

The Largest Cnidaria Among the Sea Anemones; Description of a New Haloclavid Species from Japan, *Haloclava hercules* (Cnidaria: Actiniaria: Enthemonae: Haloclavidae)

Takato Izumi

Molecular Invertebrate Systematics and Ecology Laboratory, Department of Biology, Chemistry, and Marine Sciences,
Faculty of Science, University of the Ryukyus, 1 Seimbaru, Nishihara, Okinawa 903-0213, Japan
E-mail: iz.takato@gmail.com

(Received 10 December 2020; Accepted 12 July 2021)

<http://zoobank.org/59F8BE91-1327-4081-B515-85DFC8A26492>

Members of the family Haloclavidae, belonging to the order Actiniaria, are characterized by the presence of a large siphonoglyph next to their actinopharynx and an aboral end without a basal disc. Members of the genus *Haloclava* Verrill, 1899 have been reported primarily from Europe and America, and have not yet been described from Japanese waters based on the collected specimen. In this study, I describe a new species, *Haloclava hercules* sp. nov., from the Pacific coast of Japan. This new species is chiefly characterized by cudgel-like blunt massive tentacles with knob-like acrospheres in the outer tentacular cycle and gigantic basitrichs over 250 μm in length, which are the largest known from sea anemones.

Key Words: Enthemonae, cnidom, acrosphere, R/V *Rinkai-Maru*, R/V *Seisui-Maru*, nematocysts, largest record.

Introduction

The most characteristic features of the sea anemone family Haloclavidae (Cnidaria: Anthozoa: Actiniaria: Enthemonae: Actinioidea) are a ventral siphonoglyph, which is usually extraordinarily strong, and a physa-like aboral end (Carlgren 1949). All species in this family have thick, blunt, and bar-like tentacles, and bury themselves in the sand or mud bottom.

Currently, approximately 30 species are described from 13 genera in this family (Daly and Fautin 2020), of which seven species in five genera are known from Japanese waters (Table 1; Yanagi 2006).

Sea anemones of *Haloclava* Verrill, 1899 are characterized by a siphonoglyph without conchula and simple columns without apertures. Though this genus includes six species, and *Haloclava chinensis* Carlgren, 1931 and *H. stimpsoni* (Verrill, 1868) have been reported from Chinese waters (Verrill 1868; Carlgren 1931; Pei 1998), there has been only one report from Japanese waters [*H. aff. producta* (Stimpson, 1856) in a field guide by H. Uchida and Soyama (2001) without detailed description of internal morphologies].

In the present study, two specimens of sea anemones of Haloclavidae, which possess the abovementioned diagnostic features of *Haloclava*, were collected from the Pacific coast of Japan. The two specimens presented a peculiar feature: extraordinarily large basitrichs in acrospheres, the knob-like structures on the tentacular tips of this haloclavid. The lengths of the basitrichs reached 273 μm , slightly shorter than 0.3 mm, which is far larger than those from any pre-

viously recorded sea anemones (previous maximum record was 202 μm in length, found in the nemathyobomes of *Edwardsia alternobomen* Izumi and Fujita, 2019). With additional several features by which these haloclavid specimens can be distinguished from other species of *Haloclava*, I describe *Haloclava hercules* sp. nov. for these specimens.

Materials and Methods

Sample collection and preservation. Examined two specimens of *Haloclava hercules* sp. nov. were collected with 50 cm biological dredges. The holotype was collected west off Misaki, Miura Peninsula, Kagawa Prefecture (Fig. 1A), at 272–370 m depth, on 9 May 2017, St. 1, during a cruise of the R/V *Rinkai-Maru* of Misaki Marine Biological Station, The University of Tokyo. The single paratype specimen was collected south off Shima, Shima Peninsula, east of Mie Prefecture (Fig. 1B), at 130–132 m depth, on 9 November 2017, St. 7, during a research cruise of R/V *Seisui-Maru* of Mie University. The two specimens of *H. hercules* sp. nov. were kept undisturbed in small cases for several hours until they spread and elongated their tentacles. Subsequently, the relaxed specimens were anesthetized with l-menthol (holotype) or magnesium chloride solution (paratype) and fixed in 5% (v/v) seawater formalin solution. The examined specimens were then deposited in the Tsukuba Research Department of the National Museum of Nature and Science, Tokyo (NSMT) and the Coastal Branch of Natural History Museum and Institute, Chiba (CMNH).

Table 1. Haloclavidae anemones from Japanese waters. Field guides are indicated as bold.

