New record of \textit{Linuche draco} (Scyphozoa, Coronatae, Linuchidae) from Japan

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Received 7 April 2020; Accepted 8 September 2020

Responsible Editor: Dhugal Lindsay
doi: 10.3800/pbr.15.327

Abstract: The order Coronatae is a unique group, with the following characters distinguishing them from other scyphozoans: a coronal furrow, a coronate pedalium, and oocytes that develop without accessory pigments. Coronatae polyps are enclosed in chitinous tubes and produce multiple ephyrae via polydisk strobilation. So far, eleven described species of Coronatae have been reported in Japanese waters: \textit{Atolla wyvillei}, \textit{Atolla vanhoeffeni}, \textit{Atolla russelli}, \textit{Atorella vanhoeffeni}, \textit{Atorella japonica}, \textit{Nausithoe punctata}, \textit{Palephyra pelagica}, \textit{Periphylla periphylla}, \textit{Periphyllopsis braueri}, \textit{Stephanoscyphistoma corniformis} and \textit{Nausithoe racemosa}. The present study reports detailed observations of the morphology of one species newly recorded in Japan: \textit{Linuche draco}. Development from ephyra to mature medusa was observed and recorded. Additional investigations are needed to understand the diversity of the order Coronatae in Japan.

Key words: development, ephyra, medusa, stings, thimble jellyfish

Introduction

To date, about 50 species in six families have been described in the order Coronatae (Scyphozoa) worldwide (Daly et al. 2007, Jarms et al. 2019). Coronatae species possess three synapomorphies that distinguish them from other scyphozoans: a coronal furrow, a coronate pedalium, and oocytes that develop without accessory pigments (Marques & Collins 2004). Many species inhabit the deep sea, however, some have been reported in shallow waters (Jarms et al. 1999, Daly et al. 2007). The order Coronatae has sexual, planktonic medusae and asexual, benthic polyps in their life cycle (Silveira & Morandini 1997, 1998). Polyps are enclosed in chitinous tubes and produce ephyrae via polydisk strobilation (Silveira & Morandini 1997). However, the pelagic species \textit{Periphyllopsis periphylla} (Péron & Lesueur, 1810) completely lacks the polyp stage, and planulae develop directly into ephyrae (Jarms et al. 1999).

Linuchidae is one of the smallest families in the Coronatae and includes two genera, \textit{Linantha} and \textit{Linuche} (Daly et al. 2007). \textit{Linuche} species are known as “thimble jellyfish,” "sea thimbles," or “button jellyfish” because of their umbrella shape (Calder 2009), and are popularly exhibited in public aquaria (Murakami 2013). Species of the genus \textit{Linuche} have two to three rows of subumbrellar protuberances which are unique morphological characters for the genus (Mayer 1910). Subumbrellar protuberances are pouch-like diverticula, hanging into the bell cavity from the oral walls of the radial pouches (Bigelow 1928). Numerous injuries to humans caused by \textit{Linuche} stings have been reported in seas off the coast of Florida, the Gulf of Mexico, the Caribbean Sea and the Philippines (Penner 1962, Segura-Puertas et al. 2001, Guevara et al. 2017). Consequently, it is important to understand the species diversity, biology, and ecology of \textit{Linuche}. Several studies on the distribution (Kramp 1961), seasonal occurrence (Larson 1992), stings (Segura-Puertas et al. 1999, 2001, Humann 2002), feeding (Larson 1976), and life cycle (Ortiz-Corp’s et al. 1987, Silveira & Morandini 1998) of the \textit{Linuche} have been carried out in the Atlantic, while studies on \textit{Linuche} are scarce in the Indo-Pacific, including Japan.

