

Supplementary Material for:

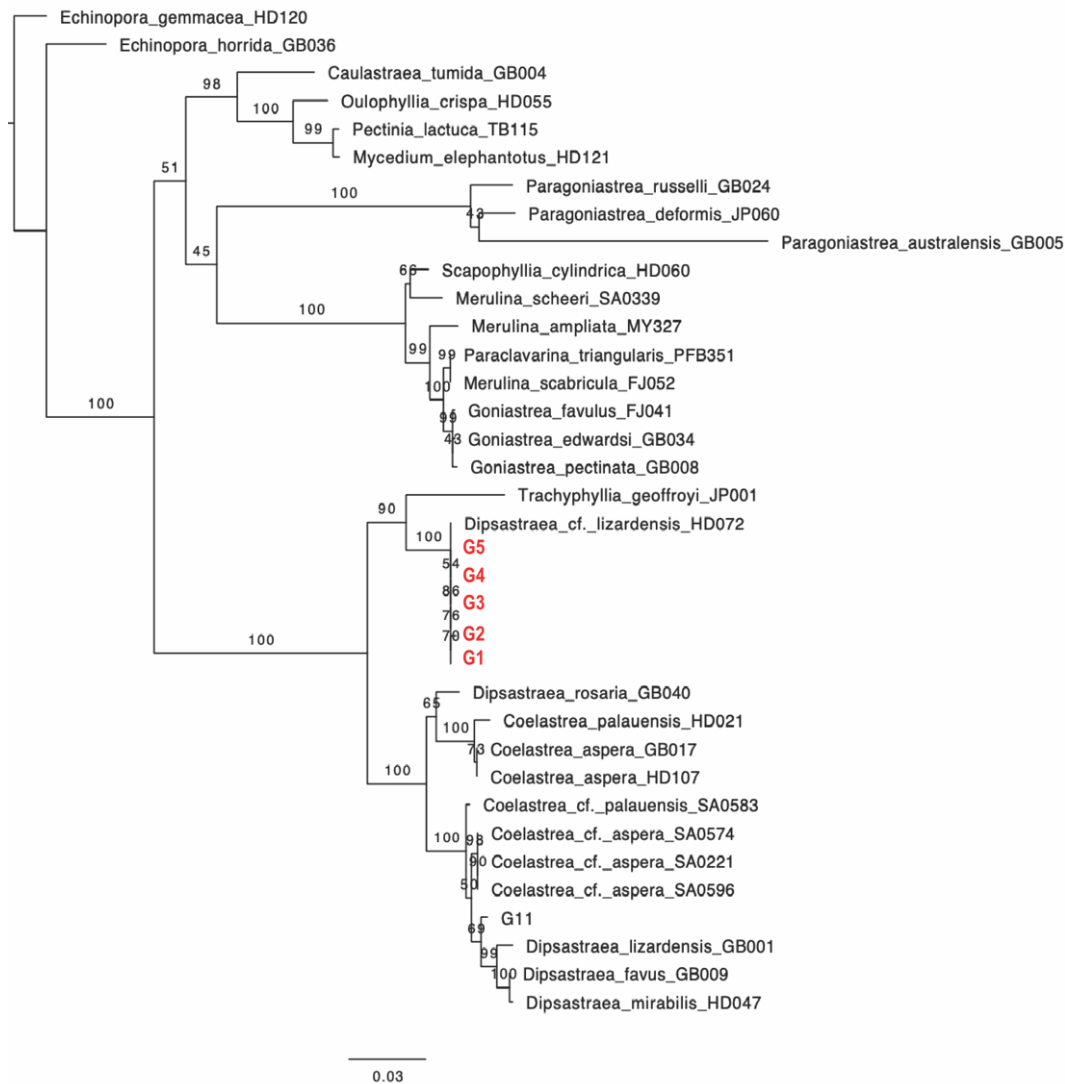
Emersion-Associated Responses of an Intertidal Coral and Its Suitability for Transplantation to Ecologically Engineered Seawalls

