. This solution was mixed with 10 µl of 100X H_2O_2 stock (199 µl of 1X PBS plus 1 µl 30% H_2O_2). Filter pieces were transferred to 2.0 ml Eppendorf tubes containing amplification buffer plus 2 µl of fluorescently labeled tyramide (Alexa488-labeled from Biomers.net GmbH, Germany) and incubated at 37°C for 15 minutes in the dark on a rotary shaker. Filter pieces were washed in 50 ml 1X PBS for 15 minutes at room temperature, then 50 ml ddH_20, followed by 96% ethanol, and air-dried, all in the dark. Filters were mounted in Citifluor/Vectashield mounting solution (5.5 parts Citifluor, 1 part Vectashield, 0.5 parts 1X PBS) with 1µg/ml final concentration of DAPI, and stored at -20°C until microscopy was performed.