Morphotaxonomy and Ecology of Branchiurans of the Genus *Argulus* (Crustacea: Argulidae), Ectoparasites of Freshwater and Marine Fishes in Japan

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Chapter 1. Introduction

1.1 Branchiurans of the genus *Argulus* in Japan

The Branchiura Thorell, 1864 is one of two subclasses in the crustacean class Ichthyostraca Zrzavý, Hypša, and Vlášková, 1997, and another subclass is the Pentastomida Diesing, 1836 (WoRMS, 2023). The Branchiura includes 166 valid species that all belong to a single family Argulidae Leach, 1819 in a single order Arguloidea Yamaguti, 1963 (Møller, 2009; Neethling and Avenant-Oldewage, 2016; Walter and Boxshall, 2023). This family is classified into four genera, which are *Argulus* Müller, 1785, *Dolops* Audouin, 1837, *Chonopeltis* Thiele, 1900, and *Dipteropeltis* Calman, 1912). *Argulus* is the most speciose genus and comprises 138 valid species. Branchiurans are ectoparasites of fishes in fresh and marine waters, with a few exceptions of their infection on amphibians (frogs and tadpoles) (Kabata, 1970). *Argulus* spp. become problems in fish-rearing facilities and in the wild.

In 1900, the first paper of the Japanese branchiurans was published by Thiele (1900), who described two new species, *A. japonicus* and *A. scutiformis,* using specimens collected in Japan. Branchiurans of this genus since have been studied in Japan, and 12 valid species have been reported from this country to date (Nagasawa, 2009, 2011a, 2023b; Uyeno *et al.*, 2017; Nagasawa *et al.*, 2022b). They are the following six freshwater and six marine species:

Freshwater species (in alphabetical order)

Argulus americanus C. B. Wilson, 1902

Argulus coregoni Thorell, 1894

Argulus japonicus Thiele, 1900

Argulus lepidostei Kellicott, 1877

Argulus mongolianus Tokioka, 1939

Argulus nobilis Thiele, 1904

Marine species (in alphabetical order)

Argulus caecus C. B. Wilson, 1922

Argulus kusafugu Yamaguti and Yamasu, 1959

Argulus matuii Sikama, 1938

Argulus onodai Tokioka, 1936

Argulus quadristriatus Devaraj and Ameer Hamsa, 1977

Argulus scutiformis Thiele, 1900.

Of the six freshwater species, only two species (*A. japonicus* and *A. coregoni*) are native to Japan, whereas the four other species were introduced to Japan: three species (*A. americanus*, *A. lepidostei*, and *A. nobilis*) were from North America (Tsutsumi, 1968; Shimura and Asai, 1984; Nagasawa, 2023b) and *A. mongolianus* most probably from China (Nagasawa *et al*., 2022b).

Yamaguti (1937) described a new species of argulid, *Argulus plecoglossi*, based on specimens of both sexes from the body surface of ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) (reported as "*P. altivelis*") (Plecoglossidae) in the Hozu River, Kyoto Prefecture, central Honshu. However, Tokioka (1965, 1979) and Shimura (1981: 347, appendix) suggested that *A. plecoglossi* is synonymous with *A. coregoni*, and the present author has followed their suggestions (Nagasawa, 2009, 2011a; this paper).

1.2 Purpose of the Study

In Japan, both *A. japonicus* and *A. coregoni* are found in freshwater fish farms, and their ecology has been studied under such fish-rearing conditions (*e.g.*, Nakazawa, 1914, Kimura, 1970 for *A. japonicus*; Shimura, 1983a for *A. coregoni*). However, since these two species and other congeneric species are rare parasites of wild fishes (Tokioka, 1936c), it is difficult to collect their specimens in the wild, and our knowledge of the Japanese *Argulus* species infecting wild fishes is still very limited. Moreover, in Japan, besides the present author's co-workers (Saito and Nagasawa, 2010; Saito *et al*., 2011; Uyeno *et al.*, 2017), some scientists have studied the branchiurans since the 1950s. They are: Hoshina (1950), Yamaguti and Yamasu (1959), Kato (1964), Tsutsumi (1968), Shimura and his colleagues (Shimura and Egusa, 1980; Inoue *et al.*, 1980; Shimura, 1981, 1983a, b; Shimura *et al.*, 1983a, b; Shimura and Inoue, 1984; Shimura and Asai, 1984), Takegami (1984), Yamauchi and his collaborators (Yamauchi *et al.*, 2011; Yamauchi and Kobayashi, 2013), and Takeda *et al.* (2000). In such a situation, more studies are needed to better understand the biology of the Japanese *Argulus* species. Thus, the present work was conducted to study the morphology of six species (*A. japonicus*, *A. coregoni*, *A. mongolianus*, *A. nobilis*, *A. caecus*, and *A. matuii*), whose specimens were obtained herein, and to clarify the distributional patterns of two species (*A. japonicus* and *A. coregoni*) in various freshwater bodies. Unfortunately, no specimens of the other *Argulus* species were available in this study.

This dissertation consists of four chapters. Chapter 1 is the Introduction, in which the purpose of the present study is mentioned.

In Chapter 2, morphological characters of four freshwater species (*A. japonicus*, *A. coregoni*, *A. mongolianus*, and *A. nobilis*) and two marine species (*A. caecus* and *A. matuii*) are reported based on a detailed examination of specimens collected from Japan. Of these species, *A. mongolianus* and *A. nobilis* are reported and described for the first time in Japan. While *A. japonicus* and *A. coregoni* are morphologically species, it is possible to differentiate them from each other by the number of plumose setae on the posterior margin of the coxa of the first pair of legs and the number of supporting rods in two sucker membranes of the first maxillae. A key is also given to identify the four freshwater species found in Japan.

Chapter 3 focuses on the distribution patterns of *A. coregoni* in mountain streams, those of *A. coregoni* and *A. japonicus* in the Lake Biwa Basin, and the occurrence of *A. japonicus* in unusual water bodies, such as brackish waters. This chapter demonstrates that *A. coregoni* infects various salmonids in the upper reaches of rivers at high elevations but parasitizes ayu in the middle and lower reaches of rivers. In the Lake Biwa Basin, *A. coregoni* similarly occurs in upper reaches of rivers flowing into the lake at higher elevations, but *A. japonicus* is found in the lake and a nearby pond. Based on this result, it is inferred that *A. coregoni* inhabits the lotic, cold, highly oxygenated streams but *A. japonicus* occurs in the still or slow-flowing (lentic) waters and is more tolerant to higher water temperatures and lower oxygen concentrations than *A. coregoni*. In this chapter, *A. japonicus* is also recorded from two brackish water lakes, where the parasite was found infecting euryhaline fishes. Furthermore, *A. japonicus* was found on a cyprinid in a river flowing through a residential area.

In Chapter 4, the results reported in the previous chapters are summarized and discussed, and several suggestions are given for future research on the biology of the Japanese *Argulus* species.

Chapter 2. Morphology and Taxonomy of *Argulus* spp. parasitic on fishes in Japan

2.1 Freshwater species

2.1.1 *Argulus japonicus* Thiele, 1900

[Based on Nagasawa, K. (2021): *Argulus japonicus* Thiele, 1900 parasitic on largemouth bass *Micropterus salmoides* in Japan, with the morphology of the adult female of the argulid. Crustacean Research, 50: 119–129]

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 *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 North American origin as its host in Japan.

2.1.1 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 and Inoue, 2012; Nagasawa and Sato, 2015).

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. 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 (1936a), 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 and 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*.

2.1.1 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 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 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 and Gooding, 1964; Benz and 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 and Otting (1996). The specimen was deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr).

2.1.1 Results

Argulus japonicus Thiele, 1900

[Japanese name: Chou]

(Figs 2.1.1.1–2.1.1.3)

Host. Largemouth bass *Micropterus salmoides* (Lacepède, 1802) (Centrarchiformes: Centrarchidae).

Attachment site. Body surface.

Locality. 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, Japan.

Material examined. One adult female (NSMT-Cr 29117) from a largemouth bass *M. salmoides*.

Adult female. *Body* dorsoventrally flattened, measuring 6.0 mm in total length (TL, from anterior tip of carapace to posterior tip of abdomen) and 3.8 mm in maximum body width (around midlength of carapace) (Fig. 2.1.1.1A, B). *Carapace* nearly circular, 4.0 mm long, comprising 66.7% of TL, with frontal region weakly delimited by anterolateral indentations, and almost totally covering first to second pairs of legs (Fig. 2.1.1.1A, B). Paired compound eyes distinct dorsally in frontal region of carapace. Naupliar eye located posterior to compound eyes along midline of frontal region of carapace. Dorsal surface of carapace smooth without spines (Fig. 2.1.1.1A). Ventral surface of frontal and lateral regions of carapace ornamented with numerous, small sharply pointed spines (Fig. 2.1.1.1B). 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. 2.1.1.1A). 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 (Figs 2.1.1.1B, 2.1.1.2D). *Thorax* indistinctly segmented (due to developed ovary filled with eggs) (Fig. 2.1.1.1A, B). *Abdomen* bilobed by anal indentation, longer than wide, with posterior margin of each lobe rounded and covered with tiny spines; anal indentation 46.2% as long as abdomen (Fig. 2.1.1.1A, B). Paired spermathecae oval in anterocentral region of abdomen. Subquadrate caudal rami located at base of anal indentation with four short naked setae on each ramus (Fig. 2.1.1.1A, C).

First antennae with four segments (Fig. 2.1.1.2A); 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. 2.1.1.2B); first segment sclerotized, with 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. 2.1.1.2A). Preoral sheath on ventral midline of frontal region of carapace, with anterior tip of stylet seen at sheath opening (Fig. 2.1.1.2B). 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. 2.1.1.2C).

First maxillae forming cup-like suckers (Figs 2.1.1.1B, 2.1.1.2E), 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. 2.1.1.2F–H); 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 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. 2.1.1.1B). Postmaxillary spines located posterior to accessory spines (Fig. 2.1.1.1B).

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 (Fig. 2.1.1.3). *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. 2.1.1.3C). First pair of legs each with single plumose seta on posterior margin of coxa and three-segmented endopod ending in three short spines (Fig. 2.1.1.3A, B). Sympods of second and third pairs of legs without setae (Fig. 2.1.1.3C, D). Endopods of *third and fourth pairs of legs* two-segmented (Fig. 2.1.1.3D, 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. 2.1.1.3E).

Color (based on 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. 2.1.1.4).

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, 1936a; Yamaguti, 1937), the argulid specimen collected in this study has a shorter carapace that does not reach the third pair of legs (Fig. 2.1.1.1A, 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, 1936a; 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 2.1.1.1B, 2.1.1.3C, 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 and 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 and Qian, 1991; Wadeh *et al.*, 2008; Yang, 2009), Vietnam (Arthur and Te, 2006; Ky and Te, 2007), Malaysia (Seng, 1986), Philippines (Cruz-Lacierda and 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; Seng, 1986; Kuang and Qian, 1991; Choi and Yang, 1998; Ky and Te, 2007; Yang, 2009; Cruz-Lacierda and 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, 1936a; Yamaguti, 1937; Hoshina, 1950; Nagasawa and Yuasa, 2020; Nagasawa and Taniguchi, 2021), Russian Far East (*e.g.*, Dogiel and Akhmerov, 1952; Smirnova, 1971; Gusev, 1987; Ermolenko, 2004a, b; Sokolov *et al.*, 2012), China (*e.g.*, Wang, 1958; Institute of Hydrobiology, Hubei Province, 1973; Kuang and Qian, 1991; Yang, 2009), and Malaysia (Everts and Avenant-Oldewage, 2009). *Argulus japonicus* is distinguished from *A. coregoni* by having an apically rounded abdomen (Fig. 2.1.1.1A, 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. 2.1.1.3A) (vs. four to eight plumose 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*.

2.1.1 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 and Vitule, 2019) but, to date, *A. japonicus* has not been recorded from this fish species 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, 2018a, 2021a; Yamauchi and Shimizu, 2013; Nagasawa, 2017b, 2018; Nagasawa and Miyajima, 2018; Nagasawa and Ishiyama, 2019b; see Nagasawa, 2009, 2011a 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 and Inoue, 2012; Nagasawa and 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 and Uyeno, 2012), and these three crustacean parasites can utilize largemouth bass of 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.

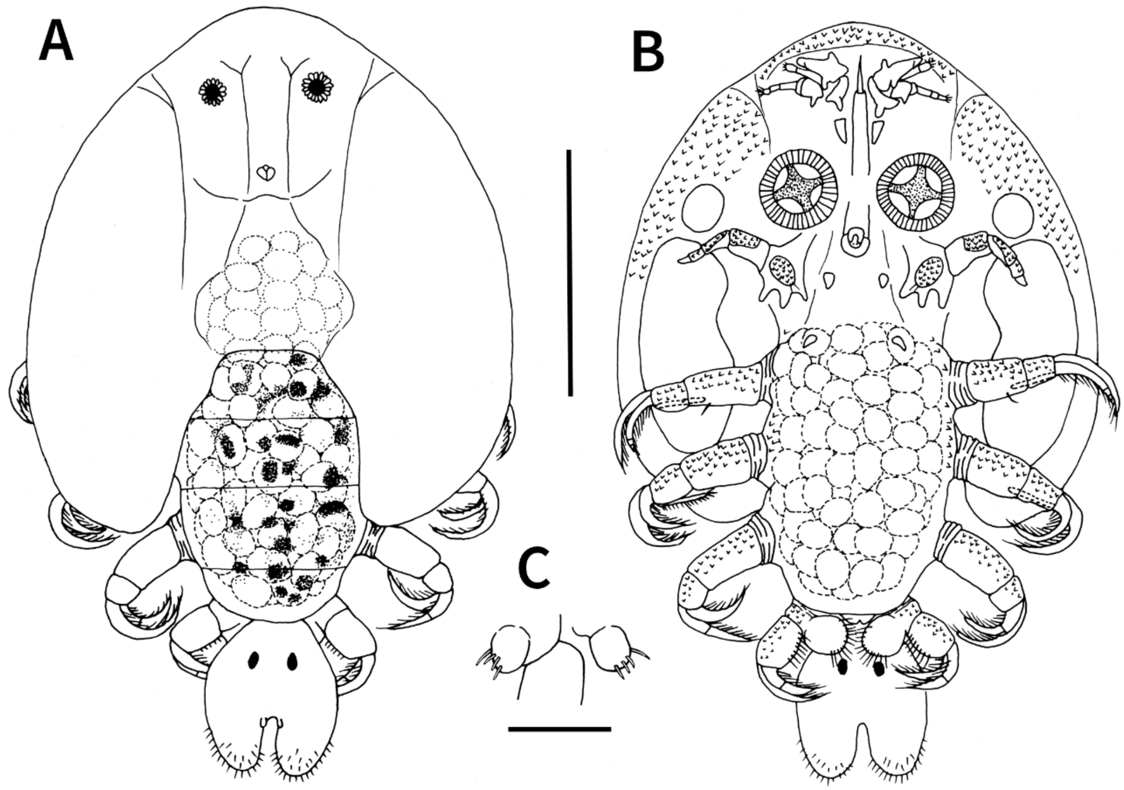

Fig. 2.1.1.1. *Argulus japonicus*, adult female (6.0 mm total length), NSMT-Cr 29117, from largemouth bass *Micropterus salmoides* in the Kako River, Hyogo Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view; C, caudal rami, dorsal view. Scale bars: A, B, 2 mm; C, 0.1 mm.

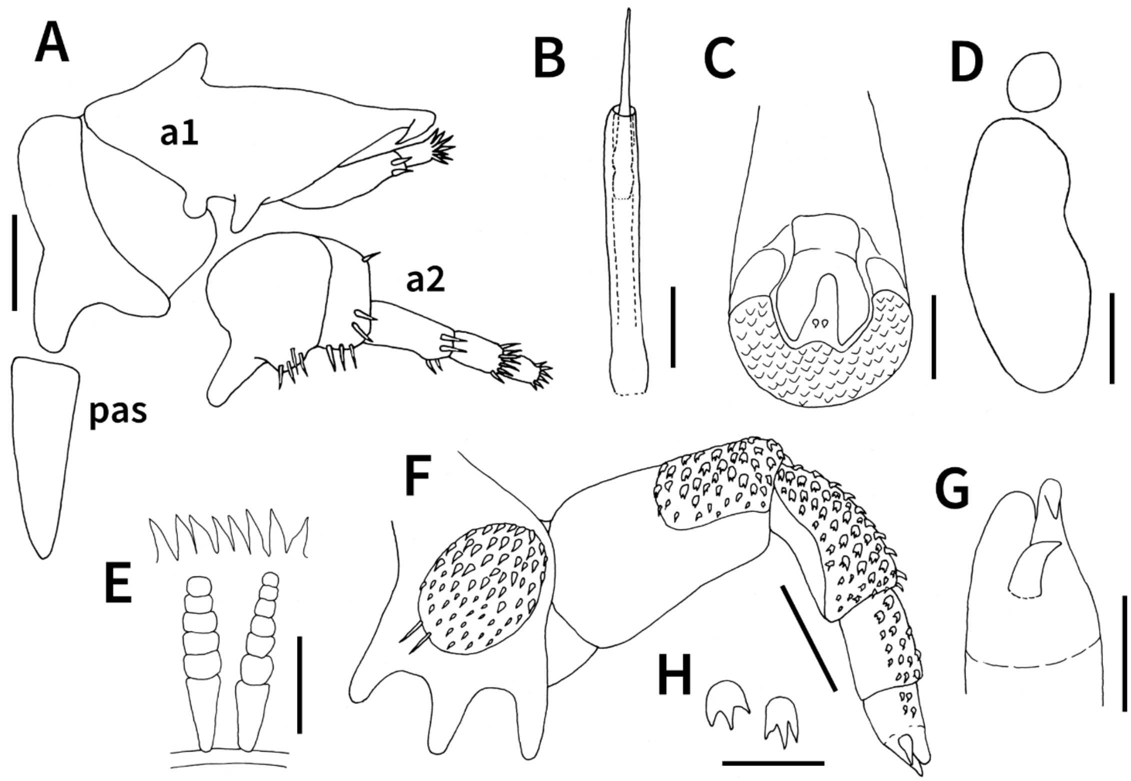

Fig. 2.1.1.2. *Argulus japonicus*, adult female (6.0 mm total length), NSMT-Cr 29117, from largemouth bass *Micropterus salmoides* in the Kako River, Hyogo Prefecture, Japan. A, first antenna (a1), second antenna (a2), and postantenal spine (pas), ventral view; B, preoral sheath and stylet, ventral view; C, mouth tube, ventral view; D, respiratory areas, ventral view; E, section of sucker membrane of first maxilla showing two supporting rods and marginal projections, ventral view; F, second maxilla, ventral view; G, tip of terminal segment of second maxilla, ventral view; H, two scale-like denticles on third segment of second maxilla, ventral view. Scale bars: A, B, 0.1 mm; C, F, 0.2 mm; D, 0.5 mm; E, G. H, 0.05 mm.

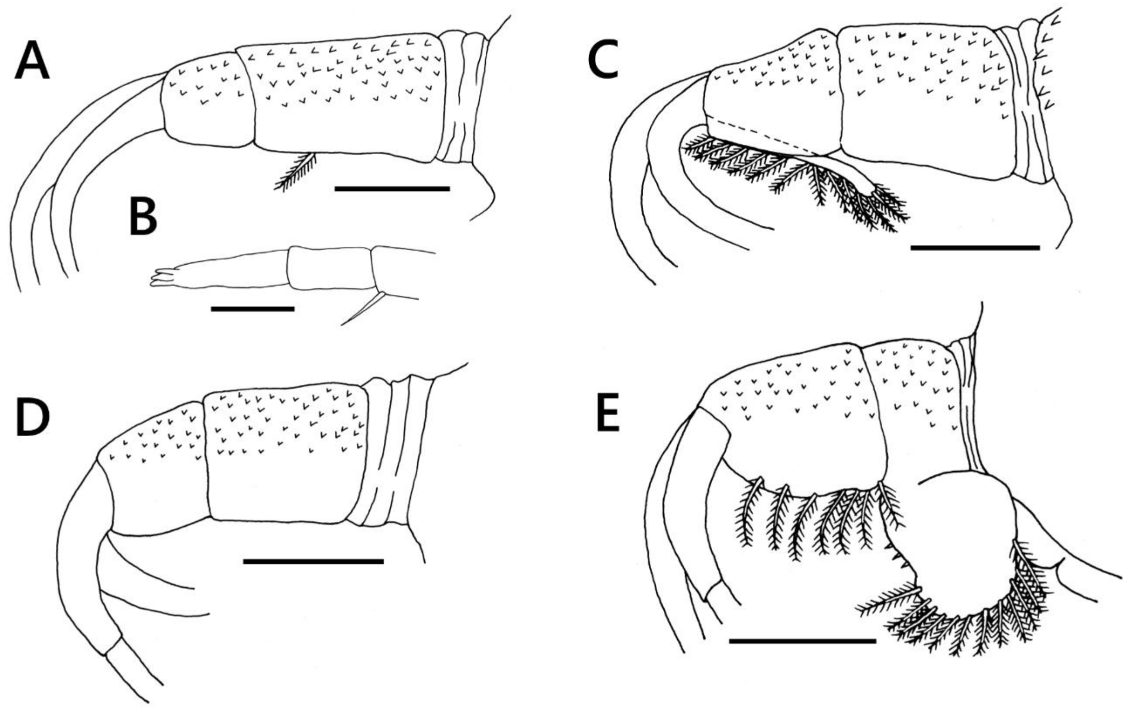

Fig. 2.1.1.3. *Argulus japonicus*, adult female (6.0 mm total length), NSMT-Cr 29117, from largemouth bass *Micropterus salmoides* in the the Kako River, Hyogo Prefecture, Japan. A, first leg, ventral view; B, second to apical segments of endopod of first leg, ventral view; C, second leg, ventral view; 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.

Fig. 2.1.1.4. *Argulus japonicus*, adult female (6.0 mm total length), NSMT-Cr 29117, from largemouth bass *Micropterus salmoides* in the the Kako River, Hyogo Prefecture, Japan. Ethanol-preserved specimen, habitus, ventral view. Scale bar: 2 mm.

2.1.2 *Argulus coregoni* Thorell, 1864

[Based on Nagasawa, K. and Taniguchi, R. (2021): Second record of *Argulus coregoni* (Branchiura: Argulidae) from acheilognathine fish (Cyprinidae): its infection of an oily bitterling *Tanakia limbata* in central Japan. Taxa―Proceedings of the Japanese Society of Systematic Zoology, 51: 29–37. *This paper was published in Japanese but is herein translated into English*]

Abstracts. An adult female of *Argulus coregoni* Thorell, 1864 was collected from the dorsal body surface of an oily bitterling *Tanakia limbata* (Temminck and Schlegel, 1846) (Cyprinidae: Acheilognathinae) in the lower reaches of the Asahi River, Okayama Prefecture, western Japan. This represents a new host record for *A. coregoni* and its new prefectural record in Japan. The external morphology of the female collected is reported in detail. *Tanakia limbata* is the second acheilognathine host of *A. coregoni*. The occurrence of *A. coregoni* on the oily bitterling is unusual because this parasite usually infects various salmonids and ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) (Plecoglossidae) in the upper and middle or lower reaches of rivers, respectively, in central and western Japan. The individual of *A. coregoni* probably parasitized the oily bitterling after detachment from ayu near the collection locality.

