Antennapeachia jambio (Cnidaria: Actiniaria: Haloclavidae), the Second Species of Genus *Antennapeachia*, with Revision of the Diagnosis of the Genus

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A new species of sea anemone, *Antennapeachia jambio* sp. nov., was collected off Jogashima, central Japan. The presence of a single, strong siphonoglyph and physa-like aboral end, and the absence of the sphincter muscle classify this sea anemone within the family Haloclavidae. Two antenna tentacles and non-paired microcnemes categorize it into genus *Antennapeachia*, where only *Antennapeachia setouchi* Izumi and Yanagi, 2016 was included. However, unlike *A. setouchi*, *A. jambio* has two more marginal tentacles and two macrocnemes between ventral directives and ventro-lateral mesenteries. On the basis of details of these new characters of *Antennapeachia*, the diagnosis of the genus was revised to accommodate both species.

Key Words: Sea anemone, Haloclavidae, *Antennapeachia*, revising genus, new species, Japan, Pacific, R/V *Rinkai-Maru*.

Introduction

Haloclavidae is a family of sea anemones included in the order Actiniaria. The most distinct features of Haloclavidae are a single, ventral, usually very strong siphonoglyph and physa-like aboral end (Carlgren 1949). All species included in this family always inhabit the sand or mud on the sea-floor. There are nine genera and around 30 species known in this family worldwide (Daly *et al.* 2007; Fautin 2007, 2013; Lauretta *et al.* 2011; Yap *et al.* 2014; Izumi *et al.* 2016). In Japan, six species in five genera of this family are known: *Anemonactis mazeli* Jourdan, 1880; *Anemonactis minutus* Wassilieff, 1908 (nomen dubium); *Haloclava* aff. *producta* Stimpson, 1856 in Uchida and Soyama (2001); *Harenactis attenuata* Torrey, 1902; *Peachia quinquecapitata* McMurrich, 1913; and *Antennapeachia setouchi* Izumi and Yanagi, 2016 (Yanagi 2006; Izumi *et al.* 2016).

The newest genus *Antennapeachia* Izumi and Yanagi, 2016 was established for an halocladid sea anemone that is characterized by having two peculiar discal tentacles, “antenna tentacles” (Izumi *et al.* 2016), whereas all the other genera have only uniformly shaped, thick, bar-like tentacles. This genus accommodates only one species, *A. setouchi*.

During dredging surveys off Jogashima, located at the southern tip of the Miura Peninsula, central Japan, specimens bearing two discal tentacles similar to *A. setouchi* were collected. In the present study, we describe these specimens as a new species of *Antennapeachia*, *A. jambio*. Izumi *et al.*

(2016) stated that the genus *Antennapeachia* has two pairs of mesenteries consisting of a macrocneme and a microcneme between ventral directives and ventro-lateral mesenteries based on *A. setouchi*, the only species in this genus. However, the new specimens have two mesenterial pairs consisting of macrocnemes and two independent microcnemes. Therefore, we revised the diagnosis of *Antennapeachia*.

Materials and Methods

Sample collection and preservation. The holotype specimen was collected off Jogashima, Kanagawa Prefecture, in the Pacific Ocean (Fig. 1), at 238–309 m depth on February 19, 2014, by using a biological dredge onboard the R/V *Rinkai-Maru* of Misaki Marine Biological Station, The University of Tokyo, during the second Coastal Organism Joint Survey of JAMBIO, Japanese Association for Marine Biology. The specimen was carried to the Coastal Branch of Natural History Museum and Institute, Chiba (CMNH) and kept undisturbed in an aquarium until the tentacles were completely spread and elongated. Then, it was anesthetized with magnesium sulfate solution. Finally, the whole body, except for two marginal tentacles, was fixed in 5% formalin solution with seawater. The two tentacles were dissected from the body and preserved in 99.5% ethanol for future DNA analysis. The paratype specimen was collected off Miura Peninsula, Kanagawa Prefecture, in the Pacific Ocean (Fig. 1), at 95–97 m depth on February 15, 2017, by using

Fig. 1. The sampling locality of *Antennapeachia jambio* sp. nov. Stars denote the sampling sites; black star indicates the locality of holotype (CMNH-ZG 06546); white one indicates the locality of paratype (NSMT-Co 1596). (Position: the left side of the page, nearby the Material and Method part.)

a biological dredge onboard the R/V *Rinkai-Maru*, during the twelfth Coastal Organism Joint Survey of JAMBIO. The specimen was processed by the completely same way as the holotype except the place where that was preserved; the all process was undergone in Misaki Marine Biological Station.