To date, eleven described Coronatae species have been reported in Japanese waters: \textit{Atolla wyvillei} Haeckel, 1880, \textit{Atolla vanhoeffeni} Russell, 1957, \textit{Atolla russelli} Repelin, 1962, \textit{Atorella vanhoeffeni} Bigelow, 1909, \textit{Atorella japonica} Kawaguti & Matsuno, 1981, \textit{Stephanoscyphistoma corniformis} (Komai, 1936), \textit{Nausithoe punctata} Kolliker, 1853, \textit{Nausithoe racemosa} (Komai, 1936), \textit{Palephyra pe-
lagica (Haeckel, 1880), Periphylla periphylla (Péron & Lesueur, 1810), and Periphyllopsis braueri Vanhöffen, 1902 (Komai 1936, Uchida 1954, Kawaguti & Matsuno 1981, Lindsay et al. 2004, Minemizu et al. 2015), and one undescribed species of Atorella (Kitamura et al. 2008). The current study outlines a morphological analysis of one Coronatae species and provides a new record of Linuche draco (Haeckel, 1880) in Japan.

Materials and Methods

Seven unidentified ephyrae were collected using a dip net (mesh size 0.2 mm) at fishing ports in Kochi and Okinawa Prefectures, southern Japan, during the day on 8 July 2015 and 13 June 2018, respectively (Table 1, Fig. 1). The ephyrae were transferred to Petri dishes (diameter 149 mm, height 91 mm, water volume 1500 mL) filled with filtered seawater (1 µm filter pore size) and were kept at 28°C. Two to three individuals were reared per dish. The ephyrae were incubated under LED lights (10 W) between 9:00 and 21:00 and in darkness between 21:00 and 9:00. Artemia nauplii were fed directly to the ephyrae on a daily basis. Culture water was replaced with fresh seawater approximately three hours after each feeding. The cultured ephyrae and medusae were fixed in 3% formalin seawater for taxonomic observations.

Morphological observations and measurements were made on live or preserved specimens according to Straehler-Pohl & Jarms (2010). Ephyrae were put on an objective slide and covered with a cover slip to make observations. Medusae were placed and flattened on a dish (diameter 50 mm). Umbrella height was measured from the apex of the umbrella to the umbrella margin. Umbrella diameter was measured across turnover of exumbrella. Measurements were made using ImageJ (NIH, USA) to the nearest 0.1 µm.

Results

Class Scyphozoa Goette, 1887
Subclass Coronamedusae Calder, 2009
Order Coronatae Vanhöffen, 1892

| No. | Collection date | Locality | Lat.& Long. | No. of individuals |
|-----|-----------------|----------|------------|-------------------|
| 1   | 2015/7/8        | Komame Fishing Port, Otsuki, Kochi | 32°47′22.36″N, 132°41′12.61″E | 2 |
| 2   | 2018/6/13       | Sesoko Island, Motobu, Okinawa | 26°38′9.48″N, 127°51′55.35″E | 5 |

Fig. 1. Map of the sampling sites: A) Okinawa Island, B) Sukumo Bay, Shikoku. 1 = Sesoko Island, 2 = Komame Fishing Port.
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Family Linuchidae Haeckel, 1880  
Genus *Linuche* Eschscholtz, 1829  
*Linuche draco* (Haeckel, 1880)

New Japanese name: Yubinuki-kurage  
Figs. 2–5

**Description.** Collected ephyrae (n=7) had flat, transparent umbrellas with yellowish brown symbiotic zooxanthellae clusters (Fig. 2A–F). Umbrella height ranged from 0.3 to 0.4 mm (mean: 0.3 mm), and total body diameter varied from 1.7 to 2.0 mm (mean: 1.8 mm). Tiny nematocyst clusters were scattered across the entire exumbrella (Fig. 2D). The ephyrae had sixteen round spoon-like marginal lappets, and a single rhopalium situated between the two lappets (Fig. 2B, C, F). Four simple gastric filaments were visible in the stomach through the umbrella (Fig. 2D, E). The marginal tentacles were not developed (Fig. 2F). The manubrium was short, about 10% of the central disk diameter (adradial diameter of the central disc), and was cruciform with four narrow, rounded lips (Fig. 2E). The coronal furrow was present midway between the center and the margin of the exumbrella (Fig. 2B–E). Circular coronal muscle present between coronal furrow and margin of umbrella (Fig. 2B, C).

