Argulus matuii (Branchiura: Argulidae) parasitic on yellowfin seabream Acanthopagrus latus in Japan, with a note on the body coloration in an ethanol-preserved argulid specimen

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Abstract.— An ovigerous female of *Argulus matuii* Sikama, 1938 was collected from the body surface of a yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782) in a cove facing the Bungo Channel off Ehime Prefecture, western Japan. This represents a new host record for *A. matuii*, and the female collected is herein described. This female, which was fixed and preserved in 70% ethanol, showed a prominent body coloration, *i.e.*, six streaks of yellow fringed with dark brown pigments, on the dorsal surface of the carapace even on 279th day (ca. nine months) after fixation, but the yellow streaks disappeared and the dark brown fringes were fading on 1675th day (ca. four years and seven months) after fixation. Thus, this paper reports on the usefulness of those yellow streaks in identification of *A. matuii* for a certain period after fixation but also emphasizes the importance of a detailed examination of morphological features in identification of long-term ethanol-preserved argulid specimens. The species is distinguished from the five marine congeners from Japan by its possession of numerous supporting rods in the marginal membranes of the first maxillae.

Key words: parasitic crustacean, influence of ethanol preservation, Sparidae

Introduction

The argulid branchiuran *Argulus matuii* Sikama, 1938 is an ectoparasite of marine fishes in Japan (Nagasawa, 2009, 2011). The species was originally described by Sikama (1938) based on specimens from chicken grunt *Parapristipoma trilineatum* (Thunberg, 1793) (type host) in the Northwestern Pacific off Futomi, Chiba Prefecture (reported as “Hutomi, Tiba prefecture”). Sikama (1938) also states that *A. matuii* infested three fish species held in an aquarium, *i.e.*, white trevally *Pseudocaranx dentex* (Bloch & Schneider, 1801) (reported as “Caranx delicalissimus”), gnomefish *Scombrops boops* (Houttuyn, 1782), and red seabream *Pagrus major* (Temminck & Schlegel, 1843) (reported as *Pagrosomus major*). Subsequently, *A. matuii* was collected from bastard halibut *Paralichthys olivaceus* (Temminck & Schlegel, 1846) cultured in Oita Prefecture (Nagasawa & Fukuda, 2009) and white trevally in the Northwestern Pacific off Kanagawa and Chiba prefectures (Saito & Nagasawa, 2010; Nagasawa *et al*., 2021). The present knowledge of *A. matuii* is based on the above four papers and still quite limited.

An ovigerous female of *A. matuii* was collected from a yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782) in coastal waters of Ehime Prefecture. This represents a new host record for *A. matuii*, and the morphology of the female collected is herein reported. It is known that the species exhibits a prominent body coloration when fresh (Sikama, 1938; Nagasawa *et al*., 2021) and such coloration was kept for nearly two years in the formalin-preserved specimens (Sikama, 1938).
In the present study, the specimen of *A. matuii* was preserved in 70% ethanol for more than four years. This paper also reports on a change in the body coloration of this ethanol-preserved specimen.

**Materials and Methods**

A yellowfin seabream (290 mm total length) was caught using hook and line on 27 May 2017 in a cove (33°10′17″N, 132°30′52″E) facing the Bungo Channel off Miura-Higashi, Uwajima, Ehime Prefecture, Japan. An argulid was carefully removed from the fish, brought to the laboratory of Ehime Prefectural Uwajima Fishery High School, Uwajima, and fixed in 70% ethanol on the same day. In early February 2018, the argulid was sent to the Aquaparasitology Laboratory, Shizuoka, where it was first examined under an Olympus SZX10 stereo microscope, then cleared in lactophenol and examined under an Olympus BX51 phase-contrast compound microscope using the wooden slide method recommended by Humes & Gooding (1964) and Benz & Otting (1996). The body coloration of the specimen was observed and photographed on 7 February 2018 and 4 December 2021. All drawings were made with the aid of drawing tubes attached to the microscopes. Morphological terminology follows Benz *et al.* (1995) and Benz & Otting (1996). The specimen has been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 30676). The scientific and common names of fishes mentioned in this paper follow those in FishBase (Froese & Pauly, 2021).