Species	References
<i>Anemonactis tohrui</i> Izumi, Yanagi, and Fujita, 2020	Izumi et al. (2020)
<i>Anemonactis minuta</i> (Wassilieff, 1908)	Izumi et al. (2020)
<i>Haloclava</i> aff. <i>producta</i> (Stimpson, 1856)	H. Uchida and Soyama (2001)
<i>Harenactis attenuata</i> Torrey, 1902	T. Uchida (1965)
<i>Peachia quinquecapitata</i> McMurrich, 1913	T. Uchida (1965)
<i>Antennapeachia setouchi</i> Izumi and Yanagi, 2016	Izumi et al. (2016)
<i>Antennapeachia jambio</i> Izumi, Fujita, and Yanagi, 2017	Izumi et al. (2017)

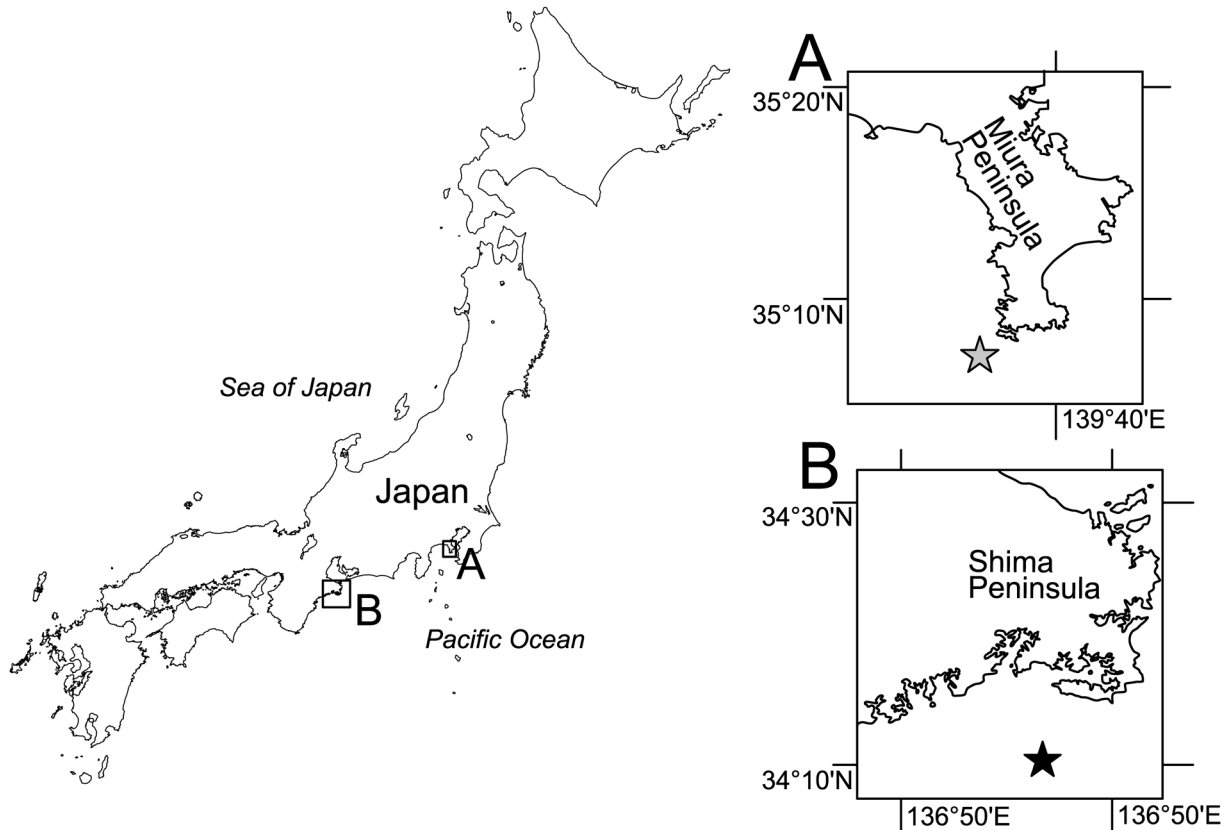


Fig. 1. Sampling localities of *Haloclava hercules* sp. nov. A, Locality of the holotype (NSMT-Co 1754); B, locality of the paratype (CMNH-ZG 09758).

