***Argulus japonicus* (Branchiura: Argulidae) parasitic on largemouth bass *Micropterus salmoides* in Japan, with the morphology of the adult female of the argulid**

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Abstract.—Largemouth bass *Micropterus salmoides* (Lacepède, 1802) was introduced from North America into Japan in 1925 and 1972. The fish has spread extensively since the late 1960s and is now found in all prefectures of Japan. An adult female of the argulid branchiuran *Argulus japonicus* Thiele, 1900 was collected from the body surface of a largemouth bass in the Kako River, Hyogo Prefecture, Japan. This represents a new host record for *A. japonicus*. The morphology of the adult female collected is reported in detail. Although *A. japonicus* is native to Japan, the species is not strictly host-specific and can utilize largemouth bass of the North American origin as its host in Japan.

Key words: *fish parasite, fish louse, new host record*

■ Introduction

Many species of freshwater fishes have been introduced from other countries to Japan for aquaculture, sport-fishing, and ornamental purposes (Chiba *et al.*, 1989). The largemouth bass *Micropterus salmoides* (Lacepède, 1802) (Centrarchiformes: Centrarchidae) is one such fish: the species was introduced in 1925 and 1972 from North America into Japan, where, since the late 1960s, it has established populations nationwide (Takamura, 2007). Nonetheless, little attention has been paid to the parasite fauna of largemouth bass from Japan. In addition to some helminth parasites and glochidial larvae of unionid bivalves, only two species of crustacean parasites are known to infect largemouth bass in Japan (Nagasawa, 2017a), both are cyclopoid copepods: *Lernaea cyprinacea* Linnaeus, 1758 (Lernaeidae) (Kasahara, 1962) and *Neoergasilus japonicus* (Harada, 1930) (Ergasilidae) (Nagasawa & Inoue, 2012; Nagasawa & Sato, 2014).

A specimen of crustacean parasite collected

from a largemouth bass in western Japan was received from a student at Hiroshima University. As described below, the specimen was identified as *Argulus japonicus* Thiele, 1900 (Branchiura: Argulidae). This represents a new host record for *A. japonicus* and its first record from largemouth bass in Japan. *Argulus japonicus* was originally described by Thiele (1900) based on a single female specimen from Tokyo (reported as “Yeddo”), Japan, but its original description was very short, without any figure and host information. Thus, the species was later redescribed by Thiele (1904) from specimens collected from goldfish *Carassius auratus* (Linnaeus, 1758) from Yokohama, Japan, and also by Nakazawa (1914), Tokioka (1936), and Yamaguti (1937) from specimens taken from Japanese freshwater fishes, including goldfish and common carp *Cyprinus carpio* Linnaeus, 1758. However, as compared with the current descriptions of *Argulus* spp. (*e.g.*, Benz *et al.*, 1995; Uyeno *et al.*, 2017; Nagasawa & Hirose, 2021), the redescriptions of *A. japonicus* by the above authors lack detailed

morphological information. Moreover, as *A. japonicus* has spread globally (Poly, 2008), it is important to accurately describe the species from Japan in order to aid in distinguishing it from morphologically similar species in other countries. Therefore, based on a detailed examination of the specimen collected, this paper reports on the morphology of *A. japonicus*.

Materials and Methods

The argulid specimen was collected from a largemouth bass *M. salmoides* (body size not measured) on 5 June 2007 in a reservoir (34°47'38"N, 134°53'35"E) created upstream of a barrage in the lower reaches of the Kako River at Yahata, Kakogawa, Hyogo Prefecture, western Japan. It was removed from the fish's body surface, fixed, and then preserved in 70% ethanol. Later, at the Aquaparasitology Laboratory, Shizuoka Prefecture, the specimen was first examined under an Olympus SZX10 stereo microscope. It was then cleared in lactophenol and examined under an Olympus BX51 phase-contrast compound microscope using the wooden slide procedure (Humes & Gooding, 1964; Benz & Otting, 1996). 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 was deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 29117).

Results

The argulid specimen collected in this study is an adult female of *A. japonicus*, measuring 6.0 mm total length (from anterior tip of carapace to posterior tip of abdomen) and 3.8 mm maximum width (around midlength of carapace).