2.1.2 Introduction

The crustacean subclass Branchiura Thorell, 1864 are parasitic on the body surface and fins of freshwater and marine fishes (Yamaguti, 1963). Branchiurans of the genus *Argulus* Müller, 1785 have been reported from Japan, where four species of this genus have so far been reported: *A. japonicus* Thiele, 1900; *A. coregoni* Thorell, 1864; *A. americanus* C. B. Wilson, 1922; and *A. lepidostei* Kellicott, 1877 or a closely related species (Nagasawa, 2009, 2011a). Of these species, the latter two species have been recored only from aquarium-held exotic fishes (Tsutsumi, 1968; Shimura and Asai, 1984), whereas the former two species are found on comercially important fishes and have been frequently studied in Japan (Nagasawa, 2009). *Argulus japonicus* is usually found on cyprinids, such as common carp *Cyprinus carpio* Linnaeus, 1758 (Nakazawa, 1914; Kimura, 1970), and *A. coregoni* is known as the parasite of various salmonids and ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) (*e.g.*, Yamaguti, 1937; Hoshima, 1950; Shimura, 1983a; Nagasawa and Morikawa, 2019a; Nagasawa and Yuasa, 2020).

Regarding the known Japanese hosts of *A. coregoni*, there is an interesting fact. When Tokioka (1936a) reported this species for the first time in Japan, he found it on a bitterling *Acheilognathus* sp. (Cyprinidae: Acheilognathinae) [reported as *A. moriokae*, see Goda *et al.* (2017) for this scientific name] in central Japan. As stated above, since *A. coregoni* parasitizes salmonids and ayu and usually does not infects cyprinids in Japan. The bitterling was taken at Otsu, Shiga Prefecture (Tokioka, 1936a), and this collection site is most probably coastal waters of Lake Biwa. As bitterlings inhabit slow-flowing water bodies and *A. coregoni* is usually found on fishes in fast-flowing streams, Tokioka's (1936a) collection of this parasite from *Acheilognathus* sp. is very unusual. Since 1936, *A. coregoni* has not been collected from fishes in Lake Biwa to date (Nagasawa, 2020).

Recently, the present author found a branchiuran-infected oily bitterling *Tanakia limbata* (Temminck and Schlegel, 1846) (Cyprinidae: Acheilognathinae) among bitterlings caught in the lower reaches of the Asahi River, Okayama Prefecture, western Japan, and the branchiuran was identified as the female of *A. coregoni*. Since this species morphologically resembles *A. japonicus* infecting cyprinids, it is important to examine the morphology of *A. coregoni* from the oily bitterling in order to differentiate from *A. japonicus*. This paper redescribes *A. coregoni* using the female specimen collected and discusses its occurrence on this fish in the Asahi River.

2.1.2 Materials and methods

Bitterlings were collected in a small pool-like area (called "wando") in the lower reaches of the Asahi River flowing in Okayama City, Okayama Prefecture, western Japan, on 8 June 2020. When they were individually identified and photographed in the field, a single individual of oily bitterling was found infected with a branchiuran on the body surface, and this parasite was carefully removed using forceps from the fish, fixed and preserved in 70% ethanol. Later, at the Aquaparasitology Laboratory, Shizuoka Prefecture, the branchiuran specimen was cleared in lactophenol and examined under an Olympus SZX10 stereo microscope an Olympus BX51 phase-contrast compound microscope using the wooden slide procedure (Humes and Gooding, 1964; Benz and Otting, 1996). All drawings were made with the aid of drawing tubes each attached to the stereo microscope (for the habitus) and the compound microscope (for the body parts). Morphological terminology follows Benz *et al.* (1995) and Benz and 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).

In addition to the first sampling on 8 June 2020, bitterlings were collected in the same pool-like area in the Asahi River at five times (19 November, 4 and 29 December, 2020; 16 April, 29 May 2021) to examine the infection of *A. coregoni* on them. These fishes were similarly identified and photographed in the field, and data on their standard length (SL) were obtained from the photographs. The scientific names of fishes stated in this paper follow Nakabo (2018).

2.1.2 Results

Argulus coregoni Thorell, 1864

[Japanese name: Chou-modoki]

(Figs 2.1.2.1–2.1.2.3)

Host. Oily bitterling *Tanakia limbata* (Temminck and Schlegel, 1846) (Cypriniformes: Cyprinidae: Acheilognathinae).

Attachment site. Body surface.

Locality. Lower-reaches of the Asahi River flowing in Okayama City, Okayama Prefecture, Japan.

Material examined. One adult female (NSMT-Cr 29034) from an oily bitterling *T. limbata*.

Occurrence on bitterlings. In total, 67 individuals of the following three species of bitterlings (the subfamily Acheilognathinae) were collected in the Asahi River on 8 June 2020: oily bittering [n = 56, 33–73 (mean, 47) mm SL], slender bitterling *Tanakia lanceolata* (Temminck and Schlegel, 1846) [n = 10, 47–74 (62) mm SL], and white tabira bitterling *Acheilognathus tabira tabira* Jordan and Thompson, 1914 (n =1, 69 mm SL). Of these fishes, one individual of oily bitterling (65 mm SL) had an infection of an adult female of *A. coregoni* on the dorsal body surface (Fig. 2.1.2.1). However, this parasite was not found on the bittelings [31 individuals of oily bitterling, 34–71 (mean, 59) mm SL, and nine individuals of slender bitterling, 60–74 (69) mm SL] from mid-November 2020 to late May 2021.

Adult female (based on one specimen). *Body* dorsoventrally flattened, measuring 9.2 mm in total length (TL, from anterior tip of carapace to posterior tip of abdomen) and 6.0 mm in maximum body width (around midlength of carapace) (Fig. 2.1.2.2A, B). *Carapace* nearly circular, 6.6 mm long (72% of TL), with paired longitudinal interocular ribs dorsally present and bifurcated at anterior ends, forming paired posterolateral lobes separated by sinus, and covering coxa and basis of first to third pairs of legs (Fig. 2.1.2.2A). Dorsal surface of carapace smooth without spines (Fig. 2.1.2.2A). Paired compound eyes present dorsally at level of anterolateral indentations of carapace (Fig. 2.1.2.2A, B). Naupliar eye located posterior to compound eyes. Dorsal surface of carapace smooth without small spines (Fig. 2.1.2.2A, B). Ventral surface of frontal and lateral regions of carapace ornamented with numerous, small spines (Fig. 2.1.2.2B). Posterolateral lobes of carapace ending in rounded margin (Fig. 2.1.2.2A). Paired respiratory areas present in individual lobes, each consisting of small, oval anterior area and large, reniform posterior area, located at levels of first and second maxillae and first and second pairs of legs, respectively (Fig. 2.1.2.2B, D). *Thorax* four-segmented (Fig. 2.1.2.2A, B). *Abdomen* bilobed by anal indentation, with posterior end of each lobe pointed (Fig. 2.1.2.2A, B). Caudal rami located at base of anal indentation with four naked setae on posterior margin of each ramus (Fig. 2.1.2.2A, C). Ventral process present near coxae of fourth pairs of legs (Fig. 2.1.2.2B).

First antennae with four segments (Fig. 2.1.2.2H); first segment sclerotized, with large projection on posterolateral margin; second segment also sclerotized, with large projection on anterior margin, apically bent hook at distal corner, and large projection on posterior margin; third segment elongate, with three naked setae near distal margin; apical segment shorter than third segment, with five setae (two apical setae slightly longer than others). *Second antennae* with five segments (Fig. 2.1.2.2H); first segment sclerotized, with large projection and small swelling with six naked setae on posterior margin and small spine near distal margin; second segment nearly round, with four and three naked setae on posterior and near distal margins, respectively; third, fourth, and apical segments each longer than wide, decreasing in length; individual segments bearing five, nine, and three setae near distal ends, respectively (Fig. 2.1.2.2H). Postantennal spines stout, each located posterior to projection of first segment of first antenna (Fig. 2.1.2.2H). Preoral sheath nearly elongate and present on ventral midline of frontal region of carapace, with anterior portion of stylet seen at sheath opening (Fig. 2.1.2.2B, F). Mouth tube located posterior to preoral sheath, longer than wide, becoming wider posteriorly; posterior portion composed of anterior labrum and posterior labium bearing pair of tiny spines (Fig. 2.1.2.2G).

First maxillae forming cup-like suckers (Fig. 2.1.2.2B, E), with 72 and 67 supporting rods each in two sucker membranes; each rod composed of six to eight (mostly seven, n = 10) sclerites; basal sclerite longer than other trapezoidal sclerites; outer margin of rim of sucker membrane with numerous tiny projections. *Second maxillae* with five segments (Fig. 2.1.2.2I); first segment robust, with three long blunt projections on posterior margin; corpus of first segment with raised oval field of denticles; second segment elongate, with denticles (some with serrated margin) on anterodistal surface; third segment narrower than second segment, with denticles on anterolateral surface; fourth segment short, with field bearing several denticles and three distal setae; terminal segment smallest, ending in three small projections. Accessory spines slightly apart from first segments of second maxillae (Fig. 2.1.2.2B). Postmaxillary spines located anterior to first segments of thorax (Fig. 2.1.2.2B).

First to fourth pairs of legs biramous; sympods two-segmented, each consisting of coxa and basis; rami each consisting of exopod and endopod, with two rows of plumose setae each on ventro- or dorsoposterior margin (Fig. 2.1.2.3). *First pairs of legs* each with six and five plumose setae on posterior margin of coxa and basis, respectively, and tiny spines on anterior margin of coxa; exopod unsegmented, with 21 ventral plumose setae; endopod three-segmented; first segment, with single naked and 16 plumose setae near distal end and on ventral surface, respectively; second segment with short naked seta at apex; apical segment ending in two small spines (Fig. 2.1.2.3A, B). *Second pairs of legs* each with one and six plumose setae on posterior margin of coxa and basis, respectively; exopod and endopod unsegmented, with 20 and 21 ventral plumose setae, respectively (Fig. 2.1.2.3C). *First and second pairs of legs* each with dorsal flagellum projecting from extreme proximal part of exopod. *Third pairs of legs* each with two and six plumose setae on posterior margin of coxa and basis, respectively; exopod unsegmented, with at least 12 ventral plumose setae; endopod two-segmented, proximal and terminal segments with 10 and 13 ventral plumose setae, respectively (Fig. 2.1.2.3D). *Fourth pair of legs* each with coxa forming natatory lobe, bearing 15 short and three long plumose setae on posterior margin of each lobe and distal margin, respectively; basis nearly quadrangle and larger those of first to third pairs of legs, bearing 16 plumose setae on posterior margin; exopod unsegmented, with at least nine ventral plumose setae; endopod two-segmented, proximal and terminal segments with 12 and 10 ventral plumose setae (Fig. 2.1.2.3E).

Color. Carapace pale dark green, and thorax pale yellow with black spots scattered on dorsal surface in the fresh specimen (Fig. 2.1.2.1). Body almost white, but black spots on dorsal surface of thorax, both compound eyes and naupliar eye black, respiratory areas fringed by black pigments, small pale brown spots near on ventral surface of mouth tube in the specimen observed in early June 2021 about one year after collection.

Remarks. The morphological characters of the branchiuran specimen examined in this study are almost identical to those of the adult female of *A. coregoni* reported by Yamaguti (1937) and Hoshina (1950), and the specimen is herein identified as this species. In particular, the basis of the fourth leg is nearly quadrangle and larger those of the first to third legs and the large natatory lobe is present on the posterior margin of the coxa of the fourth leg are ones of the features in the female of the species (Yamaguti, 1937: fig. 19; Hoshina, 1950), and these features are confirmed in the female specimen examined (Fig. 2.1.2.3E). Hoshina (1950) reported the number of plumose setae each on the coxa, basis, exopod, and endopod of the first to fourth legs of *A. coregoni*, and the specimen studied herein shows a similar number of those setae. Furthermore, *A. coregoni* is differentiated from *A. japonicus* by having more supporting rods in two sucker membranes of the first maxillae (more than 60 rods vs. ca. 50 rods) and more plumose setae on the posterior margin of the coxa of the first leg (4–8 setae vs. one seta) (Tokioka, 1936a; Yamaguti, 1937; Hoshina, 1950; this paper).

When the present specimen of *A. coregoni* was observed using the stereo microscope, eggs were seen through the thoracic wall (Fig. 2.1.2.2B), and more eggs were clearly recognized in the specimen in lactophenol. This observation shows that the specimen is the adult female.

Although only the adult female of *A. coregoni* was collected in this study, the adult male of this species can be differentiated from the that of *A. japonicus* by having one digitiform process on the posterodorsal margin of the coxa of the second leg (Tokioka, 1936a, 1965; Yamaguti, 1937). The morphology of the peg on the anterior margin of the basis of the fourth leg is slightly different between *A. coregoni* and *A. japonicus*: the peg in the former species bears two blunt spines on the anterior margin, while that in the latter species has chitinous thickenings along the outer edge and ends in a very short spine (Tokioka, 1936a: pl. 21, figs 2, 3). Furthermore, a protrusion is present on anterodistal margin of the coxa of the third leg in the adult male of *A. japonicus* (Tokioka, 1936a: pl. 21, fig. 2; Yamaguti, 1937: fig. 8) but not in that of *A. coregoni*.

Tokioka (1965) showed figures of the habitus of the adult *A. coregoni* and *A. japonicus* and stated that the former species is a longer abdomen than the latter species and has sharply pointed abdominal lobes, which gives us an impression to differentiate the two species from each other by the length and morphology of their abdomen. However, the abdominal lobes of *A. coregoni* from Japan were previously illustrated to be long oval shaped (Yamaguti, 1937: figs 10, 11; Tokioka, 1965) or oval shaped (Hoshina, 1952: fig. 1A, B), and the specimen examined in this study has oval abdominal lobes (Fig. 2.1.2.2A, B). This indicates that the relative length-to-width ratio of the abdominal lobes varies among individuals of *A. coregoni* and is not appropriate to use as a key in identifying the species, and it is desirable to examine variations in the morphology of the abdomen of the species.

In Japan, the adult female of *A. coregoni* has been described by Yamaguti (1937) and Hoshina (1950) and herein, and the adult male of the species by Tokioka (1936a), Yamaguti

(1937), and Hoshina (1950). Larval development of *A. coregoni* was studied by Shimura (1981). Yamaguti (1937) described a new species *Argulus plecoglossi*, which has been relegated to a junior synonym of *A. coregoni* (Tokioka, 1965; Shimura, 1981).

Oily bitterling represents a new host record for *A. coregoni*. In Japan, this parasite was previously collected from a bitterling (*Acheilognathus* sp., see the Introduction for this scientific name) at Otsu, Shiga Prefecture (Tokioka, 1936a). The present collection from oily bitterling is the second record of *A. coregoni* from Japanese acheilognathine fishes and also the first record of the species from Okayama Prefecture. This parasite has been recorded from neighboring Hyogo and Hiroshima prefectures (Nagasawa *et al.*, 2009).

2.1.2 Discussion

As stated in the Introduction, the infection of *A. coregoni* on Japanese bitterlings is rare. In this study, 107 individuals of three species of bitterlings (oily bitterling, n = 87; slender bitterling, n = 19; white tabira bitterling, n =1) were collected at the same site in the lower-reaches of the Asahi River from June 2020 to May 2021, and only one of the 56 oily bitterling examined in June 2020 was found infected with *A. coregoni*. This situation is similar to the fact that the parasite has not been found from fishes in Lake Biwa and adjacent regions since the collection from a bitterling at Otsu (Tokioka, 1936a). Regarding such a rare occurrence of *A. coregoni* on bitterlings in Japan, the present author infers that the collected specimen of the species might have first detached from ayu inhabiting the Asahi River, swam to the collection site, and parasitized the oily bitterling. This is because ayu serves as the major host for *A. coregoni* in the middle and lower-reaches of rivers in various regions of Japan (Yamaguti, 1937; Nagasawa and Ikeda, 2011; Nagasawa *et al.*, 2015, 2018b, 2019a, 2020a; Nagasawa and Morikawa, 2019a, b, c; Katahira *et al.*, 2021) and it is highly probable that this fish plays the same role for the parasite in the Asahi River. In other words, this paper suggests that *A. coregoni* usually parasitizes ayu and accidentally infected oily bittering in the lower reaches of the Asahi River.

The *A. coregoni*-infected bitterling reported by Tokioka (1936a) was most probably collected in coastal waters of Lake Biwa at Otsu, Shiga Prefecture (see the Introduction). To date, this parasite was found from ayu in the lower-reaches of the Ado River flowing into the lake (Nagasawa *et al*., 2018b) but has not been collected from fishes in the lake since 1936 (Nagasawa, 2020). Thus, there is the possibility that the specimen of *A. coregoni* reported by Tokioka (1936a) detached from ayu in an inflowing river, entered Lake Biwa, and attached to the bitterling. Moreover, *A. coregoni* is known to infect red-spotted masu salmon *Oncorhynchus masou ishikawae* (Jordan and McGregor, 1925) in the upper reaches of rivers flowing into Lake Biwa (Nagasawa, 2009; Nagasawa and Kawai, 2019) but it is unlikely that this parasite can move or drift to the lake from the upper reaches of the inflowing rivers and find a new host fish in the lake because of its mortality or reinfection during movement or drifting from the upper reaches to the lake.

Recently, the knowledge of the hosts of *A. coregoni* has been increasing in Japan, where this parasite has been recorded from not only salmonids, ayu, and bitterling but also fluvial dark sleeper *Odontobutis hikimius* Iwata and Sakai, 2002 (Perciformes: Odontobutidae) (Nagasawa *et al.*, 2014) and torrent reddish bullhead *Liobagrus reini* Hilgendorf, 1878 (Siluriformes: Amblycipitidae) (Nagasawa and Ishikawa, 2015). In the upper and middle reaches of rivers in Japan, salmonids are the major hosts of *A. coregoni*, and the known wild salmonid hosts are red-spotted masu salmon, masu salmon *Oncorhynchus masou masou* (Brevoort, 1856), spotted head char *Salvelinus leucomaenis imbrius* (Jordan and McGregor, 1925), Japanese char *S. leucomaenis japonicus* (Oshima, 1938), and white-spotted char *S. leucomaenis leucomaenis* (Pallas, 1814) (*e.g.*, Kato, 1964; Takegami, 1984; Nagasawa and Kawai, 2008, 2015, 2016, 2019; Tamura and Maruyama, 2009; Nagasawa *et al.*, 2009, 2020c; Nagasawa, 2017c). In constract, ayu serves as the major host for *A. coregoni* in the middle and lower reaches of Japanese rivers, where only two fish species (ayu and oily bitterling) have been reported as the hosts of this parasite. Thus, it is necessary to study the host range of *A. coregoni* in those reaches and to distinguish this species from a morphologically similar species *A. japonicus* in order to better understand the host utilization and longtudinal distribution along rivers each of the two parasites.

Although *A. coregoni* has not so far been reported from Hokkaido, the northernmost major island of Japan, the species is likely to occur on this island because it has been reported from Sakhalin Island north of Hokkaido (Sokolov *et al.*, 2012) and Akita Prefecture, northen

Honshu south of Hokkaido (Nagasawa *et al*., 2020c). In rivers in Hokkaido, masu salmon and whtespotetd char usually inhabit in the upper reaches of rivers but also often occur in the middle and lower reaches. This longitudinal distrubitonal pattern of these salmonids is different from that on the three southern major islands (Honshu, Shikoku, and Kyushu), and it is desirable to study whether *A. coregoni* shows a different longtudinal distributional pattern in Hokkaido from that in the southern major islands.

Fig. 2.1.2.1. *Argulus coregoni*, adult female (9.2 mm total length, arrow), NSMT-Cr 29034, parasitic on the dorsal surface of the anterior trunk of an oily bitterling *Tanakia limbata* (65 mm standard length), from the lower reaches of the Asahi River, Okayama Prefecture, western Japan. Fresh specimen. Scale bar: 10 mm.

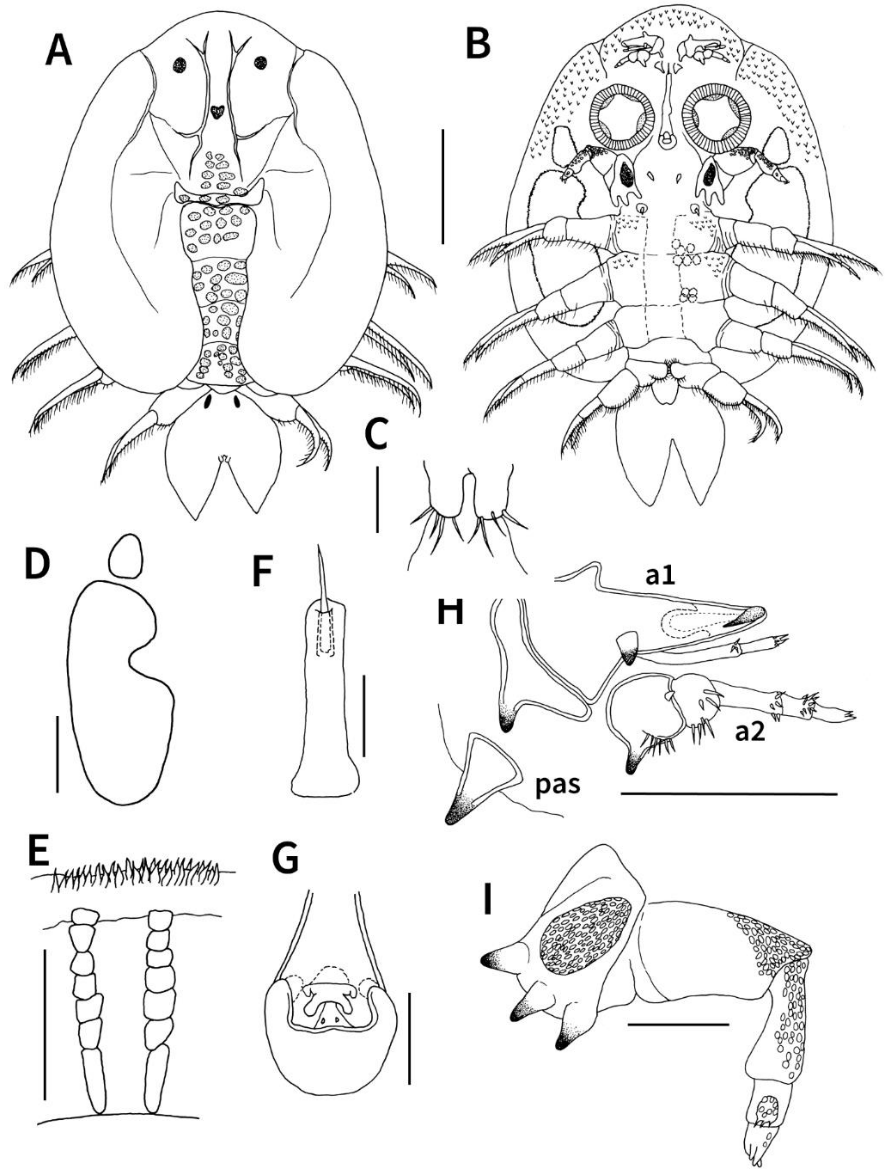

Fig. 2.1.2.2. *Argulus coregoni*, adult female (9.2 mm total length), NSMT-Cr 29034. A, habitus, dorsal view; B, habitus, ventral view; C, caudal rami, dorsal view; D, respiratory area, ventral view; E, two supporting rods in marginal membrane of first maxilla, ventral view; F, preoral stylet and sheath, ventral view; G, mouth tube, ventral view; H, first antenna (a1), second antenna (a2), and postantennal spine (pas), ventral view; I, second maxilla, ventral view. Scale bars: A, B, 2 mm; C, E, 0.1 mm; D, 1 mm; F, G, 0.2 mm; H, I, 0.5 mm.