Observation of specimens and preparation of histological sections. The holotype specimen was dissected and observed using a stereoscope, and the dissected tissues were sectioned as following standard protocols (Presnell and Schreibman 1997): dissected tissues were dehydrated by ethanol and xylene, embedded in paraffin, sliced into 10 μ m thick serial sections using a microtome (HistoCore AUTOCUT R; Leica), mounted on glass slides, and stained by hematoxylin and eosin.

Cnidae observation. Tissue with cnidae were extracted from the tentacle (acrosphere and other parts), actinopharynx, column, and filament tissues of the holotype (NSMT-Co 1754) and smeared to glass slides. Images of the cnidae were obtained by differential interference contrast microscopy (Zeiss Axio Imager; Zeiss), following the method of Yanagi (2017). For each capsule, the length and width on the images were measured using the software ImageJ v. 1.49

(Rasband 1997–2012). Size distributions were processed, and values of means and standard deviations were calculated using Microsoft Excel 2013. Cnidae nomenclature referred to Mariscal (1974).

Taxonomy

Order **Actiniaria** Hertwig, 1882
 Superfamily **Actinoidea** Rafinesque, 1815
 [Japanese name: umeboshi-isoginchaku-jouka]
 Family **Haloclavidae** Verrill, 1899
 [Japanese name: kombo-isoginchaku-ka]

Haloclavidae Verrill, 1899: 41; Carlgren 1949: 29.

Type genus. *Haloclava* Verrill, 1899.