Description of adult female

Body dorsoventrally flattened (Fig. 1A, B). Carapace nearly circular, 4.0 mm long, comprising 66.7% of total length, with frontal region weakly delimited by anterolateral indentations, and almost totally covering first to second pairs of legs (Fig. 1A, B). Paired compound eyes distinct dorsally in frontal region of carapace (Fig. 1A). Naupliar eye located posterior to compound eyes along midline of frontal region of carapace (Fig. 1A). Dorsal surface of carapace smooth without spines (Fig. 1A). Ventral surface of frontal and lateral regions of carapace ornamented with numerous, small sharply pointed spines (Fig. 1B). Posterolateral lobes of carapace 1.1 mm long, comprising 27.5% of carapace length, ending in rounded margin, separated by sinus nearly 1/3 length of carapace (Fig. 1A). Paired respiratory areas each consisting of small, oval anterior area and large, reniform posterior area, located at levels of first maxillae and second maxillae to first legs, respectively (Fig. 1B, D). Thorax indistinctly segmented (due to developed ovary filled with eggs) (Fig. 1A, B). Abdomen bilobed by anal indentation, longer than wide, with posterior margin of each lobe rounded and covered with tiny spines (Fig. 1A, B); anal indentation 46.2% as long as abdomen. Paired spermathecae oval in anteroventral region of abdomen (Fig. 1A, B). Subquadrate caudal rami located at base of anal indentation with four short naked setae on each ramus (Fig. 1A, C).

First antennae with four segments (Fig. 1E); first segment sclerotized, with large projection on posterolateral margin; second segment also sclerotized, with large projection on anterior margin, apically bent hook at lateral corner, and large projection and knob-like swelling on posterior margin; third segment longer than wide, with two naked spiniform setae near distal margin; apical segment shorter than third segment, with seven short naked spiniform setae apically. Second antennae with five segments (Fig. 1E); first segment sclerotized, with

Fig. 1. *Argulus japonicus*, adult female, 6.0 mm total length, NSMT-Cr 29117, from a largemouth bass *Micropterus salmoides* in the the Kako River, Hyogo Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view; C, caudal rami, dorsal view; D, respiratory areas, ventral view; E, 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 sucker membrane of first maxilla showing two supporting rods and maginal projections, ventral view; I, second maxilla, ventral view; J, tip of terminal segment of second maxilla, ventral view; K, two scale-like denticles on third segment of second maxilla, ventral view. Scale bars: A, B, 2 mm; C, E, F, 0.1 mm; D, 0.5 mm; G, I, 0.2 mm; H, J, K, 0.05 mm.

Fig. 2. *Argulus japonicus*, adult female, 6.0 mm total length, NSMT-Cr 29117, from a largemouth bass *Micropterus salmoides* in the the Kako River, Hyogo Prefecture, Japan. A, first leg, ventral view; B, second to apical segments of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view. Distal part of exopods and endopods and setae projecting from these rami are not illustrated. Scale bars: A, C–E, 0.3 mm; B, 0.05 mm.

large projection and small swelling with four naked spiniform setae on posterior margin; second segment shorter than first segment, with three and three naked spiniform setae, respectively, near distal and on posterior margins; third, fourth, and apical segments each longer than wide, decreasing in length; third segment with two and two naked spiniform setae, respectively, near distal and on posterior margins; fourth segment with seven naked spiniform setae on distal margin; apical segment ending in six naked spiniform setae. 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 tip of stylet seen at sheath opening (Fig. 1F). Mouth tube located posterior to preoral sheath, longer than wide, becoming wider posteriorly, composed of anterior labrum and posterior labium furnished with scales and pair of tiny spines

(Fig. 1G).

First maxillae forming cup-like suckers (Fig. 1B, H), with 50 and 52 supporting rods each in two sucker membranes; each rod composed of six to seven (mostly seven, $n=10$) sclerites; basal sclerite nearly three times as long as wide, widening distally; other sclerites trapezoidal or oval, decreasing in size distally; outer margin of rim of sucker membrane with numerous triangular projections. Second maxillae with five segments (Fig. 1I–K); first segment robust, with three basally separated, subequally long blunt projections on posterior margin; corpus of first segment bearing two posteriorly directed spiniform setae and furnished with raised field of scale-like denticles; second segment nearly two times as long as wide, with raised patch of serrate scale-like denticles on anterodistal surface; third segment shorter than second segment, with patch of serrate scale-like denticles on anterolateral surface; fourth

Fig. 3. *Argulus japonicus*, adult female, 6.0 mm total length, NSMT-Cr 29117, from a largemouth bass *Micropterus salmoides* in the the Kako River, Hyogo Prefecture, Japan. Ethanol-preserved specimen, habitus, ventral view. Scale bar: 2 mm.

segment subquadrate, with small denticles anteriorly; terminal segment smallest, ending in one club-like and two spiniform projections. Accessory spines near first segments of second maxillae (Fig. 1B). Postmaxillary spines located posterior to accessory spines (Fig. 1B).