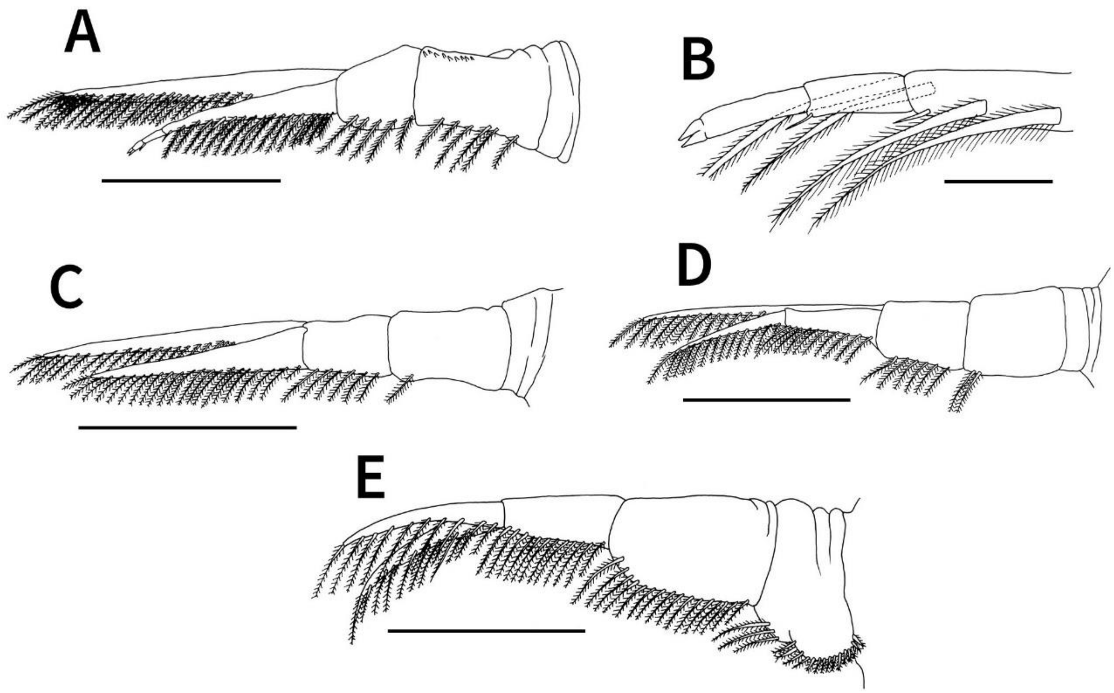

Fig. 2.1.2.3. *Argulus coregoni*, adult female (9.2 mm total length), NSMT-Cr 29034. A, first leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view. Flagella projecting from the exopods of the first and second legs are not seen in ventral view. Plumose setae on the dorsal side of the exopods and endopods are omitted in Fig. 2.1.2.3A, C–E. Scale bars: A, C–E, 1 mm; B, 0.1 mm.

2.1.3 *Argulus mongolianus* Tokioka, 1939

2.1.3.1 Redescription of *Argulus mongolianus* with its first record from Japan

[Based on Nagasawa, K., Asayama, T., and Fujimoto, Y. (2022b): Redescription of *Argulus mongolianus* (Crustacea: Branchiura: Argulidae), an ectoparasite of freshwater fishes in East Asia, with its first record from Japan. Species Diversity, 50: 119–129]

Abstract. Both adult females and males of *Argulus mongolianus* Tokioka, 1939 were collected from the buccal cavity wall and body surface of freshwater fishes in Lake Izunuma and Lake Uchinuma, Miyagi Prefecture, northen Honshu, Japan. The original description of the species was insufficient, based on a single female from Inner Mongolia, China, and no further description has been made to date. Thus, this paper redescribes *A. mongolianus* based on the newly-collected specimens, including the first description of the male. This represents the first record of *A. mongolianus* from Japan. The infected fishes were largemouth bass *Micropterus salmoides* (Lacepède, 1802), common carp *Cyprinus carpio* Linnaeus, 1758, Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846, and silver crucian carp *Carassius* sp. The three species other than common carp are new host records for *A. mongolianus*. This parasite was most probably introduced with an unspecified freshwater fish from China into Japan.

2.1.3.1 Introduction

Two species of the argulid branchiuran genus *Argulus* Müller, 1785 are known to infect wild freshwater fishes in Japan (Nagasawa, 2009, 2011a). They are *A. japonicus* Thiele, 1900 and *A. coregoni* Thorell, 1864. These branchiurans are important parasites of farmed fishes as well, and their biology has been extensively studied in Japan (*e.g.*, Nakazawa, 1914; Tokioka, 1936b; Kimura, 1970; Ikuta and Makioka, 1997 for *A. japonicus*; Hoshina, 1950; Shimura, 1981, 1983a; Nagasawa and Yuasa, 2020; Katahira *et al*., 2021 for *A. coregoni*).

During an ecological study of freshwater fishes in two small lakes, Lake Izunuma and Lake Uchinuma, Miyagi Prefecture, northern Japan in 2020, some of the fishes caught were found to be infected with argulids on the buccal cavity wall and body surface. The argulids were later identified as two species of *Argulus*, *A. japonicus* and *A. mongolianus* Tokioka, 1939. The former species was originally described from Japan (Thiele, 1900), where the species has been reported mainly from various cyprinids (*e.g*., Thiele, 1904; Tokioka, 1936a; Yamaguti, 1937; Nagasawa, 2009, 2011a, 2021; Nagasawa *et al*., 2012, 2018a, 2021a). In contrast, *A. mongolianus* was described as a new species from Inner Mongolia, China (Tokioka, 1939) and subsequently recorded three times, once each from Vietnam (Ky and Te, 2007), China (Wadeh *et al*., 2008, incorrectly reported as "*A. mongolisnus* Tokioka, 1938"), and the Russian Far East (Shedko *et al.*, 2018). Thus, the collection of *A. mongolianus* in this study represents its first record from Japan.

The original description of *A. mongolianus* was based on a single adult female that had been brought from Inner Mongolia to Japan (Tokioka, 1939). No further description was made in the subsequent papers (Ky and Te, 2007; Wadeh *et al*., 2008; Shedko *et al.*, 2018) and, in particular, no information is available on the male. Therefore, based on the specimens collected in this study, the female is redescribed, and the male is described in detail.

When Tokioka (1939) worked at the Seto Marine Biological Laboratory (SMBL) of the Kyoto Imperial University [now, Kyoto University (KU)], he described *A. mongolianus* but did not register the specimen used in the original description. Then, we thought that the specimen might have been deposited at the SMBL Museum, Shirahama, or the KU Museum, Kyoto. However, no specimen of *A. mongolianus* has been found in the collections of these two museums (Michitaka Shimomura, SMBL; Takafumi Nakano, KU, personal communications). Further, no specimen of the species has been deposited in the crustacean collection of the National Museum of Nature and Science, Tsukuba (NSMT) (Hironori Komatsu, NSMT, personal communication). These facts indicate that the specimen used in the original description has been lost.

2.1.3.1 Materials and methods

Specimens of *A. mongolianus* were collected in April to June 2020 from the buccal cavity wall and body surface of four species of fishes [largemouth bass *Micropterus salmoides* (Lacepède, 1802); common carp *Cyprinus carpio* Linnaeus, 1758; Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846; and silver crucian carp *Carassius* sp.] caught by electrofishing in two small nearby lakes, Lake Izunuma (surface area: 2.89 km^2, maximum depth: 1.6 m) and Lake Uchinuma (0.98 km^2, 1.6 m), Miyagi Prefecture, northern Honshu, Japan. The specimens were fixed and preserved in 70% ethanol. They were studied using an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope. One adult female specimen from *M. salmoides* was used to make drawing of dorsal and ventral views of its habitus, and two specimens (one adult male and one adult female) from the same fish species were cleared in lactophenol and examined using the wooden slide procedure (Humes and Gooding, 1964; Benz and 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 and Otting (1996). Voucher specimens of *A. mongolianus* have been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture, Japan (NSMT-Cr).

2.1.3.1 Results

Argulus mongolianus Tokioka, 1939

[Japanese name: Mouko-chou]

(Figs 2.1.3.1.1– 2.1.3.1.6)

Argulus mongolianus Tokioka, 1939: 42–47, figs 1–2, pl. 1; Tokioka, 1940: 306–308, figs 3–4; Yamaguti, 1963: 325, pl. 323, fig. 2 (monograph); Song and Kuang, 1980: 82, unnumbered figs (monograph); Kuang and Qian, 1991: 166–107, fig. 103 (monograph); Ky and Te, 2007: 299, fig. 316; Wadeh *et al.*, 2008: 765 (misspelled as *A. mongolisnus*); Neethling and Avenant-Oldewage, 2016: 1341 (monograph); Shedko *et al*., 2018: 7.

Hosts. Largemouth bass *Micropterus salmoides* (Lacepède, 1802) (Centrarchiformes: Centrarchidae); common carp *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae); Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846 (Cypriniformes: Cyprinidae); and silver crucian carp *Carassius* sp. (Cypriniformes: Cyprinidae).

Attachment sites. Buccal cavity wall and body surface.

Localities. Lake Izunuma (38°43′09″N, 141°06′09″E at center) and Lake Uchinuma (38°42′41″N, 141°04′33″E at center), Miyagi Prefecture, Japan.

Material examined. One adult female (NSMT-Cr 29369) and three adult males (NSMT-Cr 29370) from the buccal cavity wall of *M. salmoides* from Lake Izunuma on 2 June 2020; five adult females (NSMT-Cr 29371) and five adult males (NSMT-Cr 29372) from the buccal cavity wall of *M. salmoides* from Lake Izunuma on 12 May 2020; two adult females and two adult males (NSMT-Cr 29373) from the buccal cavity wall of *Cyprinus carpio* from Lake Uchinuma on 12 May 2020; two adult females and one adult male (NSMT-Cr 29374) from the body surface of *Carassius cuvieri* from Lake Uchinuma on 12 May 2020; one adult male (NSMT-Cr 29375) from the body surface of *Carassius* sp. from Lake Uchinuma on 28 April 2020.

Adult female (based on six specimens from *M. salmoides*). *Body* dorsoventrally flattened, measuring 6.6–8.3 (mean = 7.3, n = 6) mm in total length (from anterior tip of carapace to posterior tip of abdomen) and 4.0–4.7 (mean = 4.4, n = 6) mm in maximum body width (around midlength of carapace).

Carapace (including posterolateral lobes) circular, covering sympods and proximal part of first to third (or fourth in large specimens) pairs of legs in dorsal view, 4.3–5.5 (mean = 4.7, n

= 6) mm long, comprising 62.5–66.3% (mean = 64.8%, n = 6) of total length (Figs 2.1.3.1.1A, B, 2.1.3.1.6A, B). Centrofrontal region of carapace protruding anteriorly; anterior margin horizontal. Anterolateral indentations shallow; central longitudinal ribs distinct, anteriorly bifurcated. Compound eyes distinct, located dorsally at level where frontal region joins main part of carapace. Nauplius eye located posterior to compound eyes along midline of carapace. Dorsal surface of carapace smooth without spines. Ventral surface of lateral region of carapace ornamented with numerous, small posteriorly directed spines (Fig. 2.1.3.1.1B). Posterolateral lobes of carapace partially overlapped (Fig. 2.1.3.1.1B) or not overlapped (Fig. 2.1.3.1.2A), ending in rounded margin, separated by sinus nearly 40% length of carapace. Respiratory areas comprising small, oval anterior area and large, reniform posterior area; former area located at level between first and second maxillae, and latter at level of second maxillae to third pair of legs (Figs 2.1.3.1.1B, 2.1.3.1.2B). *Thorax* with four segments, bearing small spiniform projections on ventral surface; third segment narrower than first and second segments (Fig. 2.1.3.1.1A, B); fourth segment narrowest, with two posteriorly directed lateral protrusions and weakly convex posterior margin in dorsal view (Fig. 2.1.3.1.2A). *Abdomen* longer than wide; anal indentation 60.0–77.3% (mean = 70.5%, n = 6) of abdomen length forming two lobes; lateral margins and posterior surface of lobes with small sharply pointed spines; each lobe oval, becoming wider toward midlength, then narrower posteriorly, ending in rounded margin (Fig. 2.1.3.1.1A, B). Paired spermathecae each oval, located anteriorly in abdomen (Figs 2.1.3.1.1A, B, 2.1.3.1.2A). Caudal rami located at base of anal indentation, each with five naked setae on posterior margin (Figs 2.1.3.1.1A, 2.1.3.1.2C).

First antennae with four segments (Fig. 2.1.3.1.2D, E): first segment heavily sclerotized in mesial and posterior regions, with large projection on mesial margin; second segment also heavily sclerotized, with triangular projection on anterior margin, strong apically bent hook at distal corner, stout projection near posterior margin, and oblique swelling near mesial margin; third segment cylindrical, with three naked setae, one of them stout and larger than others; apical segment shorter than third segment, with seven naked setae of different sizes at tip. *Second antennae* with five segments (Fig. 2.1.3.1.2D, F): first segment sclerotized, with large rounded protrusion bearing eight naked setae on posterior margin and short spine in centroventral region; second segment shorter than first segment, with four naked setae on posterior margin, three naked setae near distal margin, and short seta on anterior margin; third, fourth, and apical segments nearly cylindrical and decreasing in length, possessing four, six, and four naked setae, respectively. Postantennal spines large and robust, each located posterior to large projection of first segment of first antenna (Figs 2.1.3.1.1B, 2.1.3.1.2D). Preoral sheath cylindrical and visible near anterior regions of first maxillae on ventral midline of carapace (Figs 2.1.3.1.1B, 2.1.3.1.2G); anterior portion of stylet protruding from opening of preoral sheath. Mouth tube located just posterior to preoral sheath, longer than wide, becoming gradually wider posteriorly (Figs 2.1.3.1.1B, 2.1.3.1.2H); mouth composed of small anterior serrated labrum and larger posterior labium with scales on ventral surface and several short naked setae on outer margin (Fig. 2.1.3.1.2H); mandibles completely covered by labrum, composed of anterior part bearing many tiny spines on both distal and mesial sides and posterior part with seven teeth on mesial side (Fig. 2.1.3.1.2I); small paired buccal spines present on ventral surface of labium (Fig. 2.1.3.1.2H).

First maxillae forming well developed cup-like suckers (Fig. 2.1.3.1.1B), with 64–71 (mean = 68, n = 10) supporting rods in sucker membrane (Fig. 2.1.3.1.2J). Supporting rods each composed of one basal elongate plus eight to ten trapezoidal sclerites, slightly wider at second to fourth sclerites, and tapering distally [of 35 supporting rods examined, commonest number of sclerites per rod is 10 (n = 15), followed by 11 and nine (n = 10 and 8, respectively), and two rods have unusual numbers of sclerites (one has six, the other 16 sclerites)] (Fig. 2.1.3.1.2K). Both outer and inner margins of rim of sucker membrane with numerous apically pointed projections (Fig. 2.1.3.1.2J, K). *Second maxillae* with five segments (Fig. 2.1.3.1.2L); first segment robust, with three basally separated, almost equally long blunt projections; corpus of first segment with two posteriorly directed naked spiniform setae between two projections and furnished with raised patch of numerous posteriorly directed scales; second segment longer than first segment, bearing some distally directed scales on anteroventral surface and small posteriorly directed scales on ventral surface; third segment shorter and narrower than second segment, with raised patch of distally directed scales on ventral surface (scales near anterior margin are slightly larger than those in other regions); fourth segment subquadrate, with raised patch of distally directed scales on ventral surface; terminal segment bearing some tiny scales on ventral surface, ending in one club-shaped protrusion and two hook-like claws. Accessory spines near ventral midline, slightly apart from first segments of second maxillae (Fig. 2.1.3.1.1B). Postmaxillary spines small and located just anterior to first segment of thorax (Fig. 2.1.3.1.1B).

First to fourth pairs of legs (Fig. 2.1.3.1.3) biramous, each with sympods composed of coxa and basis, sympods and rami of first to fourth legs ventrally covered with small spiniform projections; rami bearing two lateral rows of plumose setae each near ventro- and dorsoposterior margins; first and second pairs of legs each possessing dorsal flagellum projecting from extreme proximal part of exopod. *First leg* (Fig. 2.1.3.1.3A, B) coxa bearing single plumose seta near posterior margin; basis nearly half as wide as coxa, with small swelling near anterior junction of coxa and basis; exopod unsegmented, with 23 plumose setae near ventroposterior margin; endopod three-segmented, proximal segment with 16 plumose setae near ventroposterior margin and short naked seta at posterodistal corner, middle segment with short naked seta at posterodistal corner, and terminal segment ending in three short spines; flagellum extending to proximal margin of coxa, with 28 plumose setae on posterior margin and no plumose setae on anterior margin. *Second leg* (Fig. 2.1.3.1.3C) sympod without setae on posterior margin; posterior margin of coxa weakly concave; basis slightly shorter than coxa; exopod and endopod unsegmented, with 30 and 22 plumose setae, respectively, near ventroposterior margin; flagellum extending to proximal margin of coxa, with 28 plumose setae on posterior margin and no plumose setae on anterior margin. *Third leg* (Fig. 2.1.3.1.3D) sympod without setae on posterior margin; basis slightly shorter than coxa; exopod unsegmented, with 29 plumose setae near ventroposterior margin; endopod two-segmented, proximal segment with 13 plumose setae near ventroposterior margin and short naked seta near distal margin, terminal segment with 11 plumose setae near ventroposterior margin. *Fourth leg* (Fig. 2.1.3.1.3E, F) coxa forming natatory lobe bearing 17 plumose setae on posterior margin and four short naked setae near posterior margin; basis longer than coxa, with 12 plumose setae near ventroposterior margin; exopod unsegmented, with 27 plumose setae near ventroposterior margin; endopod two-segmented, proximal segment with 13 plumose setae near ventroposterior margin and short naked seta near distal margin, terminal segment with 11 plumose setae and short naked seta near ventroposterior proximal margin.

Adult male (based on eight specimens from *M. salmoides*). *Body* dorsoventrally flattened, measuring 4.2–5.6 (mean = 4.8, n = 8) mm in total length and 2.5–3.2 (mean = 2.8, n = 8) mm in maximum body width.

Carapace (including posterolateral lobes) nearly circular, covering sympods and proximal portions of first to third pairs of legs in dorsal view, 2.8–3.9 (mean = 3.2, n = 8) mm long, comprising 63.0–69.6% (mean = 66.1%, n = 8) of total length (Figs 2.1.3.1.4A, B, 2.1.3.1.6C, D). Centrofrontal region of carapace protruding anteriorly; anterior margin widely rounded or horizontal. Anterolateral indentations shallow; central longitudinal ribs distinct, anteriorly bifurcated. Compound eyes distinct, dorsally located at level where frontal region joins main portion of carapace. Nauplius eye located posterior to compound eyes along midline of carapace. Dorsal surface of carapace smooth without spines. Ventral surface of lateral region of carapace ornamented with numerous, small posteriorly directed spines (Fig. 2.1.3.1.4B). Posterolateral lobes of carapace not overlapped, ending in rounded margin, separated by sinus nearly 1/3 length of carapace. Respiratory areas comprising small, oval anterior area and large, reniform posterior area; former area located at level between first and second maxillae, and latter at level of second maxillae to third pair of legs (Fig. 2.1.3.1.4B, C). *Thorax* with four segments, bearing small spiniform projections on ventral surface; third segment shorter and narrower than first and second segments; fourth segment shortest and narrowest, ending in weakly concave posterior margin (Fig. 2.1.3.1.4A, B). *Abdomen* longer than wide; anal indentation 42.9–61.1% (mean = 51.8%, n = 8) of abdomen length forming two lobes; lateral margins and posterior surface of lobes with small sharply pointed spines; each lobe oblong, ending in rounded margin (Fig. 2.1.3.1.4A, B). Testes elliptical, located in anterior to middle portion of abdomen (Fig. 2.1.3.1.4A, B). Caudal rami located at base of anal indentation, each with five naked setae on posterior margin (Fig. 2.1.3.1.4D).

First antennae with four segments (Fig. 2.1.3.1.4E, F): first segment heavily sclerotized in mesial and posterior regions, with large projection on mesial margin; second segment also heavily sclerotized, with triangular projection on anterior margin, strong apically bent hook at distal corner, stout projection near posterior margin, and oblique swelling near mesial margin; third segment cylindrical, with three naked setae, one of them stout and larger than others; apical segment shorter than third segment, with seven naked setae of different sizes at tip.

Second antennae with five segments (Fig. 2.1.3.1.4E, G): first segment sclerotized and ovoid, with large rounded protrusion bearing eight naked setae on posterior margin and short spine in centroventral region; second segment shorter than first segment, with four naked setae on posterior margin, three naked setae near distal margin, and short seta on anterior margin; third, fourth, and apical segments nearly cylindrical and decreasing in length, possessing four, six, and four naked setae, respectively. Postantennal spines large and robust, each located posterior to large projection of first segment of first antenna (Fig. 2.1.3.1.4E). Preoral sheath cylindrical, located between first maxillae on ventral midline of carapace (Fig. 2.1.3.1.4B, H); anterior portion of stylet protruding from opening of preoral sheath. Mouth tube located just posterior to preoral sheath, longer than wide, becoming gradually wider posteriorly (Fig. 2.1.3.1.4B, I); mouth composed of small anterior serrated labrum and larger posterior labium with scales on ventral surface and several short naked setae on outer margin (Fig. 2.1.3.1.4I); mandibles completely covered by labrum, composed of anterior part bearing many tiny spines on both distal and mesial sides and posterior part with five teeth on mesial side (Fig. 2.1.3.1.4J); small buccal paired spines present on ventral surface of labium (Fig. 2.1.3.1.4I).

First maxillae forming cup-like suckers (Fig. 2.1.3.1.4B), with 58–64 (mean = 61, n = 10) supporting rods in sucker membrane. Supporting rods each composed of one basal elongate plus six to eight trapezoidal sclerites, slightly wider at second to fourth sclerites, and tapering distally [of 30 supporting rods examined, commonest number of sclerites per rod is eight (n = 17), followed by seven and nine (n = 8 and 5, respectively)] (Fig. 2.1.3.1.4K). Both outer and inner margins of rim of sucker membrane with numerous apically pointed projections (Fig. 2.1.3.1.4K). *Second maxillae* with five segments (Fig. 2.1.3.1.4L); first segment robust, with three basally separated, almost equally long blunt projections; corpus of first segment with two posteriorly directed naked spiniform setae between two projections and furnished with raised patch of numerous posteriorly directed denticles; second segment longer than first segment, bearing some apically bifurcated denticles on anteroventral surface, some posteriorly directed denticles on centroventral surface, and small denticles on posteroventral surface; third segment shorter and narrower than second segment, with raised patch of some apically bifurcated denticles on anterodorsal surface and small distally or posteriorly directed denticles on other ventral surface; fourth segment subquadrate, with raised patch of distally directed denticles on ventral surface; terminal segment bearing some tiny denticles on ventral surface, ending in one club-shaped and two hook-like projections. Accessory spines near ventral midline, slightly apart from first segments of second maxillae (Fig. 2.1.3.1.4B). Postmaxillary spines small and located anterior to first segment of thorax (Fig. 2.1.3.1.4B).

First to fourth pairs of legs (Fig. 2.1.3.1.5) biramous, with each sympod composed of coxa and basis; sympods and rami of first to fourth legs ventrally covered with small spiniform projections; rami bearing two lateral rows of plumose setae each near ventro- and dorsoposterior margins; first and second pairs of legs each possessing dorsal flagellum projecting from extreme proximal part of exopod. *First leg* (Fig. 2.1.3.1.5A, B) coxa bearing single plumose seta near posterior margin; basis nearly half as long as coxa, with small swelling near anterior junction of coxa and basis; exopod unsegmented, with 20 plumose setae near ventroposterior margin; endopod three-segmented, proximal segment with 12 plumose setae near ventroposterior margin and short naked seta at posterodistal corner, middle segment with short naked seta at posterodistal corner, terminal segment ending in three short spines; flagellum extending to proximal margin of coxa, with 18 plumose setae on posterior margin and no plumose setae on anterior margin. *Second leg* (Fig. 2.1.3.1.5C) sympod without setae on posterior margin; posterior margin of coxa weakly concave, densely armed with small projections; basis slightly shorter than coxa; exopod and endopod unsegmented, with 17 and 15 plumose setae, respectively, near ventroposterior margin; flagellum extending to proximal margin of coxa, with 20 plumose setae on posterior margin and no plumose setae on anterior margin. *Third leg* (Fig. 2.1.3.1.5D) sympod without setae on posterior margin, possessing swelling (= socket) at posterior junction of coxa and basis; basis nearly as long as coxa, bearing apically rounded projection on anterior margin; exopod unsegmented, with 18 plumose setae near ventroposterior margin; endopod two-segmented, proximal and terminal segments with eight and nine plumose setae, respectively, near ventroposterior margin. *Fourth leg* (Fig. 2.1.3.1.5E) coxa forming natatory lobe bearing 12 plumose setae on posterior margin and two short naked setae on ventroposterior surface; basis longer than coxa, bearing stout peg with distally bifurcated, rounded ends covered by minute scales on anterior margin and five plumose setae on posterior margin; exopod unsegmented, with 15 plumose setae near ventroposterior margin; endopod two-segmented, proximal segment with eight plumose setae near ventroposterior margin and short naked seta near distal margin, terminal segment with eight plumose setae near ventroposterior margin.