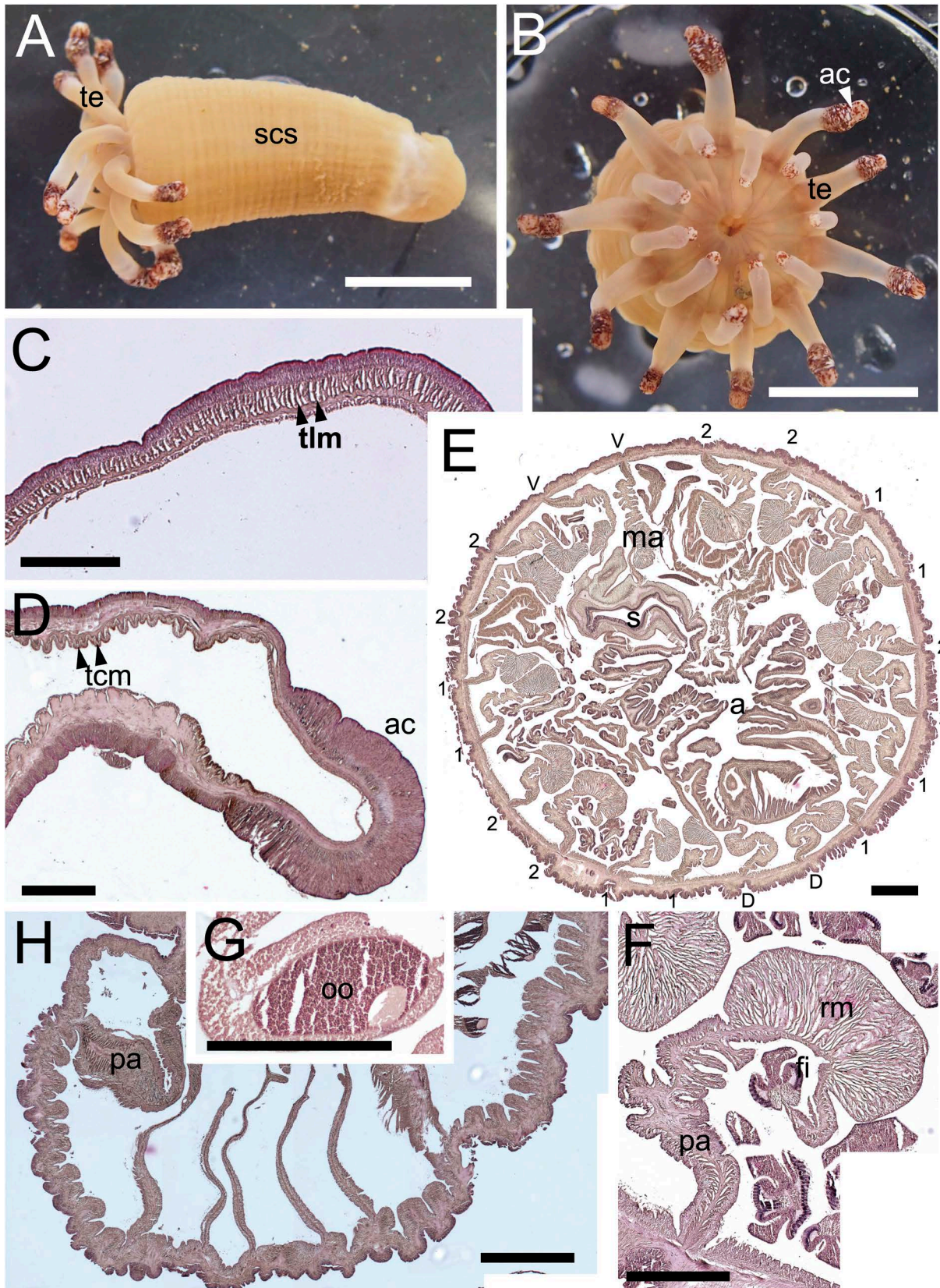


Fig. 2. *Haloclava hercules* sp. nov., holotype, NSMT-Co 1754, external morphology in living state (A, B) and internal morphology of the preserved specimen (C–H, histological section stained by hematoxylin and eosin). A, Lateral view of the whole body; B, oral view; C, transverse section of a tentacle; D, longitudinal section of a tentacle; E, transverse section of the column; F, enlarged view of the transverse section of the column; G, enlarged view of the transverse section of an oocyte; H, longitudinal section of the aboral end. Abbreviations: a, actinopharynx; ac, acrosphere; D, dorsal directive; fi, filament; ma, macrocneme; oo, oocyte; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; scs, scapus; tcm, tentacular circular muscle; te, tentacles; tlm, tentacular longitudinal muscle; V, ventral directive; 1, first cycle mesentery; 2, second cycle mesentery. Scale bars: 10 mm in A and B, 1 mm in E, F, H, 500 μ m in D, G, and 100 μ m in C.

