Some symbionts of the sea cucumber *Stichopus cf. horrens* with notes on the behavior of pearlfish *Carapus mourlani*

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ABSTRACT

Sea cucumbers are known to host a variety of symbiotic organisms such as fishes and invertebrates. This paper is the first report on some of the endo- and epibionts of the sea cucumber *Stichopus* cf. *horrens* collected in the Bolinao-Anda reef complex in Pangasinan, northwestern Philippines, which includes the pearlfish *Carapus mourlani*, the polychaete *Gastrolepidia clavigera*, the gastropod *Melanella sp.*, the harlequin crab *Lissocarcinus orbicularis*, and unidentified amphipods. Some behaviors of *C. mourlani* were also described, including its re-entry strategies into the host. Not much is known about the implications of these symbionts on their host sea cucumbers, especially those that are cultured. Thus, future studies on host-symbiont interactions and their implications on cultured sea cucumbers are warranted.

Keywords: Stichopus cf. *horrens,* symbiont, *pearlfish,* invertebrates

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SOME SYMBIONTS OF THE SEA CUCUMBER *STICHOPUS CF. HORRENS* WITH NOTES ON THE BEHAVIOR OF PEARLFISH *CARAPUS MOURLANI*

Sea cucumbers are ecologically and economically important echinoderms commonly found in seagrass and coral reef ecosystems (Purcell et al. 2012). These organisms play several important ecological roles in benthic ecosystems, which include (1) improvement of sediment quality through bioturbation, (2) nutrient recycling, (3) increasing total alkalinity of water, (4) facilitation of energy transfer in the food chain as prey for predators in higher trophic levels, and (5) increasing biodiversity through symbiotic associations (Purcell et al. 2016). Sea cucumbers serve as hosts to a variety of symbiotic species. Symbionts refer to organisms that are either found within the body cavity (endobiont) or on the body surfaces (epibiont) of its host organism (Jangoux 1987). Among sea cucumbers, common forms of host-symbiont relationships include commensalism and parasitism. Commensalism involves symbionts benefiting from its association with the host with neither positive nor negative effect on the latter, while parasitism involves harm to the host by competition for nutrients and feeding on host tissues (Combes 1995). Sea cucumber symbionts belong to a wide range of taxa including annelids, crustaceans, gastropods, and fish, which are found either on the surface of the body wall or inside the buccal, cloacal, or coelomic cavities of sea cucumbers (Hamel et al. 2022). Available literature on sea cucumber symbionts in the Philippines is very limited. Some of the previously published papers include studies by Trott (1970) and Trott and Trott (1972) on pearlfishes collected from sea cucumbers in Puerto Galera, Mindoro Island. Meanwhile, there are no existing published literature on invertebrate symbionts in sea cucumbers in the Philippines.

Aside from its ecological function, sea cucumbers are important fishery resources for many coastal communities that depend on them as food and supplemental source of livelihood (De Guzman et al. 2019). Commercially important sea cucumbers in the Philippines include *Holothuria scabra* and *Stichopus horrens*. Dried sea cucumber products known as trepang or beche-de-mer command high prices locally and abroad, and thus provide additional income to households involved in sea cucumber gathering (Olavides et al. 2010). Meanwhile, *S. horrens* is known for its pharmaceutical and nutraceutical potential, thus another potential source of livelihood for fishers (Eriksson et al. 2007). In recent years, there has been an increase in research interest on the biology, ecology, and development of culture technologies for *S. horrens* in the Philippines as a means to prevent overfishing and help rebuild stocks (Juinio-Meñez et al. 2024). A review by Hamel et al. (2022) found that some endoparasitic symbionts of sea cucumbers may cause harm to

the host's internal organs such as the respiratory tree, stomach, and gonads. These could affect physiological and reproductive functions as well as produce wounds that could be the site of bacterial infection. Understanding symbiont biology, distribution, and interaction with their host will be useful in formulating measures to mitigate possible impacts of parasitic infestations on cultured sea cucumbers.

