

# THE ASSOCIATION BETWEEN THE SEA STAR SHRIMP *PERICLIMENES SOROR* AND ITS ASTEROID HOSTS: ASSESSING PREFERENCES IN HOST SPECIES AND FOOD CHOICE

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**Abstract.** *Periclimenes soror* is a Pontoniid shrimp that lives symbiotically with at least 16 species of sea star, including *Culcita novaeguineae* and *Linckia laevigata*. *Periclimenes soror* has been known to switch hosts intraspecifically, but whether it switches between host species is unknown. Feeding preferences of *P. soror* and their effects on hosts have also not been described. The present study aims to clarify these aspects of the association by addressing the following questions: (I) Does *P. soror* has a preference for host species? (II) What are *P. soror*'s feeding preferences? Host species preference was determined by giving shrimp a choice of hosts and recording settlement in several immediate and long-term experiments. *Periclimenes soror* was found to prefer *Culcita* over *Linckia*, indicating that interspecific host-switching is unlikely. Feeding preferences were determined by presenting shrimp with a choice between several food items and observing feeding behaviors. A survivorship experiment in different filtered seawater treatments determined that *P. soror* is a filter-feeding planktivore. An examination of appendage morphology identified several filter-feeding structures. It was determined that *P. soror* is a commensal associate of its hosts and demonstrates host species fidelity.

**Key words:** symbiosis; commensalism; host-switching; feeding preference; *Periclimenes soror*; *Culcita novaeguineae*; *Linckia laevigata*

## INTRODUCTION

Symbiotic relationships are one of the most common interspecific interactions in nature and have been successful strategies for many species (Roughgarden 1975, Bruce 1982, Criales 1984, Gherardi 1991, Rice and Miller 1991, Ates 2003, Buhl-Mortensen 2004, Marin et al. 2005, Hayes 2007, Leung and Poulin 2008, Horká 2016). A symbiosis is defined as an association between two species, where at least one is obligatorily dependent on the other during some stage of its life history (Leung and Poulin 2008). These associations can be parasitic, mutualistic, or commensal, depending on the effects of the relationship on the associates (Leung and Poulin 2008). Symbioses that benefit both participants are known as mutualisms, while parasitic relationships occur when one organism benefits to the detriment of the other (Roughgarden 1975). Commensal relationships benefit one associate without any noticeable effect on the other. However, these categorizations should be treated cautiously since the costs and benefits to the associates are often difficult to assess and may change throughout their life histories (Leung and Poulin 2008).

The evolution of a symbiotic lifestyle is one of the most important adaptations of marine crustaceans (Horká et al. 2016). Symbioses between crustaceans and echinoderms are especially common (Castro 1971, Criales 1984, Rice and Miller 1991, Hayes 2007). Many of these associations are thought to be commensal, with the larger echinoderm host sheltering the smaller crustacean symbiont without being affected by its presence (Castro 1971). Shrimps of the subfamily Pontoniinae almost always occur in permanent commensal associations with other macroinvertebrates, often with echinoderms (Bruce 1968). The genus *Periclimenes* is the only Pontoniid shrimp associated with multiple classes of host, including eleven species associated with crinoids, seven with echinoids, two with asteroids, two with holothurians, and one with ophiuroids (Bruce 1982). The sea star shrimp *Periclimenes soror* lives symbiotically with at least sixteen species of sea star that cover a diverse range of habitats (Bruce 1982, Crandall et al. 2008). *Culcita novaeguineae* and *Linckia laevigata* are hosts to *P. soror* and occur throughout the Indo-Pacific, where they live in shallow coral reefs (Crandall et al. 2008).

Populations of *P. soror* living symbiotically with *Culcita* change frequently as shrimp switch hosts throughout their life cycles (Gontang 1999, Olliff 2011). Colors of individual *P. soror* correlate with their hosts, but they can change color and do not show individual host preference or fidelity (Gontang 1999). Although conspecific host-switching occurs in *P. soror*, switching between species of host during an individual shrimp's life cycle has not yet been described. Host-switching has played a significant role in the diversification of Palaemonidae, a largely symbiotic family to which *P. soror* belongs and the most speciose family of Caridean shrimp (Horká 2016). Symbiotic species that associate with different host species and demonstrate host fidelity undergo limited gene flow and are effectively reproductively isolated (Futuyma and Moreno 1988). Sympatric speciation can occur in these species when conspecific subpopulations associate with preferred host species, which are different enough that these subpopulations develop host-associated adaptations (Feder et al. 1994, Dres and Mallet 2002).

