Supplementary Material

Molecular genetic biodiversity assessment of the Wallis Island sponge fauna in the Tropical Pacific

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This file contains:

- Supplementary Methods S1: Details on morphological identification

Supplementary Methods S1: Detailed description of the procedures for the initial morphological identification of collected sponge specimens.

Morphological Species Identification

Specimens collected for morphological analyses in the Queensland Museum and subsequent molecular analyses were immediately stored in 70% ethanol after collection for preservation. Tissue preparations for light microscopy were made by cutting thin sections through the ectosome and choanosome with a scalpel. These sections were then cleared in Histoclear (National Diagnostics, Atlanta, USA) for 12 h, dried, mounted on a microscope slide, and fixed in Histomount (National Diagnostics, Atlanta, USA). Spicules and fibers were also prepared by dissolving the tissue in 12.5% sodium hypochlorite to remove the soft tissue, monitoring the tissue as it dissolves, assisted by dexterous use of forceps to mediate the removal of collagen, neutralizing the reaction with distilled water and then rinsing twice in 70% ethanol and then 100% ethanol.

Fibers were examined using an Olympus SZ60 dissection microscope with a Tucsen 3.0 camera. The spicules were air dried and mounted on microscope slides and fixed with Histomount. The fixed microscope slides of the sections and spicules were examined using an Olympus BH2 with an optical stage micrometer and photographed with a Nikon CoolPix 5400 mounted camera. Twenty five random spicules of each type were measured with maximum, mean and minimum calculated from those random spicules. Preserved specimens were photographed with a Canon G5X. Surface characteristics were examined using an Olympus SZ60 dissection microscope with a Tucsen 3.0 camera. The majority of Queensland Museum underwater photographs were captured using a Canon G10 or G5X.

Scanning Electron Microscope (SEM) spicule preparations were made by dissolving the tissue in 12.5% sodium hypochlorite to remove the soft tissue, and neutralized in distilled water, rinsed twice in 70% ethanol and then finally rinsed twice in 98% ethanol and then air-dried. SEM preparations were sputter coated in gold to improve resolution. The scanning electron micrograph photos and measurements were made using a Hitachi TM-1000 SEM. Sponges were classified using morphological characters to genus and species level where possible and assigned an OTU matching with corresponding species at the Queensland Museum.

Additional pictures of specimens (in-situ or ex-situ), tissue sections, and spicules are available in the Sponge Barcoding Database (accession numbers SBD#2566 - 2878).

The World Porifera Database (de Voogd et al. 2023) was used as the systematic index.