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First report of the chelonophilic tanaidid *Hexapleomera urashima* from an environment other than the surface of sea turtles

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**Abstract.** We report the tanaidid *Hexapleomera urashima* Tanabe, Hayashi, Tomioka & Kakui, previously known only as an epibiont on the loggerhead sea turtle, *Caretta caretta* (Linnaeus), from a fouling community on an old tether line in a fishery harbor, Yakushima Island, Ryukyu Islands, southern Japan. We found a single, female specimen whose species identity based on morphology was confirmed with a cytochrome *c* oxidase subunit I (COI) DNA barcode sequence. This is the first molecularly supported evidence of a chelonophilic *Hexapleomera* species from an environment other than the surface of sea turtles. However, as the fishing harbor where we collected the specimen was not far from the beach where numerous sea turtles nest, and only the one specimen was found in the fouling community despite considerable sampling efforts, we cannot rule out the possibility that the specimen had drifted from a turtle into the fouling community.

**Introduction**

Sea turtles serve as hosts to a wide variety of epibionts (Frick & Pfaller 2013). The most diverse epibiotic faunal community has been reported on the loggerhead sea turtle, *Caretta caretta* (Linnaeus) (Domènech et al. 2015), with echinoderms, platyhelminths, annelids, gastropods, kinorhynchs, nematodes, pycnogonids, mites, insects, and crustaceans occurring as mobile epibionts (Tanabe et al. 2017). Based on the degree of their association to sea turtles, epibiotic taxa can be classified as 1) “obligate commensals”, which are found almost exclusively on sea turtles; 2) “chelonophilic facultative commensals”, which show a marked association with sea turtles; and 3) “facultative commensals”, which typically occur on inanimate substrates, or predominantly as free-living forms (Frick & Pfaller 2013; Domènech et al. 2015).

The order Tanaidacea is a group of tiny aquatic crustaceans, with ca. 1400 species known to date (Anderson 2017). Most species are free-living, but a few have also been reported as epibiotic species on sea turtles. The genus *Hexapleomera* Dudich includes the most common sea-turtle epibiotic tanaidacean, *Hexapleomera robusta* (Moore, 1894); in addition, among eight species in the genus, *H. edgari* Bamber, 2012 and *H. urashima* Tanabe, Hayashi, Tomioka & Kakui, 2017, have also been reported from the carapaces of sea turtles (Tanabe et al. 2017; Wi et al. 2018). On carapaces, *H. robusta* and *H. urashima* have a tubicolous mode of life (Moore 1894; Y. Tanabe unpublished data; unknown in *H. edgari*). The degree of association of these tanaidaceans with sea turtles remains unclear. *Hexapleomera edgari* and *H. urashima* have been reported only from the surface of sea-turtles, although the information is based on a single report in each case (Edgar 2008; Tanabe et al. 2017). In contrast, *Hexapleomera robusta* has been reported from the surfaces of sea-turtles and manatees, among fouling communities on nearshore fish-farms, among algae, and in soft sediments on shallow bottoms (Moore 1894; Morales-Vela et al. 2008; Bamber et al. 2009; Esquete & Fernandez-Gonzalez 2017). These varied occurrences suggest that this species is a chelonophilic facultative commensal or facultative commensal. However, taxonomists have recently realized that *Hexapleomera* has far higher species richness than previously assumed: prior to 2012, only one cosmopolitan *Hexapleomera* species (*H. robusta*) was recognized, whereas now the genus contains eight species (Wi et al. 2018). At least two previous reports of “*H. robusta*” by tanaidacean taxonomists were actually misidentifications of congeners (Bamber 2012), and so previous records of “*H. robusta*” need to be carefully reexamined morphologically and/or with molecular data.

During qualitative sampling of small crustaceans in a fishing harbor on the Ryukyu Islands, southern Japan, we collected a single specimen that we identified morphologically as *H. urashima*. In this study, we obtained a COI barcode sequence to...
confirm the identification based on morphology and showed conclusively that *H. urashima* occurred in a harbor fouling community as well as a commensal on the loggerhead sea turtle.