*Periclimenes soror* is an obligate associate of its host, which may provide food in addition to protection from predators (Olliff 2011). Feeding preferences of *P. soror* and the effects of these on hosts are unknown. *Periclimenes soror* may feed on ectoparasites living on its host, but the surfaces of sea stars in tropical waters have been found to be free of fouling organisms (Guenther et al. 2007a, 2007b). An organism's feeding preferences can often be deduced by examining the morphology of its feeding structures (Bruce 1982, Olliff 2011). The Pontonine genus *Coralliocaris* beats its exopods through the water, which may enable it to feed on planktonic organisms (Bruce 1982). In another Pontonine species, *Levicaris mammillata*, a modified second maxilliped may allow it to scrape off and feed on its host's spines (Bruce 1982). The tissue and mucus covering of the host may be an important food source to *P. soror*, and one study found that the shrimp's stomach contents are similar in color to its host, suggesting that shrimp feed on host tissues (Horká 2016). *Periclimenes soror* may benefit its host by feeding on ectoparasites or harm its host by feeding on host tissues. Alternatively, the shrimp may feed on epibenthic felt or plankton and have no effect on its host. The effects of the shrimp's feeding preferences on its host, whether positive or negative, are unknown.

The present study was designed to clarify several aspects of the association between *Periclimenes soror* and its asteroid hosts. Two major questions were addressed. (I) Does *P. soror* prefer *Culcita novaeguineae* or *Linckia laevigata* as a host, and does inter-specific host-switching occur? Host species preference may reflect host quality, which could shed light on how shrimp benefit from the association. (II) What are the feeding preferences of *P. soror*, and how do they affect the host? Ascertaining *P. soror*'s feeding preferences would help determine whether *P. soror* is a mutualistic, parasitic, or commensal associate of its host.

## METHODS

### Lab population setup

Five *Culcita novaeguineae* and *Linckia laevigata* individuals and between 20 and 30 *Periclimenes soror* individuals were collected opportunistically from the reefs surrounding Cook's Bay on Moorea, French Polynesia (Fig. 1). The stars and shrimp were housed in a single large table aquarium of dimensions 2.45x1.05x0.17 meters divided into ten compartments. A star was kept in each compartment and separated by a mesh divider that allowed water flow but prevented shrimp from passing through. *Culcita* were fed with *Acropora* corals and *Linckia* were fed with rocks covered in algae and epibenthic felt. An additional two *Culcita* that did not participate in experiments were used to house the shrimp between experiments. The aquarium was constantly cycled with seawater directly from Cook's Bay, allowing the animals to be kept as close as possible to the temperature and salinity of their natural habitat. Experiments were performed in low light in a clear plastic bin of dimensions 0.70x0.40x0.16 meters with an open top and surrounded with tarpaulin,

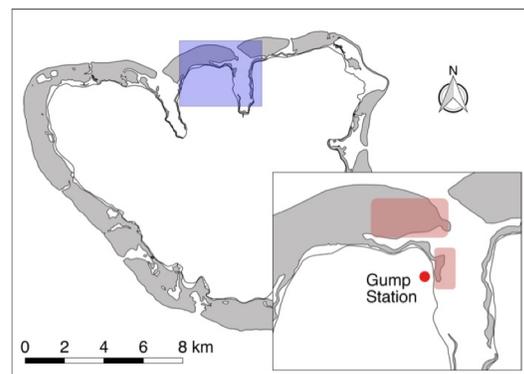


FIG. 1. Map of Moorea, French Polynesia, with collection sites indicated in red.

minimizing disturbance by the unfamiliar lab setting.