**Results**

The argulid collected was an ovigerous female of *A. matuii*, measuring 7.9 mm total length (TL, from anterior tip of carapace to posterior tip of abdomen) and 3.8 mm in maximum width (around 2/5 length of carapace from its anterior tip). It was found on the body surface of the host near its left pectoral fin.

**Description of ovigerous female**

Body dorsoventrally flattened. Carapace elliptical, 6.2 mm long, comprising 78% of TL, almost totally covering first to second pairs of legs (Fig. 1A, B). Frontal region of carapace slightly protruding anteriorly, and delimited by anterolateral indentations. Posterolateral lobes of carapace 5.1 mm long, comprising 82% of carapace length, separated by sinus, and ending each in rounded margin; mesial margins of both lobes slightly overlapping, with quite narrow interspace between lobes. Compound eyes weakly visible dorsally but distinct ventrally in frontal region of carapace. Naupliar eye well visible dorsally along midline of carapace. Dorsal surface of marginal frontal zone of carapace covered with small sharply pointed spines. Ventral surface of frontal and lateral regions of carapace ornamented with numerous, small sharply pointed spines. Respiratory areas consisting of small, oval anterior area and large, reniform posterior area, located at levels of second maxillae and first to second legs, respectively (Fig. 1C). Thorax with four segments; ventral surface covered with numerous minute scales (Fig. 1B). Abdomen longer than wide, bilobed by anal indentation; each lobe ending in pointed tip (Fig. 1A, B). Paired spermatiche oval in anterior region of abdomen (Fig. 1B). Caudal rami small and rounded, located at base of anal indentation (no setae visible on each ramus) (Fig. 1D).

First antennae with four segments (Fig. 1E); first segment sclerotized, with two large projections on posterolateral margin; second segment also sclerotized, with apically bent hook on anterior margin, strong posteroventrally directed hook at lateral corner, and large projection and knob-like swelling on posterior margin; third segment longer than wide; apical segment shorter than third segment (due to
Fig. 1. *Argulus matuii*, ovigerous female, NSMT-Cr 30676, 7.9 mm TL, from a yellowfin seabream *Acanthopagrus latus* in coastal waters of Ehime Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view; C, right respiratory areas, ventral view; D, caudal rami at base of anal indentation, dorsal view; E, left first antenna (a1), second antenna (a2), and postantennal spine (pas), ventral view; F, preoral sheath and stylet, ventral view; G, mouth tube, ventral view; H, section of marginal membrane of first maxilla showing two supporting rods and marginal projections, ventral view; I, left second maxilla, ventral view. Scale bars: A, B, 2 mm; C, 1 mm; D, 0.1 mm; E–H, 0.2 mm; I, 0.05 mm.
damage, no observation possible on setae of third and apical segments). Second antennae with five segments (Fig. 1E); first segment sclerotized, with large projection and small swelling on posterior margin; second segment shorter than first segment, bulbous; third, fourth, and apical segments each longer than wide, decreasing in length (due to damage, no observation possible on setae of third to apical segments). Postantennal spines large and stout, located posterior to projections of first segments of first antennae (Fig. 1E). Preoral sheath on ventral midline of frontal region of carapace, with anterior part of stylet protruding from sheath opening (Fig. 1F). Mouth tube located posterior to preoral sheath, longer than wide, composed of anterior labrum and posterior labium with pair of tiny spines; anterior surface covered with nearly circular scales (Fig. 1G).

First maxillae forming cup-like suckers (Fig. 1B, H), with 90 and 91 supporting rods each in two marginal membranes; each rod composed of 13 or 14 (the latter more often, n=10) sclerites; basal sclerite longer than wide; other sclerites oval or trapezoidal, decreasing in size distally. Second maxillae with five segments (Fig. 1I); first segment stout, with three basally separated, almost equally long projections on posterior margin; corpus of first segment furnished with raised field of scale-like denticles; second segment quite thick at base, covered with scale-like denticles on anterolateral surface; third segment longer than wide, covered extensively with scale-like denticles; terminal segment smallest, covered with small denticles, ending in one club-like and two spiniform projections. Accessory spines each near posterior margin of mouth tube (Fig. 1B). Postmaxillary spines located just in front of first thoracic segment (Fig. 1B).

