**Reproductive isolation and evolution of the gamete recognition proteins bindin and speract in sympatric species of *Echinothrix* from the Indo-Pacific**

Supervisor: [Dr Simon Coppard](https://www.queensu.ca/bisc/academics/teaching-and-learning/faculty/faculty-coppard), Lecturer in Biology & Science Coordinator.

**Background**

Understanding the mechanisms by which sister-species maintain their integrity and avoid hybridization is a primary goal of evolutionary biology. *Echinothrix* *calamaris* (Pallas, 1774) has long been considered a single trans Indo-Pacific species, but recent research by Coppard *et al.* (2021) has shown that *E. calamaris* contains three discrete species (see Fig. 1), one endemic to the Red Sea and the Gulf of Oman, a second species distributed from Malaysia in the West to Moorea in the East and a third species distributed across the entire Indo-Pacific biogeographic region. Two species therefore have widely overlapping distributions, so what prevents hybridization and genetic introgression among these broadcast spawners? Both species are living side-by-side on coral reefs with no spatial isolation (Coppard & Campbell, 2005a), their genetic divergence may well therefore be the result of reproductive incompatibility. In Fiji, where both species occur, both spawned around the time of the new moon (Coppard & Campbell, 2005b). Given the lack of temporal reproductive isolation or spatial isolation between species, possible barriers to genetic intermingling could be molecules involved in species-specific recognition of gametes. Specifically, these could proteins (speract) involved in sperm and egg attraction (Jagadeeshan *et al.* 2015) and sperm and egg fusion. Bindin is a sperm protein that mediates attachment and membrane fusion of gametes (Zigler, 2008). The mode of bindin evolution has been shown to vary across sea urchin genera studied to date depending on evolutionary drivers that prevent hybridizations.

**Significance**

The mechanisms by which species avoid hybridization is key to our understanding of evolution and speciation. Understanding levels of species interaction across the World’s oceans is of increasing importance for making informed conservation decisions in a changing world.

**Aim of Summer project.**

This investigation aims to amplify and sequence the genes of speract (sperm egg attraction) and bindin in the two newly identified species of *Echinothrix*. Primers will be designed to amplify these genes based on a transcriptome already sequenced by the supervisor. The mode of molecular evolution and levels of divergence will be assessed to determine the role of these genes in reproductive isolation. The aquarium trade will be used to acquire both species which are readily available. ATPase-6 and ATPase-8 will also be sequenced to act as a reference relative to the phylogeny of Coppard *et al.* (2021).

**Echinothrix-tree-talk.tif**

Fig. 1. Phylogeography of the *Echinothrix*. Clade 2 and Clade 3 of *E. calamaris* represent discrete species with sympatric distributions throughout the western and central Pacific Ocean.

**References**

**Coppard, S.E.**& Campbell, A.C.**(2005a).**Distribution and abundance of regular echinoids on two coral reefs in Fiji. Micronesica, 37 (2), 249-269.

**Coppard, S.E.** & Campbell, A.C. (2005b).Lunar periodicities of diadematid echinoids breeding in Fiji. *Coral Reefs*, 24, 224-332.

**Coppard, S.E.,** Jessop, H. & Lessios, H.A. (2021). Phylogeography, colouration, and cryptic speciation across the Indo-Pacific in the sea urchin genus *Echinothrix*. *Sci Rep* 11,16568 https://doi.org/10.1038/s41598-021-95872-0

Jagadeeshan, S., **Coppard S.E.,** & Lessios, H.A. (2015). Evolution of gamete attraction molecules: the sperm activating peptide and its receptor are neutrally evolving molecules in the pantropical sea urchin *Diadema*. *Evolution and Development* 17(1): 91-108.

Zigler, K.S. (2008). The evolution of sea urchin sperm bindin. *Int. J. Dev.* 52, 791−796. doi: 10.1387/ijdb.072521kz.

**Expected Outcomes.**

The student researcher will present their findings at an end of term research conference (either remotely or in person) at the BISC during the summer of 2022. A blog post will be written weekly to discuss how the project is proceeding. The major outcome of this project will be the submission of a peer reviewed publication after completion of the summer project. The student research will gain skills and expertise in designing primers for polymerase chain reaction (PCR), conducting DNA extractions, running PCRs, and analysing sequence data. They will learn how to align sequences using MacClade 4.08a and how to test for evidence of selection using PAML and HyPhy. This will include Fixed Effects Likelihood (FEL), Random Effects Likelihood (REL) and Fast Unconstrained Bayesian Approximate (FUBAR) methods to find codons with signatures of positive or negative selection. The Branch-site REL test will also be used to identify sites with evidence of pervasive positive selection using the Mixed Effects Model of Evolution (MEME).

The project supervisor has extensive experience in conducting this type of research. It should be noted that an investigation to obtain the data and preliminary analyses has already been carried by the supervisor. The chance of success by the student researcher is therefore high.

# Paper Co-Authored by Coppard Awarded Smithsonian Research Prize