Color (based on ethanol-preserved specimens). Carapace, legs, and male thorax white; female thorax and centroposterior region of cephalothorax pale or dark yellow with irregularly shaped black spots unevenly scattered on dorsal surface; respiratory areas fringed weakly by black pigment (Fig. 2.1.3.1.6).

Remarks. *Argulus mongolianus* was originally described based on a single ovigerous female from Lake Dalai-nor, Inner Mongolia, China (Tokioka, 1939). The host was not reported. Subsequently, *A. mongolianus* was reported from two provinces (Bắc Kạn and Lào Cai), northern Vietnam (Ky and Te, 2007), Guangdong Province, China (Wadeh *et al*., 2008), and the Primorye Territory, Russian Far East (Shedko *et al*., 2018) but no further description has been made to date. Tokioka (1940) published a report in which the original description of the species was translated from English into Japanese.

The adult females of the argulid collected in the present study are almost completely identical to the adult female of *A. mongolianus* described by Tokioka (1939). The adult males were also collected and as described above, their morphology is quite similar to that of the females. Therefore, both the females and males collected in this study are identified as *A. mongolianus*. The male is described for the first time. The species is characterized by the anterior protrusion of the centrofrontal carapace in both sexes (Figs 2.1.3.1.1A, B, 2.1.3.1.4A, B, 2.1.3.1.6A–D), a circular carapace and oval abdominal lobes in the female (Figs 1A, B, 6A, B), oblong abdominal lobes in the male (Figs 2.1.3.1.4A, B, 2.1.3.1.6C, D), and the presence of a single plumose seta near the posterior margin of the coxa of the first pair of legs in both sexes (Figs 2.1.3.1.3A, 2.1.3.1.5A).

There are several differences in the morphology of *A. mongolianus* between the original and present descriptions. The ventral surface of the frontal region of the carapace of the female was figured to have many small spines (Tokioka, 1939, pl. 3, fig. A), but both the female and male of the species examined in this study have no such spines. While the dorsoposterior margins of the female's thoracic segments were reported to be "complexly corrugated" (Tokioka, 1939, pl. 3, fig. C), no similar structure was observed in the specimens of both sexes examined in this study. Further, the number of supporting rods in the sucker membrane of the female was reported to be “70–80” (Tokioka, 1939), but the females and males examined herein have fewer [64–71 (mean = 68) and 58–64 (mean = 61)] supporting rods, respectively. It seems likely that Tokioka (1939) incorrectly counted the number of supporting rods.

There are morphological differences between the sexes of *A. mongolianus*. The female's carapace is more circular than the male's, and the female's abdominal lobe is oval but the male's is oblong (Figs 2.1.3.1.1A, B, 2.1.3.1.4A, B, 2.1.3.1.6A–D). Only the male has accessory copulatory structures, *i.e.*, the socket and peg on the third and fourth pairs of legs, respectively (Fig. 2.1.3.1.5D, E). The male possesses both posteriorly directed and apically bifurcated scales on the second and third segments of the second maxilla (Fig. 2.1.3.1.4K), but the female has only posteriorly directed scales on these segments (Fig. 2.1.3.1.2K). The posterior margin of the coxa of the male's second leg is densely armed with small projections (Fig. 2.1.3.1.5C), but the female has no such ornamentation on the same margin (Fig. 2.1.3.1.3C). Moreover, as stated above, the female's first maxilla has more supporting rods (64–71) than the male's (58–64). Similarly, the number of sclerites per supporting rod in the female (9–11) is more numerous than in the male (7–9). These sex-related differences in the number of supporting rods and sclerites per rod are probably related to differences in the carapace length of the females [4.3–4.8 (mean 4.6, n = 5) mm] and males [2.8–3.2 (mean 2.9, n = 5) mm] examined. It is known, for example, that larger individuals of *Argulus melanostictus* C. B. Wilson, 1935 have more supporting rods and sclerites per rod than smaller ones (Benz *et al.*, 1995).

As reported by Boxshall and Jaume (2009) in *A. japonicus*, *A. mongolianus* also has a flagellum projecting from the extreme proximal part of the exopod of each of the first and second pairs of legs (Figs 2.1.3.1.3A, B, 2.1.3.1.5A, B).

Information on the hosts of *A. mongolianus* is quite limited. In the original description of the species from Lake Dai-nor, Inner Mongolia, the host was reported as unknown (Tokioka, 1939). Wadeh *et al.* (2008) later listed *A. mongolianus* as one of eight congeneric species infecting farmed fishes in Guangdong Province, China, but no fish name was reported. In contrast, two and one species of fishes have been reported as the hosts of *A. mongolianus*, respectively, from northern Vietnam (Ky and Te, 2007) and Russian Far East (Shedko *et al.*, 2018): the two hosts are *Spinibarbus denticularis* (Oshima, 1926) (Cypriniformes:

Cyprinidae) and *Bagarius bagarius* (Hamilton, 1822) (Siluriformes: Sisoridae), and another host is common carp. In the present study, *A. mongolianus* was collected from largemouth bass, common carp, Japanese white crucian carp, and silver crucian carp. Excluding common carp, the three other species represent new host records. Based on the host species reported in this and previous papers (Ky and Te, 2007; Shedko *et al*., 2018), *A. mongolianus* is not strictly host-specific but may commonly utilize cyprinids as its hosts.

Largemouth bass was introduced in 1925 and 1972 from North America into Japan (Takamura, 2007), where this fish species is known as a host of *A. japonicus* as well (Nagasawa, 2021). Similarly, *A. japonicus* has been reported in Japan from common carp (Nagasawa *et al*., 2012, 2013; Nagasawa, 2018; Nagasawa and Ishiyama, 2019b; see Nagasawa 2009, 2011a for the earlier literature), Japanese white crucian carp (Takeda *et al.*, 2000; Nagasawa, 2011b; Nagasawa *et al*., 2012), and silver crucian carp (Takeda *et al.*, 2000, reported as *Carassius auratus langsdorfii*; Nagasawa, 2011b, reported as *C. a. langsdorfii*).

Key to *Argulus mongolianus*, *A. japonicus*, and *A. coregoni* from Japan

Two species of *Argulus*, *A. japonicus* and *A. coregoni*, are known to parasitize wild freshwater fishes in Japan (Nagasawa, 2009, 2011a). *Argulus mongolianus* represents the third congeneric species from Japanese wild freshwater fishes. A key to the species of *Argulus* parasitic on Japanese wild freshwater fishes is given below and can be used for both sexes (Nagasawa, 2021; Nagasawa and Taniguchi, 2021; this paper):

1. Centrofrontal region of carapace protruding anteriorly
   .... *A. mongolianus* Tokioka, 1939
   − Centrofrontal region of carapace not protruding anteriorly 2
2. A single plumose seta on posterior margin of coxa of first leg
   .... *A. japonicus* Thiele, 1900
   − Four to eight plumose setae on posterior margin of coxa of first leg
   .... *A. coregoni* Thorell, 1864

2.1.3.1 Discussion

Both *A. mongolianus* and *A. japonicus* were found to infect the fishes in the two small lakes investigated. At present, it is unknown whether these parasites, especially *A. mongolianus*, are native to the lakes because 14 (39%) of 36 fish species inhabiting the lakes and several inflowing rivers were introduced from other localities in Japan (Fujimoto *et al.*, 2008). Nonetheless, *A. japonicus* has a wide distribution in Japan ranging from Hokkaido to Kyushu islands (Nagasawa, 2009, 2017b, 2018; Nagasawa *et al.*, 2012; Nagasawa and Miyajima, 2018), and therefore it is reasonable to infer that the species was not introduced from other localities but naturally occurs in the lakes. Ten species of cyprinids are known to be native to the lakes and inflowing rivers (Fujimoto *et al.*, 2008) and cyprinid fishes are the hosts commonly utilized by *A. japonicus* in Japan (Nagasawa, 2009, 2011a). Thus, in addition to the three cyprinid species reported herein, *A. japonicus* may also infect other cyprinid species in the lakes and inflowing rivers.

Despite the fact that branchiurans of the genus *Argulus* have been studied in Japan for more than 120 years since 1900 (Thiele, 1900; see Nagasawa, 2009, 2011a; Nagasawa and Yuasa, 2020), *A. mongolianus* was discovered in this study for the first time in Japan. This implies that the species is not native to Japan and was recently introduced from an East Asian country, most likely from China, to Japan and then to the lakes. Since 1878, 19 species of freshwater fishes have been introduced from China to Japan, consisting of eight cyprinids [silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844); bighead carp *Hypophthalmichthys nobilis* (Richardson, 1845); black carp *Mylopharyngodon piceus* (Richardson, 1846); grass carp *Ctenopharyngodon idella* (Valenciennes, 1844); rosy bitterling *Rhodeus ocellatus ocellatus* (Kner, 1866); giant bitterling *Acheilognathus macropterus* (Bleeker, 1871); Wuchang bream *Megalobrama amblycephala* Yih, 1955; stone moroko *Pseudorasbora parva* (Temminck and Schlegel, 1846)] (Maruyama *et al.*, 1987; Hagiwara, 2002, 2017; Hirashima, 2006), four cobitids [pond loach *Misgurnus anguillicaudatus* (Cantor, 1842); large-scale loach *Paramisgurnus dabryanus* Dabry de Thiersant, 1872; eightbarbel loach *Lefua costata* (Kessler, 1876); an unidentified cobitid] (*e.g.*, Hirashima, 2006; Kitagawa *et al.*, 2011; Mukai *et al.*, 2011; Nakajima and Uchiyama, 2017), one bagrid [yellow catfish *Tachysurus fulvidraco* (Richardson, 1846)] (Arayama *et al.*, 2012), one gasterosteid [Amur stickleback *Pungitius sinensis* (Guichenot, 1869)] (Hirashima, 2006), one odontobutid [yokoshima-donko *Micropercops swinhonis* (Günther, 1873)] (Hirashima, 2006; Arao *et al.*, 2010), three gobiids [two unidentified species of *Rhinogobius* Gill, 1859 and one unidentified gobiid] (Hirashima 2006), and one osphronemid (round tail paradisefish *Macropodus ocellatus* Cantor, 1842) (Hirashima, 2006). Moreover, based on a recent molecular work, common carp from Japan is separated into two (native and introduced) groups, the latter partially consisting of descendants of the species from Chinese origin (Mabuchi *et al.*, 2008). Unfortunately, there is no definite record of introduction of common carp from China into Japan (Maruyama *et al.*, 1987; Mabuchi *et al.*, 2008), but such introduction was probably conducted before 1910 because the descendants of introduced common carp were found in the early 1910s in Lake Biwa, the largest lake in Japan (Anonymous, 1915) and those of Chinese origin have been a major cyprinid in the lake (Mabuchi *et al.*, 2008, 2010). Of the above 19 species, five cyprinids (silver carp, bighead carp, black carp, rosy bitterling, and grass carp) were introduced more than 40 years ago at least: the first four species and grass carp were introduced in 1878 and from 1878 to 1955, respectively (Maruyama *et al.*, 1987). Both these cyprinids and common carp of Chinese origin have become established in Japan (Maruyama *et al.*, 1987; Mabuchi *et al.*, 2008; Hosoya, 2015) but, to date, no argulid infection has been documented in relation to them, which indicates that *A. mongolianus* was not introduced with them from China into Japan.

In contrast, the remaining 14 fish species (giant bitterling, Wuchang bream, stone moroko, pond loach, large-scale loach, eightbarbel loach, an unidentified cobitid, yellow catfish, Amur stickleback, yokoshima-donko, three unidentified gobiids, and round tail paradisefish) have been introduced more recently from China to Japan. However, nothing is known about the Chinese hosts of *A. mongolianus*, and it is impossible to specify which fish species was the likely host for the introduction of the argulid into Japan. Nevertheless, the parasite is currently found in only a limited region (the two lakes reported herein), which implies that it was not introduced into multiple localities with multiple fish species but first into a single locality with one fish species and then into other localities. Some of the introduced fishes have become well established since their introductions into Japan: for example, two cobitids (pond loach and large-scale loach) have spread widely in Japan (Kitagawa *et al.*, 2011; Mukai *et al.*, 2011; Nakajima and Uchiyama, 2017) and three species (giant bitterling, yellow catfish, and eightbarbel loach) have been expanding their ranges in central Honshu (Hagiwara, 2002; Sakamoto, 2009; Arayama *et al.*, 2012; Ohama *et al.*, 2013; Kanazawa, 2014; Hosoya, 2015; Nitta *et al.*, 2017; Nakajima and Uchiyama, 2017). Based on these facts, it is inferred that: 1) *A. mongolianus* was introduced into Japan with an unspecified species of freshwater fish from China; 2) with the establishment and spread of this unspecified host species in Japan, *A. mongolianus* has also expanded its range in Japan and, due to its low host specificity, utilizes various fish species as hosts in translocated localities; 3) *A. mongolianus* was introduced with an unspecified host (for example, large-scale loach or largemouth bass, see below) from somewhere in Japan into the two lakes; and 4) *A. mongolianus* has established and maintained its populations in the lakes, where, as reported herein, adult females and males of the species currently infect at least four fish species. Since around 2010, large-scale loach has been recognized as a new invasive species in the lakes (Y. Fujimoto, Miyagi Prefectural Izunuma-Uchinuma Environmental Foundation, personal communication), and largemouth bass was illegally stocked into a pond upstream from one of the lakes around 2019 (Saitoh *et al.*, 2021). Therefore, there is also the possibility that *A. mongolianus* was introduced very recently into the lakes through such fish invasion or illegal fish stocking.

Argulus mongolianus represents a threat to freshwater fish culture. The species infected unspecified fishes farmed in small ponds near Lake Dai nor, Inner Mongolia, China, and caused serious damage to the host skin (Tokioka, 1940). This parasite was also found at fish farms in Guangdong Province, China (Wadeh *et al.*, 2008). Further, it was reported to have killed more than 90% of the common carp individuals cultured in the suburb of Vladivostok, Russian Far East (Shedko *et al.*, 2018). No information has so far been published on the biology of *A. mongolianus* and in order to aid in finding efficient control measures, it is necessary to study various aspects of the biology of the species, including its growth and maturation, spawning, larval development, seasonal occurrence, feeding, and pathological impacts. Moreover, as discussed above, *A. mongolianus* is likely to infect various fish species in localities other than the two lakes investigated. It is desirable to study the geographical distribution and host range of the species in Japan.

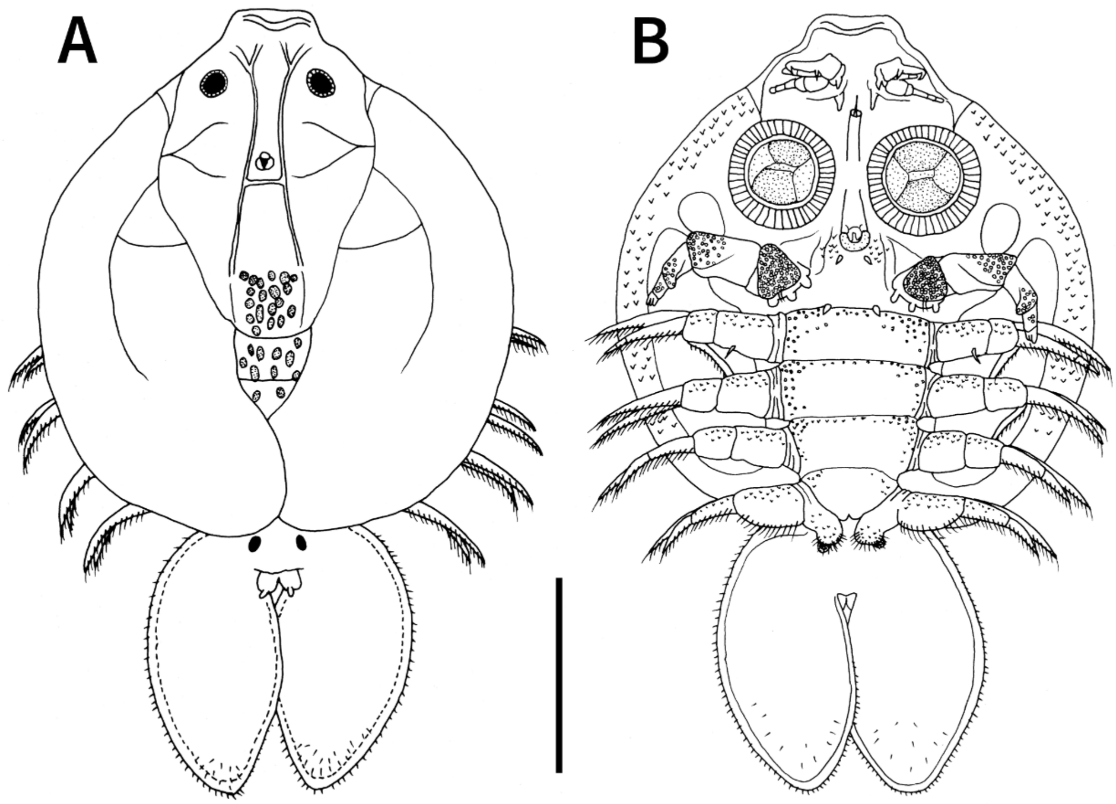

Fig. 2.1.3.1.1. *Argulus mongolianus*, adult female, NSMT-Cr 29369, from largemouth bass *Micropterus salmoides* in Lake Izunuma, Miyagi Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view. Scale bar: 2 mm.

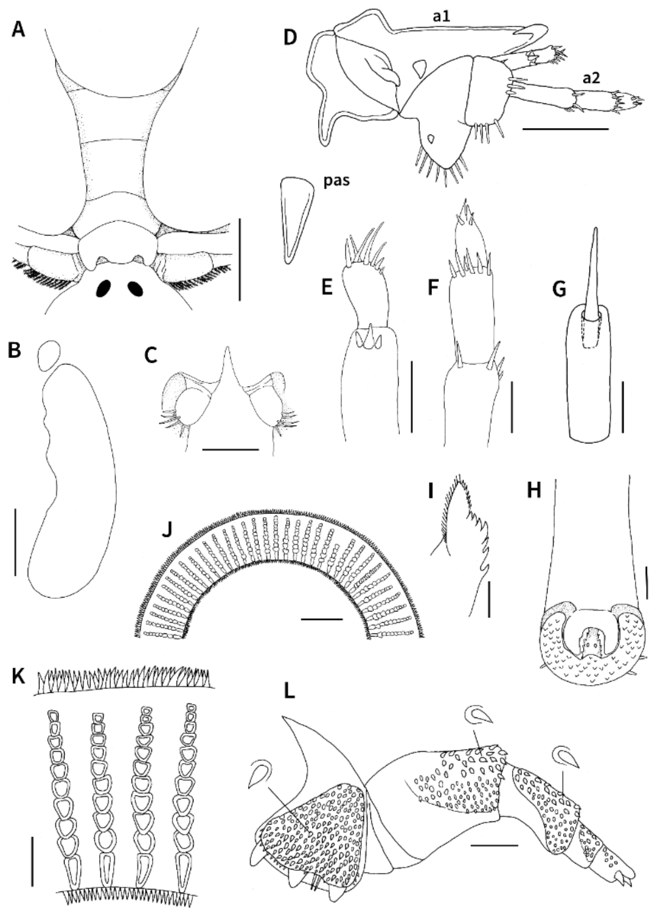

Fig. 2.1.3.1.2. *Argulus mongolianus*, adult female (different specimen shown in Fig. 2.1.3.1.1), NSMT-Cr 29371, from largemouth bass *Micropterus salmoides* in Lake Izunuma, Miyagi Prefecture, Japan. A, thorax partially covered with carapace, anterior portion of abdomen, and sympods of fourth pair of legs, dorsal view; B, respiratory areas, ventral view; C, caudal rami, dorsal view; D, first antenna (a1), second antenna (a2), and postantennal spine (pas), ventral view; E, distal part of first antenna, ventral view; F, distal part of second antenna, ventral view; G, preoral sheath and stylet, ventral view; H, mouth tube, ventral view; I, mandible, ventral view; J, part of sucker membrane of first maxilla, ventral view; K, four supporting rods and maginal projections, ventral view; L, second maxilla and denticles on first, second, and third segments, ventral view. Scale bars: A, B, 1 mm; C, G, H, 0.1 mm; D, H, J, L, 0.2 mm; E, F, K, 0.05 mm; I, 0.02 mm.

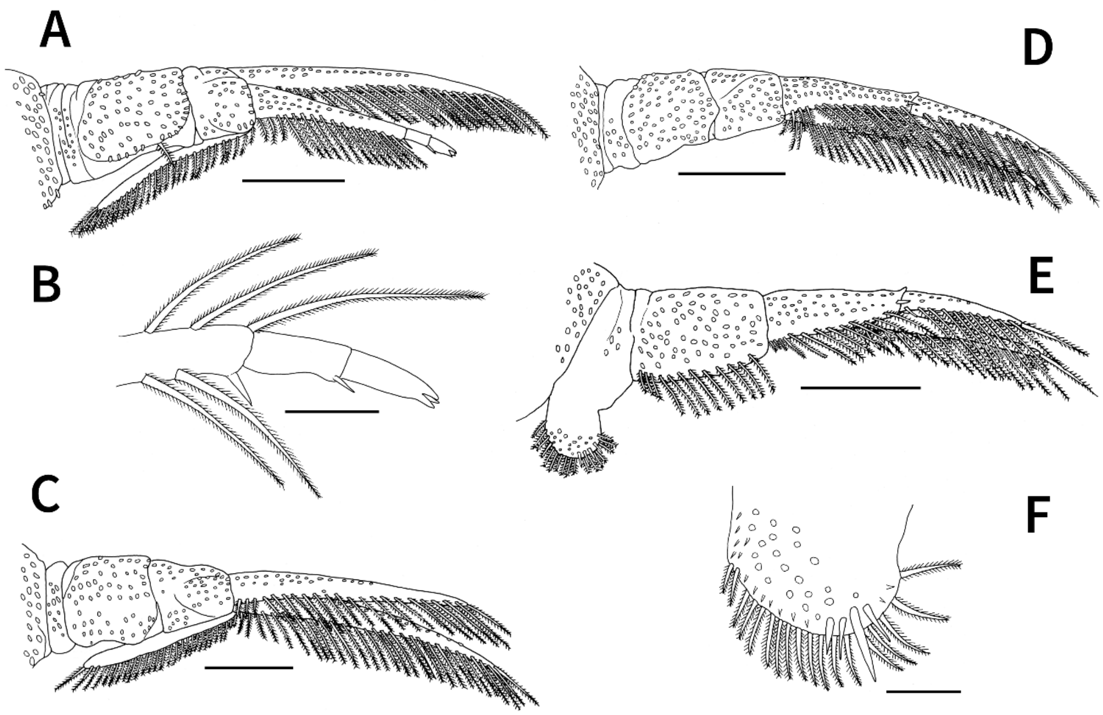

Fig. 2.1.3.1.3. *Argulus mongolianus*, adult female (different specimen shown in Fig. 2.1.3.1.1), NSMT-Cr 29371, from largemouth bass *Micropterus salmoides* in Lake Izunuma, Miyagi Prefecture, Japan. A, first leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view; F. natatory lobe, ventral view. Scale bars: A, C–E, 0.5 mm; B, F, 0.1 mm.