Fourteen days post initiation of culture, the ephyrae (n=7) were about 6.2 mm (6.1 to 6.3 mm) in total body diameter and about 1.8 mm (1.7 to 2.0 mm) in umbrella height (Fig. 3A–C). Mesoglea thickened and umbrella became dome-shaped. One whorl of more or less round clusters of zooxanthellae was observed halfway between the center and the margin of the subumbrella, and more peripherally, many irregularly-shaped clusters occurred (Fig. 3B, C, E), while many clusters were distributed around the umbrella margin and manubrium. Marginal lappets became broader (Fig. 3F), and eight gastric cirri were visible in the stomach (Fig. 3C, E). The manubrium was elongated to the bottom of the umbrella (Fig. 3D), and mouth was squared and lips rounded (Fig. 3D). Very short club-shaped tentacles were situated on the adradii of the umbrella margin (Fig. 3F). Four pairs of thin thread-like gonads appeared on the interradii of the upper part of the umbrella (Fig. 3E).

Twenty-one days post initiation of culture, the medusae (n=7) were about 7.4 mm (7.0 to 8.0 mm) in umbrella diameter and about 4.2 mm (4.0 to 4.5 mm) in height (Fig. 4A–C). Umbrella was much wider than tall and gonads were elongated and thickened (Fig. 4A, B). Apex of the umbrella thickened (Fig. 4A). Sixteen to twenty gastric cirri were present in the stomach (Fig. 4D). The cirri became yellowish brown due to symbiotic zooxanthellae clusters. The manubrium was elongated and thickened (Fig. 4E). Marginal lappets became wider than that of ephyrae, bent inwards (Fig. 4G, H).

Twenty-eight days post initiation of culture, the medusae were 6.3 mm in umbrella diameter and 6.2 mm in height (Fig. 5A–C). Umbrella was elongated and the shape became thimble-like (Fig. 5A). Tiny nematocyst clusters were scattered across the entire exumbrella. The coronal furrow was present just below apex of the umbrella, and was shal-
low (Fig. 5A). Stomach pouches sixteen, extended from the stomach to nearly to the edge of the lappets. Ring canal was observed between stomach pouches formed on their margin. Twenty to twenty-four gastric cirri were present in stomach (Fig. 5D). Tentacles were very short, club-shaped (Fig. 5E). Gonads were developed and became crescentic in shape (Fig. 5F). Subumbrellar protuberances were not observed. Circular coronal muscle developed near margin of subumbrella (Fig. 5A, C).

Nematocysts
Not examined in this study.

Discussion
*Linuche draco* was described by Haeckel (1880) (as *Lingereis draco*) from the China Sea, western Pacific. The species has been reported from the China Sea and New Britain, West Pacific (Haeckel 1880, Maas 1903, Thiel 1928). The species is up to 20 mm in diameter, and up to 18 mm high; exumbrella smooth, with a nearly flat central disc and vertical peripheral margin; 16 marginal lappets short, broad, and bluntly oval; eight tentacles very short, filiform; eight rhopalia; subumbrellar sacs arranged in two circles; four perradial gonads horseshoe-shaped, composed of two flanks each; medusa coloration golden-brown due to many zooxanthellae embedded in the tissues (Jarms et al. 2019). Medusae of the species from Japan were 6.3 mm in diameter, and 6.2 mm in high; exumbrella with tiny nematocyst clusters across the entire exumbrella; 16 marginal lappets, eight tentacles very short, club-shaped; eight rhopalia; sub-umbrellar protuberances not observed; gonads crescentic in shape; circular coronal muscle developed radially near the margin of the subumbrella. The morphological inspection of *L. draco* individuals collected in Japan agrees with the morphological descriptions found in Haeckel (1880), Thiel (1928) and Jarms et al. (2019). However, the exumbrella was reported smooth in Jarms et al. (2019). Thiel (1928) reported that the coronal furrow was absent in his specimen. Subumbrellar protuberances or sacs could not be observed in the present study. These protuberances have never been observed in cultured ephyrae or medusae of *Linuche unguiculata* (Ortiz-Corpa’s 1987, Silveira and Morandini 1998). The number and arrangement of subumbrellar protuberances has been reported to vary depending on the specimen (Bigelow 1928).

