Widening the host range of the ectosymbiotic scale-worm *Asterophilia culcitae* (Annelida: Polynoidae) to three echinoderm classes, with data on its body color variation

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Abstract: The Polynoidae, commonly known as “scale-worms” due to the scale-like elytra on the dorsal surface, contains many species living in symbioses with other invertebrates. Most of these symbionts are host-specific, but some have a wide range of hosts. The genus *Asterophilia* includes two species living in shallow subtropical to tropical waters in the Pacific Ocean as ectosymbionts of asteroids and, more rarely, crinoids. Here, we recorded *Asterophilia culcitae* from asteroid hosts (*Culcita novaeguineae*, *Linckia laevigata*, *L. guildingi*, and *Leiaster leachi*) and, for the first time, from holothurian hosts [*Stichopus chloronotus*, *Holothuria atra*, *H. (Stauropora) pervicax*, and *Bohadschia argus*] along warm Japanese Pacific coasts. The cytochrome *c* oxidase subunit I sequences of the specimens from holothurians did not differ significantly from those on asteroid hosts, proving that *A. culcitae* has a wide host range across three different echinoderm classes: asteroids, holothurians, and crinoids. The general body color of *A. culcitae* was constantly reddish (female) or whitish (male), regardless of the host body color, although a previous study suggested that it differs in accordance with the host body color. However, we found that one individual from a holothurian host showed a different color pattern: *A. culcitae* typically shows three whitish or yellowish elytral mounds that have been suggested to mimic the tube foot of the asteroid hosts, whereas one individual from *S. chloronotus* had reddish brown translucent mounds, which we suggest might be cryptic on its holothurian host.

Key words: Asteroidea, color variation, Holothuroidea, host specificity, symbiosis

Introduction

The Polynoidae, which comprises 18 subfamilies and about 900 species, is one of the largest families in the phylum Annelida and is known to prevail from intertidal waters to deep seas worldwide (Bonifácio & Menot 2018, Read & Fauchald 2019). Most polynoids have elytra (or scales) on their dorsal side and, thus, are commonly known as scale-worms. This family contains many symbiotic species associated with other marine invertebrates, including Cnidaria, Echinodermata, and Arthropoda (Martin & Britayev 1998, 2018). The host range of these worms differs among species (Martin & Britayev 1998, 2018), for example, *Harmothoe oculinarum* (Storm, 1879) has a high specificity toward the single annelid host *Eunice norvegica* (Linnaeus, 1767) (Martin & Britayev 2018), whereas *Arectonoe vittata* (Grube, 1855) has a wide host range (i.e., Asteroidea, Cnidaria, Echinodermata, Holothuroidea, Gastropoda, Annelida, Polyplacophora, and Decapoda) (Britayev et al. 1999). However, in many symbiotic polynoids, the host range remains poorly understood.

The polynoid genus *Asterophilia* Hanley, 1989 is characterized by reddish elytra with yellowish or whitish spots (mounds) and dorsal cirri with whitish inflated tips. *Asterophilia* consists of two species: *A. carlae* Hanley, 1989 and *A. culcitae* Britayev & Fauchald, 2005, the former occurring from Fiji to Southeast Asia (Hanley 1989) and the latter from Southeast Asia to Japan (Britayev & Antokhina 2012, Britayev & Fauchald 2005, Okinawa General Bureau 2013). Both species are well-known symbionts of a wide range of Asteroidea, although they have recently been...
found with an unidentified crinoid (Britayev & Antokhina 2012, Martin & Britayev 1998, 2018, Britayev & Fauchald 2005). The whitish or yellowish spots on the elytra, the whitish inflated tips of the antenna, and the tentacular and dorsal cirri have been suggested to mimic the tube foot of the host asteroids to avoid being detected by predators (Britayev & Fauchald 2005, Hanley 1989).