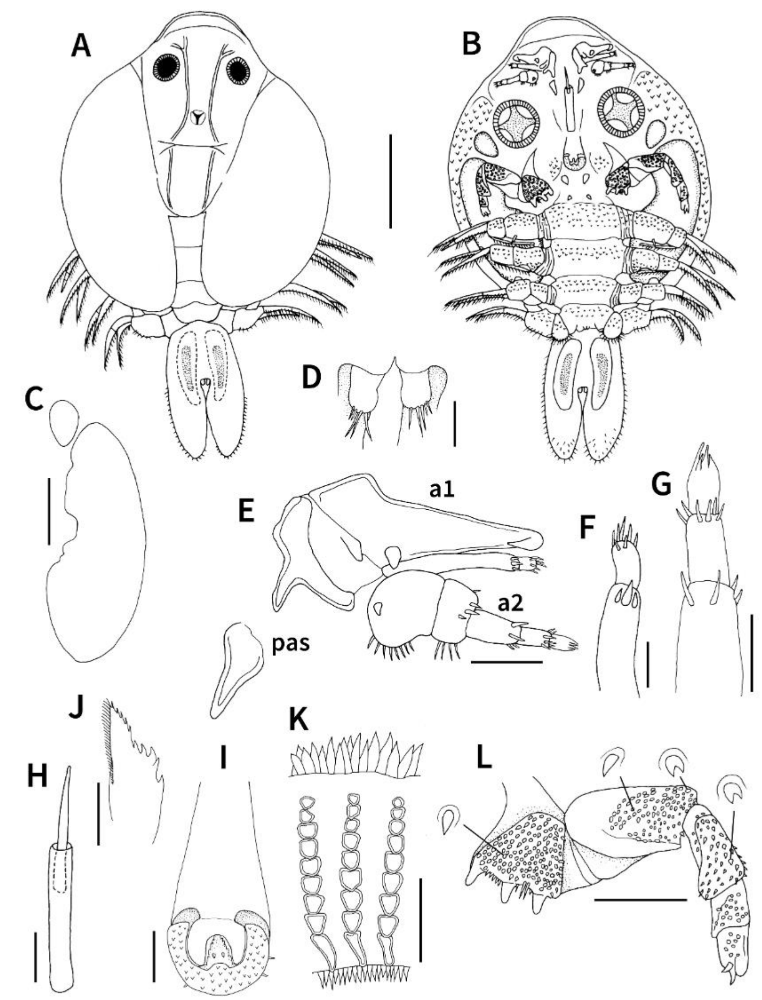

Fig. 2.1.3.1.4. *Argulus mongolianus*, adult male, NSMT-Cr 29372, from largemouth bass *Micropterus salmoides* in Lake Izunuma, Miyagi Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view; C, respiratory areas, ventral view; D, caudal rami, dorsal view; E, first antenna (a1), second antenna (a2), and postantennal spine (pas), ventral view; F, distal part of first antenna, ventral view; G, distal part of second antenna, ventral view; H, preoral sheath and stylet, ventral view; I, mouth tube, ventral view; J, mandible, ventral view; K, section of sucker membrane of first maxilla showing three supporting rods and maginal projections, ventral view; L, second maxilla and denticles on first, second, and third segments, ventral view. Scale bars: A, B, 1 mm; C, 0.5 mm; D, F, G, K, 0.05 mm; E, H, I, 0.1 mm; L, 0.3 mm; I, 0.03 mm.

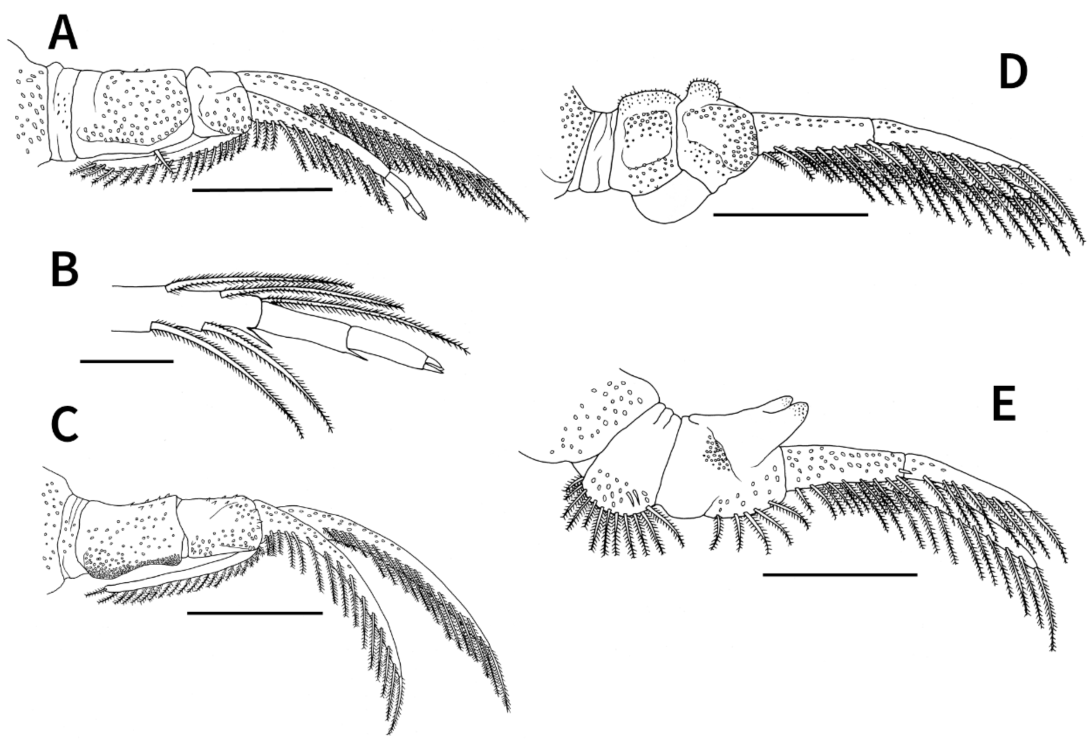

Fig. 2.1.3.1.5. *Argulus mongolianus*, adult male, NSMT-Cr 29372, from largemouth bass *Micropterus salmoides* in Lake Izunuma, Miyagi Prefecture, Japan. A, first leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view. Scale bars: A, C–E, 0.5 mm; B, 0.1 mm.

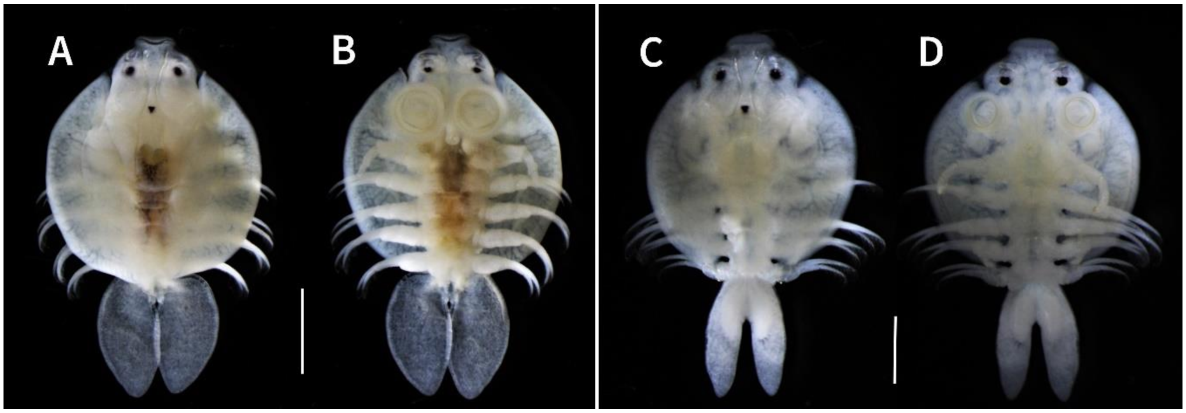

Fig. 2.1.3.1.6. *Argulus mongolianus*, adult female (A and B), NSMT-Cr 29369, and adult male (C and D), NSMT-Cr 29370, from largemouth bass *Micropterus salmoide*s in Lake Izunuma, Miyagi Prefecture, Japan. Ethanol-preserved specimens. A, C, habitus, dorsal view; B, D, habitus, ventral view. The adult female and male were both collected on 2 June 2020 and photographed on 2 October 2021. Scale bars: A, 2 mm; B, 1 mm.

2.1.3.2 Second record of *Argulus mongolianus* in Japan

[Based on Nagasawa, K. and Okamoto, Y. (2023): Second record of *Argulus mongolianus* Tokioka, 1939 (Branchiura: Argulidae), an ectoparasite of freshwater fishes, in Japan. Crustacean Research, 52: 91–95.]

Abstract. Examination of Japanese eels *Anguilla japonica* Temminck and Schlegel, 1846 from Kadota Reservoir, Fukuoka Prefecture, western Japan, revealed the infection by *Argulus mongolianus* Tokioka, 1939 in the eel buccal cavity. This is the second record of *A. mongolianus* in Japan and extends its distributional range from Miyagi Prefecture, northern Honshu, southwestward to Fukuoka Prefecture, northern Kyushu. *Anguilla japonica* represents a new host record. The specimens of both sexes of *A. mongolianus* are briefly described. This paper suggests that the parasite has been co-introduced with non-native fish hosts into Kadota Reservoir and other inland waters in northern Kyushu.

2.1.3.2 Introduction

Argulus mongolianus Tokioka, 1939 was originally described by Tokioka (1939) based on an adult female collected in Inner Mongolia, China. This species has since been reported from northern Vietnam (Ky and Te, 2007) and the Russian Far East (Shedko *et al*., 2018). Recently, Nagasawa *et al*. (2022b) collected *A. mongolianus* from freshwater fishes in Miyagi Prefecture, northern Honshu, Japan, and redescribed it. These authors suggested that this parasite was introduced from China into Japan and has spread to various localities in Japan.

During a parasitological survey of fishes in Fukuoka Prefecture, northern Kyushu, the present author collected specimens of *A. mongolianus* from Japanese eels *Anguilla japonica* Temminck and Schlegel, 1846 (Anguilliformes: Anguillidae). This collection represents the second record of *A. mongolianus* in Japan.

2.1.3.2 Materials and methods

One and two Japanese eels were caught using hook and line with small fish bait in Kadota Reservoir (33°51'23.8"N, 130°35'58.3"E at center, ca. 62420 m^2) at Yoshiki in Okagaki, Fukuoka Prefecture, northern Kyushu, western Japan, on 14 May and 4 June 2023, respectively. Fish collection was made following the Fukuoka prefectural regulation for fishery coordination (Regulation No. 62 of 20 November 2020). Eels were transported alive to a home of the second author, Kitakyushu, where they were measured for total length (TL, mm) and examined for ectoparasites. As five argulid branchiurans were found on the first eel, the other two eels were preliminarily examined by the second author and then transported frozen to the Aquaparasitology Laboratory, Shizuoka, where they were thawed and carefully examined by the first author for ecto- and endoparasites. The argulid specimens collected on 14 May 2023 were fixed in 10% formalin but soon later preserved in 70% ethanol, but two argulid specimens from a Japanese eel collected on 4 June 2023 were fixed and preserved in 70% ethanol. These specimens were soaked in lactophenol for two hours and observed using an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope. Identification was made using the wooden slide procedure recommended by Humes and Gooding (1964) and Benz and Otting (1996), and drawings were made with the aid of a drawing tube attached to the compound microscope. After the specimens were identified, they were recorded for their sex and total length (TL, from the anterior tip of the carapace to the posterior tip of the abdomen). Morphological terminology follows Benz *et al*. (1995) and Benz and Otting (1996). The specimens have been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 31504 and 31505).

2.1.3.2 Results

Two of the three Japanese eels examined were found to be infected with *A. mongolianus*. Five and two specimens of *A. mongolianus* were collected from the buccal cavity of Japanese eels (650 and 767 mm TL) collected on 14 May and 4 June 2023, respectively. The remaining Japanese eel (815 mm TL) was not infected with *A. mongolianus*.

The seven specimens of *A. mongolianus* comprised five adult females and two adult males, measuring 6.6–10.9 (mean = 9.2, n = 5) mm TL and 5.0–5.5 (mean = 5.3, n = 2) mm TL, respectively.

The specimens of both sexes have a dorsoventrally flattened body; a nearly circular carapace with an anteriorly protruding centrofrontal region; compound eyes visible in the frontal region of the carapace; a naupliar eye present dorsally along midline of the carapace; posterolateral lobes of the carapace separated by a sinus, ending each in a rounded margin; paired respiratory areas, each comprising a small anterior area and a much longer posterior area; the thorax consisting of four segments, each bearing a pair of legs; the first and second antennae found beneath the frontal region of the carapace; the preoral sheath and stylet located along the midline posterior to the second antennae; the mouth tube nearly cylindrical; the first maxillae forming cup-like suckers; the second maxillae each with a robust first segment bearing three large projections; the first to fourth pairs of legs biramous, each comprising the coxa, basis, exopod, and endopod; and the fourth pair of legs each with the coxa forming a natatory lobe (Fig. 2.1.3.2.1). The male has a swelling at the posterior junction of the coxa and basis of the third leg and a peg on the anterior margin of the basis of the fourth leg. The female has oval abdominal lobes, whereas the male has oblong abdominal lobes. Each of the first pair of legs has a single plumose seta near the posterior margin of the coxa in both sexes (Fig. 2.1.3.2.2). The number of supporting rods per first maxilla ranges from 60–71 (mean = 65, n = 6) in the female and 54–63 (mean = 58, n = 4) in the male.

The body color of the specimens of *A. mongolianus* observed five days after being fixed in 10% formalin was white, while the compound eyes and naupliar eye were black and the respiratory areas were dark green (Fig. 2.1.3.2.1). The thorax of the female was pale yellow and its dorsal surface had dark brown speckles.

Remarks The morphology of the specimens collected in this study almost corresponds to that of *A. mongolianus* described by Tokioka (1939) and redescribed by Nagasawa *et al*. (2022b), and the specimens are identified as this species. In particular, the species is characterized by the anterior protrusion of the centrofrontal carapace in both sexes, a circular carapace with oval and oblong abdominal lobes in the female and male, respectively, and the first pair of legs each with a single plumose seta near the posterior margin of the coxa in both sexes (Nagasawa *et al*., 2022b), which are confirmed in the specimens examined (Figs 2.1.3.2.1, 2.1.3.2.2). Moreover, *A. mongolianus* is readily distinguished by the presence of an anteriorly protruding centrofrontal region of the carapace from two congeneric species, *Argulus japonicus* Thiele, 1900 and *A. coregoni* Thorell, 1894, which are also known to infect wild freshwater fishes in Japan (Nagasawa *et al*., 2022b).

In the original description of *A. mongolianus*, the number of supporting rods per first maxilla was reported to be "70–80" (Tokioka, 1939). However, the redescribed specimens had fewer supporting rods [64–71 (mean = 68) in the female; 58–64 (61) in the male] than the specimen originally described (Nagasawa *et al.*, 2022b), and the specimens examined in this study similarly have fewer supporting rods [60–71 (mean = 65) in the female; 54–63 (mean = 58) in the male]. Nagasawa *et al*. (2022b) have suggested that Tokioka (1939) incorrectly counted the number of supporting rods.

In Japan, *A. mongolianus* has been reported only from two small lakes, Lake Izunuma and Lake Uchinuma, Miyagi Prefecture, northern Honshu (Nagasawa *et al.*, 2022b). The present collection of *A. mongolianus* markedly extends its distributional range from Miyagi Prefecture, northern Honshu southwestward to Fukuoka Prefecture, northern Kyushu, in Japan.

The known Japanese hosts of *A. mongolianus* are largemouth bass *Micropterus salmoides* (Lacepède, 1802) (Centrarchiformes: Centrarchidae), common carp *Cyprinus carpio* Linnaeus, 1758, Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846, and silver crucian carp *Carassius* sp. (all Cypriniformes: Cyprinidae) (Nagasawa *et al*., 2022b). While no information is present on the hosts of *A. mongolianus* in China (Tokioka, 1939; Song and Kuang, 1980; Kuang and Qian, 1991; Wadeh *et al*., 2008), one and two species of freshwater fishes were reported as the hosts of this parasite in Russian Far East and northern Vietnam, respectively. They are common carp from Russia (Shedko *et al.*, 2018); phoenix barb *Spinibarbus denticularis* (Oshima, 1926) (Cypriniformes: Cyprinidae) and goonch *Bagarius bagarius* (Hamilton, 1822) (Siluriformes: Sisoridae) from Vietnam (Ky and Te, 2007). The finding of *A. mongolianus* in Japanese eels represents a new host record. It is apparent that this parasite shows no strict host specificity.

The body coloration of *A. mongolianus* reported by Tokioka (1939) is similar to that of the specimens observed in this study (Fig. 2.1.3.2.1).

2.1.3.2 Discussion

When Nagasawa *et al*. (2022b) redescribed *A. mongolianus* using the specimens collected from Lake Izunuma and Lake Uchinuma, northern Japan, these authors made the following inference: 1) this parasite was introduced from China into Japan with an unspecified species of freshwater fish; 2) with the establishment and spread of this unspecified host species in Japan, the parasite has also expanded its range in Japan and, due to its low host specificity, utilizes various fish species as hosts in translocated localities; 3) it was introduced with an unspecified host from somewhere in Japan into the two lakes; and 4) it has established and maintained its populations in the lakes. If this is the case, it is possible to infer that *A. mongolianus* was also introduced from somewhere in Japan to Kadota Reservoir. While there is no information on the fish fauna of this reservoir, non-native largemouth bass is very likely to have been released and established its population there because the reservoir is known for its largemouth bass fishing, and those released largemouth bass may have brought *A. mongolianus* into the reservoir. Furthermore, since largemouth bass serves as one of the hosts for *A. mongolianus* in the two lakes, northern Japan (Nagasawa *et al.*, 2022b), this fish may harbor the parasite in Kadota Reservoir as well.

According to Nakajima *et al*. (2008), 13 species of non-native freshwater fishes were found between 1998 and 2008 in numerous rivers, reservoirs, and creeks of northern Kyushu including Fukuoka and adjacent prefectures, and Japanese white crucian carp was most frequently collected, followed by largemouth bass and bluegill *Lepomis macrochirus* Rafinesque, 1819 (Centrarchiformes: Centrarchidae). We infer from these facts that *A. mongolianus* was also co-introduced with those non-native fishes into various water bodies in northern Kyushu other than Kadota Reservoir investigated. As this parasite is not host-specific, it is desirable to study the geographical distribution and occurrence of *A. mongolianus* parasitic on various freshwater fishes in a wide area of this region.

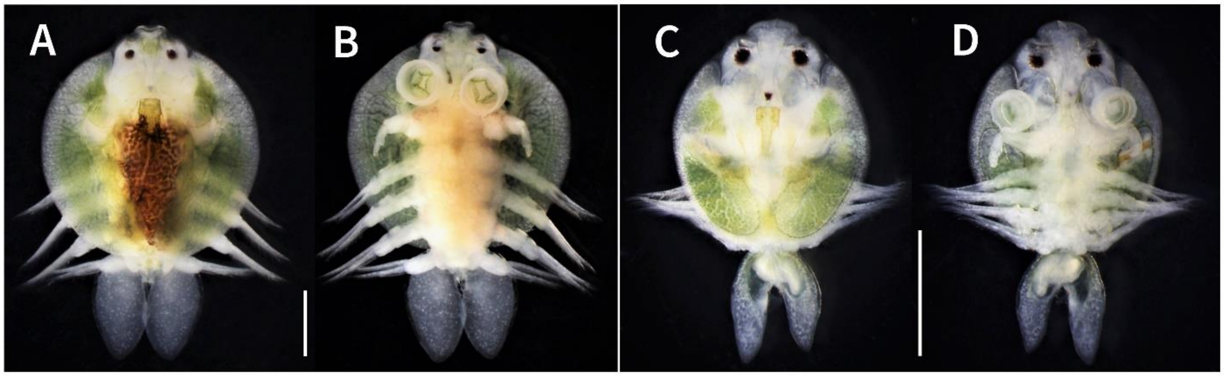

Fig. 2.1.3.2.1. *Argulus mongolianus*, adult female (A and B, 10.9 mm total length) and adult male (C and D, 5.5 mm total length), NSMT-Cr 31504, from Japanese eel *Anguilla japonica* in Kadota Reservoir, Fukuoka Prefecture, northern Kyushu, Japan. The female and male specimens were photographed five days after being fixed in 10% formalin. The testes of the male were damaged. A and C, habitus, dorsal view; B and D, habitus, ventral view. Scale bars: A and B, 2 mm; C and D, 2 mm.

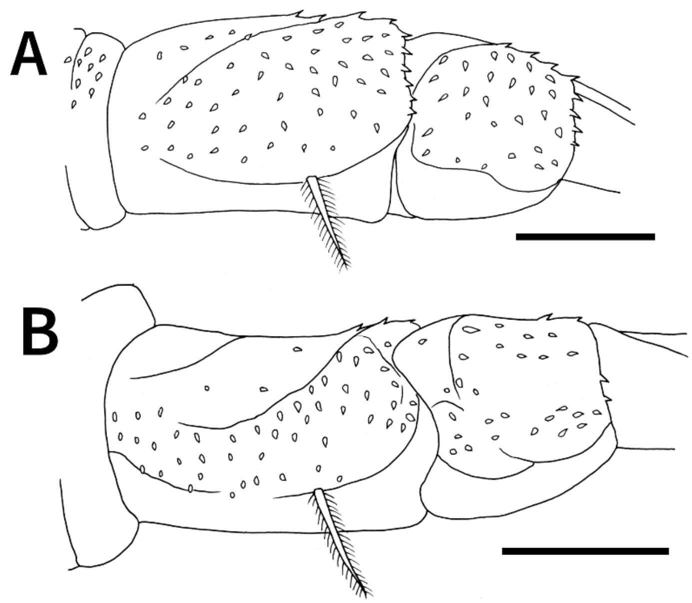

Fig. 2.1.3.2.2. Coxa (with a plumose seta) and basis of the first leg in the female (A, 6.6 mm total length, NSMT-Cr 31504) and male (B, 5.0 mm total length, NSMT-Cr 31505) of *Argulus mongolianus* from Japanese eel *Anguilla japonica* in Kadota Reservoir, Fukuoka Prefecture, northern Kyushu, Japan. Ventral view. Scale bars: A and B, 0.2 mm.

2.1.4 *Argulus nobilis* Thiele, 1904

[Based on Nagasawa, K. (2023e): First Japanese record of *Argulus nobilis* (Crustacea: Branchiura: Argulidae), an ectoparasite of gars of North American origin. Species Diversity, 28: 205–215]

Abstract. *Argulus nobilis* Thiele, 1904 is an ectoparasite of gars (Lepisosteidae) in the U.S.A. Five female specimens of *A. nobilis* were found in the collection of the Lake Biwa Museum, Japan. This is the first record of the species from Japan and outside the U.S.A. The specimens were collected from an unidentified gar and shortnose gar *Lepisosteus platostomus* Rafinesque, 1820. Based on a detailed examination of the specimens, the adult female of *A. nobilis* is redescribed. The finding from shortnose gar represents a new host record for this parasite. The specimens of *A. nobilis* were collected in 1992, and it is unknown whether they were taken from gars soon after imported from the U.S.A. or those that had been held in Japan.