First to fourth pairs of legs biramous; sympods two-segmented, consisting of coxa and basis, with small scale-like projections on ventral surface; rami each consisting of exopod and endopod, with two rows of plumose setae, respectively, near ventro- and dorsoposterior margins (Figs. 1, 2). First and second pairs of legs each with dorsal flagellum projecting from extreme proximal part of exopod (flagella usually not seen in ventral view, but flagellum bearing 13 plumose setae ventrally visible on second leg of specimen examined, Fig. 2C). First pair of legs each with single plumose seta on posterior margin of coxa and three-segment-

ed 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 natatory lobe bearing 12 plumose and three short naked setae, respectively, on posterior and distal margins, and basis bearing seven plumose setae on posterior margin (Fig. 2E).

Color in ethanol-preserved specimen: Carapace, abdomen, and legs white; thorax pale yellow with irregularly shaped black spots unevenly scattered on dorsal surface; respiratory areas fringed by continuous black pigment (Fig. 3).

Remarks

Although the carapace of *A. japonicus* from Japan has been reported to cover the first to third or fourth pairs of legs (Nakazawa, 1914; Tokioka, 1936; Yamaguti, 1937), the argulid specimen collected in this study has a shorter carapace that does not reach the third pair of legs (Fig. 1A, B). In spite of this difference, other morphological characters of the specimen correspond to those of *A. japonicus* reported by the above authors, and the specimen is identified as *A. japonicus*.

Only one adult female of *A. japonicus* was examined in this study, but several morphological differences are known between both sexes of the species (Nakazawa, 1914; Tokioka, 1936; Yamaguti, 1937). The male of *A. japonicus* has two posteriorly directed finger-like projections on the posteroventral margin of the coxa of the second leg, a swelling (=socket) on the posterior margin of the basis of the third leg, and a peg on the anterior margin of the basis of the fourth leg. A knob-like projection is also found at the anteroventral side of the third segment of the thorax of the male. These structures are not found on the sympods and thorax of the female (Figs. 1B, 2C, D, E).

Argulus japonicus has been introduced to other continents from east/southeast Asia (Poly,

2008), in which it has been reported from Japan, Russian Far East (Gusev, 1987), Korea (Choi & Yang, 1998; Han *et al.*, 1998; De Zoysa *et al.*, 2017), China (*e.g.*, Tokioka, 1939; Hsiao, 1950; Wang, 1958; Institute of Hydrobiology, Hubei Province, 1973; Kuang & Qian, 1991; Wadeh *et al.*, 2008; Yang, 2009), Vietnam (Arthur & Te, 2006; Ky & Te, 2007), Malaysia (Leong, 1986), Philippines (Cruz-Lacierda & Nagasawa, 2017), and Indonesia (*e.g.*, Inaya *et al.*, 2015; Idris *et al.*, 2020). The species has been identified using a traditional microscopic technique (Tokioka, 1939; Hsiao, 1950; Wang, 1958; Institute of Hydrobiology, Hubei Province, 1973; Leong, 1986; Kuang & Qian, 1991; Choi & Yang, 1998; Ky & Te, 2007; Yang, 2009; Cruz-Lacierda & Nagasawa, 2017) and a scanning electron microscope (Wadeh *et al.*, 2008; De Zoysa *et al.*, 2017). Although no morphological information exists on *A. japonicus* from Far East Russia and Indonesia, there is no significant difference in the morphology of the species from Japan, China, Korea, Philippines, and Malaysia (see the above literature).

