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Intraspecific variation in the turtle barnacle, *Cylindrolepas sinica* Ren, 1980 (Cirripedia, Thoracica, Coronuloidea), with brief notes on habitat selectivity

Ryota Hayashi[1,2]

1 *International Coastal Research Center, Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa, Chiba, 277-8564, Japan* **2** *Current address: Seikai National Fisheries Research Institute, Fisheries Research Agency 1551–8, Tairamachi, Nagasaki, 851–2213, Japan*

Corresponding author: *Ryota Hayashi* (bubobubo32@gmail.com)

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Abstract
Specimens of the turtle barnacle *Cylindrolepas sinica* Ren, 1980 were collected from sea turtles in Japanese waters. The specimens were hexagonal in shape and were found burrowing into the sea turtle plastron. Specimens were dissected and the hard and soft parts were compared with the original description.

Keywords
Turtle barnacle, *Cylindrolepas*, epibiont, variation, redescription, mitochondrial genes 12S and 16S

Introduction

Ren (1980) described a new species of barnacle, *Cylindrolepas sinica*, collected from green sea turtles, *Chelonia mydas* (Linnaeus, 1758). Hayashi (2009) subsequently recorded *Cylindrolepas sinica* from three species of sea turtle: the green sea turtle, *C. mydas*, loggerhead, *Caretta caretta* (Linnaeus, 1758), and hawksbill, *Eretmochelys imbricata* (Linnaeus, 1766). Subsequently, 13 species of the superfamily Coronuloidea, including *C. sinica*, were recorded during a 10-year (2002–2011) survey of epibionts attached to marine vertebrates from Japanese waters (Hayashi 2012). As shown in previous studies (Hayashi 2009, 2012), *C. sinica* is a common species on Japanese sea turtles.

This species was described as a cylindrical and rounded barnacle in previous studies (as shown in Fig. 1A and 1B). This study describes the intraspecific variation occurring in *C. sinica* and emphasises the morphological differences between *C. sinica* and related species. Brief comments on host selectivity are also presented.

Materials and methods

Epibiotic barnacles were sampled from sea turtles on breeding beaches, in bycatch, and from strandings in Japanese waters (see Hayashi 2012). Specimens of *C. sinica* were collected from the turtle (skin and plastron) and preserved in 99% ethanol. The specimens were dissected and their soft parts mounted on slides with a drop of glycerine. The specimens examined have been deposited in Fujukan, the Museum of the University of Ryukyus, under accession numbers RUMF-ZC.

Systematics

Class Maxillopoda Dahl, 1956
Subclass Cirripedia Burmeister, 1834
Superorder Thoracica Darwin, 1854
Order Sessilia Lamarck, 1818
Suborder Balanomorpha Pilsbry, 1916
Superfamily Coronuloidea Newman & Ross, 1976
Family Platylepadidae Newman & Ross, 1976
Genus *Cylindrolepas* Pilsbry 1916

***Cylindrolepas sinica* Ren, 1980**
http://species-id.net/wiki/Cylindrolepas_sinica
Figs 1, 2

Cylindrolepas sinica Ren, 1980: 194, fig. 6; pl. 2 figs 12–20. Hayashi 2009: 1, fig. 1A, B. Hayashi 2012: 118, figs 10, 15g, pl. 3d.
Platylepas decorata Zardus & Balazs, 2007: 1303, figs 7–9. Frick and Zardus 2010: 294.

Material examined. From the plastron of a green sea turtle stranded on Ishigaki Island, Okinawa, Japan, November 25, 2001, Ryota Hayashi coll. (RUMF-ZC-02047); from the plastron of a living green sea turtle, April 17, 2004, Hahajima Island, Ogasawara, Tokyo, Japan, Ryota Hayashi coll. (RUMF-ZC-02045); from a plastic tag on a stranded loggerhead sea turtle, September 26, 2010 at Yomitan, Okinawa, Japan, Ryota Hayashi coll. (RUMF-ZC-02048).

Additional description of intraspecific variation in the parietal wall. Wall outline stellate, parietes concave (Fig. 1C); translucent between external ornamentation, external