2.1.4. Introduction

Argulus nobilis Thiele, 1904 is an ectoparasite of gars (the family Lepisosteidae) in the U.S.A. (Hoffman, 1967, 1999; Cressey, 1972; Neethling and Avenant-Oldewage, 2016). This species was originally described by Thiele (1904) based on female and male specimens in the collection of the Berlin Zoological Museum (currently the Natural History Museum, Berlin). The specimens were collected in Dallas, Texas. Based on the museum label, "*Lepisosteus aculeatus*" was reported as the host of *A. nobilis*. However, the author doubted the validity of this scientific name and suggested that the host was "*Lepisosteus viridis*," which was later inferred by Wilson (1916) to be alligator gar, whose current scientific name is *Atractosteus spatula* (Lacepède, 1803). Without mentioning Thiele's work, Wilson (1912) described a new species *Argulus ingens* C. B. Wilson, 1912, using female and male specimens from alligator gar [reported as "*Lepisosteus tristoechus* (Bloch and Schneider, 1801)"] in Moon Lake, Mississippi. However, because both *A. nobilis* and *A. ingens* were morphologically quite similar, Wilson (1924) regarded the latter taxon as a variety of *A. nobilis* and redescribed the species based on specimens from alligator gar in Lake Calcasieu, Louisiana. Later, Meehean (1940) reported morphological characters of *A. nobilis* from alligator gar (reported as "*Atractosteus tristoechus*") from Louisiana, Texas, and Mississippi, and longnose gar *Lepisosteus osseus* (Linnaeus, 1758) from Ocean Pond, Lake City, Florida. Causey (1960) added the White River, Arkansas, and the Golden Gate Park Aquarium, California, as new localities for *A. nobilis*. Hoffman (1999) reported this parasite from spotted gar *Lepisosteus oculatus* Winchell, 1864 in Arkansas and also regarded the type host ("*Lepisosteus aculeatus*") as spotted gar without any comment. Molecular data on *A. nobilis* from longnose gar in an unknown locality (perhaps Florida or Louisiana based on the authors' addresses) were analyzed for a phylogenetic analysis on the status of tongue worms (the crustacean subclass Pentastomida) (Abele *et al.*, 1989). An illustrated identification key to 23 species of *Argulus* O. F. Müller, 1785 (including *A. nobilis*) of the U.S.A. was provided by Cressey (1972). There is no published record of *A. nobilis* collected in countries other than the U.S.A.

Gars are popular as exotic ancient fishes in Japan (Taki *et al.*, 1995), and they had been imported alive from North America to Japan until 2018, when the Japanese government placed a ban on the importation of all gar species and their hybrids from other countries to Japan. Tsutsumi (1968) identified the branchiuran as *Argulus foliaceus* (Linnaeus, 1758) taken from the body surface of a spotted gar (reported as "*Lepisosteus productus*") that had been imported alive from North America to Tokyo and then transported to the Shimonoseki Municipal Aquarium, western Japan. Subsequently, this collection was briefly reported again by the same author (Tsutsumi, 1977). However, because *A. foliaceus* does not occur in North America (Cressey, 1972), Shimura and Asai (1984) doubted Tsutsumi's identification and suggested that the branchiuran might be identifiable as *Argulus lepidostei* Kellicott, 1877 or a closely related species. Following this suggestion, it was later reported as "*Argulus lepidostei* or *Argulus* sp. cf. *lepidostei*" (Nagasawa, 2009) or "*Argulus lepidostei*" (Nagasawa, 2011a). No further argulid infection has been reported from gars held in Japan.

Recently, the present author had the opportunity to examine some branchiuran specimens in the collection of the Lake Biwa Museum, Japan, and found female specimens of *A. nobilis*. In this paper, based on a detailed examination of the specimens, the adult female of *A. nobilis* is redescribed. This represents the first record from Japan and outside the U.S.A. for the species.

2.1.4 Materials and methods

Five specimens of *A. nobilis* preserved in 70% ethanol were received on loan from the Lake Biwa Museum, Kusatsu, Shiga Prefecture, Japan. The specimens composed one female collected from “garpike” on 20 June 1992 and four females from shortnose gar *Lepisosteus platostomus* Rafinesque, 1820 on 17 July 1992. At the Aquaparasitology Laboratory, Shizuoka Prefecture, the specimens were studied using an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope. One specimen was dissected, cleared in lactophenol, and examined using the wooden slide procedure (Humes and Gooding, 1964; Benz and 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 and Otting (1996). The scientific and common names of fishes mentioned in this paper follow Froese and Pauly (2023), except for those of ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) and silver crucian carp *Carassius* sp., which are based on Hosoya (2015).

2.1.4 Results

Argulus nobilis Thiele, 1904

[New Japanese name: Odeko-chou]

(Figs 2.1.4.1–2.1.4.5)

Argulus nobilis Thiele, 1904: 28–32, pl. 7, figs 64–66, pl. 8, figs 67–76; Wilson, 1916: 354; Wilson, 1924: 2–4, pl. 1, figs 6–9; Meehean, 1940: 515–517, fig. 45; Causey, 1960: 74, 75, figs 3–5, 8, 9; Yamaguti, 1963: 326, pl. 324, fig.1; Hoffman, 1967: 303; Cressey, 1972: 4, 10, figs 1B, 5I; Hoffman, 1977: 3; Abele *et al.*, 1989: 685–687; Hoffman, 1999: 316; Neethling and Avenant-Oldewage, 2016: 1254, 1290, 1297.

Argulus ingens C. B. Wilson, 1912: 253–255, pl. 30, figs 1–6, pl. 31, fig. 7; Wilson, 1916: 354; Yamaguti, 1963: 324, pl. 318, fig. 2; Hoffman, 1967: 302; Hoffman, 1999: 316; Neethling and Avenant-Oldewage, 2016: 1254, 1277, 1297.

Argulus nobilis var. *nobilis*: Wilson, 1924: 2; Yamaguti, 1963: 326.

Argulus nobilis var. *ingens*: Wilson, 1924: 2; Meehean, 1940: 515; Yamaguti, 1963: 326.

Hosts. Shortnose gar *Lepisosteus platostomus* and an unidentified lepisosteid fish (recorded as "garpike" on the label of the specimen, LBM 1430006167).

Attachment site. No record on the labels.

Localities. In Japan. No record on the labels, but they were most probably collected in Shiga Prefecture, where Lake Biwa Museum is located, or its adjacent region.

Material examined. Five adult females: LBM1430006167 for one specimen [10.2 mm in total length (TL, from anterior tip of carapace to posterior tip of abdomen)]; LBM1430006168 for three specimens (9.2, 9.9, and 10.5 mm in TL) and one dissected specimen (10.1 mm in TL).

Adult female (based on five specimens, including one dissected specimen). *Body* dorsoventrally flattened, measuring 9.2–10.5 (mean = 9.9, n = 5) mm in TL and 6.0–7.0 (mean = 6.4, n = 5) mm in maximum body width (around midlength of carapace).

Carapace (including posterolateral lobes) nearly circular, covering almost totally first pair of legs and partially second and third pairs of legs in dorsal view, 6.6–8.5 (mean = 7.4, n = 5) mm long, comprising 65.3–81.5% (mean = 74.5%, n = 5) of total length (Figs 2.1.4.1, 2.1.4.5). Frontal region of carapace delimited by pronounced anterolateral indentations and protruding anteriorly; anterior margin rounded (Fig. 2.1.4.2A). Central longitudinal ribs distinct, curved laterally near compound eyes; transverse ribs evident behind nauplius eye (Fig. 2.1.4.1A). Compound eyes well visible, dorsally located at level of anterolateral indentations of carapace (Figs 2.1.4.1A, 2.1.4.2A, 2.1.4.5). Nauplius eye located posterior to compound eyes on midline of carapace. Dorsal surface of carapace smooth without spines. Ventral surface of frontal and anterolateral regions of carapace ornamented with numerous, small posteriorly directed spines (Fig. 2.1.4.2A). Posterolateral lobes of carapace not overlapping, ending in rounded margin, separated by sinus, 34.1–45.4% (mean = 40.6%, n = 5) length of carapace (Figs 2.1.4.1A, 2.1.4.5A). Respiratory areas located at level between second maxillae and second pairs of legs; smaller anterior area nearly oval, located mesially in big anterior notch of posterior area; larger posterior area composed of three portions, *i.e.*, slightly expanded anterior portion, narrow neck-like portion, and large, nearly inverted triangular portion with small notch on anterior margin (Figs 2.1.4.1B, 2.1.4.2B). *Thorax* with four segments, bearing small spiniform projections laterally; fourth segment tapering posteriorly (Figs 2.1.4.1, 2.1.4.2B). *Abdomen* heart-shaped, with some small spiniform projections on ventral surface; anal indentation 27.6–38.5% (mean = 32.0%, n = 5) as long as abdomen to form two lobes; each lobe becoming wider toward one-quarter length of abdomen, then tapering posteriorly, ending in pointed margin (Figs 2.1.4.1, 2.1.4.2B, 2.1.4.5). Paired spermathecae each round, located in anterocentral portion of abdomen (Fig. 2.1.4.1A); paired spermathecal spines each torpedo-shaped and tapering posteriorly, located on ventral midline between natatory lobes (Figs 2.1.4.1B, 2.1.4.2B, D, 2.1.4.5B). Caudal rami located at one-third length of anal indentation, each with five naked setae on posterior margin (Figs 2.1.4.1, 2.1.4.2B, E).

First antennae with four segments (Fig. 2.1.4.3A, B): first segment heavily sclerotized in mesial and posterior regions, with large blunt projection on posterior margin; second segment largest and heavily sclerotized, with strong posteroventrally directed hook on anterior margin, strong lateroventrally directed hook at distal corner, and ventral projection near posterior margin; third segment cylindrical and unarmed; apical segment shorter than third segment, with four naked setae at tip. *Second antennae* with five segments (Fig. 2.1.4.3A, C, D): first segment sclerotized, with sharp, strong posteroventral spine and apically rounded protrusion bearing six naked setae on posterior margin; second segment shorter than first segment, with small protrusion bearing four naked setae on posterior margin; third, fourth, and apical segments nearly cylindrical and decreasing in length; third segment with four and four naked setae on lateral and distal margins, respectively; fourth segment with two and four naked setae on lateral and distal margins, respectively; apical segment with two short and three naked setae near and at tip, respectively. Postantennal spines large and robust, each located lateral to spine of first segment of second antenna (Figs 2.1.4.2A, 2.1.4.3A). Preoral sheath cylindrical and visible on ventral midline of carapace near anterior region of first maxillae (Figs 2.1.4.1B, 2.1.4.2A, 2.1.4.3E); anterior portion of stylet protruding from opening of preoral sheath. Mouth tube without ornamentation located just posterior to preoral sheath, longer than wide; posterior portion composed of anterior labrum and posterior labium (Figs 2.1.4.1B, 2.1.4.3F).

First maxillae forming well developed cup-like suckers (Fig. 2.1.4.1B), with 59–65 (mean = 62, n = 4) supporting rods in sucker membrane. Supporting rods each composed of 10–12 sclerites [in 30 supporting rods examined, 11 sclerites per rod is the commonest (n = 24, 80.0%), followed by 10 (n = 4, 13.3%) and 12 (n = 2, 6.7%) sclerites, respectively]; sclerites oblong and decreasing in size distally (Fig. 2.1.4.3G). Outer margin of rim of sucker membrane with numerous apically pointed projections. *Second maxillae* with five segments (Fig. 2.1.4.3H): first segment robust, with three, almost equally long spines on posterior margin; corpus of first segment with larger papilla bearing five long naked setae, smaller papilla bearing one long and one short naked setae, three short naked setae near outer margin, and several minute denticles; second segment longer than first segment, with distally directed unidentate, bidentate, or tridentate spinules and one long naked seta on ventral surface; third segment shorter and narrower than second segment, with distally directed unidentate, bidentate, or tridentate spinules on ventral surface and short naked setae on posterior margin; fourth segment subquadrate, with two short naked setae on ventral surface and some longer naked setae on ventral surface; terminal segment bearing several setae on posterior marine, ending in three projections (central one stout, lateral ones short). Accessory spines near ventral midline, each located slightly apart from first segment of second maxilla (Figs 2.1.4.1B, 2.1.4.3H). Postmaxillary spines small, located on anterior portion of first segment of thorax (Fig. 2.1.4.1B).

First to fourth pairs of legs (Fig. 2.1.4.4) biramous, with sympods each composed of coxa and basis; sympods and rami of first to fourth legs ventrally covered with small spiniform projections; rami each issuing two rows of plumose setae; first and second pairs of legs each possessing flagellum projecting from extreme proximal part of exopod. *First leg* (Fig. 2.1.4.4A, B) coxa bearing 15 long plumose setae and one short plumose seta on posterior margin; basis nearly half as wide as coxa, bearing seven plumose setae on posterior margin; exopod unsegmented, with 23 and 20 plumose setae near anterior and posterior margins, respectively; endopod three-segmented, proximal segment long, with 16 and 19 plumose setae near anterior and posterior margins, respectively, and one naked seta in ventrodistal portion, middle segment much shorter than proximal segment, with one short naked seta at posterodistal corner, and terminal segment tapering distally, ending in two short spines; flagellum extending to proximal margin of coxa, with 21 and 13 plumose setae on anterior and posterior margins, respectively. *Second leg* (Fig. 2.1.4.4C) coxa with seven plumose setae on posterior margin; basis slightly shorter than coxa, bearing seven plumose setae on posterior margin; exopod and endopod unsegmented, with two rows of plumose setae near anterior and posterior margins (24 and 21 setae on exopod and 20 and 19 setae on endopod); flagellum extending to proximal margin of coxa, with 23 and 14 plumose setae on anterior and posterior margins, respectively. *Third leg* (Fig. 2.1.4.4D) coxa with six plumose setae on posterior margin; basis slightly shorter than coxa, bearing six plumose setae on posterior margin; exopod unsegmented, with 23 and 25 plumose setae on dorsolateral and ventrolateral sides, respectively; endopod two-segmented, proximal segment with a small process at anterodistal corner, 12 and six plumose setae near dorso- and ventroposterior margins, respectively, terminal segment with 12 and 12 plumose setae near dorso- and ventroposterior margins, respectively. *Fourth leg* (Fig. 2.1.4.4E) coxa forming natatory lobe bearing 11 plumose setae and a group of short spines on proximal, rounded posterior margin, 39 plumose setae on weakly curved, posterior margin, and four plumose setae on anterodistal margin; basis slightly shorter than main portion of natatory lobe, with six plumose setae on posterodistal margin; exopod unsegmented, bearing 19 and 19 plumose setae on dorsolateral and ventrolateral sides, respectively; endopod two-segmented, proximal segment with a small process at anterodistal corner, one naked short seta and 13 plumose setae near dorsoposterior margin and eight plumose setae near ventroposterior margin, and terminal segment with one naked short seta and 12 plumose setae near dorsoposterior margin and 12 plumose setae near ventroposterior margin.

Color (based on ethanol-preserved specimens). Carapace and abdomen brownish white; legs white; thorax pale brown with irregularly shaped, dark brown spots scattered on dorsal surface; respiratory areas weakly fringed by dark brown pigment (Fig. 2.1.4.5).

Japanese name. The new Japanese name for *A. nobilis* is formed as a compound of "odeko" (meaning "bulging forehead"), referring to the protruding frontal region of the carapace and "chou" (meaning "freshwater branchiuran").

Remarks. The morphology of the female argulid examined in this study is identical to the original and subsequent descriptions of female *A. nobilis* given by Thiele (1904), Wilson (1912, 1924), and Meehean (1940), and the specimens are thus identified as *A. nobilis*. This species is characterized by the anterior protrusion of the frontal region of the carapace (Figs 2.1.4.1, 2.1.4.2A, 2.1.4.5), a heart-shaped abdomen (Figs 2.1.4.1, 2.1.4.2B, 2.1.4.5), the transverse ribs of the carapace (Figs 2.1.4.1A, 2.1.4.5A), the boot-shaped natatory lobes of the fourth pairs of legs (Figs 2.1.4.1B, 2.1.4.2B, 2.1.4.4E, 2.1.4.5B), the peculiar nested shape of the respiratory areas (Fig. 2.1.4.2C), the presence of 10 and more sclerites per supporting rod of the first maxillae (Fig. 2.1.4.3G), the small processes at each anterodistal corner of the proximal segment of the endopod of the third and fourth legs (Fig. 2.1.4.4D, E), the prominent torpedo-shaped spermathecal spines (Fig. 2.1.4.2B, D), and the papillae bearing naked setae of different lengths on the corpus of the first segments of the second maxillae (Fig. 2.1.4.3H). Although Cressey (1972) stated that only setae exist on this corpus, Meehean (1940) recognized both a small rounded papilla and long setae on it. In the present study, very tiny denticles were also found on the corpus (Fig. 2.1.4.3H). Moreover, the unusual shape of the respiratory areas was were reported by Wilson (1912: pl. 30, fig. 5), Meehean (1940: fig. 45a), and Cressey (1972: fig. 1B). According to Cressey (1972), more than 10 sclerites are found in each supporting rod of the first maxillae: their number was reported to be ca. 13 (Thiele, 1904) and 12–14 (Meehean, 1940) and, in this study, ranges from 10–12 (mostly 11). The presence of a small process at the anterodistal end of the proximal segment of the endopod of the third and fourth legs was first noticed by Wilson (1924) and later confirmed by Meehean (1940: fig. 45c). The well-developed spermathecal spines were figured by Thiele (1904: pl. 8, fig. 76) and reported as "tactile papillae" by Wilson (1912). The presence of the transverse ribs of the carapace was previously noted by Wilson (1924). In this study, the present author could not observe the mandibles because he lost them during my dissection of the mouth tube. In the original description of *A. nobilis*, Thiele (1904) found six teeth each on the mesial margins of the mandibles. No subsequent observations have been reported on the mandibles or their number of teeth.

There are several minor differences in the morphology of *A. nobilis* between the previous and present descriptions. It has been reported that two distal spines on the first segment of the second maxilla are close together and slightly separated from the remaining one (Thiele, 1904: pl. 8, fig. 68; Wilson: 1912, 1924). In the present study, a similar arrangement is found in two of the five specimens examined, whereas in the other three specimens the three spines are almost equally separated (Fig. 2.1.4.3H). The ventral surface of the third segment of the second maxilla was figured to have a swelling or a lamellar process (Thiele, 1904: pl. 8, fig. 69; Wilson, 1912: pl. 30, fig. 3; Meehean, 1940), but no such structure was observed in the specimens examined in this study (Fig. 2.1.4.3H). The size of the notch on the anterior or mesial margin of the main portion of the posterior respiratory area appears variable: it was figured to be larger (Wilson, 1912: pl. 30, fig. 5; Meehean, 1940: fig. 45a) than observed in this study (Fig. 2.1.4.2C), whereas Cressey (1972: fig. 1B) recognized almost no notch on that mesial margin. Furthermore, the number of setae on each caudal ramus was previously reported to be “three” (Wilson, 1912), but the specimen dissected in this study has five setae (Fig. 2.1.4.2E).

Although only female specimens of *A. nobilis* were examined in this study, several morphological differences are known between both sexes of the species. The male has a more anteriorly protruding frontal region of the carapace and longer posterolateral lobes overlapping the anterior region of the abdomen than the female (Wilson, 1912: pl. 30, fig. 1, pl. 31, fig. 7) and the abdomen of the male has its anterior corners forming narrow knobs or processes (Thiele, 1904: pl. 7, fig. 65). The male also has accessory copulatory structures, i.e., a finger-like flap and the socket on the coxa and basis of the third pair of legs, respectively; and the peg on the basis of the fourth pair of legs (Thiele, 1904: pl. 8, figs. 71, 79; Wilson, 1912: pl. 30, fig. 4; Meehean, 1940: fig. 45c).

In some branchiurans of the genus *Argulus*, the flagella are known to project each from the extreme proximal part of the exopod of both the first and second pairs of legs (Boxshall and

Jaume, 2009), and two species from Japan, *A. japonicus* Thiele, 1900 and *A. mongolianus* Tokioka, 1936, have been reported to have such flagella (Nagasawa, 2021; Nagasawa *et al*., 2022b). Their presence is confirmed in *A. nobilis* in this study (Fig. 2.1.4.4A, B).

Morphologically, especially in the habitus and the outline of the respiratory areas, *A. nobilis* resembles three congeneric species (*A. lepidostei*; *A. meehani* Cressey, 1971; and *A. mexicanus* Pineda, Páramo, and Del Río, 1995), all of which are parasites of gars. However, *A. nobilis* can be separated from these three species by having 10 or more sclerites per supporting rod of the first maxilla: 10–14 in *A. nobilis* (Thiele, 1904; Meehean, 1940; Cressey, 1972; this paper) vs 7 (Wilson, 1916: pl. 62, fig. 18) and 8 or 9 in *A. lepidostei* (Meehean, 1940), less than 10 in *A. meehani* (Cressey, 1971, 1972), and 7–9 (usually 8) in *A. mexicanus* (Pineda *et al*., 1995). The position of the respiratory areas on the carapace is also different between *A. nobilis* and *A. lepidostei*: these areas are located nearly at midlength of the posterolateral lobes in *A. nobilis* (Fig. 2.1.4.1A), whereas they are close to the posterior end of the lobes in *A. lepidostei* (Wilson, 1916). Also a patch of readily visible denticles (reported as scales) is found on the corpus of the first segment of the second maxillae in *A. lepidostei* (Cressey, 1972: fig. 6B), such denticles are not present in *A. nobilis* (Fig. 2.1.4.3H).

Argulus nobilis is known as one of the largest species in this genus (Wilson, 1912). Females reach 18 mm (Thiele, 1904), 21.5–25 mm (Wilson, 1912), and up to 25 mm (Meehean, 1940) in total length. While the five female specimens examined herein are considerably smaller (9.2–10.5 mm) than those previously reported, four of them possess eggs in the ovary (Figs 2.1.4.1B, 2.1.4.5B). Males are smaller than females, reaching 16 mm in total length (Wilson, 1912; Meehean, 1940). Wilson (1924) collected "young" female and male (ca. 4 mm long) of the species.

The hosts of *A. nobilis* reported in this paper are shortnose gar (*L. platostomus*) and an unidentified gar. In the U.S.A., three species of gars, *i.e.*, alligator gar (*At. spatula*), longnose gar (*L. osseus*), and spotted gar (*L. oculatus*), are known as the hosts of *A. nobilis* (see the Introduction for the literature). The shortnose gar represents a new host record for *A. nobilis*.

The specimens of *A. nobilis* examined in this study were collected in 1992. It is unknown whether those specimens were taken from gars soon after being imported from abroad or those that had been held in Japan because gars had been imported alive from North America to Japan prior to 2018.

In this study, no information was available on the attachment site of the specimens of *A. nobilis*. However, as its congeneric species are usually found on the body surface of their host fishes, *A. nobilis* is very likely to be a skin parasite, although there is a record of the species from the mouth of alligator gar (Wilson, 1912).

Now, six species of *Argulus* (*A. japonicus*; *A. coregoni* Thorell, 1864; *A. americanus* Wilson, 1902; *A. mongolianus*; *A. lepidostei* or a related species; and *A. nobilis*) are known to parasitize freshwater fishes in Japan (Nagasawa, 2009, 2011; Nagasawa *et al.*, 2022b; this paper). The first two species are native to Japan, whereas the other four species were introduced from other countries. Both *A. japonicus* and *A. coregoni* are found on both wild and farmed fishes across a wide area of Japan: *A. japonicus* usually infects cyprinids (*e.g.*, Nakazawa, 1914; Tokioka, 1936a; Yamaguti, 1937; Takeda *et al*., 2000; Nagasawa, 2017b; Nagasawa *et al.*, 2018a, 2021a, 2023b, c) but, due to its low host specificity, also occurs on fishes of other families, such as silurids and centrarchids (Nagasawa *et al.*, 2010; Yamauchi *et al.*, 2011; Nagasawa, 2021), and *A. coregoni* parasitizes mainly salmonids and ayu *P. a. altivelis* (*e.g.*, Yamaguti, 1937; Hoshina, 1950; Shimura, 1983a; Nagasawa *et al.*, 2018b, 2022a; Nagasawa and Yuasa, 2020) but is also found on cyprinids, odontobutids, and amblycipitids (Tokioka, 1936a; Nagasawa *et al.*, 2014; Nagasawa and Ishikawa, 2015; Nagasawa and Taniguchi, 2021). *Argulus americanus* was found at a public aquarium in Tokyo, where it occurred on bowfin *Amia calva* Linnaeus, 1766 imported from North America and its hatched larvae experimentally infected Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852) (reported as *Tilapia mossambica*) (Shimura and Asai, 1984). *Argulus mongolianus* is most probably of Chinese origin and was recently discovered in two lakes in northern Honshu, Japan, where it was found to parasitize four species of freshwater fishes: largemouth bass *Micropterus salmoides* (Lacepède, 1802); common carp *Cyprinus carpio* Linnaeus, 1758; Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846; and silver crucian carp *Carassius* sp. (Nagasawa *et al*., 2022b).