First to fourth pairs of legs biramous; sympods two-segmented, consisting of coxa and basis, covered with small scales; rami each consisting of exopod and endopod, with two rows of plumose setae (Fig. 2). First pair of legs each with dorsal flagellum with 15 plumose setae on posterior margin, coxa with single plumose seta on posterior margin, and three-segmented endopod ending in three short...
spines (Fig. 2A, B). Sympods of second and third pairs of legs without setae (Fig. 2C, D). Endopods of third and fourth pairs of legs two-segmented (Fig. 2D, E). Fourth pair of legs each with coxa forming large, ventrally foot-shaped natatory lobe bearing 14 plumose setae on posterior margin and basis bearing seven plumose setae near posterior margin (Fig. 2E).

**Coloration in ethanol-preserved specimen**

The specimen, which was fixed on 27 May 2017 and then preserved in 70% ethanol, showed the following body coloration on 7 February 2018 (279th day or ca. nine months after fixation) and 4 December 2021 (1675th day or ca. four years and seven months after fixation).

Coloration on 7 February 2018 (Fig. 3A, B): Six streaks of yellow fringed with dark brown pigments found on dorsal surface of posterolateral lobes of carapace (three streaks of yellow on each lobe). Outer yellow streaks wider and longer than the others, extending from anterolateral indentation to posterior end of posterolateral lobe. Two inner yellow streaks present on each posterolateral lobe connected both anteriorly and posteriorly, joining outer streak at posterior end of posterolateral lobe. Brown streak running dorsally along lateral margin of each posterolateral lobe. Paired irregularly shaped streaks of yellow fringed with dark brown pigments found along midline of frontal zone of carapace and anterior region of posterolateral lobe. Mouth tube laterally fringed with dark brown pigments. Two streaks of dark brown present along lateral margins of abdomen, with three streaks of brown dorsally on abdomen. Respiratory areas fringed with dark brown pigments. Anterior and posterior margins of sympods of legs partially fringed with dark brown pigments.

Coloration on 4 December 2021 (Fig. 3C, D): Color fading progressed: no yellow streaks on dorsal surface of carapace, and dark brown fringes remaining but fading both dorsally and ventrally.

**Remarks**

The morphological characters of the female specimen collected in this study correspond, more or less, to the descriptions of female *A. matuii* given by Sikama (1938) and Saito & Nagasawa (2009), and the specimen is thus identified as *A. matuii*. As mentioned below, the species is characterized by numerous supporting rods in the marginal membranes of the...
first maxillae.

Sikama (1938) stated that *A. matuii* is readily differentiated from its congeners by the characteristic body coloration. However, as reported above, such coloration remained for a certain period after fixation but was fading with time, indicating that the coloration cannot be always used for identification of the species (see also the Discussion below). In contrast, the number of supporting rods per first maxilla is one of the reliable morphological characters in identification of *Argulus* spp. and can be used to differentiate *A. matuii* from the five marine congenic species from Japan. *Argulus matuii* has more supporting rods (70–95 and 75–83 rods, respectively, in the female and male: Sikama, 1938; Saito & Nagasawa, 2010; this paper) than the other species: 66 or 67 rods in the female of *A. scutiformis* Thiele, 1900 (Yamaguti & Yamasu, 1959); 54 or 55 rods in the female of *A. caecus* C. B. Wilson, 1922 (Nagasawa & Hirose, 2021); 54–62 rods in the female of *A. kusafugu* Yamaguti & Yamasu, 1959 (Yamaguti & Yamasu, 1959; Saito et al., 2011); and 58–61 rods in the male of *A. quadristriatus* Devaraji and Ameer Hamsa, 1977 (Uyeno et al., 2017). Although no exact information is available on the number of supporting rods in *A. onodai* Tokioka, 1936, it has been reported to be similar to that in *A. caecus* (Tokioka, 1938).

Several minor morphological differences are found between the present and previously reported specimens of *A. matuii*. For example, the female shown by Saito & Nagasawa (2010, fig. 1) had a “pentagon-shaped” carapace, but the females reported by other scientists (Sikama, 1938; Nagasawa et al., 2021; this paper) had an “elliptical” carapace. The four females reported by Saito & Nagasawa (2010) were as large as 16.2–18.7 mm TL, but the two females reported by Nagasawa et al. (2021) and in this paper were smaller, being 12.0 and 7.9 mm TL, respectively. Although no body size was given to the female illustrated by Sikama (1938, fig. 2), it is herein estimated to be 8.1 mm TL from a figure’s scale bar. Thus, taking account of these total lengths of the specimens, there is the possibility that the above difference in the shape of the carapace is dependent on the body size of the females examined. It is thus desirable to examine morphological variations in *A. matuii* in relation to changes in its body size or growth.