Symbionts of *S. cf. horrens* reported in this paper were obtained through chance encounters during the conditioning and spontaneous spawning observations of wild broodstock in the hatchery and while doing field surveys and laboratory experiments, and thus there was no systematic sampling scheme involved. Epi- and endobionts were observed from wild adult *Stichopus cf. horrens* collected in Bolinao-Anda Reef Complex, particularly in Brgy. Lucero, Bolinao (16°23'24" N, 119°54'51" E) and Brgy. Sablig, Anda (16°16'51" N, 120°1'42" E), Pangasinan on May 2021 (Anda), February 2022 (Bolinao), and February 2023 (Bolinao and Anda). Symbiont location, prevalence, and some behaviors such as mode of entry of pearlfish into the host were noted.

Symbionts

This study recorded fish and invertebrate symbionts of *S. cf. horrens* (Table 1). Pearlfish (6.65 ± 1.20 cm) was the only obligate endobiont and was observed during the conduct of the spawning observations in May 2021 and February 2022. A total of six pearlfish individuals were observed, with a single individual inhabiting each one of the six adult sea cucumber hosts. Pearlfish samples from the May 2021 broodstock were collected and preserved in 95% ethanol for species identification at the DNA Barcoding Laboratory of the Institute of Biology, University of the Philippines Diliman. DNA barcoding of three pearlfish samples from Anda revealed that these were all *Carapus mourlani*. Pearlfishes (family Carapidae; Table 1) are widely distributed in the temperate and tropical regions where sea cucumbers and other echinoderm hosts are also found (Parmentier et al. 2006). Previously, *C. mourlani* was reported in the cushion star *Culcita novaguineae* among echinoderms collected in Puerto Galera, Mindoro Island by Trott and Trott (1972), and in *H. argus* and *S. variegatus* in the same island by Trott (1970). Neither *C. homei* nor *Jordanicus gracilis* was found in sea cucumbers collected in Anda in the present study, unlike in other sea cucumbers (e.g., *Actinopyga*, *Holothuria*, *Stichopus,* and *Thelenota*) previously collected by Trott and Trott (1972). Pearlfishes are often found in the respiratory tree and coelomic cavity (Hamel et al. 2022). Meanwhile, the sea cucumber's cloaca serves as shelter for the pearlfish, which locates its host's anus through the excurrent resulting from the sea cucumber's respiration (Parmentier

and Vandewalle 2005). Pearlfish collected in Guam had maturing gonads from October to July, indicating that spawning occurs during summer and an intense competition for hosts may also occur (Smith 1964). *C. mourlani* and other species such as *C. boraborensis* and *C. homei* are commensals that feed outside their hosts and not their tissues, while another pearlfish *Encheliophis gracilis* likely feeds on its host (Parmentier and Das 2004; Parmentier and Vandewalle 2005). While *C. mourlani* does not feed on its host's tissues, its movement to the coelomic cavity, which tears the respiratory tree, may be detrimental to its host (Parmentier et al. 2006).

During tissue sample collection in February 2023 broodstock, invertebrate symbionts clinging to the sea cucumbers' body wall were collected and preserved in 95% ethanol for future use. The symbionts were also photographed using an Olympus TG-5 underwater camera. These epibionts are also summarized in Table 1, which include a gastropod, a polychaete, a crab, and unidentified amphipods, and were identified based on the description of Hamel et al. (2022) on sea cucumber symbionts. The polychaete *Gastrolepidia clavigera* (4 cm; family Polynoidae; Figure 1a-b) were found exclusively in sea cucumber hosts collected in Bolinao, with one from broodstock collected in the wild and the other from a juvenile reared in submerged hapa in the ocean nursery. This polychaete was observed to be moving all over the sea cucumber's body surface, although some studies showed that it is also found within the buccal and cloacal cavities of other sea cucumber species (Britayev et al. 1999; Britayev and Lyskin 2002). This species camouflaged with the body wall of *S. cf. horrens* so they were difficult to spot. This polychaete is also gonochoric with females being larger than the males (Britayev and Zamishliak 1996). While polychaetes are considered commensals, Britayev and Lyskin (2002) found that *G. clavigera* may be parasitic.