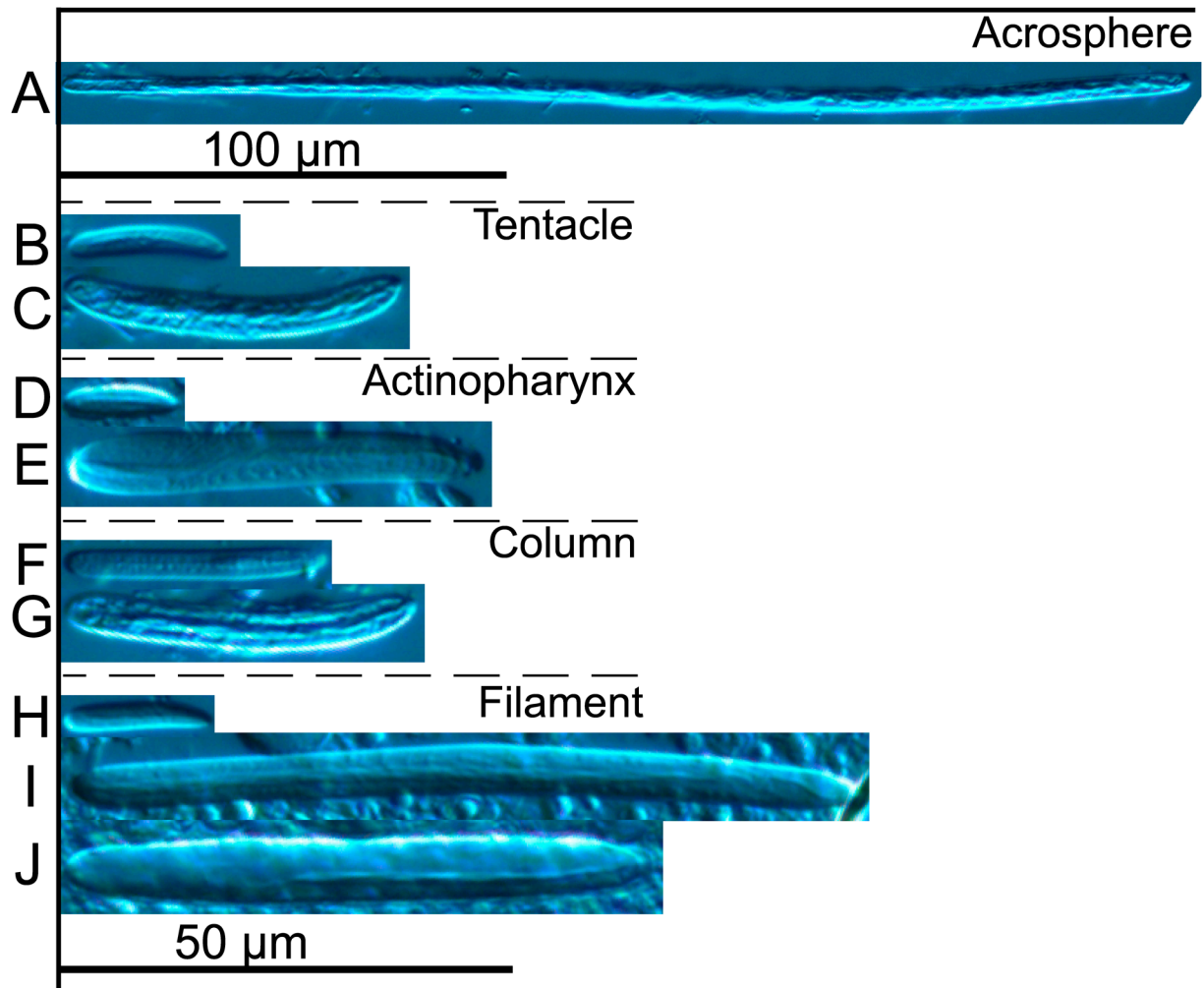


Fig. 3. Cnidom of *Haloclava hercules* sp. nov., holotype, NSMT-Co 1754. A, Basitrich of an acrosphere; B, small basitrich in a tentacle; C, large basitrichs in a tentacle; D, small basitrich in the actinopharynx; E, large basitrich in the actinopharynx; F, small basitrich in the column; G, large basitrich in the column; H, small basitrich in a filament; I, large basitrich in a filament; J, microbasic *b*-mastigophore in a filament. Scale bars: 100 µm for A, and 50 µm for all other sub-figures.

Genus *Haloclava* Verrill, 1899

[Japanese name: konbo-isoginchaku-zoku]

Haloclava Verrill, 1899: 41; Carlgren 1949: 30.

Type species. *Actinia producta* Stimpson, 1856.

Haloclava hercules sp. nov.

[New Japanese name: herakuresu-no-konbou]

(Figs 2, 3; Table 2)

Material examined. NSMT-Co 1754 (the holotype): dissected specimen, embedded tissues in paraffin, histological sections, prepared nematocysts; 9 May 2017, off Jogashima, Misaki, Kanagawa Pref., 35°06.838'N, 139°34.063'E (St. 1), at 272–370 m depth, collected by Hiroshi Namikawa using a 50 cm biological dredge, kept in a tank of Misaki Marine Biological Station, and fixed and preserved by Takato Izumi; CMNH-ZG 09758 (a paratype): dissected specimen, embedded tissues in paraffin, histological sections,

prepared nematocysts; 9 November 2017, south off Shima, Shima Peninsula, Mie Pref, 34°10.109'N, 136°44.644'E (St. 7), at 130–132 m depth, collected by Itaru Kobayashi using a 50 cm biological dredge.

Description of the holotype (NSMT-Co 1754) and a paratype (CMNH-ZG 09758). *External feature:* Column barrel or corn-like, not differentiated into parts, with high degree of expansibility, length ca. 15–20 mm, diameter ca. 7–12 mm in live specimens, and length ca. 15 mm, diameter ca. 7–10 mm in preserved specimens. Body pale brownish orange (Fig. 2A, B), with transversal wrinkles (Fig. 2A), and mesenterial insertions. Small papillae lows on the column surface, but no apertures (Fig. 2A). Aboral end physa-like, rounded, with a tiny pore in the center, same in color as column, and slightly sticky and adheres to substrate. Oral disc with 20 tentacles in two indistinct cycles of 10 each (Fig. 2B). Outer tentacles protruding outside or hanging along the column, and inner ones protruding upwards. Morphological differences between the inner and outer tentacles followings: outer ones more robust and longer (ca. 9–12 mm in

Table 2. Cnidom of *Haloclava hercules* sp. nov. (NSMT-Co 1754). Abbreviations: n, numbers of measured cnidae; SD, standard deviations.