2.1.4 Discussion

In Japan, all gar species of the family Lepisosteidae, including longnose gar, shortnose gar, spotted gar, alligator gar, Florida gar *L. platyrhincus* DeKay, 1840, Cuban gar *At. tristoechus* (Bloch and Schneider, 1801), and tropical gar *At. tropicus* Gill, 1863, are reared by hobbyists and at public aquaria (Taki *et al*., 1995) and, since the late 1990s, abandoned gars have been often caught in natural waters [Takahashi (1999) and Yanai *et al*. (2008) for longnose gar; Yanai *et al.* (2008), Yamakawa and Senou (2016), and Mukai *et al.* (2019) for shortnose gar; Yanai *et al.* (2008), Mukai *et al.* (2019), and Uchida *et al.* (2021) for spotted gar; Yanai *et al*. (2008), Kawase *et al*. (2017), Noro *et al*. (2018, 2019), and Mukai *et al*. (2019) for alligator gar; Sakamoto (2010) for Florida gar; Sakamoto (2010) for Cuban gar; Yanai *et al*. (2008) for tropical gar). In North America, gars are known to harbor various parasites (*e.g.*, Bangham 1940; Bangham and Venard, 1942; Castro and McDaniel, 1967; Moravec and Salgado-Maldonado, 2002; Salgado-Maldonado *et al*., 2004; Tkach *et al*., 2010; Tkach and Kinsella, 2011) and, for example, 13 species of metazoan parasites including one monogenean, three trematodes, one cestode, five nematodes, one acanthocephalan, and two crustaceans have been reported from Florida gar in Florida (Bangham, 1940). However, in Japan, no systematic parasitological study has been conducted for gars, and no information is available on their parasites, apart from the branchiuran *Argulus* (Tsutsumi, 1968, 1977; this paper). It is thus desirable to study the parasite fauna of both captive and wild-caught gars in Japan in order to clarify whether North American parasites were introduced to Japan with their gar hosts.

Key to the Japanese freshwater *Argulus* species

As stated in the above Remarks, six species of *Argulus* have been recorded from wild and captive freshwater fishes in Japan. Of these species, a branchiuran reported as *A. foliaceus* by Tsutsumi (1968, 1977) was clearly misidentified (Shimura and Asai, 1984) and needs re-examination of its specimens, but these specimens could not be located by the present author, which indicates that they most probably have been lost. A key to the remaining five species of *Argulus* reported from freshwater fishes in Japan is given here, based on the present and previous papers (Wilson, 1912, 1924 for male *A. nobilis*; Meehean, 1940 for *A. americanus*; Hoshina, 1950; Shimura and Asai, 1984; Nagasawa, 2021; Nagasawa and Taniguchi, 2021; Nagasawa *et al*., 2022b). Previously, four to “eight” plumose setae on the posterior margin of the coxa of the first leg were reported to be important for identification of *A. coregoni* (Nagasawa and Taniguchi, 2021; Nagasawa *et al*., 2022b), “eight” is herein changed to “nine” because the male and female of the species has four to eight and five to nine plumose setae, respectively (Hoshina, 1950, table 1). The key can be used for both sexes.

1. Frontal region of carapace protruding anteriorly 2
  Frontal region of carapace not protruding anteriorly 4
2. Horizontal anterior margin of frontal region .... *A. mongolianus* Tokioka, 1939
– Rounded anterior margin of frontal region 3
3. Two or three sclerites per supporting rod of first maxilla
  .... *A. americanus*
– Ten or more sclerites per supporting rod of first maxilla .... *A. nobilis* Thiele, 1904
4. A single plumose seta present on posterior margin of coxa of first leg
  .... *A. japonicus* Thiele, 1900
– Four to nine plumose setae present on posterior margin of coxa of first leg
  .... *A. coregoni* Thorell, 1864

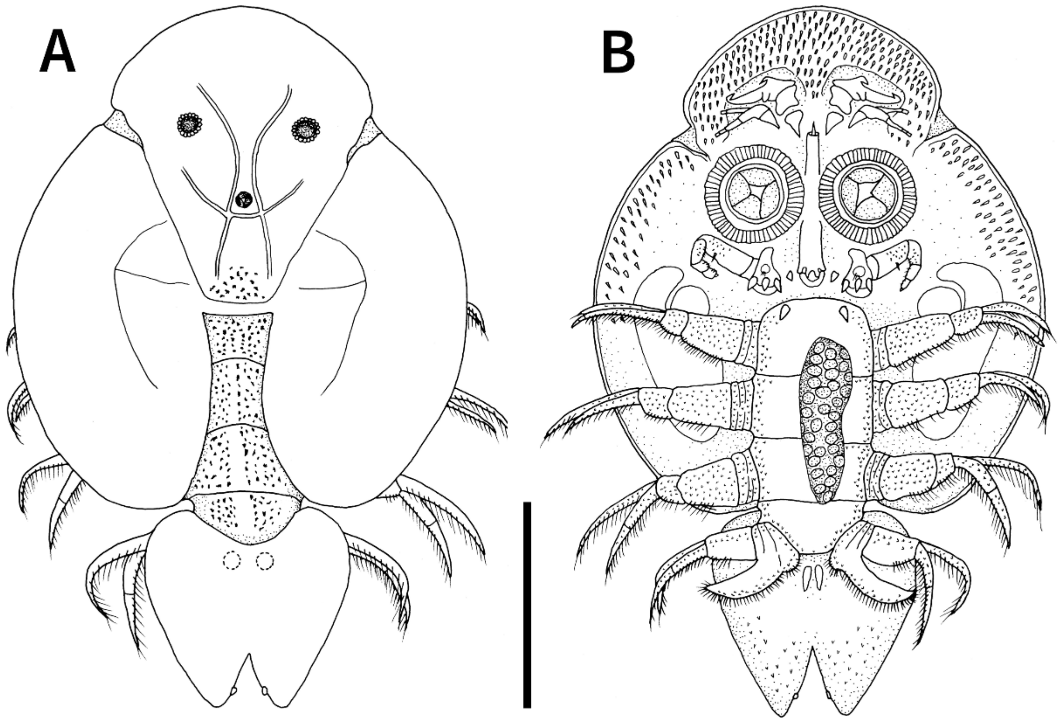

Fig. 2.1.4.1. *Argulus nobilis*, adult female, LBM1430006168. A, habitus, dorsal view; B, habitus, ventral view. Part of the ventral surface of the thorax was damaged and eggs were seen (Fig. 2.1.4.1B). Scale bar: 3 mm.

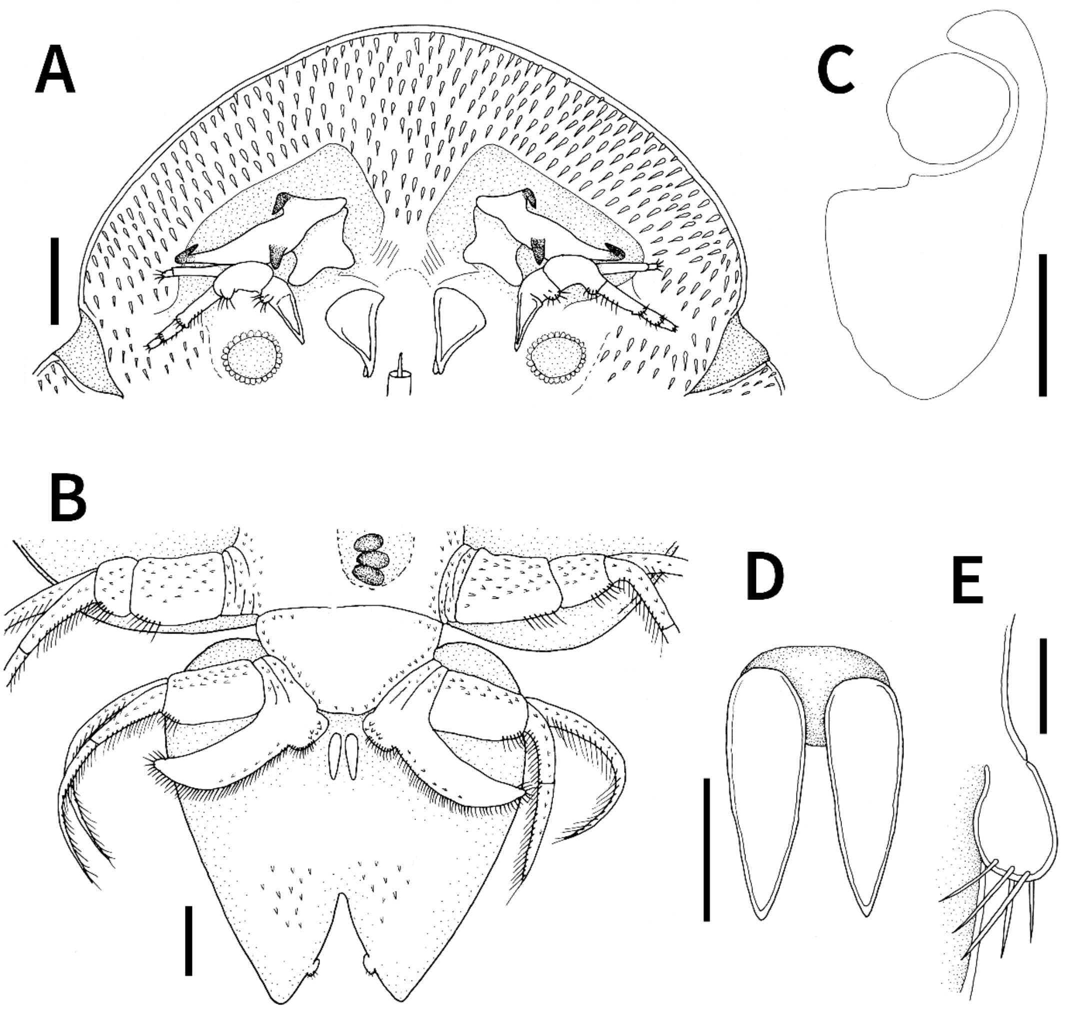

Fig. 2.1.4.2. *Argulus nobilis*, adult female, LBM1430006168. A, frontal region of carapace, ventral view; B, posterior region of body, ventral view; C, respiratory areas, ventral view; D, spermathecal spines, ventral view; E, caudal ramus, ventral view. Scale bars: A, B, 0.5 mm; C, 1 mm; D, 0.2 mm; E, 0.1 mm.

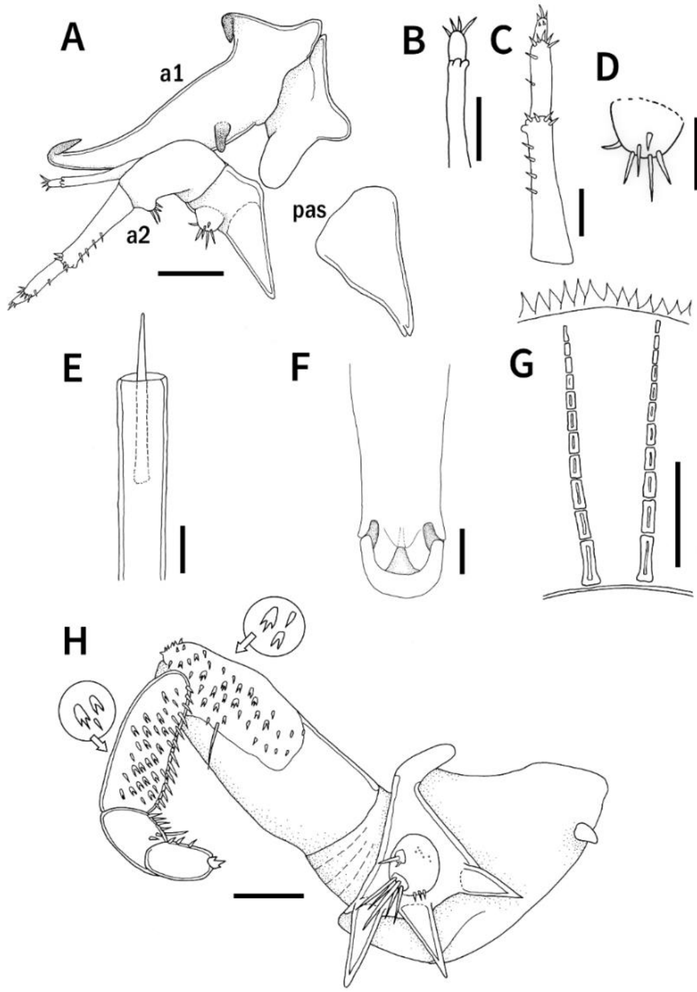

Fig. 2.1.4.3. *Argulus nobilis*, adult female, LBM1430006168. A, first antenna (a1), second antenna (a2), and postantennal spine (pas), ventral view; B, third and apical segments of first antenna, ventral view; C, third, fourth, and apical segments of second antenna, ventral view; D, swelling bearing six naked setae on posterior margin of first segment of second antenna, ventral view; E, preoral sheath and stylet, ventral view; F, mouth tube, ventral view; G, two anterior supporting rods and marginal projections from rim of first maxilla sucker, ventral view; H, second maxilla and spinules (circled) on second and third segments, ventral view. Scale bars: A, F, H, 0.2 mm; B–E, G, 0.1 mm.

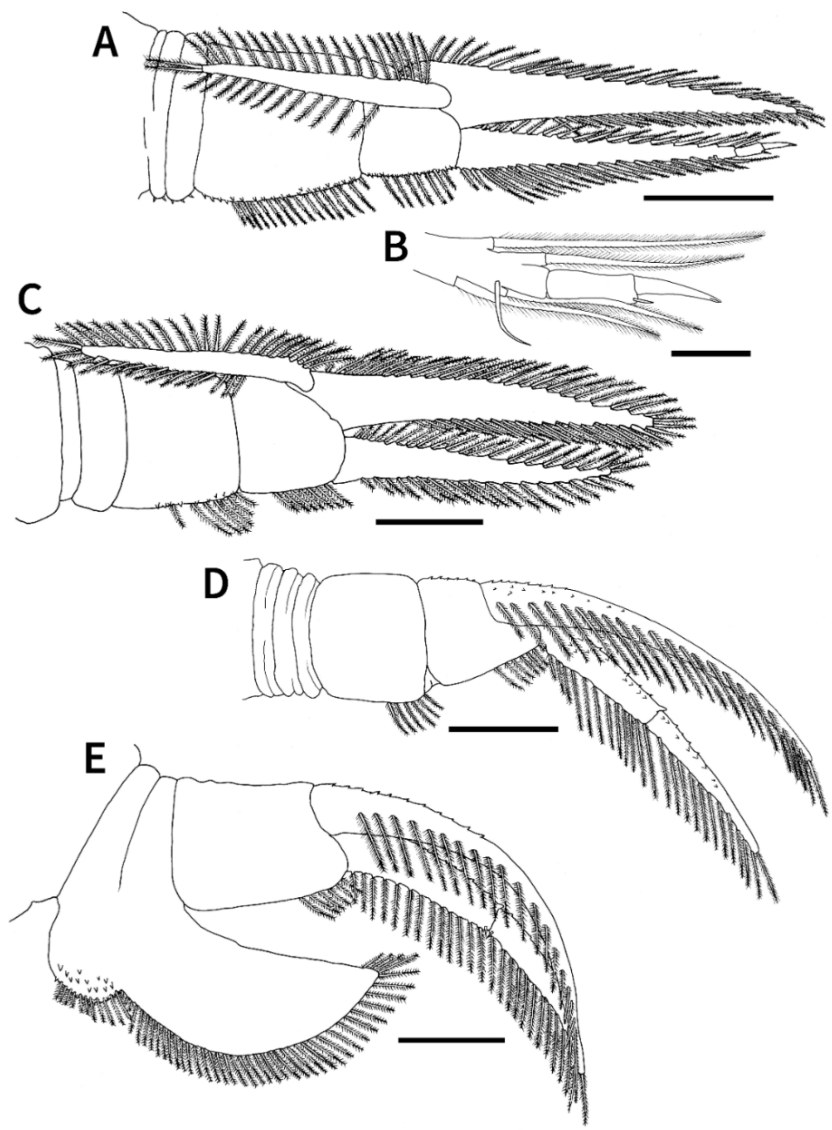

Fig. 2.1.4.4. *Argulus nobilis*, adult female, LBM1430006168. A, first leg, dorsal view; B, distal part of endopod of first leg, dorsal view; C, second leg, dorsal view; D, third leg, dorsal view; E, fourth leg, dorsal view. Ventral rows of plumose setae on rami of third and fourth legs not shown (Fig. 4D, E). Scale bars: A, C–E, 0.5 mm; B, 0.1 mm.

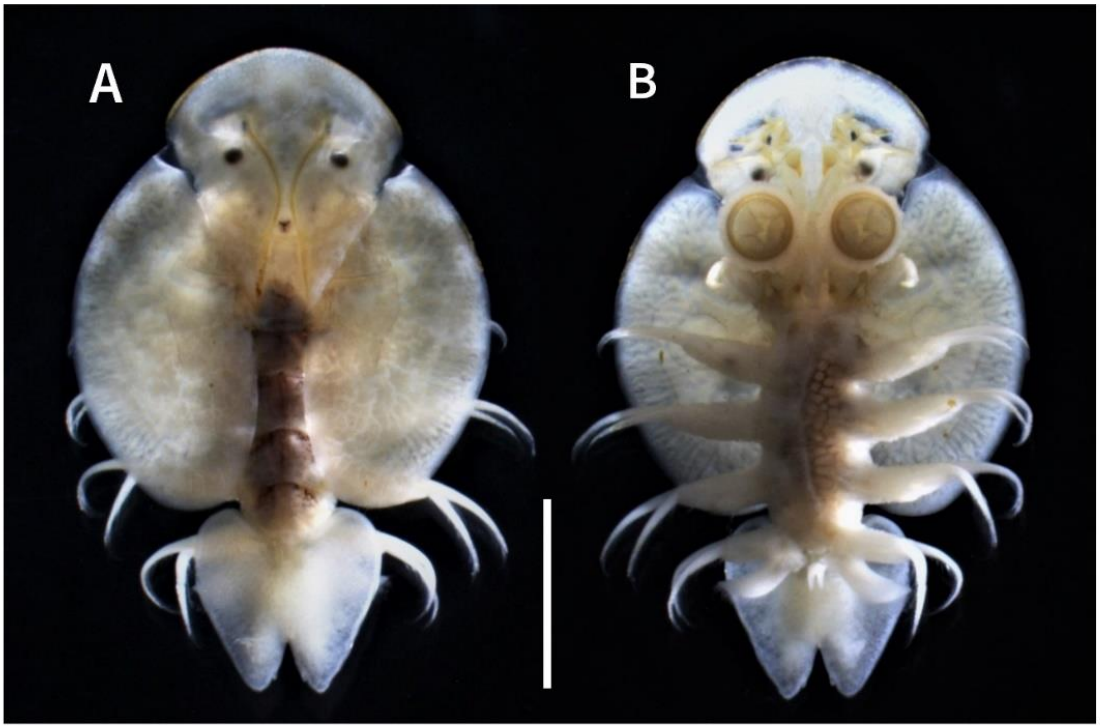

Fig. 2.1.4.5. *Argulus nobilis*, adult female, LBM1430006168, ethanol-preserved specimen. A, habitus, dorsal view; B, habitus, ventral view. This specimen was collected on 17 July 1992 and photographed on 2 October 2022. Scale bar: 3 mm.

2.2 Marine species

2.2.1 *Argulus caecus* C. B. Wilson, 1922

[Based on Nagasawa, K. and Hirose, M. (2021): Marine fish parasite *Argulus caecus* (Crustacea: Branchiura: Argulidae) accidentally collected from a fixed net caught squid in northern Japan. Species Diversity, 26: 289–296]

Abstract. An ovigerous female of a fish ectoparasite *Argulus caecus* C. B. Wilson, 1922 was collected from a squid, probably *Todarodes pacificus* (Steenstrup, 1880), from a fixed net installed in Otsuchi Bay, an inlet of the northwestern Pacific Ocean, Iwate Prefecture, northen Japan. Since the original description of *A. caecus* was insufficient, this paper reports on the morphology of the species based on a detailed examination of the female. In particular, the first and second antennae, the first and second maxillae, and four pairs of legs, whose features were poorly known, are reported in detail. The specimen of *A. caecus* is inferred to have detached itself and moved from a fish host, perhaps a coastal puffer, to the squid while these two animals were trapped in the net or when they were removed from the net. Following *Argulus scutiformis* Thiele, 1900, *A. caecus* is the second species of *Argulus* found from northern Japan located in the northern temperate or subarctic region, and its occurrence in this region is likely to be affected by the Tsushima Warm Current and its branch, the Tsugaru Warm Current, both of which flow off the coast of the region.

2.2.1 Introduction

Branchiurans of the argulid genus *Argulus* Müller, 1785 are skin parasites of freshwater and marine fishes (Yamaguti, 1963; Neethling and Avenant-Oldewage, 2016). To date, six species of the genus have been reported from marine fishes of Japan: *Argulus scutiformis* Thiele, 1900; *A. caecus* C. B. Wilson, 1922; *A. onodai* Tokioka, 1936; *A. matuii* Sikama, 1938; *A. kusafugu* Yamaguti and Yamasu, 1959; and *A. quadristriatus* Devaraj and Ameer Hamsa, 1977 (Yamaguti, 1963; Nishimura, 1995; Nagasawa, 2009, 2011a; Uyeno *et al.*, 2017). All of these species are known to occur in southern and central Japan, but only one species, *A. scutiformis*, has been reported from northern Japan (Thiele, 1904; Tokioka, 1936a).

An argulid branchiuran was found on the mantle of a squid from a fixed net in coastal Pacific waters of Iwate Prefecture, northern Japan (Kado *et al.*, 2021). Because argulids are fish parasites and can detach themselves from fish hosts, the argulid found is inferred to have moved from a fish host to the squid while these two animals were trapped in the net or when they were removed from the net. As described below, the argulid is identified as *A. caecus*. The species was originally described by Wilson (1922) based on a single specimen from central Japan and has been reported from Japan on a few occasions (Tokioka, 1936a; Anonymous, 1997; Kuramochi and Takahashi, 2010; Kondo *et al.*, 2013; Kado *et al.*, 2021). Nonetheless, the original description was insufficient, and the subsequent description by Tokioka (1936a) had only one figure showing a ventral view of the habitus. No morphological information was provided by the other authors (Anonymous, 1997; Kuramochi and Takahashi, 2010; Kondo *et al.*, 2013; Kado *et al.*, 2021). Therefore, based on a detailed examination of the newly collected specimen, this paper reports on the morphology of *A. caecus*, which is the second marine species of the genus from northern Japan.