Many individuals of the gastropod *Melanella sp.* (family Eulimidae; Figure 1c) were found in many hosts and were distributed all over the body including the mouth and the anus. The occurrence of such gastropod epibiont was also noted in juveniles reared in a submerged hapa in an ocean nursery in Silaki Island (16°26'41" N, 119°55'16" E) all year round with peak occurrence during summer months (April-June). Although the exact spawning periodicity of the species is unknown, there are indications that tropical eulimid snails spawn continuously year-round (Waren 1983). *Melanella sp.* is parasitic as it attaches its boring proboscis on the host's body surface, which allows it to feed from the coelomic cavity or the haemal system (Jangoux 1984).

The harlequin crab *Lissocarcinus orbicularis* (Family Portunidae; Figure 1d) also moved all over the sea cucumbers' bodies and tended to camouflage with the body

wall, although it may also be observed in the buccal or cloacal cavities of other sea cucumber species (Caulier et al. 2014). This species is a parasite that feeds on its host's integuments (Caulier et al. 2014). In previous studies, *L. orbicularis* and symbiotic crabs *Hapalonotus reticulatus* and *Pinnotheres halingi* were also observed in other sea cucumber species such as *H. scabra* (Nigam and Sivaperuman 2020; Tresnati et al. 2021; Vandenspiegel et al. 1992), while other symbiotic crab species in the Family Eumedonidae were found in other echinoderms such as crinoids and sea urchins (Ng and Jeng 1999). Caulier et al. (2012) found that there was at least one crab per host species, although there were heterosexual couples in 30% of the samples studied. Moreover, gravid females and juveniles were found all throughout the year suggesting that these crabs reproduce year-round with the largest females found from April to August (Caulier et al. 2012).

Amphipods were prevalent all over the body wall, and were camouflaged and concealed within the folds of the integument of *S. cf. horrens*. While the study was not able to identify the amphipods found in *S. cf. horrens*, Scholtz et al. (2018) showed that some amphipods like *Tritaeta gibbosa* found in corals, ascidians, and echinoderms are commensals.

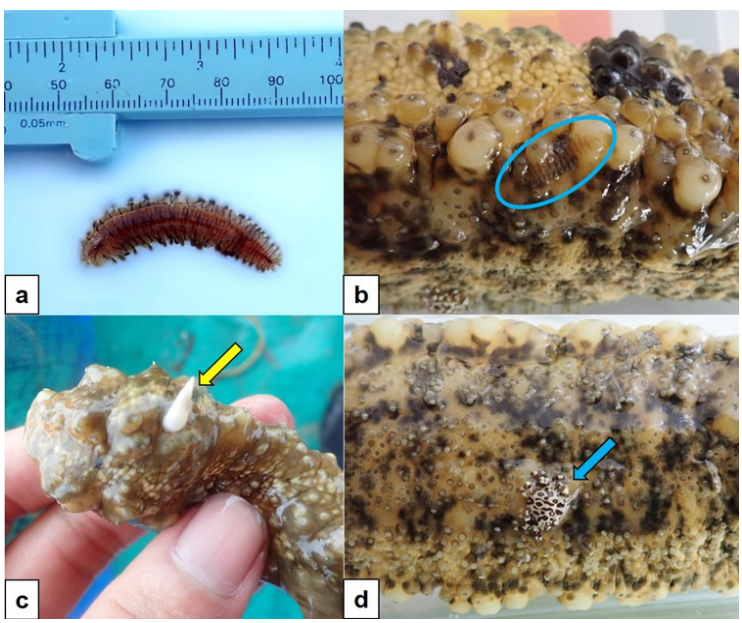

Figure 1. Some of the invertebrate symbionts of *Stichopus cf. horrens*. (a) The coloration of the polychaete *Gastrolepidia clavigera* allows (b) concealment on the surface of the sea cucumber's host body surface (in blue circle). (c) The gastropod *Melanella sp*. (yellow arrow) was found along the mouth its sea cucumber host, while (d) the harlequin crab *Lissocarcinus orbicularis* moves around the body surface of its host.