		<i>Haloclava hercules</i> sp. nov. NSMT-Co 1754 (holotype)				
		Length×Width (μm)	Mean (μm)	SD (μm)	n	frequency
Acrosphere						
large basitrichs		158.9–273.8×3.3–7.0	218.4×5.4	29.53×0.62	56	numerous
Tentacle						
basitrichs	S	10.0–19.0×2.4–3.9	16.0×3.1	2.31×0.34	41	numerous
	L	31.1–42.4×4.3–6.2	36.2×5.3	3.13×0.51	17	numerous
Actinopharynx						
basitrichs	S	10.2–17.9×2.7–3.9	14.0×3.2	1.41×0.32	47	numerous
	L	47.1–70.9×6.6–8.7	63.1×7.4	6.35×0.55	23	numerous
Column						
basitrichs	S	13.5–32.8×3.1–4.1	28.6×3.5	3.97×0.29	33	numerous
	L	27.7–41.5×5.1–6.8	36.0×5.8	3.49×0.56	12	few
Filament						
basitrichs	S	16.2–20.6×2.7–4.0	17.9×3.4	1.28×0.32	17	numerous
	L	73.5–94.6×4.8–6.6	85.0×5.7	4.56×0.45	39	numerous
microbasic <i>b</i> -mastigophores		62.2–78.8×7.0–8.9	67.1×7.9	5.12×0.58	8	few

length) than inner ones (ca. 5–7 mm in length) when fully expanded; outer ones apparently capitate with large robust acrosphere-like knobs though inner ones with indistinct small acrospheres on the tips (Fig. 2B). Both inner and outer tentacles uniform sizes and shapes in its middle part. Tentacles pale orange, translucent, smooth (Fig. 2A, B). Acrospheres patchy pattern with dark red and white, surface rough. Oral disc diameter ca. 12 mm, uniformly orange, in live specimens. Mouth at the center of oral disc, not swollen; conchula absent (Fig. 2B).

Internal anatomy. Twenty mesenteries, all macrocnemes. Ten pairs; 6 pairs of them in the first cycle and 4 pairs in the second cycle (Fig. 2E). All macrocnemes perfect, fertile, and continuing along the whole-body length. Microcnemes absent. All tentacles arise from each endocoel and exocoel. Tentacular longitudinal muscles ectodermal (Fig. 2C); tentacular circular muscles endodermal (Fig. 2D). Acrosphere on tentacle tips with densely arranged large basitrichs (Fig. 2D; Table 2). Retractor muscles restricted, limited at the center of each macrocneme (Fig. 2E), with approximately 25–40 multiple-branched muscular processes (Fig. 2F); parietal muscles of macrocnemes distinct, in peculiar shape: muscle with 10 slightly branched processes on each side bared from thin mesenteries by body wall, and additional muscle with simple 10–20 processes bared from thick mesoglea between the body wall and the retractor muscle (Fig. 2F). Moreover, several small slightly-branched muscular processes on opposite side of retractor muscle on macrocnemes. Mesoglea in the body wall far thicker than the ectoderm and remarkably thicker than the endoderm of body wall, actinopharynx and siphonoglyph (Fig. 2E). Actinopharynx smooth, grooved, approximately 2/3–3/4 the column length. Single distinct siphonoglyph on the ventral side of actinopharynx, always connected to it, and sustained by the ventral pair of directive mesenteries (Fig. 2E). Sphincter muscle absent. Filaments limited near the aboral end. All specimens dioecious; mature ovary contains several oocytes

in the mesoglea of each mesentery (Fig. 2G).

Cnidom. Basitrichs, microbasic *b*-mastigophores. See Table 2 for size and distribution.

Etymology. The new specific epithet “*hercules*” is a noun referring to blunt outer tentacles with apparent acrosphere-like cudgels containing gigantic strong basitrichs of this new species, which remains me of the thorns on the cudgel of Hercules, the famous hero of Greek Mythology.

Derivation of Japanese name. The genus *Haloclava* had been named “konbo-isoginchaku-zoku” in H. Uchida and Soyama (2001); “konbo” means the cudgel because anemones in this group have blunt tentacles with acrospheres. Thus, I omit the phrase and added Hercules at the beginning of the name, following the pattern in *Antennapeachia jambio* Izumi, Fujita, and Yanagi, 2017 (“misaki-no-antenna”).

Remarks. *Haloclava hercules* sp. nov. is the first unquestionable specimen-based record of the genus *Haloclava* from Japanese waters [H. Uchida and Soyama (2001) recorded *H. aff. producta* from Japanese waters, but the identification in this field guide is uncertain since its description is too short and lacking diagnostic characters of *Haloclava* below-mentioned]. This new species falls within the genus *Haloclava* by virtue of having following two features: the simple siphonoglyph without a conchula (Fig. 2B; strong acrospheres exist only in the genera *Haloclava* and *Anemonactis* Andres, 1881, and are thus distinguished from the other genera, e.g., *Antennapeachia* Izumi and Yanagi, 2016, *Harenactis* Torrey, 1902, *Metapeachia* Carlgren, 1943, *Peachia* Gosse, 1855, *Stephanthus* Rodríguez and López-González, 2003, *Synpeachia* Yap, Fautin, Ramos, and Tan, 2014, and *Tenactis* Barragán, Sánchez, and Rodríguez, 2019) and the surface of the body without any apertures (Fig. 2A; distinguished from the genus *Anemonactis*).