### *Feeding preferences*

Shrimp were separated from their hosts and placed in seawater that was passed through a 50- $\mu\text{m}$  filter. After 10 hours of starvation, shrimp were transferred to the center of a petri dish containing a layer of filtered seawater and food items placed at regular intervals around the circumference of the dish. The shrimp were observed under a dissecting microscope for ten minutes, and their behaviors were observed and recorded. The food choices were algae, pieces of host prey (crushed pieces of *Acropora* coral), tissue scrapings from the aboral surface of both sea star species (collected in a cotton q-tip), and tube feet from both sea star species.

Plankton feeding was tested separately. After 10 hours of starvation in the 50- $\mu\text{m}$  filtered water, the shrimp were transferred to a petri dish containing unfiltered seawater and their feeding behaviors were observed under a dissecting microscope. Zooplankton feeding was further tested by isolating shrimp in three seawater treatments and following survivorship over time. The seawater treatments were:

1. Unfiltered seawater containing planktonic organisms.
2. Seawater boiled for 10 minutes and passed through a 20- $\mu\text{m}$  filter twice. Benthic detritus was added to the water via pipette and was the only material present in the water.
3. Seawater boiled for 10 minutes and passed through a 20- $\mu\text{m}$  filter twice. Nothing was added to the water.

Seawater was heated to a boil and covered for ten minutes. The boiled water was then passed through two layers of a 20- $\mu\text{m}$  filter and cooled to ambient seawater temperature. The three seawater treatments were separated into open Tupperware containers covered with a 50- $\mu\text{m}$  mesh and kept partially submerged in cycling seawater to regulate temperature without allowing seawater to flow into the container. Five shrimp of random sizes were housed in each container under low light conditions. About 50 mL of fresh seawater from each treatment was added to the respective containers daily. Shrimp were monitored over six days and the time and treatment group of each shrimp death was recorded. Shrimp survivorship was plotted and results were analyzed in R using

an analysis of covariance at a predetermined significance level of  $p < 0.05$ .

Shrimp were dissected and examined through a dissecting microscope for feeding structures that could function as filters. The scaphocerite, antennule, and maxilla were isolated and observed under a compound light microscope at 100x and 400x magnification and photographs were taken of each structure.

### *Host species preference*

Individual shrimp were placed via pipette in the experiment aquarium between one *Culcita* and one *Linckia*, and the shrimp's host choice was recorded after the shrimp settled horizontally on the star's surface. The immediate choice between the oral and aboral surface was also recorded. Stars were placed on opposite ends of the experiment aquarium and exchanged between trials. Each shrimp was given a choice between one of three *Culcita* and one of three *Linckia* individuals, assigned in six random combinations. Each unique combination was repeated three times, for a total of 18 trials per shrimp and an overall total of 180 trials. Between the three repeated trials, the shrimp were kept with the *Culcita* holding stars.

This experiment was repeated, replacing *Linckia* with a dark basaltic rock of dimensions 15x15x20 cm, the approximate size and shape of *Culcita*. Five *Culcita* individuals were combined with the rock, and three shrimp participated in three trials for each *Culcita*, for a total of 45 trials.

A third experiment was conducted to determine long-term host preference. Ten shrimp were placed in the experiment aquarium with one *Culcita*, one *Linckia*, and the aforementioned rock. The shrimp were left undisturbed in the aquarium for at least two hours. The number of shrimp settled on each host after the acclimatization period was recorded. Five *Culcita* and five *Linckia* individuals were assigned in five unique combinations, each of which was repeated several times for a total of 15 trials. Ten shrimp participated in each trial, for a total of 150 recorded host choices.

For each of these experiments, host preference was analyzed in R with a chi-squared test at a predetermined significance level of  $p < 0.05$ . After all experiments were completed, the shrimp were returned with their hosts to the locations they were collected.

## RESULTS

### *Feeding preferences*

After being transferred to a petri dish with food choices, the shrimp moved around the dish but did not feed on any of the items. All shrimp, when placed in the petri dish, immediately moved to the edges of the dish. They then either moved around the dish's circumference or they settled on a food item and remained motionless. Three shrimp were observed for ten minutes, but the experiment was abandoned when no feeding behaviors were observed.