Twenty-one days after culturing began, young medusae of *L. draco* were 7.4 mm in total body diameter and 4.2 mm in umbrella height and displayed adult morphological characters with gonads. The gonads of *L. unguiculata* were observed after 13 days and 47 days in cultured medusae from Puerto Rico and Brazil (Ortiz-Corpa’s et al. 1987, Silveira & Morandini 1998), respectively. Ephyrae of another Coronatae species, *Nausithoe hagenbecki* Jarms, 2001, matured in two months (Jarms 2001).

The ephyrae of *L. draco* were collected in southern Japan from June to July, while *L. unguiculata* were collected in February and March from Puerto Rico and the Bahamas (Mayer 1910, Ortiz-Corpa’s 1987). In the Mexican Caribbean, the annual occurrence of the *L. unguiculata* ephyra generation appears in late January (Segura-Puertas et al. 2001, 2008). The ephyrae grow and mature into adults be-
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between March and May, and in May and early June the planktonic planulae of the next generation appear in abundance. Strobilation of *L. unguiculata* has been observed under warm water temperature conditions between 21 to 30°C and with a sudden drop of 5°C from 29±2°C in the laboratory (Silveira & Morandini 1998, Segura-Puertas et al. 2008).

Wild polyps of *L. unguiculata* have been found on calcareous fragments of the stony coral *Mussismilia hispida* (Verrill, 1902) at 2–6 m depth in a channel of a rocky shore in Brazil (Silveira & Morandini 1998), while collected from chaetopterid tubes (Leloup 1937), calcareous rocks (Allwein 1968) and coral rubble at 15–18 m depth in Puerto Rico (Ortiz-Corpus et al. 1987). These substrates were present near the sampling sites in Okinawa and Kochi prefectures, thus the polyps may inhabit shores near to the sampling sites.

Eleven described species of Coronatae have previously been reported in Japanese waters (Komai 1936, Uchida 1954, Kawaguti & Matsuno 1981, Lindsay et al. 2004, Fig. 4. Medusae of *Linuche draco* twenty-one days after collection: A) lateral view, B) apical view, C) oral view, D) gastric filaments, E) manubrium, F) mouth, G) rhopalium and tentacle, H) marginal lappets. Scale bars=2 mm (A–C), 0.5 mm (D–H). G=gonad, R=rhopalium, T=tentacle.
Minemizu et al. 2015). This study reported *L. draco* as the twelfth described species from Japan. However, unidentified Coronatae species have been reported but remain uninvestigated (Iwao 2002, Kitamura et al. 2008, Minemizu et al. 2015). Additional sampling and taxonomic investigations are needed to understand the diversity of Coronatae in Japan.

Acknowledgements

I would like to express our sincere thanks to Aki Hashimoto, Junko Fukada, Dr. Takuma Mezaki, Dr. Keita Koeda, Tatsuki Koido, Takaya Kitamura, Kaori Yamashita (Kuroshio Biological Research Foundation), and the staff at the Sesoko Tropical Biosphere Research Center. I am also grateful to Dr. Andre Carrara Morandini for providing references. This manuscript was greatly improved by the constructive comments of two anonymous reviewers. The research was financially supported by Showa Seitoku Memorial Foundation, Suzuki Shohei Marine Biological Research Grant from The University of the Ryukyus Foundation, and the JSPS KAKENHI Grant Number JP18K14791.

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