Haloclava hercules sp. nov. has a peculiar retractor muscle shape, not only for the genus, but also for the family (Fig. 2F). Moreover, the difference in shape between the inner and outer tentacles is considerably apparent in this species

Table 3. Comparison of *Haloclava hercules* sp. nov. and the other species of the genus *Haloclava*.

	<i>Haloclava hercules</i> sp. nov.	<i>Haloclava producta</i> (Stimpson, 1856)	<i>Haloclava brevicornis</i> (Stimpson, 1856)	<i>Haloclava capensis</i> (Verrill, 1868)	<i>Haloclava chinensis</i> Carlgren, 1931	<i>Haloclava stimpsonii</i> (Verrill, 1868)
Characters						
Number of inner tentacles	10	10	10	6	10	10
Form of tentacles	Not uniform	Not uniform	Uniform	Unknown	Not uniform	Not uniform
Acrospheres	Developed	Developed	Developed	Developed	Developed	Not developed
Surface of the column	Smooth	Sticky papillae	Unknown	Unknown	Smooth	Unknown
Shape of retractor muscle	Restricted	Restricted	Unknown	Unknown	Strongly circumscribed	Unknown
Shape of parietal muscle	Separated into two parts	No peculiar shape	No peculiar shape	No peculiar shape	No peculiar shape	No peculiar shape
References	The present study	Stimpson (1856)	Stimpson (1856)	Verrill (1868)	Carlgren (1931)	Verrill (1868)

(Fig. 2B). Previously, no *Haloclava* species with such differentiated tentacles have been described (Stimpson 1856; Verrill 1868; Carlgren 1931).

The other minor differences of this new species from other congeners are as follows: *Haloclava capensis* (Verrill, 1865) has six inner tentacles (Verrill 1865), in contrast to the 10 of *H. hercules*; *H. stimpsonii* (Verrill, 1868) has not developed acrospheres whereas *H. hercules* has an outer cycle with distinct acrospheres and an inner cycle with indistinct acrospheres (Verrill 1868); *H. brevicornis* (Stimpson, 1856) has 20 uniform blunt tentacles, not resembling the tentacles of *H. hercules* in shape; *H. producta* Stimpson, 1856 has 20 lines of sticky papillae on its column (Pei 1998), while the surface of *H. hercules* is smooth, and the body length of *H. producta* reaches 70 mm, larger than that of *H. Hercules* (15–20 mm); *H. chinensis* Carlgren, 1931 most strongly resembles *H. hercules*, but the shapes of retractors of *H. chinensis* are more circumscribed and strongly restricted (Carlgren 1931; see also Table 3).

The basitrichs of the acrosphere of *H. hercules*, reaching 273 µm, are extremely large for Actiniaria, for which it is rare that basitrichs reach over 200 µm (e.g., *Edwardsia alternobomen*). This is the maximum length record of basitrich among sea anemone.

Acknowledgements

I would like to thank the researchers, technician, and institutes as below: Hiroshi Namikawa (NSMT) for the collection of holotype specimens from Misaki and helping me deposit the holotype specimen in NSMT; Mamoru Sekifuji and Hisanori Kohtsuka, and R/V *Rinkai-Maru* (Misaki Marine Biological Station, The University of Tokyo) for helping in the collection of the holotype; Itaru Kobayashi (NSMT; The University of Tokyo) for the collection of the paratype; the captain and crews of the R/V *Seisui-Maru*, Taeko Kimura (Mie University), and the participants of research cruise No. 1722 for their assistance in collecting the paratype specimen; Kensuke Yanagi (CMNH) for providing me comments on the taxonomy of sea anemones, and helping me in deposition of the paratype at CMNH; Toshihiko Fujita (NSMT) for providing me the environment for research.

James Davis Reimer (University of the Ryukyus) is thanked for English editing.

This study was supported by a JSPS KAKENHI grant (Grant Number JP17J03267) to me.