A second experiment comparing shrimp survival in different seawater treatments found that shrimp kept in boiled and filtered seawater had significantly higher mortality rates than shrimp kept in unfiltered seawater (Fig. 2). The linear models of the three treatments differed significantly between both filtered and the unfiltered seawater treatments ( $p < 0.05$ ). The two filtered seawater treatments did not differ significantly ( $p = 0.2$ ).

Shrimp placed in a dish of unfiltered seawater after a 12-hour period of starvation

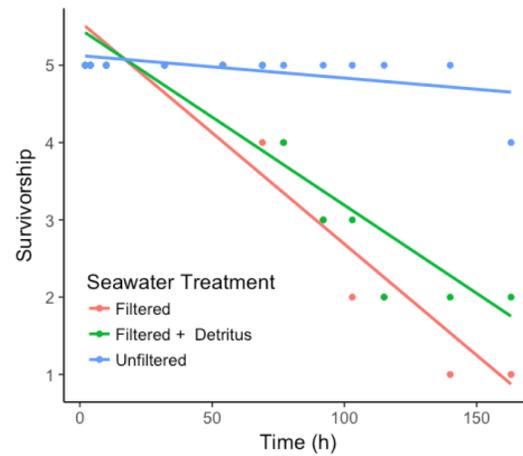


FIG. 2. Results of the survivorship experiment, with each colored line representing a seawater treatment group. Shrimp in the unfiltered seawater group had significantly higher survivorship ( $p < 0.05$ ) than both filtered seawater groups, which did not differ significantly.

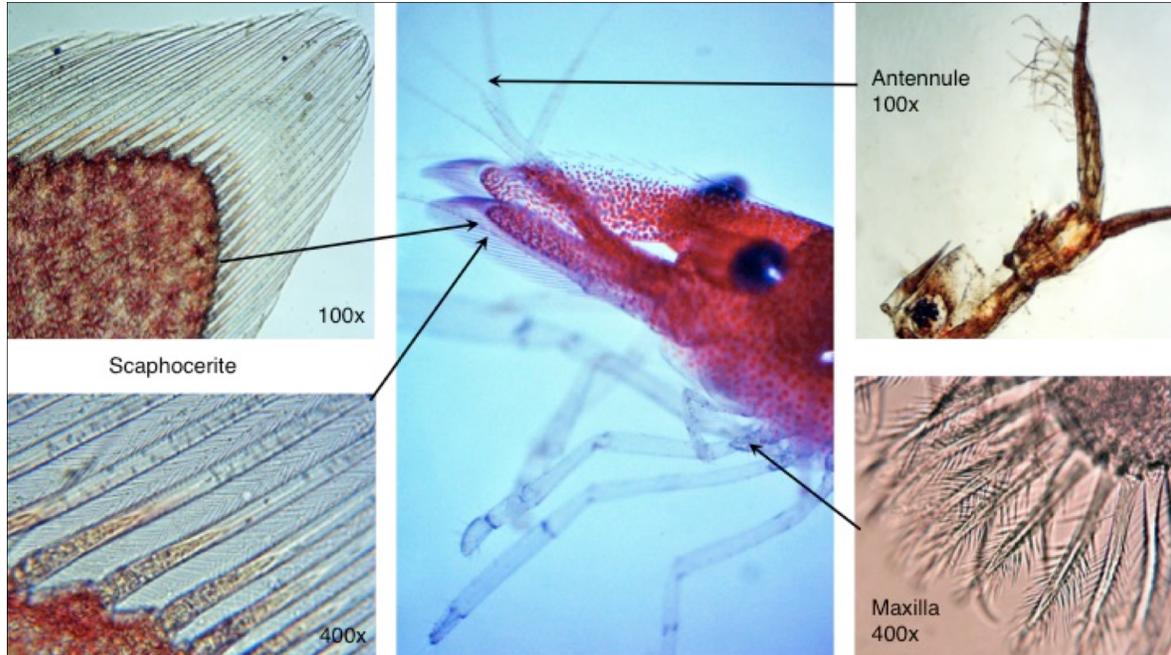


FIG. 3. Photographs of shrimp feeding structures taken at 100x magnification (upper left, upper right) and at 400x magnification (lower left, lower right). The upper and lower left images both depict the scaphocerite. The antennule is shown in the upper right, with setae visible on its distal end. The maxilla is shown in the lower right, with finely branching setae visible.