Table 1. List of symbionts found among adult *Stichopus cf. horrens* collected from the Bolinao-Anda reef complex.

Phylum	Class	Species	Location	Number of individuals found	Number of hosts observed	Dates observed	Strictly found in sea cucumbers?	Type based on location	Type of symbiosis	Effect on sea cucumbers
Chordata	Actinopterygii (Carapidae)	*Carapus mourlani*	Anus	6	6	May 2021	No[a]	Endobiont	Commensal[b]	Feeds outside host but movement may affect respiratory tree[b]
Annelida	Polychaeta (Polynoidae)	*Gastrolepidia clavigera*	Body wall, near mouth	2	2	December 2022; February 2023	Yes[c]	Epibiont*; Endobiont[b]	Parasite[d]	Ingest host's integument[d]
Mollusca	Gastropoda (Eulimidae)	*Melanella sp.*	Mouth, anus, body wall	~10-15 per host	Almost all sampled individuals	Year-round, peak in April to June	Yes[e]	Epibiont	Parasite[a]	Use proboscis to feed on host's internal fluids[a]
Arthropoda (Subphylum Crustacea)	Malacostraca (Portunidae)	*Lissocarcinus orbicularis*	Dorsal body wall (may also move to the ventral portion)	2	2	February 2023	Yes[f]	Epibiont*; Endobiont[b]	Parasite[f]	Feed on sea cucumber integument as ossicles and sea cucumber DNA were found in their gut[f]
	Malacostraca	Unidentified amphipods	Body wall	Many, not counted	Almost all sampled individuals	Year-round, peak in April to July	No[g]	Epibiont	Possibly commensal[g]	Unknown; Other amphipod species do not cause damage to host's tissue[g]

* This study
[a] Trott and Trott (1972)
[b] Hamel et al. (2022)
[c] Britayev et al. (1999)
[d] Britayev and Lyskin (2002)
[e] Warén (1999)
[f] Caulier et al. (2014)
[g] Scholtz (2018)

Notes on pearlfish behavior

The pearlfish leave its hosts at night to forage (Smith 1964; Parmentier et al. 2006). Video recordings of pearlfish behavior taken using an Olympus TG-5 underwater camera showed that there are two possible ways in which a pearlfish re-enters its host, which it does whenever the host's anus opens to respire. During a night observation in the hatchery, a pearlfish, previously collected from Bolinao, Pangasinan, swam around the mouth and body of its host, possibly foraging for food particles inw the tank or on the host's body surface. It remained outside the host for a few minutes and eventually re-entered when the sea cucumber opened its anus to breathe. During entry, the pearlfish aligned its head with the host's anus, waited for it to open, and entered the anus headfirst. Meanwhile, pearlfish from the same sea cucumber species collected from Anda, Pangasinan used a different re-entry strategy (Figure 2). When presented with one host, five pearlfish individuals competed for re-entry (Figure 2a). Once the sea cucumber opened its anus to breathe, a pearlfish swam forward and prodded the tip of its head into the anus (Figure 2b), bent in a swift motion to insert its tail into the anal opening (Figure 2c), and slowly entered its entire body into the cloaca of its host (Figure 2d). Trott (1970) reported that pearlfish enters their host either head or tail first, but this depends on the size of the fish and the sea cucumber host. The tail first entry allows the pearlfish to use its body as guide to insert its tail and eventually penetrate the cloaca while the sea cucumber releases exhalant respiratory water (Parmentier and Vandewalle 2003).