References

- Carlgren, O. 1931. Zur Kenntnis der Actiniaria Abasilaria. *Arkiv für Zoologi* 23: 1–48.
- Carlgren, O. 1949. A survey of the Ptychodactylaria, Corallimorpharia and Actiniaria. *Kungliga Svenska Vetenskapsakademiens Handlingar* 1: 1–121.
- Daly, M. and Fautin, D. 2020. World List of Actiniaria. Haloclavidae Verrill, 1899. Accessed through: World Register of Marine Species. Available at <http://marinespecies.org/aphia.php?p=taxdetails&id=100671> (24 November 2020).
- Hertwig, R. 1882. *Die Actinien der Challenger Expedition*. Gustav Fischer, Jena, 119 pp.
- Izumi, T., Yanagi, K., and Fujita, T. 2016. The 'Antenna Balloon Anemone' found in the Seto Inland Sea: new genus and species of sea anemone, *Antennapeachia setouchi* (Cnidaria, Actiniaria, Haloclavidae). *Zoological Science* 33: 448–454.
- Izumi, T., Fujita, T., and Yanagi, K. 2017. *Antennapeachia jambio* (Cnidaria: Actiniaria: Haloclavidae), the second species of genus *Antennapeachia*, with revision of the diagnosis of the genus. *Species Diversity* 22: 109–115.
- Izumi, T., Yanagi, K., and Fujita, T. 2020. Comprehensive revision of *Anemonactis* (Cnidaria: Anthozoa: Actiniaria: Haloclavidae) in Japan: reestablishment of *Anemonactis minuta* (Wassilieff, 1908) comb. nov. and description of *Anemonactis tohrui* sp. nov. *Marine Biodiversity* 50: 73.
- Mariscal, R. N. 1974. Nematocysts. Pp. 129–178. In: Muscatine, L. and Lenhoff, H. M. (Eds) *Coelenterate Biology: Reviews and New Perspectives*. Academic Press, New York.
- Pei, Z. 1998. *Fauna Sinica Coelenterata Actiniaria Ceriantharia Zoanthidea*. Science Press, Beijing, 286 pp. [In Chinese]
- Presnell, J. K. and Schreibman, M. P. 1997. *Humason's Animal Tissue Techniques 5th Edition*. Johns Hopkins University Press, Baltimore, 600 pp.
- Rafinesque, C. S. 1815. *Analyse de la Nature ou Tableau de l'Univers et des Corps Organisés*. C. S. Rafinesque, Palerme, 224 pp.
- Rasband, W. S. 1997–2012. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. Available at <http://imagej.nih.gov/ij/>.
- Stimpson, W. 1856. Descriptions of some of the new marine invertebrata from the Chinese and Japanese seas. *Proceedings of the Acad-*

- emy of Natural Sciences of Philadelphia 7: 375–384.
- Uchida, H. and Soyama, I. 2001. *Sea Anemones in Japanese Waters*. TBS-Britannica, Tokyo, 157 pp. [In Japanese]
- Uchida, T. 1965. Actiniaria. P. 269. In: Okada, Y., Uchida, S., and Uchida, T. (Eds) *New Illustrated Encyclopedia of the Fauna of Japan (Vol. 1)*. Hokuryu-Kan, Tokyo. [In Japanese]
- Verrill, A. E. 1865. Classification of polyps (Extract condensed from a Synopsis of the Polypi of the North Pacific Exploring Expedition, under Captains Ringgold and Rodgers, U.S.N.). *Proceedings of the Essex Institute* 4: 145–152.
- Verrill, A. E. 1868. Synopsis of the polyps and corals of the North Pacific Exploring Expedition, under Commodore C. Ringgold and Capt. John Rodgers, U. S. N., from 1853 to 1856. Collected by Dr. Wm. Stimpson, naturalist to the expedition. Part IV. Actiniaria [First part]. *Communications of the Essex Institute* 5: 315–330.
- Verrill, A. E. 1899. Descriptions of imperfectly known and new Actinians, with critical notes on other species, II. *American Journal of Science, Fourth Series* 7: 41–50.
- Yanagi, K. 2006. Sea anemones around the Sagami Sea with the list of Japanese species. *Memoirs of the National Science Museum* 40: 113–173. [In Japanese]
- Yanagi, K. 2017. [Chapter 4, Actiniaria]. Pp. 53–87. In: The Sessile Organisms Society of Japan (Ed.) *Shin Fuchaku Seibutsu Kenkyu-ho [How to Study Sessile Organisms: Revised]*. Kouseisha Kouseikaku, Tokyo. [In Japanese]