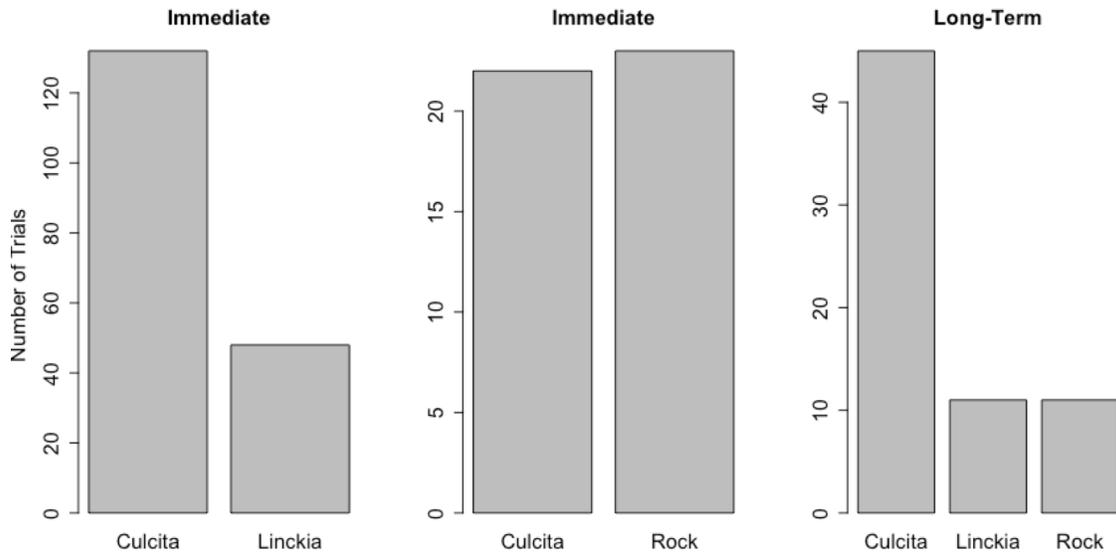


FIG. 4. Results of the host species preference experiments. When hosts were immediately chosen, *Culcita* was chosen significantly more often than *Linckia* but equally often as a similarly sized rock. When hosts were chosen over a longer time period, *Culcita* was chosen significantly more often than either.

were observed beating their antennules through the water and feeding on particles trapped by antennular setae. Antennules were observed at 100x magnification, where setae attached to the distal end of the antennule could be seen (Fig. 3). Scaphocerites observed at 400x magnification showed finely branching setae (Fig. 3) and were observed rotating laterally. Maxilla closer to the oral cavity were also lined with fine setae and were observed to flutter during feeding.

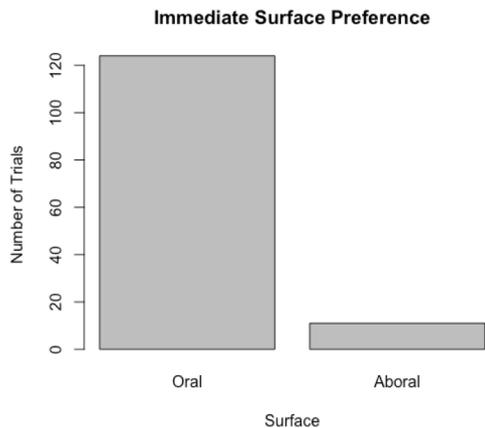


FIG. 5. The oral surface of the host was chosen for immediate settlement significantly more often than the aboral surface ( $p < 0.05$ ).