The holotype specimen was deposited at the CMNH (CMNH-ZG 06546). The paratype one was at the National Museum of Nature and Science, Tsukuba (NSMT-Co 1596).

Preparation of histological sections. The holotype specimen (CMNH-ZG 06546) was processed to histological sections by standard methods (Presnell and Schreibman 1997). The dissected specimen preserved in 5% formalin was dehydrated with ethanol, dissected some tissues by tweezers and scissors, dehydrated by xylene, embedded in paraffin, sliced into serial sections (8 μ m thick) by using a microtome, mounted on glass slides, and stained with hematoxylin and eosin.

Observation of cnidae. Cnidae on the tentacles (both antenna tentacles and marginal tentacles), column, actinopharynx (above tissues were from both specimens), and filaments (only from CMNH-ZG 6456, because the NSMT-Co 1596 was too damaged in proximal end to take tissue of filament for analysis) were observed. Images of the cnidae were obtained by differential interference contrast microscopy (Yanagi *et al.* 2015), and the length and width were measured using the software ImageJ v. 1.49 (Rasband 1997–2012). Cnida nomenclature followed Mariscal (1974).

Results

Order **Actiniaria** Hertwig, 1882

Family **Haloclavidae** Verrill, 1899

Antennapeachia Izumi and Yanagi, 2016

[New Japanese name: Antena-isoginchaku-zoku]

Diagnosis. See below discussion.

Type species. *Antennapeachia setouchi* Izumi and Yanagi, 2016.

Antennapeachia jambio sp. nov.

[New Japanese name: Misaki-no-antena]

(Figs 2–4, Table 1)

Material examined. Holotype: CMNH-ZG 06546; dissected specimen, embedded tissues in paraffin, histological sections (3 slides), prepared nematocysts (5 slides); February 19, 2014, off Jogashima, Kanagawa Prefecture, 35°06.082'N, 139°34.232'E, at 238–309 m in depth collected by Kensuke Yanagi. Paratype: NSMT-Co 1596; whole specimen, somewhat damaged in proximal end; February 15, 2017, off Miura Peninsula, Kanagawa Prefecture, 35°08.0383'N, 139°33.731'E, at 95–97 m in depth collected by Takato Izumi.

Etymology. This species was named, because the type specimens were collected in the second and twelfth JAMBIO Coastal Organism Joint Survey. JAMBIO stands for “Japanese Association for Marine Biology.”

Description. *External anatomy.* Column smooth, cylindrical or barrel-like, rich in expansibility, length *ca.* 9 mm, diameter *ca.* 7 mm in alive, and length 8 mm diameter 6 mm in preserved specimens. Color of body orange (Fig. 2A, B). Column surface smooth, papillae absent, with numerous discontinuous wavy wrinkles running direction of transversal and pale white patches (Fig. 2B). Aboral end flattened or slightly concaved, like a shape of donut, with tiny hole in center, semitransparent, so that line of mesenteries visible from outer side. Pedal disk absent, but somewhat sticky so that particles of mud adhered to column or aboral end (Fig. 2B). Two circles of tentacles on oral disc; 16 tentacles. Fourteen marginal tentacles in outer circle; two antenna tentacles in inner circle (Fig. 3A). Marginal tentacles protruding outside or hanging along column. Antenna tentacles rising straight upward. Marginal tentacles approximately 4–5 mm in length when fully expanded. Antenna tentacles 1–2 mm in length, far shorter than marginal tentacles. Both tentacles simple, without acrospheres. Coloration of marginal tentacles brownish, obscure cross-bands of yellow and brown; surface complicatedly wrinkled. Antenna tentacles without cross-bands; surface wrinkled (Fig. 2A). Oral disk diameter *ca.* 4 mm, same color as tentacles. Mouth at center of oral disk, a little swelled, with well-extended and robed conchula on ventral side (Fig. 2A).