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1. Species identification

Genomic DNA (gDNA) from six *Dipsastraea* samples were extracted using ReliaPrep™ gDNA tissue miniprep (Promega, Wisconsin, USA) following the manufacturer's protocol. Polymerase chain reaction (PCR), Labcycler Gradient and Thermoblock 96, Sensoquest, Germany) was performed to amplify the mitochondrial intergenic region (MNC1f: 5'-GAGCTGGGCTTCTTTAGAGTG-3', MNC1r: 5'-GTGAGACTCGAACTCAC-TTTTC-3') [44,77]. Each PCR reaction (25.0 µl volume) consisted of 12.5 µl of GoTaq Green Master Mix (Promega, Wisconsin, USA), 1.0 µl of each forward and reverse primer (10 µM), 8.5 µl of water and 2.0 µl of diluted gDNA. PCR reaction steps were (1) denaturation: 95 °C 45 s (2) annealing: start at 55 °C, increasing in increments of 1 °C to annealing temperature (60 °C) for 45 s (3) extension: 72 °C for 5 min, repeated for 30 cycles. Gel electrophoresis was performed to verify the amplification. Amplified products were purified using SureClean Plus (Bioline Inc., London, UK) according to the manufacturer's protocol. Sequencing of the purified DNA products was performed with the dye terminator method on an Applied Biosystems 3130XL Genetic Analyzer.

DNA sequences obtained here were combined with those in [44] that have been reduced to species level (see [78]) and compiled in Mesquite 3.6 [79]. Alignments were carried out using the E-INS-i option under default parameters in MAFFT 7.205 [80–82]. The maximum likelihood phylogeny under the GTRGAMMA model was inferred using RAxML 8.0.9 [83,84] and 100 random starting trees, with 1000 bootstrap replicates.



Supplementary Figure S1. Maximum likelihood tree of coral species under the family Merulinidae based on the mitochondrial intergenic region (iGR), showing five tagged colonies (G1-G5, in red) clustered under *Dipsastraea cf. lizardensis*.