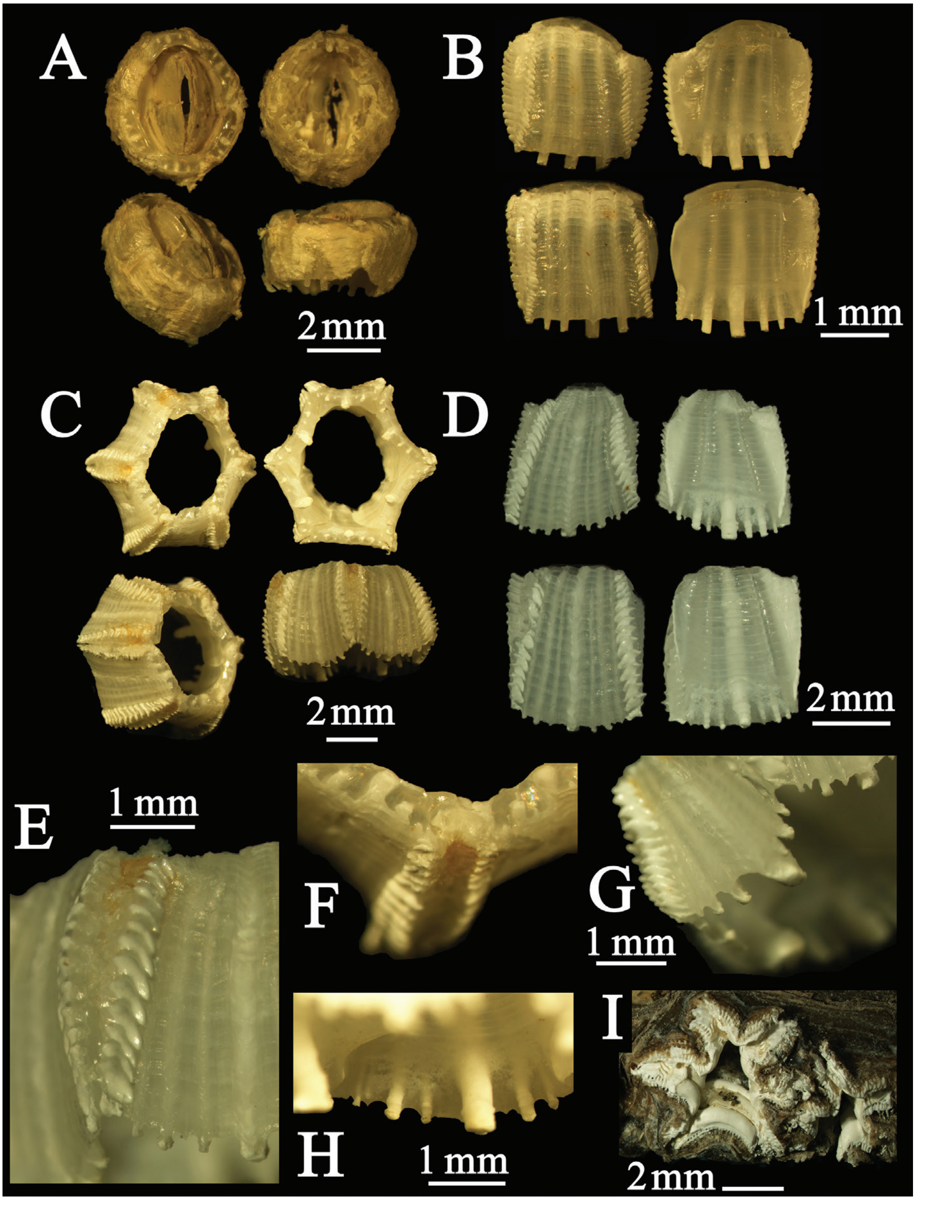

Figure 1. *Cylindrolepas sinica* Ren, 1980. **A–B** original variant occurring on the soft skin of sea turtles (RUMF-ZC-02030) **C–H** hexagonal variant occurring on the plastron of sea turtles (RUMF-ZC-02045) **A** and **C**, upper, basal, upper oblique and lateral views **B** and **D** parietal plates (surface and back view) **E** sutural elaboration of the parietal wall **F** upper view of radii **G** inner view of basal margin **H** basal margin with parietal ribs **I** hexagonal shell wall of *Xenobalanus globicipitis*.

longitudinal ridges low, broad, poorly defined, growth ridges numerous, fine, closely spaced; (Fig. 1D); sutural elaborations opaque, erect, irregular ridges slightly directed toward apex, not cupped (Fig. 1E); radii very narrow, externally teeth partly concealed