Sikama (1938, fig. 10) found five long sharp setae on the raised field of the first segment of the second maxilla. However, Saito & Nagasawa (2010) did not comment on the presence of such setae, and no similar setae were present on the specimen examined in this study (Fig. 11). Further, the endopods of the second and third legs illustrated by Saito & Nagasawa (2010, fig. 2J, K) were two- and non-segmented, respectively, but those of individual legs of the specimen examined herein show a different segmentation, being non- and two-segmented (Fig. 2C, D). These differences in the morphology of *A. matuii* between the previous and present papers should be examined in future research using additional specimens of the species.

The male of *A. matuii* has been poorly described. In the original description of the species, Sikama (1938) focused on its female with seven figures, but for the male, provided only three figures (first and second antennae and two posterior pairs of legs). Only female specimens were used in the redescriptions of the species (Saito & Nagasawa, 2010). However, the male and female of *A. matuii* photographed by Nagasawa et al. (2021) showed a clear difference in the shape of the carapace and abdomen. A redescriptions of male *A. matuii* is required to aid in identifying the species and distinguishing it from the other Japanese marine congeners.

The known hosts of *A. matuii* consist of five fish species (chicken grunt, white trevally, gnomefish, red seabream, and bastard halibut), the first two of which are wild hosts (Sikama, 1938; Nagasawa & Fukuda, 2009; Saito & Na-
New Host and Body Coloration of *Argulus Matuii*

Gasawa, 2010; Nagasawa *et al.*, 2021). Yellow-fin seabream, from which the specimen of *A. matuii* was collected in this study, represents the third wild host as well as a new host of the parasite.

**Discussion**

Sikama (1938) emphasized that the body coloration in fresh specimens of *A. matuii* was “very characteristic” and had “several prominent streaks of deep yellow-ochre bordered by dark brown (burnt sienna) on the ground color of light yellow-ochre” on the dorsal side of the carapace. Recently, a similar body coloration was confirmed by Nagasawa *et al.* (2021), who have suggested that such coloration can be used to differentiate fresh specimens of *A. matuii* from those of the five other marine congeners from Japan. Interestingly, the bright coloration was reported to be “kept for nearly two years in 10% formalin” (Sikama, 1938).

In the present study, the specimen of *A. matuii* was preserved in 70% ethanol after fixed on 17 May 2017. Although not examined for its fresh coloration, the specimen observed on 7 February 2018 (ca. nine months after fixation) still had six streaks of yellow fringed with dark brown pigments on the posterolateral lobes of the carapace (Fig. 3A, B). However, on 4 December 2021 (ca. four years and seven months after fixation), the yellow streaks disappeared and the dark brown fringes were fading (Fig. 3C, D). This observation demonstrates that the six yellow streaks are useful for identification of the species for a certain period after fixation but cannot be constantly used as a species-specific feature.

Invertebrate specimens including argulids are usually preserved in ethanol (Levi, 1966; Simmons, 1999; Poly, 2016), and Sikama’s (1938) method to keep his specimens in 10% formalin is currently regarded as uncommon. In order to identify ethanol-preserved argulid specimens, especially long-term stored ones, it is important to make a detail examination of their morphological features and, as necessary, to employ molecular techniques. Benz & Otting (1996) recommended the use of the wooden slide procedure for a detailed examination using light microscopy.

Since 1938 when *A. matuii* was originally described (Sikama, 1938), the species has been mainly studied on its taxonomy and morphology (Saito & Gasawa, 2010; Nagasawa *et al.*, 2021), thus, much remains unknown about its biology. More studies are needed to understand various aspects of the biology of the species, including its geographical distribution, host range, prevalence and intensity, growth and maturation, and pathological impact on a host fish. The six fish species reported as the hosts of *A. matuii* include both wild and captive hosts, both of which belong to different taxonomic groups. Hence, the species is not host-specific and likely to be found on more fish species in the wild and captivity, as well.

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