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

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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 chinesis* 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 *Haloclava* Verrill, 1899 are characterized by a siphonoglyph without conchula and simple columns without apertures. Though this genus includes six species, and *Haloclava chinesis* 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 *Haloclava* Verrill, 1899 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.

**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).
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 µm 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; Carlsgren 1949: 29.

Type genus. Haloclava Verrill, 1899.
A new species of *Haloclava* from Japan

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 µm in D, G, and 100 µm in C.
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. 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.

**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.
A new species of Haloclava from Japan

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

| Acrosphere                  | Length × Width (µm) | Mean (µm) | SD (µm) | n  | frequency |
|----------------------------|---------------------|-----------|---------|----|-----------|
| 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 b-mastigophores | 62.2–78.8×7.0–8.9   | 67.1×7.9  | 5.12×0.58 | 8  | few       |

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 siphoglyph without a conchula (Fig. 2B); strong acrospheres exist only in the genera Antennapeachia and Anemonaecis. This new species lacks the following diagnostic characters of Haloclava: the simple siphoglyph without a conchula (Fig. 2B); strong acrospheres exist only in the genera Antennapeachia and Anemonaecis. 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 Tán, 2014, and Tenactis Barragán, Sánchez, and Rodríguez, 2019) and the surface of the body without any apertures (Fig. 2A; distinguished from the genus Anemonaecis).

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.

| Characters                        | Haloclava hercules sp. nov. | Haloclava producta (Stimpson, 1856) | Haloclava brevicornis (Stimpson, 1856) | Haloclava capensis (Verrill, 1868) | Haloclava chinensis Carlgren, 1931 | Haloclava stimpsonii (Verrill, 1868) |
|----------------------------------|-----------------------------|-------------------------------------|---------------------------------------|----------------------------------|----------------------------------|-------------------------------------|
| 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                             | Unknown                          | Developed                        | Not developed                       |
| Surface of the column            | Smooth                      | Sticky papillae                     | Unknown                               | Unknown                          | Smooth                           | Unknown                             |
| Shape of retractor muscle        | Restricted                  | Restricted                          | Restricted                            | 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)

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