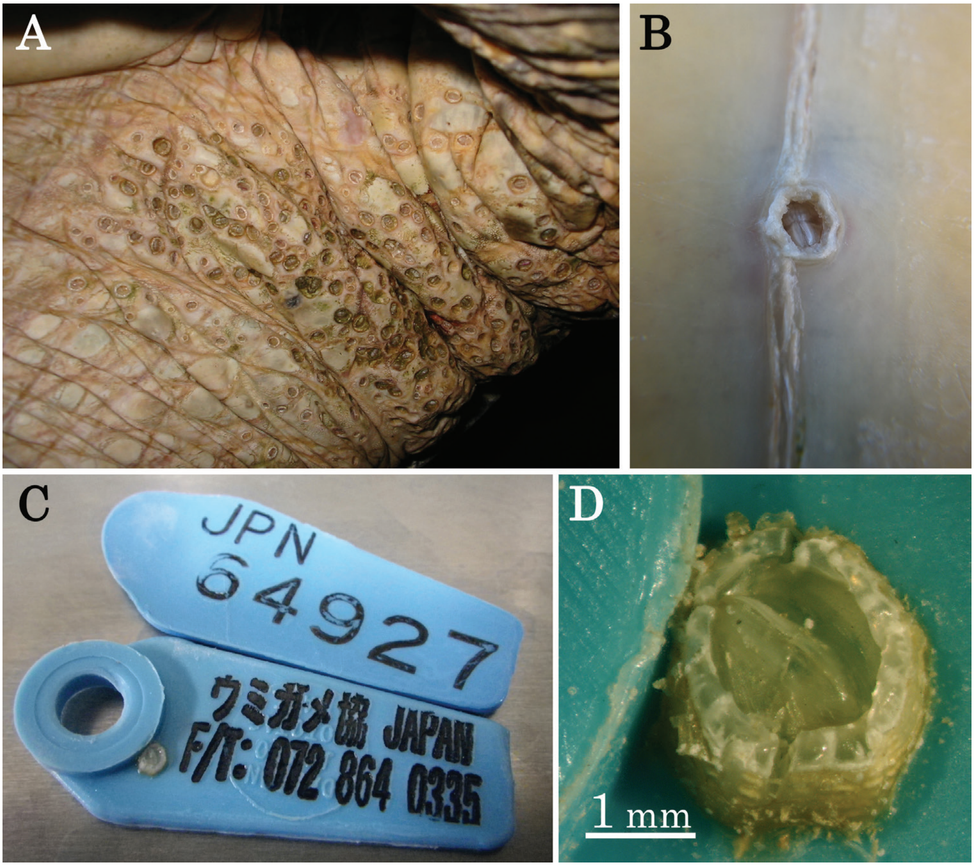

Figure 2. Habitat of *Cylindrolepas sinica* Ren, 1980. **A** the original rounded form aggregated on the tail of a green sea turtle **B** the hexagonal variant burrowing into a green sea turtle plastron **C** an unusual specimen attached to a plastic tag on a loggerhead sea turtle **D** close-up view of the individual attached to the plastic tag.

by sutural elaborations (Fig. 1F); internal midrib broad, short, flaring terminally or club-shaped, directed more downward than medially (Fig. 1G); internal lateral ribs well developed, moderately broad, short, extending below the basal margin, approximately same size and number of ribs on each side of midrib on all plates (Fig. 1G–H); sheath about two-thirds height of wall, basally terminating abruptly, not depending (Fig. 1D). Opercular valves and soft parts as described in Ren (1980) and Hayashi (2012).

Remarks. The original description of *C. sinica* described the rounded, cylindrical form and is accurate for individuals occurring on the soft skin of sea turtles. The general morphology of this species is as described by Ren (1980) and Hayashi (2012) and illustrated in the present work in Figs 1A, 1B, and 2A. Frick and Zardus (2010) and Frick (2013) regarded *C. sinica* as a junior synonym of *Platylepas decorata* Darwin, 1854. However, morphological differences between *C. sinica* and *P. decorata* are clearly detailed by Monroe and Limpus (1979), Ren (1980), Young (1991), and Hayashi (2012). *C. sinica* can be distinguished from other species easily by the morphological

Table 1. Comparative features of *Cylindrolepas* spp. and *Platylepas* spp.

	Labrum	Basal margin of sheath	Ornamentation of suture	Longitudinal ridges on parietes	Radii	Midrib folds	Secondary ribs	Inner surface of parietes
Cylindrolepas sinica	with a few teeth on each crest	continuous with inner laminae	present	absent	not visible	not visible	present	smooth
Cylindrolepas darwiniana	multidentate	continuous with inner laminae	rudimentary	rudimentary	not visible	not visible	present	smooth
Platylepas decorata	multidentate	depending	present	present	not visible	conspicuous	present	smooth
Platylepas hexastylos	with a few teeth on each crest	depending	absent	absent	visible, narrow	conspicuous	absent	with longitudinal ridges

Table 2. List of the materials examined. The GenBank accession numbers are from Hayashi et al. (2013).