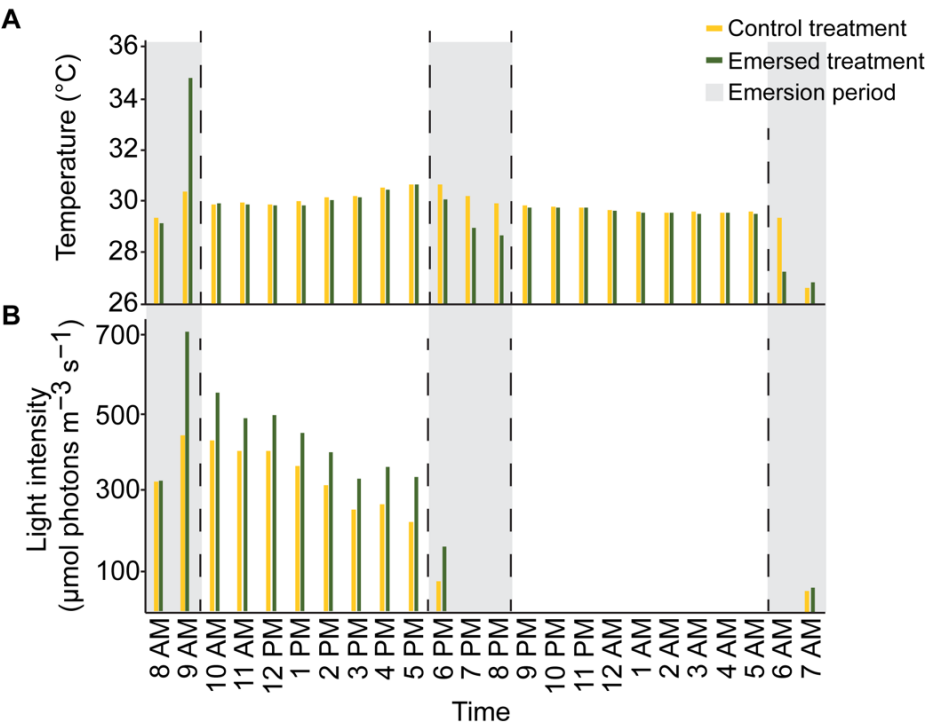
2. Genotyping

Genomic DNA (gDNA) from five confirmed *Dipsastraea cf. lizardensis* colonies (i.e., G1-G5, Supplementary Figure S1) were extracted using Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. The internal transcribed spacer 2 (*ITS2*) was amplified using coral-specific (forward) primer A18S: 5' – GATCGAACGGTTTAGTGGG – 3' [85] and universal (reverse) primer "ITS-4: 5' – TCCTCCGCT TATTGATATGC – 3' [86]. Downstream processes (i.e., gel electrophoresis, amplicon cleanup and Sanger sequencing) followed the same procedures described above. Sequences were aligned and pairwise distances of the samples were obtained using the Geneious software package (<http://www.geneious.com> (accessed on 1 October 2021)) to determine that distinct genotypes were present.

Out of the five colonies, only G1, G3 and G4 were successfully sequenced, and these were found to be genetically distinct. The average pairwise similarity between the three genotypes was 97.87 ± 0.41 %. (Supplementary Table S1). The sequence qualities for G2 and G5 were poor (~20%) and thus uninformative.

Supplementary Table S1. Genetic identity matches between pairs of genotypes.

| | G1 | G3 | G4 |
|----|--------|--------|--------|
| G1 | - | 97.54% | 97.39% |
| G3 | 97.54% | - | 98.69% |
| G4 | 97.39% | 98.69% | - |



Supplementary Figure S2. Temperature (A), light intensity (B) and estimated emersion period profile.

References

44. Huang, D.; Licuanan, W.Y.; Baird, A.H.; Fukami, H. Cleaning up the 'Bigmessidae': Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. *BMC Evol. Biol.* **2011**, *11*, 37, <https://doi.org/10.1186/1471-2148-11-37>.

77. Fukami, H.; Budd, A.F.; Levitan, D.R.; Jara, J.; Kersanach, R.; Knowlton, N. Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. *Evolution* **2004**, *58*: 324–337. <https://doi.org/10.1111/j.0014-3820.2004.tb01648.x>.

78. Huang, D.; Benzoni, F.; Arrigoni, R.; Baird, A.H.; Berumen, M.L.; Bouwmeester, J.; Chou, L.M.; Fukami, H.; Licuanan, W.Y.; Lovell, E.R.; et al. Towards a phylogenetic classification of reef corals: the Indo-Pacific genera *Merulina*, *Goniastrea* and *Scapophyllia* (Scleractinia, Merulinidae). *Zool. Scr.* **2014**, *43*, 531–548. <https://doi.org/10.1111/zsc.12061>.

79. Maddison, W.P.; Maddison, D.R. Mesquite: A Modular System for Evolutionary Analysis, version 3.7. Available online: <http://mesquiteproject.org> (accessed on 5 October 2021).

80. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. <https://doi.org/10.1093/molbev/mst010>.

81. Katoh, K.; Asimenos, G.; Toh, H. Multiple alignment of DNA sequences with MAFFT. *Methods Mol. Biol.* **2009**, *537*, 39–64. https://doi.org/10.1007/978-1-59745-251-9_3.

82. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. <https://doi.org/10.1093/nar/gkf436>.

83. Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *J. Bioinform.* **2006**, *22*, 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>.

84. Stamatakis, A.; Hoover, P.; Rougemont, J. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **2008**, *57*, 758–771. <https://doi.org/10.1080/10635150802429642>.

85. Takabayashi, M.; Carter, D.; Ward, S.; Hoegh-Guldberg, O. Inter- and Intra-Specific Variability in Ribosomal DNA Sequence in the Internal Transcribed Spacer Region of Corals. In Proceedings of the Australian Coral Reef Society 75th Anniversary Conference, Heron Island, Australia, 2–6 October 1997; pp.241–248.

86. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications*; Academic Press: Cambridge, MA, USA, 1990; 38, pp. 315–322. Available online: <http://pdf.xuebalib.com:1262/3x0d5gC6z4eF.pdf> (accessed on 7 October 2021).