**Supplemental File 1**

**Mass Spectrometry Methods**

LC-MS/MS was performed with an Ultimate 3000 RSLC-nano (U3000) HPLC system coupled to the Nanospray Flex Ion Source of an Orbitrap Fusion Lumos Tribrid mass spectrometer (ThermoScientific, Waltham, MA) as previously detailed (Dean et al., 2020). The U3000 system was configured for one-dimensional nanoflow separations with on-line desalting. By way of loading pump flow of 2% solvent B (0.1% formic acid in acetonitrile) at 5 µL/min, the autosampler injected and concentrated peptide sample onto a trap column (PepMap 100, C18, 300 µm ID x 5 mm, 5 µm, 100 Å). After desalting for 3 minutes, the flow was diverted in-line at 300 nL/min for separation across a reverse phase analytical column (Acclaim PepMap RSLC, 75 µm ID x 150 mm, C18, 2 µm, 100 Å) for a total duration of 120 min. A two-step gradient of increasing solvent B (18% over first 80 min, followed by an increase of 60% over 15 min). Nanoelectrospray voltage was applied via the ion source with a stainless-steel emitter tip.

Mass spectrometry data were recorded on the Orbitrap Fusion Lumos Tribrid in data dependent mode with dynamic exclusion enabled. XCalibur (v4.2) was used to acquire profile (or untargeted) measurements and targeted measurements for observed mass-to-charge (m/z) ratios. Common settings for both methods are as follows: 20 scans per cycle were performed with a survey scan range of 400-1,600 Da using the Orbitrap detector (resolution 120K) for MS1 scans; the maximum injection time was set to 100 ms and the automatic gain control (AGC) target was 1.06; the most intense ions with charges of 2-5 were fragmented using assisted HCD (higher-energy collisional dissociation; 15%, 30%, and 45%); ions were excluded for 15 sec from subsequent MS/MS submission after 1 time with a +50 ppm error tolerance; fragment ions were measured in the Orbitrap detector (60,000 resolution) for a maximum injection time of 200 milliseconds, AGC target of 1.06; EASY-IC was enabled for data-dependent MS and MS/MS scans. For the untargeted fragmentation method, the intensity threshold was set to 5.05 to trigger MS/MS. For the targeted method, XCalibur was programmed to fragment ions with the precursor molecular weight of 30 peptides from AaCP19-1 (n=16) and AaCP43-1 (n=14) with a charge range of 2-5 (**Supplemental Table 1**) with a mass tolerance of +50 ppm. The intensity threshold was lowered to 5.04 to trigger MS/MS.

Peptide-spectrum matching (PSM) of MS/MS spectra to *in silico* predicted proteins was conducted with Mascot (v. 2.6.2, Matrix Science, LTD, London, UK) and Scaffold (v. 4.8.9, Proteome Software, Portland, OR). Primary data was converted to Mascot generic-formatted (MGF) peak lists by ProteoWizard (msconvert version 3.0.19070) via automated scripting (Hervey IV et al., 2009; Tackett et al., 2005). A restricted, manually-curated list of 106 predicted barnacle protein sequences based on transcriptomic data from sub-mantle tissue (Wang et al., 2015) was used as the primary sequence database reference, as previously described (So et al., 2016; So et al., 2017; Wang et al., 2018). Only proteins previously identified in *A. amphitrite adhesive* (Schultzhaus et al., 2019) and several other likely highly abundant proteins (i.e., actin, collagen, vitellogenin) were included in this reference as barnacle proteins. These sequences were concatenated with an in-house list of 191 contaminant, mass standard, and reagent peptide sequences (eg. trypsin, keratins, etc.) for a total of 296 sequences. Database search parameters included variable modifications previously described (Schultzhaus et al., 2019), <3 missed tryptic cleavage sites per peptide, and precursor and fragment ion tolerances set to +100 ppm and +0.6 Da, respectively. Mascot PSM results were imported into Scaffold for downstream analysis and manual validation.