Internal anatomy. Eight pairs of macrocnemes, six of first cycle and extra two pairs between ventro-lateral mes-

Fig. 2. *Antennapeachia jambio* sp. nov., holotype, CMNH-ZG 06546, external morphology in living state (A, B) and internal morphology of the preserved specimen (C–I). (A) Oral view; the conchula is located on the ventral side of the mouth. (B) Lateral view of the whole body. (C) Cross section of the column. (D) Longitudinal section of a tentacle. (E) Cross section of a tentacle showing the ectodermal longitudinal muscles (indicated by arrowheads). (F) Cross section of the column, enlargement of a part of (C). (G) Longitudinal section of the oral end of the column. (H) Cross section of the mesentery with filament. (I) Longitudinal section of the aboral end. Abbreviations: a, actinopharynx; at, antenna tentacle; c, conchula; f, filament; me, mesoglea; mi, microcneme; mt, marginal tentacle; pm, parietal muscle; rm, retractor muscle; s, siphonoglyph; tcm, tentacular circular muscle; tlm, tentacular longitudinal muscle. Scale bars indicate 5 mm in A and B, 1 mm in C, and 100 μ m in D–I. (Position: the center of upper or lower of the page, nearby the Result part.)

Fig. 3. Schematic representation of the arrangements of the tentacles (A) and mesenteries (B) in *Antennapeachia jambio* sp. nov. There are two antenna tentacles and fourteen marginal tentacles in the cycles, and one tentacle communicates with each endo- and exocoel (A and M means the position of antenna and marginal tentacle, respectively). The antenna tentacles are between the ventro-lateral mesenteries. Two microcnemes (broken line) disappear near the mouth. (Position: the left or right of the page, nearby the Result part.)

enteries and ventral directives; two independent microcnemes between ventro-lateral mesenteries and ventral directives (Figs 2C, 3B). All macrocnemes perfect and continuing along whole length of body. Microcnemes present from proximal end to middle part of body, but absent near oral end. Antenna tentacles between ventro-lateral endocoels (Fig. 3B). Seven marginal tentacles of outer cycle on dorsal half of body; one of them arised from dorsal endocoel; two (one on each side) from dorso-lateral exocoels; two from dorso-lateral endocoels; other two from lateral exocoels. Other seven on ventral half of body; one of them arised from ventral endocoel; two from endocoel between two extra pairs of macrocnemes; the other four in ventro-lateral exocoel (Figs 2A, 3B). Tentacular circular muscle endodermal (Fig. 2D); tentacular longitudinal muscle ectodermal (Fig. 2E). Retractor muscles strongly circumscribed, limited at the center of each macrocneme (Fig. 2C, F), with approximately 15–30 a little branched muscular processes; parietal muscles of macrocnemes distinct, with around ten simple muscular processes (Fig. 2F, H). Mesoglea in body wall far thicker than ectoderm and endoderm, thinner in actinopharynx and siphonoglyph (Fig. 2F). Actinopharynx smooth, comparatively long in column, with huge siphonoglyph on ventral side, which always connected to it (Fig. 2C). Sphincter muscle absent (Fig. 2G). On aboral end, basilar muscle absent (Fig. 2I). Filaments at the tips of macrocnemes, small and limited near aboral end. Matured gametes not observed, so sexuality of specimens not clear.

Cnidom. Spirocyst in both tentacles, basitrich in every tissue, microbasic amastigophore in actinopharynx and filament, microbasic *p*-mastigophore in actinopharynx and filament (Table 1, Fig. 4). Basitrichs in filament distinguished as two types by their size. Microbasic *p*-mastigophore in actinopharynx morphologically distinguished as two types

Fig. 4. Cnidae of *Antennapeachia jambio* sp. nov., holotype, CMNH-ZG 06546. (A) Spirocyst in an antenna tentacle. (B) Basitrich in an antenna tentacle. (C) Spirocyst in marginal tentacles. (D) Basitrich in marginal tentacles. (E) Microbasic amastigophore in marginal tentacles. (F) Basitrich in the actinopharynx. (G) Microbasic amastigophore in the actinopharynx. (H) Microbasic *p*-mastigophore type-1 in the actinopharynx. (I) Microbasic *p*-mastigophore type-2 in the actinopharynx. (J) Basitrich in the column. (K) Short basitrich in the filament. (L) Long basitrich in the filament. (M) Microbasic amastigophore in the filament. (N) Microbasic *p*-mastigophore in the filament. (Position: the center of upper or lower of the page, nearby the Result part.)