Argulus japonicus is morphologically similar to *Argulus coregoni* Thorell, 1864, which also occurs in east/southeast Asia, including Japan (*e.g.*, Tokioka, 1936; Yamaguti, 1937; Hoshina, 1950; Nagasawa & Yuasa, 2020; Nagasawa & Taniguchi, 2021), Russian Far East (*e.g.*, Dogiel & Akhmerov, 1952; Smirnova, 1971; Gusev, 1987; Ermolenko, 2004; Sokolov *et al.*, 2012), China (*e.g.*, Wang, 1958; Institute of Hydrobiology, Hubei Province, 1973; Kuang & Qian, 1991; Yang, 2009), and Malaysia (Everts & Avenant-Oldewage, 2009). *Argulus japonicus* is distinguished from *A. coregoni* by having an apically rounded abdomen (Fig. 1A, B) (*vs.* apically pointed abdomen in *A. coregoni*), about 50 supporting rods in the sucker membrane of the first maxilla (*vs.* 60 or more supporting rods in *A. coregoni*), and a single plumose seta on the posterior margin of the coxa of the first leg (Fig. 2A) (*vs.* four to eight plu-

mose setae in *A. coregoni*). The morphological characters of *A. coregoni* have been reported from Japan, China, and Malaysia (see the above literature).

No information is available on the native hosts of *A. japonicus* in the Kako River, where the specimen was collected in this study. Previously, two specimens of *A. japonicus* were collected from the river, but one specimen was found on an unknown host and another specimen was a detached, free-swimming individual (Nagasawa *et al.*, 2009). In this study, the infected largemouth bass was collected in the lower reaches of the river, where 18 species of cyprinids are found (Nishiguchi, 2007) and likely to serve as native hosts for *A. japonicus*.

Discussion

Largemouth bass is native to North America, where the species is known as the hosts of several *Argulus* species (*A. appendiculosus* C. B. Wilson, 1907, *A. flavescens* C. B. Wilson, 1916, *A. mississippiensis* C. B. Wilson, 1916, and an unidentified species) (Hoffman, 1999; McAllister *et al.*, 2016). Although *A. japonicus* was introduced from Asia including Japan into the U.S.A. (Meehean, 1940; Wilson, 1944; Cressey, 1978), it has not been reported from largemouth bass (*e.g.*, Amin, 1981; Poly, 1998; Hoffman, 1999). For sport fishing, largemouth bass has been transplanted from North America into other regions, such as South America, Europe, South Africa, and Far East Asia (Pereira & Vitule, 2019) but, to date, *A. japonicus* has not been recorded from the fish in these regions. Thus, the collection of *A. japonicus* in this study represents the first record of the species from largemouth bass worldwide.

In Japan, various species of cyprinids (Cypriniformes) serve as the hosts for *A. japonicus* (*e.g.*, Nagasawa *et al.*, 2012, 2013, 2018, 2021; Yamauchi & Shimizu, 2013; Nagasawa, 2017b, 2018; Nagasawa & Miyajima, 2018; Nagasawa & Ishiyama, 2019; see Nagasawa, 2009, 2011

for the earlier literature), and a non-cyprinid fish, *i.e.*, Amur catfish *Silurus asotus* Linnaeus, 1758 (Siluriformes: Siluridae) is also known as a host for the parasite (Nagasawa *et al.*, 2010; Yamauchi *et al.*, 2011). Largemouth bass is the second species of non-cyprinid host of *A. japonicus* in Japan. Moreover, two species of parasitic copepods, *L. cyprinacea* and *N. japonicus*, have been reported from largemouth bass in Japan (Kasahara, 1962; Nagasawa & Inoue, 2012; Nagasawa & Sato, 2015), and *A. japonicus* is the third crustacean parasite from this fish species. Like *A. japonicus*, both *L. cyprinacea* and *N. japonicus* show no strict host specificity (Nagasawa *et al.*, 2007; Nagasawa & Uyeno, 2012), and these three crustacean parasites can utilize largemouth bass of the North American origin as their host in Japan.

Recently, using GenBank, molecular identification has been applied to *A. japonicus* and its populations (Wadeh *et al.*, 2010; Tandel *et al.*, 2021) but considerable variation is found between populations within the same country and those in different countries (Wadeh *et al.*, 2010). There is, however, the possibility that molecular data of misidentified congeneric species have been registered at GenBank as those of *A. japonicus*. Thus, for better understanding such variations, accurate morphological identification of *A. japonicus* from each collection locality is essential in submitting its sequence data to GenBank.

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