In this study, we report, for the first time, on *A. culcitae* from holothuroid hosts along the warm Pacific coasts of Japan and compare it (morphologically and molecularly) with *A. culcitae* specimens collected from asteroid hosts.

**Materials and Methods**

**Sampling and observation**

Eleven specimens of *Asterophilia* were collected from holothurian and asteroid hosts in the Japanese and Ryukyu Islands from 2014 to 2020 (Table 1). The specimens were relaxed in 7% MgCl₂ solution before being photographed with digital cameras (Olympus Stylus TG-4 Tough or Olympus Tough TG-5) and fixed with 99.5% ethanol. Their body size, sex, and other morphological characteristics were examined under a binocular microscope (Nikon ECLIPSE E800). Sex was determined by checking for the presence of sperm or oocytes.

**DNA extraction and molecular phylogenetic analyses**

The genomic DNA was extracted from elytra, which were dissected from the eleven specimens, with the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the kit extraction protocol. Partial sequences of the cytochrome c oxidase subunit I (COI) gene were amplified by polymerase chain reaction (PCR) as follows. We placed 2.5 µL DNA extraction in a PCR tube and added 17.5 µL distilled water, 2.5 µL Ex Taq buffer, 2.0 µL dNTP mix, 0.3 µL of forward and reverse primers (10 µM each), and 0.13 µL TaKaRa Ex Taq (Takara Bio Inc, Kusatsu, Japan). The following primers were used: LCO1490 (5′-GGTCAAATCATATAAGATACTG-3′) and HCO2198 (5′-TAACTTCGGGGTAGATGTG-3′) (Folmer et al. 1994). The mixture was then heated for 1 min at 94°C, followed by 35 cycles in three stages (94°C for 45 s, 45°C for 1 min, and 72°C for 1 min), and incubation for 4 min at 72°C. ExoSAP-IT (Thermo Fisher Scientific K. K., Tokyo, Japan) was used for purification of the PCR products. The sequencing reactions were performed at Eurofins Genomics (Tokyo, Japan) with the same primers as those used in the PCR. The resulting sequences were registered in GenBank with the accession numbers LC538222–LC538232 and aligned using the MUSCLE program of SeaView (Galtier et al. 1996, Gouy et al. 2010). A median-joining haplotype network of all haplotypes was constructed using PopART (Leigh & Bryant 2015) to visually depict the genetic structure. The pairwise genetic distances were calculated using the Kimura 2-parameter (K2P) model in MEGA X (Kumar et al. 2018).

**Table 1.** Sampling locality, host and accession numbers of *Asterophilia culcitae* collected in this study.

| ID | Sampling locality       | Latitude     | Longitude     | Host                        | accession number |
|----|-------------------------|--------------|---------------|-----------------------------|-----------------|
| 1  | Yakatakatabaru, Okinawa Island, Japan | 26°29′26″N | 127°50′17″E | Holothurian (Stichopus chloronotus) | LC538222        |
| 2  | Yakatakatabaru, Okinawa Island, Japan | 26°29′29″N | 127°50′20″E | Holothurian (Holothuria atra) | LC538223        |
| 3  | Sakimotobu, Okinawa Island, Japan | 26°38′07″N | 127°52′56″E | Holothurian (Bohadschia argus) | LC538224        |
| 4  | Kushimoto, Wakayama, Japan | 33°28′44″N | 135°44′45″E | Holothurian (Holothuria pervicax) | LC538225        |
| 5  | Kashiwa Island, Kochi, Japan | 32°46′26″N | 132°37′47″E | Asteroid (Culcita novaeguineae) | LC538226        |
| 6  | Kashiwa Island, Kochi, Japan | 32°46′26″N | 132°37′47″E | Asteroid (Culcita novaeguineae) | LC538227        |
| 7  | Kushimoto, Wakayama, Japan | 33°27′08″N | 135°45′09″E | Asteroid (Linckia guildingi) | LC538228        |
| 8  | Kushimoto, Wakayama, Japan | 33°27′08″N | 135°45′09″E | Asteroid (Linckia guildingi) | LC538229        |
| 9  | Zamami Island, Okinawa, Japan | 26°14′29″N | 127°18′26″E | Asteroid (Linckia laevigata) | LC538230        |
| 10 | Kushimoto, Wakayama, Japan | 33°27′44″N | 135°47′45″E | Asteroid (Leiaster leachi) | LC538231        |
| 11 | Kushimoto, Wakayama, Japan | 33°27′44″N | 135°47′45″E | Asteroid (Leiaster leachi) | LC538232        |
Fig. 1. *Asterophilia culcitae* from holothuroid hosts. (A) A female on *Holothuria atra*, (B) a female on *Bohadschia argus*, (C) a female on *Stichopus chloronotus*, and (D, E) a female on *Holothuria pervicax*. Scale bars: approximately 10 mm (A, B, D, E) and 5 mm (C). Photo credits: T. Sugiyama (A–C) and I. Hirabayashi (D, E).
Results and Discussion