#### Host species preference

Shrimp placed between one *Culcita* and one *Linckia* chose *Culcita* for immediate settlement in 73% of trials, significantly preferring *Culcita* ( $p < 0.05$ ) (Fig. 4). Shrimp settled on the oral surface of the host in 92% of trials, a significant preference over the aboral surface ( $p < 0.05$ ) (Fig. 5). Shrimp placed between a large rock and a *Culcita* chose the rock for immediate settlement in 51% of trials, with no significant difference between the two ( $p = 0.8$ ) (Fig. 4). In a third experiment, shrimp placed in a tank with a large rock, a *Linckia*, and a *Culcita* and given at least two hours to settle on a host chose *Culcita* in 83% of trials, with a significant preference for *Culcita* ( $p < 0.05$ ) (Fig. 4).

## DISCUSSION

### Host preference

When given an immediate choice, shrimp chose *Culcita* significantly more often than *Linckia*. When given an immediate choice between *Culcita* and a similarly sized rock, the shrimp showed no preference. These results suggest that *P. soror's* immediate choice of host depends on the host's ability to cover and protect the shrimp. *Culcita* and a similarly

sized rock provide equal cover, while *Culcita* is much bulkier and has a greater oral surface area than *Linckia*, making it a more effective shelter. The shrimp's immediate settlement on the host's oral surface, hidden from view, further indicates that the shrimp's immediate impulse when placed in open water is to conceal itself.

When given at least two hours to acclimatize to the aquarium environment, shrimp settled on *Culcita* significantly more often than on *Linckia* or on the rock. Although the shrimp's immediate choice of host is driven by a need for concealment, long-term host preference must be influenced by other factors. In addition to shelter and protection from predators, long-term host preference could be influenced by host mobility, increasing the shrimp's access to varied feeding or mating opportunities.

Due to a scarcity of other host species in Moorea, all shrimp used in this study were collected from *Culcita* hosts. Preference for *Culcita* could implicate one of two possibilities. (I) *Periclimenes soror* prefers the host species from which it was originally collected and does not readily switch between host species within its lifetime. (II) *Culcita* may be a preferable host regardless of original host species. It is larger, bulkier, and provides better protection than *Linckia*. Future studies should clarify this distinction by experimenting with *P. soror* collected from several host species and determining host preference across species.

If shrimp prefer original host species, host-switching events are unlikely. *Periclimenes soror* could occur in many subpopulations that specialize on different host species and do not interact. Specialization on different host species may drive morphological differences in subpopulations, and a lack of gene flow may be actively driving speciation. Molecular and morphological studies should compare *P. soror* subpopulations associated with different host species to determine whether different host races exist and whether sympatric speciation is occurring.

*Periclimenes soror* is associated with many host species, and host-switching is unlikely to occur during an individual's life cycle. Host-switching may occur over successive generations if larvae from shrimp associated with one host species settle on another host species. Future studies should determine whether host species preference is genetically

determined or whether it is established upon initial settlement.

Shrimp isolated in seawater with no other source of food did not feed on any of the presented food items. *Periclimenes soror* therefore does not preferentially feed on host tissues, host surface scrapings, algae, or *Acropora* corals.

Shrimp kept in unfiltered seawater had significantly higher survivorship than shrimp kept in unfiltered seawater. The shrimp must have survived by feeding on living organisms suspended in the unfiltered seawater that were not present in the boiled and filtered seawater. Mortality rates were equal in the two boiled and filtered seawater treatments regardless of whether detritus was present in the water. Detritus and other dead organic material was therefore not sufficient to sustain the shrimp, which died at the same rate as shrimp isolated without any food source. These results indicate that shrimp are planktivores, feeding on living organisms present in the water.

Shrimp appendages were examined to identify structures that may function to filter plankton from the water. Three potential filter-feeding structures were identified. Scaphocerites are rotating fan-like appendages anterior to the oral cavity and are highly branched and lined with fine setae. They can be cleared of food particles with chelated pereopods, although this behavior was not observed. The antennule is another structure that may function in filter feeding. Shrimp were observed to feed on particles collected on antennular setae, but these setae were not distinctly branched, which may rule out filter feeding. Finally, the maxilla and the maxillipeds are small structures near the oral cavity that are lined with well-developed branching setae. These structures were observed to flutter through the water during feeding. Shrimp may use a combination of these structures to collect plankton from the water. Future studies should attempt to narrow down these possibilities.