(“Type 1” and “Type 2”; Fig. 4H, I): Type 1 comparatively shorter than Type 2 (Figs 4, 5); ratio of shaft length for capsule length of Type 1 smaller than that of Type 2 (0.65–0.76 [$n=10$] vs. 0.92–1.01 [$n=5$]); and slight middle expansion on shaft present in Type 1 but not in Type 2.

Remarks. This species resembles *A. setouchi* in having antenna tentacles, forming a siphonoglyph (which is connected to conchula) beside their actinopharynx and having wrinkled orange color column. *Antennapeachia jambio*, however, has many different features from *A. setouchi*; antenna tentacles are far smaller than marginal tentacles, in contrast those of *A. jambio* are bigger and more prominent than marginal ones (Fig. 2, Izumi *et al.* 2016); *A. jambio* has a different type of cnidae from *A. setouchi*, especially the microbasic *p*-mastigophores are only in actinopharynx and filament of *A. jambio* (Table 1, Figs 4, 5); the two pairs of macrocnemes between ventro-lateral mesenteries and ventral directives are only formed in the column of *A. jambio*. The last difference is most characteristic, that is why we judged that *A. jambio* is appropriate to be treated as new species, and we revised the diagnosis of genus (see the Discussion part).

Discussion

Revision of the diagnosis of *Antennapeachia*. *Antennapeachia* was founded to accommodate *A. setouchi* Izumi and Yanagi, 2016 (Izumi *et al.* 2016). *Antennapeachia setouchi* has a unique tentacular arrangement compared with the other haloclavids; two antenna tentacles located in the inner circle and 12 marginal tentacles in the outer circle. *Anten-*

napeachia setouchi has two independent macrocnemes between ventral directives and ventro-lateral mesenteries in its column except six pairs of macrocnemes in the first cycle. Each of the two independent macrocneme apparently paired with each microcneme. *Antennapeachia jambio*, however, had two additional marginal tentacles at the outer circle, and accordingly, two additional macrocnemes in its column. Each of the additional macrocnemes was located between the macrocneme and microcneme and paired with the independent macrocnemes; the eight macrocneme pairs—six pairs in the first cycle and two more pairs—were in its column (Fig. 6), and two microcnemes were independent. As a result, the diagnosis of the genus was revised to include both types of mesentery arrangement and tentacular arrangement found in these two species of *Antennapeachia*. We also found an additional type of cnidae, microbasic *p*-mastigophores, in *A. jambio*, which was not found in *A. setouchi* (Fig. 6). Thus, the diagnosis of cnidom was also revised.

Finally, the diagnosis of *Antennapeachia* is emended as followed due to accommodating this new haloclavid species.

Emended Diagnosis. Elongate haloclavid with well-developed, rounded or tapered aboral end. Column indistinctly divisible into regions, without tenaculi, but with minute adherent areas on surface. Sphincter absent. Tentacles 14–16 (two discal tentacles [or antenna tentacles] near mouth and 12 or 14 marginal tentacles), without acrospheres. Single, very deep siphonoglyph, with oral end drawn out in lobate conchula, never forming distinct tube separated from cavity of actinopharynx by ectodermal fusion. Pairs of macrocnemes six or eight; two independent microcnemes between ventral directives and ventro-lateral mesenteries. All mesenteries, except two microcnemes in second cycle perfect,

Table 1. Size and distribution of cnidae in *Antennapeachia jambio* sp. nov., holotype, CMNH-ZG 06546 and paratype, NSMT-Co 1596. A–N in parentheses indicate the figures of each cnidae in Fig. 4. Concerning the paratype specimen, filament was too damaged to observe nematocysts.