**Materials and Methods**

Sampling of tanaidaceans was conducted at the Kurio fishing harbor, Yakushima Island, Japan (30°16'08.0"N 130°25'09.7"E), on 18 December 2015 and 1 July 2016. The tanaidacean specimen was collected on one of the old tether lines investigated by the first author in 2015 (Fig. 1). The tether lines hung down from harbor walls, with most of each line continually submerged and encrusted by various sessile organisms, including ascidians, bryozoans, and hydroids. The fouled parts of the lines were rinsed in seawater to detach epibionts, and the water was sieved through a 0.3 mm-mesh plankton net. The tanaidacean was picked from the debris with forceps, and fixed and preserved in 99% ethanol. Total DNA was extracted from cephalothorax muscle tissue by using the silica method (Boom et al. 1990). Part of the cytochrome *c* oxidase subunit I (COI) gene was amplified by PCR with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Methods for PCR amplification, sequencing, and sequence assembly were as described by Tomioka et al. (2016). The COI sequence obtained was deposited in the International Nucleotide Sequence Database (INSD) under accession number LC411960. The methods used for dissection, preparation of slides, and light microscopy were as described by Kakui & Anguspanich (2012). The specimen was deposited in the University Museum Fujukan, University of the Ryukyus (catalogue number RUMF-ZC-5351).

**Results and Discussion**

Our specimen (Fig. 2) was a female. Although it was damaged, the morphologies of the labium, maxillipeds, pleopods 1–3, and uropods could be observed. The labial process was fused to the outer lobe, the maxillipedal endite bore two tiny dorsodistal and two distal spiniform setae, the maxillipedal coxa had two simple setae, the basal article of pleopod 3 had two outer setae but lacked inner setae, and the uropod consisted of a basal article and three-articulate ramus. This suite of characters indicated that the specimen was *H. urashima*. The COI sequence data supported this conclusion: the sequence obtained (655 nt long) was identical to one of two haplotype sequences reported for *H. urashima* by Tanabe et al. (2017) (accession number LC322246). This is the first DNA barcode confirmation of a presumed obligate commensal *Hexapleomera* species found in an environment other than on sea turtles’ surfaces.

Fig. 1. An old, fouled tether line from Kurio fishing harbor, Yakushima Island, Ryukyu Islands, southern Japan, where the specimen studied was collected.

図1. 標本を採集した屋久島栗生漁港の係留ロープと付着物.

Fig. 2. Preserved female specimen of *Hexapleomera urashima* collected from a fouling community, dorsal (A) and ventral (B) views, both chelipeds broken.

図2. 採集されたウラシマタナイス (*Hexapleomera urashima*) のメス個体 (両鉗脚は破損している ). 固定標本の背側 (A) と腹側 (B) 写真．
Hexapleomera urashima is not an obligate commensal. Yakushima Island, where the specimen was collected, is the primary nesting site for the North Pacific population of the loggerhead sea turtle (Hamann et al. 2013). Although we surveyed more than ten lines in the harbor on Yakushima and many additional lines in harbors on several other islands of the Ryukyus, the specimen recorded here was the only H. urashima specimen we found (Y. Tanabe unpublished data). This low abundance in the non-commensal environment may indicate that the single specimen became separated from a sea turtle accidentally and drifted haphazardly into the fouling community. To elucidate the association between H. urashima and loggerhead sea turtles, it would be necessary to examine more environmental samples from many sites, including sites on the western coast of North America, which is the easternmost edge of the distribution range of the North Pacific population of the loggerhead sea turtle (Wallace et al. 2010).

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References


[Report] Kakui & Tanabe: Hexapleomera urashima not from sea turtle
ウラシマタナイスのウミガメ体表以外からの初報告

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要旨. 屋久島栗生漁港の古い係留ロープの付着物から採集されたウラシマタナイス属(Hexapleomera Dudich)に属するメス1個体について、形態とCOI遺伝子の部分配列情報に基づき、アカウミガメ体表性種であるウラシマタナイス(Hexapleomera urashima Tanabe, Hayashi, Tomioka & Kakui)であることを明らかにした。本報告により、ウミガメ体表性タナイス類がウミガメ体表以外の環境からも見つかり得ることが初めて示された。今回得られた1個体は、1) 同個体がウミガメの産卵地として有名な永田浜から遠くない場所から採集されたこと、2) これまで琉球列島の様々な島で係留ロープ付着物上の生物調査を行ってきたものの本種の標本は今回1個体しか採集できていないことから、アカウミガメ体表上から偶然離れてしまった個体である可能性が高いと考えられる。

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