We collected the specimens of *Asterophilia* from four species of holothurians, namely, *Stichopus chloronotus* Brandt, 1835, *Holothuria atra* Jaeger, 1833, *H. (Stauropsis) pervicax* Selenka, 1867, and *Bohadschia argus* Jaeger, 1833 (Fig. 1), as well as from four species of asteroids, namely, *Culcita novaeguineae* Müller & Troschel, 1842, *Linckia laevigata* (Linnaeus, 1758), *L. guildingi* Gray, 1840, and *Leiaster leachi* (Gray, 1840) (Fig. 2, Table 1). Most specimens were found attached to the ventral side of the host body (Table 2).

The species of *Asterophilia* can be distinguished from those of the closely related genus *Gastrolepidia* Schmarda, 1861, a holothurian ectosymbiont, by their fifteen pairs of elytra (Hanley 1989) and lack of ventral lamellae [i.e., ventral lobes in Britayev & Zamishliak (1996)]. All of our specimens (i) had elytra on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 26, 29, and 32, except for a juvenile that had only 31 segments and (ii) lacked ventral lamel-
lae. Thus, we identified all the specimens as belonging to *Asterophilia*.

All the specimens had elytra with frontal pockets, instead of an anterior fold (Fig. 3A, D), and scattered micro-papillae (Fig. 3B, E), as well as middle and lower neurochaetae with serrated edges that lacked semilunar pockets (Fig. 3C, F). These characteristics accord with the original description of *A. culcitae* (Britayev & Fauchald 2005).

We identified four haplotypes among the 582 bp COI fragments from our specimens, with only one being shared by the specimens from holothurians and asteroids (Fig. 4). The maximum intraspecific difference was three nucleotides. The intraspecific K2P genetic distance was 0.17–0.52%, which is much smaller than the interspecific distances reported for other polynoids [e.g., 17–20% in *Chevaldonné et al.* (1998); 16–19% in *Gastaldi* (2019)]. Accordingly, all the specimens belonged to *A. culcitae*, proving that this species has a wider host range than previously accepted, covering three different echinoderm classes.

Some other symbiotic polynoids also show a wide host range. For example, *Arctonoe vittata* was recorded with eight invertebrate classes (Martin & Britayev 1998, 2018), while *Hololepidella nigropunctata* (Horst, 1915) occurs in seven classes among four phyla (Martin & Britayev 1998). Some polynoids may change their hosts as they grow (Martin & Britayev 2018); however, *A. culcitae* exhibited different sizes in each of the holothurian and asteroid host individuals (Table 2), allowing us to discard their developmental stages as a cause of the host switch.

In Vietnamese asteroid hosts, there were multiple single (male) and paired (male and female) infestations by *A. culcitae* (Britayev & Fauchald 2005); the latter also occurred in the asteroid hosts studied herein. Conversely, holothurian hosts in this study always harbored a single symbiont (either male or female). Therefore, we presume that *A. culcitae* only forms male–female pairs on asteroid hosts, while holothurians act as intermediary hosts. However, further evidence is needed to validate this hypothesis.