Cnidae type	Size (μm)						N	Size (μm)						N
	Length	mean	SD	Width	mean	SD		Length	mean	SD	Width	mean	SD	
CMNH-ZG 06546 (Holotype)								NSMT-Co 1596 (Paratype)						
Antenna tentacle														
Spirocysts (A)	16.0–37.4	27.7	5.40	2.5–5.3	3.8	0.57	64	20.1–38.3	29.0	4.41	2.6–4.3	3.6	0.47	79
Basitrichs (B)	18.6–26.9	23.2	1.54	2.9–4.2	3.5	0.31	51	14.0–24.7	20.8	2.90	2.5–3.8	3.0	0.33	38
Marginal tentacle														
Spirocysts (C)	16.6–39.4	29.7	5.06	2.7–4.9	3.8	0.51	82	18.3–36.0	29.1	4.58	2.2–4.2	3.3	0.37	71
Basitrichs (D)	20.4–27.5	24.1	1.42	2.4–4.2	3.3	0.37	62	15.4–27.7	22.7	2.06	2.2–3.7	3.1	0.31	44
Microbasic amastigophores (E)	44.6–47.9	46.5	1.42	9.9–11.6	10.6	0.71	3	45.6–51.3	47.8	2.52	10.7–12.2	11.4	0.63	3
Actinopharynx														
Basitrichs (F)	20.7–30.2	25.8	1.65	2.6–4.1	3.2	0.36	48	13.2–35.1	28.5	5.18	2.5–4.6	3.38	0.39	75
Microbasic amastigophores (G)	38.3–45.8	42.7	1.80	9.3–12.0	10.7	0.68	62	40.0–50.2	44.7	3.16	9.3–13.0	11.0	1.09	12
Microbasic <i>p</i> -mastigophores (Type 1) (H)	44.2–51.8	47.8	1.96	9.5–13.5	11.7	0.87	38	38.8–48.7	43.1	2.49	8.1–11.9	9.65	0.81	33
Microbasic <i>p</i> -mastigophores (Type 2) (I)	51.0–61.7	54.0	3.91	10.9–14.9	12.7	1.63	5	Not observed						
Column														
Basitrichs (J)	12.9–17.6	15.6	1.06	2.6–4.4	3.4	0.41	65	14.6–18.3	16.7	0.78	2.2–3.9	3.1	0.33	88
Filament														
Basitrichs (short) (K)	10.5–13.2	11.6	1.10	2.6–3.2	2.8	0.25	5	No data						
Basitrichs (long) (L)	20.3–27.9	23.7	1.74	2.3–4.2	3.5	0.40	55							
Microbasic amastigophores (M)	51.0–52.9	51.9	0.93	11.9–12.8	12.3	0.43	2							
Microbasic <i>p</i> -mastigophores (N)	26.1–38.9	34.9	3.38	5.6–9.4	8.1	0.87	26

Fig. 5. Size distribution of nematocysts of *Antennapeachia jambio* sp. nov., holotype, CMNH-ZG 06546. For letter abbreviations (A–N) refer to Fig. 4. (Position: under the Fig. 4, the center of upper or lower of the page.)

fertile, with filaments and strong diffuse retractors. Cnidom: spirocysts, basitrichs, microbasic *b*-mastigophores, microbasic *p*-mastigophores, microbasic amastigophores, macrobasic mastigophores.

Role of the antenna tentacles of *Antennapeachia*

Antennapeachia setouchi has two prominent antenna tentacles raised upward (Izumi *et al.* 2016), whereas the antenna tentacles of *A. jambio* were tiny and scanty and tend to be shrunk into its column. Izumi *et al.* (2016) suggested that the antenna tentacles of *A. setouchi* might have a unique role: some kind of sensor in the upward direction, unlike

the other functionally specialized tentacles, *e.g.*, catch tentacles of *Metridium senile* (Purcell 1977). However, the antenna tentacles of *A. jambio* may not be useful as a sensor. The antenna tentacles may have similar function to marginal tentacles, and only their position is different from the latter. To clarify whether the antenna tentacles have a special function or not, we need further observation of living individuals of both species in the field and aquarium.

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Fig. 6. Comparison of the mesenterial arrangement of two *Antennapeachia* species. The difference of the mesenterial arrangement of *A. jambio* sp. nov. and *A. setouchi* is the number of macrocneme pairs; *A. jambio* has two additional pairs of macrocnemes. Both species have two independent microcnemes. (Position: the center of upper or lower of the page, nearby the Discussion part.)

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