Shrimp preferred the host's oral surface, where seawater flow is markedly lower than on the aboral surface. Shrimp were therefore observed in areas with reduced access to plankton. *Periclimenes soror* may be nocturnally active, hiding on the oral surface during the day or when threatened and venturing to the aboral surface during the night to feed. Future studies should examine *P. soror's* feeding habits and observe its behavior nocturnally.

Because *P. soror* is a planktivore, its feeding preferences do not affect its host. *Periclimenes soror* is therefore a commensal associate of its hosts with respect to feeding. Nevertheless, *P. soror* may affect its hosts in other ways, which should be further investigated in future studies. Because *P. soror* is a planktivore, its hosts do not provide it with food. Hosts provide immediate concealment, but since they are selected over rocks that provide equal protection, they must be providing the shrimp with additional benefits. One major benefit may be host mobility, increasing the shrimp's access to varied feeding or mating opportunities. Further study should clarify these aspects of the symbiosis.

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## APPENDIX

All *Culcita novaeguineae* and *Linckia laevigata* individuals used in this study are pictured below (Fig. 6).

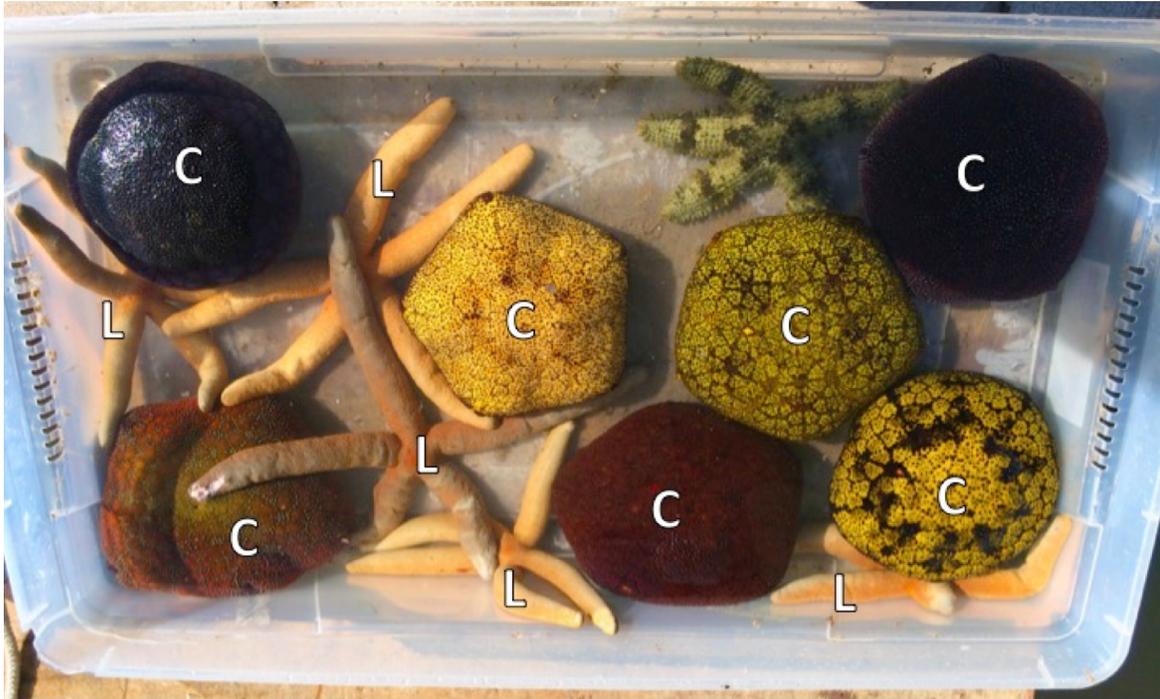


FIG. 6. Individuals marked "C" were identified as *Culcita novaeguineae* due to the rounded, inflated appearance. Individuals representing several color morphs were used in this study. Individuals marked "L" were identified as orange morphs of *Linckia laevigata* due to their five long, slender arms, the distinct pattern on the aboral surface, and the bright orange color. The unmarked individual in the upper right of the image was identified as a juvenile *C. novaeguineae* and was not used in the study.