*Gastrolepidia clavigera* Schmarda, 1861, a polynoid that is eptosymbiotic with holothurians, shows several different color morphs mimicking its hosts (Britayev & Zamishlika 1996). In contrast, *A. culcitae* was constantly reddish (females) or whitish (males), independent of the hosts’ body color, for example, black of the skins of *S. chloronotus* and *H. atra* and brown or white of the skins of *H. pervicax* and *B. argus* (Figs. 1, 2). The different color of males and females here is not owing to that of the intracoelomic gametes, but mainly to that of the trunk, elytra, antennas, and tentacular and dorsal cirri (e.g., Fig. 1E, F). The reddish coloration of starfish-associated specimens of *A. culcitae* apparently mimics the host’s body color (Britayev & Fauchald 2005). However, we posit that if the worms always tend to be reddish, mimicry can only occur when they are associated with reddish hosts (e.g., *Culcita novaeguineae*). Actually, a reddish individual of *A. culcitae* was reported from the bluish starfish *L. laevigata* (Britayev & Antokh-

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**Table 2.** Ecological and morphological information of *Asterophilia culcitae* collected in this study.

| ID | Host | Attachment site on host | Host color (the color of the attachment site) | Sex | Length (mm) | Width without parapodia (mm) | No. of segments | Lifestyle |
|----|------|-------------------------|---------------------------------------------|-----|-------------|-----------------------------|----------------|----------|
| 1  | Holothurian (†Stichopus chloronotus) | lateral side | black | male | 8.8 | 0.9 | 33 | solitary |
| 2  | Holothurian (†Holothuria atra) | ventral side | dark purple | female | 24.8 | 2.3 | 33 | solitary |
| 3  | Holothurian (†Bohadschia argus) | dorsal side | brown and white | female | 20.7 | 1.8 | 34 | solitary |
| 4  | Holothurian (†Holothuria pervicax) | lateral side | brown and white | female | 25.5 | 2.2 | 43 | solitary |
| 5  | Asteroid (†Culcita novaeguineae) | ventral side | red | female | 26.1 | 2.4 | 41 | paired |
| 6  | Asteroid (†Culcita novaeguineae) | ventral side | red | male | 12.2 | 1.2 | 37 | paired |
| 7  | Asteroid (†Linckia guildingi) | ventral side | magenta | female | 16.1 | 1.9 | 39 | paired |
| 8  | Asteroid (†Linckia guildingi) | ventral side | magenta | juvenile | 6.9 | 0.7 | 31 | paired |
| 9  | Asteroid (†Linckia laevigata) | ventral side | orange | female | 20.5 | 1.9 | 37 | solitary |
| 10 | Asteroid (†Leiaster leachi) | ventral side | red and yellow | female | 23.6 | 2.0 | 40 | paired |
| 11 | Asteroid (†Leiaster leachi) | ventral side | red and yellow | male | 9.3 | 1.1 | 35 | paired |
na 2012), which supports our presumption.

One of our specimens found on *S. chloronotus* had reddish brown translucent mounds on its elytra, instead of the whitish or yellowish mounds typical of *A. culcitae* (Britayev & Fauchald 2005) (Figs. 1, 2). The typical mound color of *A. culcitae*, together with its shape and color of the antennae and cirri, have been suggested to mimic the tube feet of their host starfish (Britayev & Fauchald 2005). However, *S. chloronotus* does not possess large standout tube feet, and so the reddish mounds may be for more effective cryptic coloring on this host (Fig. 1C). In contrast, the remaining three individuals associated with holothurians possessed the typical mound color (Fig. 1A, B, D). Thus, it remains unclear what causes the body color variation of *A. culcitae*.

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