	Host animal	Collected Locality	Materials deposited number	GenBank accession numbers
Cylindrolepas sinica (hexagonal form)	*Chelonia mydas*	Hahajima I., Ogasawara, Tokyo	RUMF-ZC-02045	AB723955
Cylindrolepas sinica (hexagonal form)	*Chelonia mydas*	Hahajima I., Ogasawara, Tokyo	RUMF-ZC-02047	AB723954
Cylindrolepas sinica (rounded form)	*Chelonia mydas*	Kanna, Ginoza, Okinawa	RUMF-ZC-02030	AB723953
Cylindrolepas darwiniana	*Caretta caretta*	Toya, Yomitan, Okinawa	RUMF-ZC-02029	AB723959
Cylindrolepas darwiniana	*Caretta caretta*	Toya, Yomitan, Okinawa	RUMF-ZC-02028	AB723960
Platylepas decorata	*Chelonia mydas*	Kanna, Ginoza, Okinawa	RUMF-ZC-02042	AB723950
Platylepas decorata	*Chelonia mydas*	Kanna, Ginoza, Okinawa	RUMF-ZC-02046	AB723951
Platylepas decorata	*Chelonia mydas*	Kanna, Ginoza, Okinawa	RUMF-ZC-02027	AB723952
Platylepas hexastylos	*Caretta caretta*	Otsuchi, Iwate	RUMF-ZC-02025	AB723956

characteristics listed in Table 1. In addition, the mitochondrial sequence variation of this and related species has been confirmed (the 12S rRNA, tRNA-Val and 16S rRNA regions, Table 2, see Appendix). Therefore, *Cylindrolepas sinica* is a valid species. In the phylogenetic analysis of Hayashi et al. (2013), *C. sinica* clustered with the whale barnacles (*Xenobalanus*, *Coronula*, and *Cryptolepas*). The pseudo-stalked barnacle *Xenobalanus globicipitis* also has hexagonal and cylindrical shell walls (Fig. 1A–I). Comparing these findings, *C. sinica* is likely ancestral to the whale barnacles (Hayashi et al. 2013).

Discussion

The rounded form (Fig. 1A and 1B) of this species (described by Ren 1980; Hayashi 2012) is found in the soft skin of sea turtles and forms colonies in proximity to other individuals, as well as in aggregations (Fig. 2A). The hexagonal variant of this species burrows into the hard parts of the turtle body (plastron) and is often found as isolated individuals (Fig. 2B). Therefore, the shell morphology of *C. sinica* exhibits phenotypic plasticity through habitat selection.

In a rare case, one individual was collected from a plastic tag attached to a loggerhead sea turtle (Fig. 2C, D). The tagged turtle was captured on June 24, 2010 in a set net at Yomitan, Okinawa, Japan, and recaptured in the same net on August 26, and finally found as a floating stranding nearby on September 26. These records indicate that this turtle was a resident in this coastal area, as reported in Hayashi (2009), and the presence of *C. sinica* is consistent with the previous report. In addition, this is the first record of the occurrence of *C. sinica* on an artificial object. This case indicates that *C. sinica* can attach to hard substrates, as well as living soft tissue, and the host selectivity of *C. sinica* is not the substrate material. Nogata and Matsumura (2006) reported the larval development and settlement of the whale barnacle, *Coronula diadema* (Linnaeus, 1767), which settled in a polystyrene Petri dish containing a small, isolated piece of skin tissue from the host whale. They suggested the involvement of a chemical cue from the host whale tissue in inducing larval settlement. In light of these findings, our finding of *C. sinica* settling on a plastic tag, and not directly on the body of the turtle, suggests that this settlement was triggered by a similar chemical cue. More information is necessary to clarify the settlement mechanism of epibiotic barnacles.

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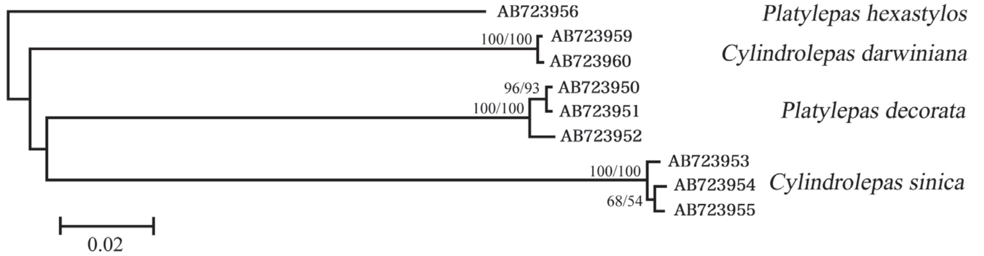
Appendix

Phylogenetic analysis



The consensus trees in the neighbour-joining and maximum-parsimony analyses were inferred from 1000 bootstrap replicates and both analyses were conducted using MEGA ver. 5.0 (Tamura et al. 2011). The sequences were aligned using MUSCLE included in MEGA ver. 5.0. At each node, the numbers separated by the slash indicate the percentage NJ and MP bootstrap support, respectively (1000 replicates).