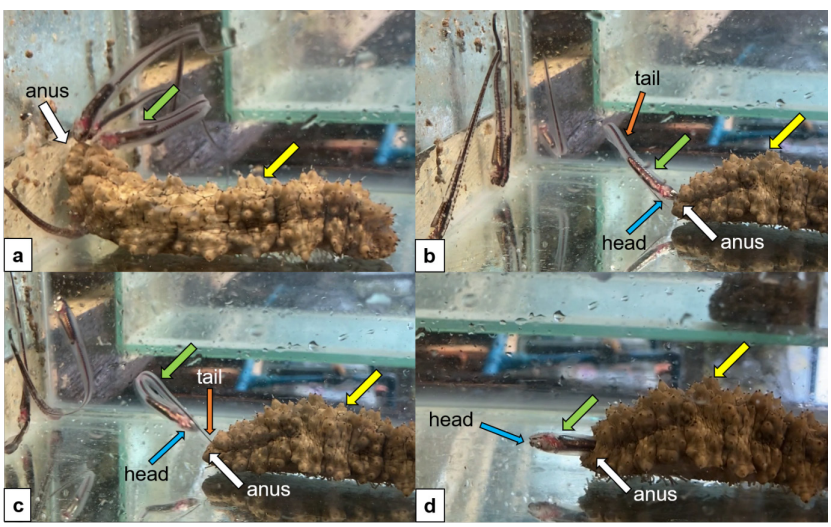

***Figure 2*. Re-entry of pearlfish into a sea cucumber host collected from Anda, Pangasinan and subsequently observed in a glass aquarium (L×W×H = 46×20×31.5 cm). (a) Five pearlfish individuals (green arrows) compete for entry when presented with one host (yellow arrows). (b) A pearlfish prods its head (blue arrows) into the anus of the sea cucumber host, (c) swiftly bends its body to insert its tail into the anal opening (orange arrows), and then (d) enters tail first into the host. Video recording by Dr. Hiyas Junio.**

CONCLUSION AND RECOMMENDATIONS

Despite the year-round prevalence of most symbionts encountered, no apparent negative effect on spawning behavior and reproductive success of *S. cf. horrens* was observed as all batches observed in the hatchery spawned successfully, producing viable larvae that later developed into juveniles. However, the effects on other physiological processes are yet to be determined. A thorough examination is still needed to establish the association of these symbionts with their host as this is influenced by other factors such as host size and habitat (Britayev and Zamishliak 1996; Caulier et al. 2012).

This study presents preliminary data on some symbionts of the sea cucumber *S. cf. horrens*, which are either commensals or parasites. The presence of obligate parasitic symbionts (e.g., *Gastrolepidia clavigera*, *Melanella sp.*, *Lissocarcinus orbicularis*) may pose serious implications on sea cucumber aquaculture, especially when there is heavy infestation. These parasites feed on the host tissues, which may affect physiological processes and reproductive success, and expose the host to infections (Hamel et al. 2022). While the presence of symbionts in wild populations is natural, heavy parasitic infestations in cultured sea cucumbers, particularly when stocked at high densities, may lead to production and economic losses. A more comprehensive study examining more samples is thus warranted to identify and conduct an inventory of symbionts, particularly parasites, and their distribution in other sea cucumber and echinoderm (e.g., starfish) species. Hosts affected by symbionts may be examined externally or dissected to determine the effect of symbionts on the host's tissues and internal organs. Identification of parasites and their distribution, prevalence, and effects on sea cucumber hosts would be vital in developing mitigation measures such as diagnosis, treatment, and timing of production cycles to avoid parasitic infestations during culture. An understanding of sea cucumber symbionts will enhance marine biodiversity studies and may become useful indicators for monitoring the health of natural populations of the diverse species of sea cucumbers found in the archipelago.

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