2.2.1 Materials and methods

The argulid specimen was collected from the mantle of a squid [most probably *Todarodes pacificus* (Steenstrup, 1880) (Cephalopoda: Teuthida: Ommastrephidae), body size not measured] on 31 July 2013 from a fixed net installed in Otsuchi Bay, an inlet of the Northwestern Pacific Ocean, off Hiraisozaki, Otsuchi, Iwate Prefecture, northern Japan. No observation was made of the argulid's attachment on the mantle. The specimen was photographed, fixed, and later preserved in 70% ethanol. It was first examined under an Olympus SZX10 stereo microscope. Then, dissected body parts were cleared in lactophenol and examined under an Olympus BX51 phase-contrast compound microscope using the wooden slide procedure (Humes and Gooding, 1964; Benz and 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 and 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). The scientific and common names of coastal puffers of the genus *Takifugu* Abe, 1949 mentioned in this paper follow Dyldin *et al.* (2016) and Matsuura (2017), and those of other puffers follow Froese and Pauly (2023).

2.2.1 Results

Argulus caecus C. B. Wilson, 1922

[Japanese name: Hoso-umichou]

(Figs 2.2.1.1–2.2.1.4)

Argulus caecus C. B. Wilson, 1922: 1–4, figs 1–5; Tokioka, 1936a: 339–340, fig. 5; Yamaguti, 1963: 322, pl. 317, fig. 4 (monograph); Tokioka, 1965: 504, unnumbered fig. (encyclopedia); Tokioka, 1979: 403, unnumbered fig. (encyclopedia); Nishimura, 1995: 113–114, fig. 21–79C (encyclopedia); Anonymous, 1997: 47; Nagasawa, 2009: 137–138 (list); Kuramochi and Takahashi, 2010: 5, figs 1–2; Nagasawa, 2011a: 19 (review); Kondo *et al.*, 2013: 157–159, figs 1–3; Neethling and Avenant-Oldewage, 2016: 1331 (monograph); Kado *et al*., 2021: 144, unnumbered fig.

Host. Unknown. The argulid specimen was found on the mantle of a squid.

Locality. Otsuchi Bay off Hiraisozaki (39°19′59.00″N, 141°55′30.00″E), Otsuchi, Iwate Prefecture, northern Japan.

Material examined. Ovigerous female (NSMT-Cr 29002), 22.0 mm in total length (TL, from anterior tip of carapace to posterior tip of abdomen), 12.5 mm in maximum body width (around midlength of carapace).

Adult female (based on one specimen). *Carapace* (including posterolateral lobes) elliptical, covering all pairs of legs in dorsal view, measuring 18.9 mm, as long as 85.9% of TL (Figs 2.2.1.1A, 2.2.1.4). Frontal region of carapace delimited by anterolateral indentations.

Compound eyes scarcely visible in freshly dead specimen, and not visible after ethanol preservation. Nauplius eye not visible. Dorsal surface of carapace smooth without spines. Ventral surface of marginal frontal and lateral regions of carapace ornamented with numerous, small sharply pointed spines (Fig. 2.2.1.1B). Posterolateral lobes of carapace 16.9 mm long, comprising 89.4% of carapace length, ending in rounded margin, separated by sinus nearly 1/2 length of carapace. Respiratory areas comprising small, elliptical anterior area and large, reniform posterior area, located at levels of second maxillae and first and second legs, respectively (Figs 2.2.1.1B, 2.2.1.2B). *Thorax* with four segments (Fig. 2.2.1.1A, B). *Abdomen* shield-shaped, slightly longer than broad (6.1 × 4.9 mm), and partially covered by posterior region of posterolateral lobes of carapace; posterior lobes with weakly pointed tips (Figs 2.2.1.1A, B, 2.2.1.1.4). Anal indentation 35.6% as long as abdomen. Paired spermathecae elongate in anterocentral region of abdomen (Fig. 2.2.1.1B). Caudal rami located at base of anal indentation with four naked setae on each ramus (Fig. 2.2.1.2A).

First antennae with four segments (Fig. 2.2.1.2C): first segment heavily sclerotized in mesial and posterior regions, with two projections on posterior margin (mesial projection larger than other); second segment also heavily sclerotized, with large projection at about middle of anterior margin, strong apically bent hook at lateral corner, and large ventral projection near posterobasal margin; third segment cylindrical, with naked seta; apical segment shorter than third, with at least four naked setae at tip. *Second antennae* with five segments (Fig. 2.2.1.2C): first segment sclerotized, with spine and small swelling bearing four naked setae on posterior margin; second segment ovoid, with two naked setae on posterior margin; third, fourth, and apical segments nearly cylindrical and decreasing in length, possessing, respectively, 14, five, and at least two apical naked setae (Fig. 2.2.1.2D). Postantennal spines large and robust, located posterior each to mesial hook of first segment of first antenna (Fig. 2.2.1.2C). Preoral sheath visible on ventral midline of frontal region of carapace between first maxillae (Fig. 2.2.1.1C). Mouth tube located just posterior to preoral sheath, nearly cylindrical, composed of small anterior labrum and larger posterior labium (Fig. 2.2.1.1C).

First maxillae forming suckers, 4.1 and 4.3 mm in diameter (32.8 and 34.4% as wide as carapace, respectively), with 54 or 55 supporting rods in sucker membrane (Fig. 2.2.1.2E).

Supporting rods becoming slightly wider toward midlength but tapering distally, composed of 27–33 (mean = 30, n = 10) sclerites per rod (Fig. 2.2.1.2F). Sclerites mostly trapezoidal and crescent-shaped, respectively, in basal and other regions of supporting rod, often imbricated around midlength of rod. Outer margin of rim of sucker membrane with numerous tiny projections.

Second maxillae with five segments (Fig. 2.2.1.2G); first segment robust, with three (one anterolateral, one nearly centrolateral, and one posterolateral) blunt projections (anterolateral one largest), corpus of first segment with raised patch of small rectangular denticles in anterior region and apically sharp simple denticles in mid- and posterior regions; second segment elongate with distal raised patch of serrated scale-like denticles (Fig. 2.2.1.2I) in anterior and mid-regions and simple acutely pointed denticles in posterior region; third segment also elongate but narrower than second, with raised patch of serrated scale-like denticles in anterobasal region and simple denticles in mid- and distal regions; fourth segment subquadrate and shorter than third, with raised patch of simple denticles; terminal segment (Fig. 2.2.1.2H) with blunt tip and two spiniform projections, partially surrounded by anterior swelling region. Accessory spines located near ventral midline, slightly apart from bases of second maxillae (Fig. 2.2.1.1C). Postmaxillary spines small and located anteriorly on first segment of thorax (Fig. 2.2.1.1C).

First to fourth pairs of legs (Fig. 2.2.1.3) biramous and of almost equal size; sympods composed of coxa and basis, those of first to third legs covered with small scale-like projections anteriorly; sympods of fourth legs and rami with small projections; first legs each possessing flagellum. *First leg* (Fig. 2.2.1.3A, B) exopod unsegmented, with 17 and 20 plumose setae, respectively, on anterior and posterior margins, and two plumose setae at tip; endopod three-segmented, with 18 and 15 plumose setae, respectively, on anterior and posterior margins of proximal segment, naked seta near anterolateral corner of middle segment, and three short spines at tip of terminal segment; flagellum projecting from exopod and extending to proximal margin of coxa, with seven plumose and two naked setae on anterior margin, 21 plumose and two naked setae on posterior margin, and plumose seta at tip. *Second leg* (Fig. 2.2.1.3C) coxa with 12 plumose setae on posterior margin; exopod unsegmented, with 17 and 20 plumose setae, respectively, on anterior and posterior margins; endopod unsegmented, with 15 and 16 plumose setae, respectively, on anterior and posterior margins. *Third leg* (Fig. 2.2.1.3D) basis bearing nine plumose setae on posterior margin; exopod unsegmented, with 16 and 18 plumose setae, respectively, on anterior and posterior margins; endopod two-segmented, proximal segment with 13 and 12 plumose setae, respectively, on anterior and posterior margins, terminal segment with naked seta and 11 plumose setae on anterior margin, 11 plumose setae on posterior margin, two short naked setae near tip, and two short naked setae at tip. *Fourth leg* (Fig. 2.2.1.3E) coxa forming posteriorly expanded natatory lobe bearing three plumose setae on distal margin; basis larger than coxa, bearing four plumose setae on posterior margin; exopod weakly two-segmented, terminal segment with at least nine plumose setae on posterior margin; endopod two-segmented, proximal segment with 11 plumose setae on posterior margin, terminal segment with six plumose setae on posterior margin.

Color (based on a freshly dead specimen)**.** Dorsal surface with dark brown pigmentation in carapace, thorax, and abdomen, forming 3 irregularly shaped longitudinal stripes in frontal region of carapace and 3 wider longitudinal stripes in each posterolateral lobe of carapace (mesial stripe widest and combined with central stripe at midlength of posterolateral lobe). Unpigmented area found between central and lateral stripes in each posterolateral lobe. Longitudinal stripes also present on thorax and abdomen. Pigmentation of body weakly visible in ethanol-preserved specimen. Respiratory areas fringed by continuous black pigment. Mouth tube with weak ventral pigmentation (Fig. 2.2.1.4).

Remarks. Without information on its host, *A. caecus* was originally described by Wilson (1922) based on a single female collected at Aburatsubo (type locality, incorrectly reported as "Aburazubo"), Misaki, Sagami (now Kanagawa Prefecture), Japan. The type locality is located on the southwest coast of the Miura Peninsula, facing Sagami Bay, an inlet of the Northwestern Pacific Ocean. There were several problems in the original description of *A. caecus*, especially regarding projections on the first and second antennae and recognition of segments of both antennae. Wilson (1922) found "two spines" on the "basis" of the "second antenna", but this is incorrect, and as described above, the "spines" are found as two projections on the posterior margin of the first segment of the first (not second) antenna (Fig. 2.2.1.2C). Wilson (1922) did not differentiate antennal segments and stated that the first antenna had “strong hooks on the anterior and lateral margins, and a stout spine on the posterior margin”. However, the former “hooks” are actually found on the second segment of the first antenna (Fig. 2.2.1.2C), and the latter “spine” is located as a large projection on the posterobasal margin of the same segment (Fig. 2.2.1.2C). In spite of such problems in the original description, *A. caecus* was characterized by a large body (19.0 mm long), invisible compound eyes, about 30 sclerites in each supporting rod, and well-armed second maxillae (Wilson, 1922). The female specimen collected in the present study has these characters and is identified as *A. caecus*.

Tokioka (1936a) also reported the morphology of female *A. caecus* from puffers at various localities on the Pacific coast of Japan. His specimens were almost identical to the original description of *A. caecus*, but the sclerites in supporting rods were fewer (20–30) than those recorded in the original description (about 30) and in this paper (27–33, mean = 30). This difference may be caused by the fact that his specimens were smaller (up to 16 mm long) than holotype (19.0 mm long) and the specimen collected in this study (22.0 mm long) because the number of sclerites per supporting rod is known to increase with increasing body size (Benz *et al.*, 1995).

Wilson (1922) reported that the tips of the posterior lobes of the abdomen were rounded, but Tokioka’s specimens had posterior lobes with “pointed or blunt” tips. Those lobes of the specimen collected in this study have weakly pointed tips (Figs 2.2.1.1, 2.2.1.4). Tokioka (1936a) also noted the presence of a large spine and a process on the first segment of the second antenna. These structures correspond in this paper to a spine and a small swelling near the spine on the posterior margin of the same segment (Fig. 2.2.1.2C). In addition, Tokioka (1936a) reported on a single seta on the posterior margin of the coxa of the first leg, but no seta was found on the specimen examined (Fig. 2.2.1.3A).

The specimen of *A. caecus* collected in this study carries flagella on the first legs. As known for other *Argulus* species (Boxshall and Jaume, 2009), the flagellum originates from the base of exopod of each leg (Fig. 2.2.1.3A).

Argulus caecus was reported to be beautifully colored when alive: the body was light blue with patterns of brown pigments (Tokioka, 1936a). However, the freshly dead specimen collected in this study did not have such a colored body, and brilliant coloration perhaps disappeared immediately after its death.

Argulus caecus resembles *A. scutiformis*, one of the six congeneric species reported from Japan, in female's large body [19.0 mm long (Wilson, 1922), up to 16.0 mm (Tokioka, 1936a), 22.0 mm long (this paper) in *A. caecus*, and more than 30.0 mm (Tokioka, 1936a), 13.6–19.8 mm long (Yamaguti and Yamasu, 1959) in *A. scutiformis*]. The female's abdomen, however, differs in shape between these two species: *A. caecus* has an elliptical or shield-shaped abdomen (Wilson, 1922; Tokioka, 1936a; this paper), whereas *A. scutiformis* has a rounded abdomen (Tokioka, 1936a; Yamaguti and Yamasu, 1959). According to Tokioka (1936a), the number of sclerites per supporting rod was also different between the two species (20–30 in *A. caecus* vs. 30–40 in *A. scutiformis*), but the specimens of *A. scutiformis* reported by Yamaguti and Yamasu (1959) had fewer sclerites (18–24 per rod), which indicates that the number of sclerites is variable in this species and cannot be always used to differentiate it from *A. caecus*. As stated above, such variations in number of sclerites per supporting rod are closely related to body size in *Argulus* species (Benz *et al.*, 1995), and Yamaguti and Yamasu's specimens were much smaller (13.6–19.8 mm) than those (more than 30.0 mm) collected by Tokioka (1936a).

Kuramochi and Takahashi (2010) showed two photographs of *A. caecus*, one is a ventral view of the habitus of one specimen that resembles the female reported herein. The authors collected their specimens from the vermiculated puffer *Takifugu snyderi* (Abe, 1988) in coastal waters of Sagami Bay off Ashina, Kanagawa Prefecture, about 8 km north from the type locality (Aburatsubo). The present record of *A. caecus* from Otsuchi Bay (Kado *et al.*, 2021; this paper) has extended its distribution range from Kanagawa Prefecture, northward to Iwate Prefecture, along the Pacific coast of Japan. Tokioka (1936a) similarly collected *A. caecus* from the Pacific coast, but no detailed information was provided on the collection localities.

There are two records of *A. caecus* from the Sea of Japan off Honshu, Japan (Anonymous, 1997; Kondo *et al.*, 2013) but much remains obscure in the identification of the species from the sea. Without any literature citation and information on its morphology and host, *A. caecus* was listed in a catalogue of the animals collected in coastal waters of Sado Island, Niigata

Prefecture (Anonymous, 1997). This species was also collected in Hibiki-nada, Yamaguchi Prefecture, from grass puffer *Takifugu alboplumbeus* (Richardson, 1845) [Kondo *et al.*, 2013, as *T. niphobles* (Jordan and Snyder, 1901); Masakazu Kondo, National Fisheries University, Shimonoseki, personal communication], but its morphology was not reported because Kondo *et al.* (2013) focused on the hemocytes of *A. caecus*. Therefore, no information has been published on the morphology of the argulid from the Sea of Japan, and it is necessary to conduct taxonomic work using the specimens previously reported as *A. caecus* and newly collected material from this sea in order to confirm whether the species actually occurs there.

2.2.1 Discussion

Argulus caecus has so far been reported only from coastal puffers (Tokioka, 1936a; Kuramochi and Takahashi, 2010; Kondo *et al.*, 2013), which suggests that these fishes are preferred hosts of the parasite. Nevertheless, only two species of coastal puffers (*Takifugu snyderi* and *T. alboplumbeus*) are known as the hosts of *A. caecus*. Tokioka (1936a) simply reported the infected coastal puffers as "*Spheroides* spp.", and it is impossible to determine the species. To date, as many as 20 species of *Takifugu* have been reported in Japan, and some of them are widely distributed in coastal waters (Yamada and Yagishita, 2013). Therefore, for clarifying the host utilization and geographical distribution of *A. caecus*, it is desirable to examine various species of coastal puffers from a wide range of Japanese waters.

In the present study, the female of *A. caecus* was taken from the mantle of a squid caught in a fixed net in Otsuchi Bay. However, as stated in the Introduction, the squid is unlikely to be a true host of *A. caecus*. Three species of *Takifugu*, *i.e.*, grass puffer *T. alboplumbeus* (= *T*. *niphobles*); panther puffer *T. pardalis* (Temminck and Schlegel, 1850) (Tatsukawa and Tanaka, 1982); and fine patterned puffer *T. pardalis* (Temminck and Schlegel, 1850) [Goto *et al*., 2017, as *T. poecilonotus* (Temminck and Schlegel, 1850)] have been recorded from Otsuchi Bay, and some other species of puffers [spottyback puffer *T. stictonotus* (Temminck and Schlegel, 1850); yellowfin puffer *T. xanthopterus* (Temminck and Schlegel, 1850); purple puffer *T. porphyreus* (Temminck and Schlegel, 1850); brown-lined puffer *Canthigaster rivulata* (Temminck and Schlegel, 1850); starry toado *Arothron firmamentum* (Temminck and

Schlegel, 1850); and stellate puffer *Ar. stellatus* (Anonymous, 1798)] are also found in the bay [Naoya Ohtsuchi, International Coastal Research Center (ICRS), Atmosphere and Ocean Research Institute, The University of Tokyo, Otsuchi, personal communication]. These coastal puffers are potential hosts for *A. caecus*.

Two species of *Argulus* have been reported from northern Japan: *A. scutiformis* from Hakodate and an unspecified locality in Hokkaido (Thiele, 1904; Tokioka, 1936a) and *A. caecus* from Otsuchi Bay, Iwate Prefecture (Kado *et al.*, 2021; this paper). Northern Japan lies in the northern temperate or subarctic region, but the seas surrounding this region are affected by two warm currents. The Tsushima Warm Current, a branch of the Kuroshio Current, flows off the west coast of northern Honshu and Hokkaido, and the Tsugaru Warm Current, a branch of the Tsushima Warm Current, flows in the Tsugaru Strait off Hakodate, southern Hokkaido and off the Pacific coast and Otsuchi Bay, northern Honshu (Yasuda *et al.*, 1988). Six species of *Argulus*, including *A. scutiformis* and *A. caecus*, have been reported from southern and central Japan influenced by two warm currents, the Kuroshio and the Tsushima Warm Current. The occurrence of *A. scutiformis* and *A. caecus* in northern Japan may be similarly affected by the Tsushima Warm Current and its branch, the Tsugaru Warm Current.

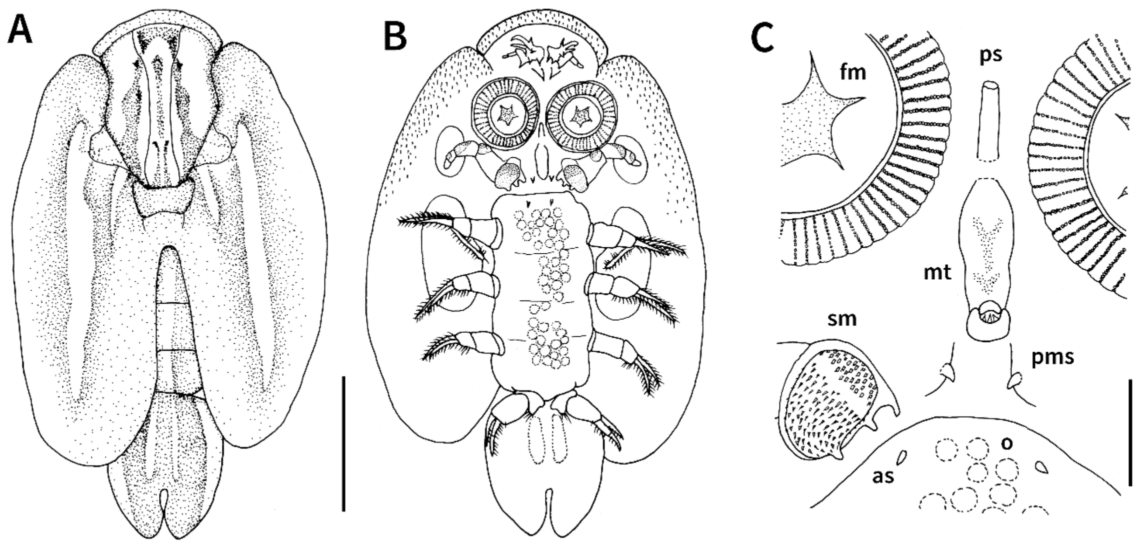

Fig. 2.2.1.1. *Argulus caecus*, ovigerous female, NSMT-Cr 29002. A, habitus, dorsal view; B, habitus, ventral view; C, central part of body, ventral view. Abbreviations: as, accessory spine; fm, first maxilla; mt, mouth tube; o, ova; pms, postmaxillary spine; ps, preoral sheath; sm, second maxilla. Scale bars: A, B, 5mm; C, 1mm.

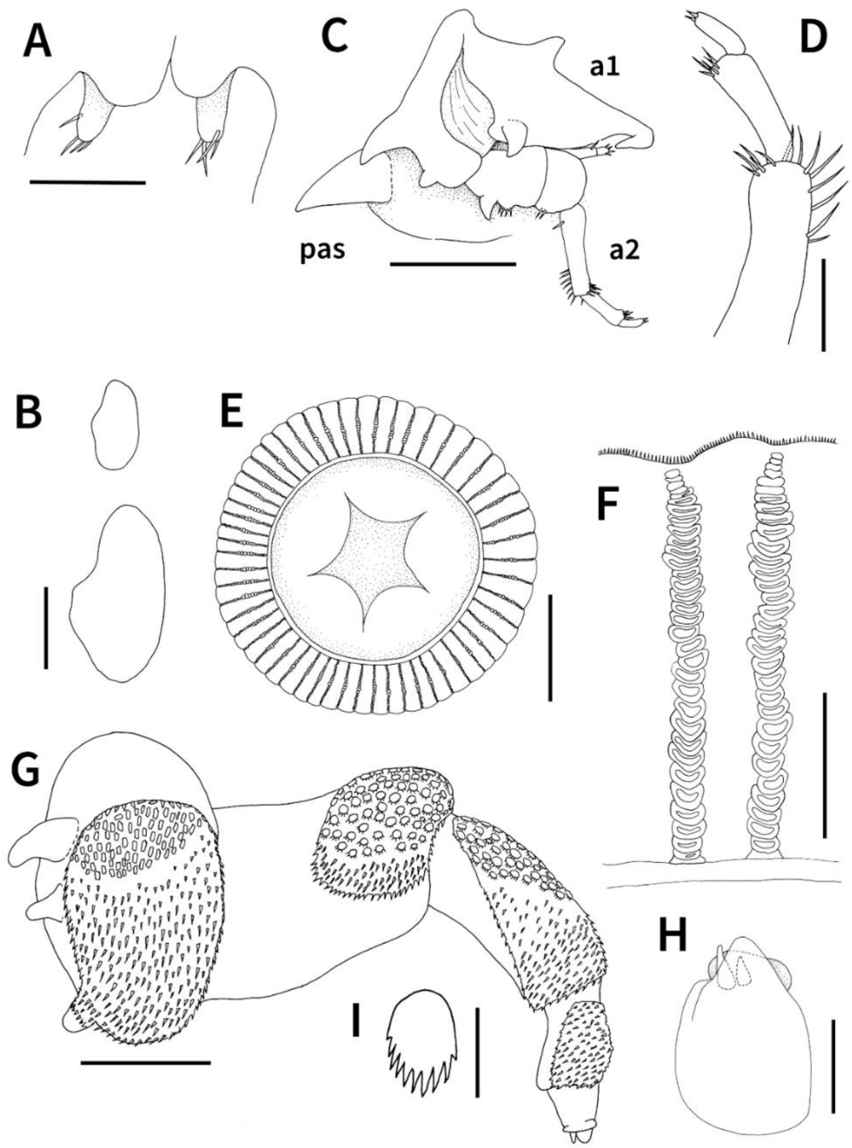

Fig. 2.2.1.2. *Argulus caecus*, ovigerous female, NSMT-Cr 29002. A, caudal rami, dorsal view; B, respiratory areas, ventral view; C, first and second antennae, ventral view; D, distal part of endopod of second antenna, ventral view; E, first maxilla, ventral view; F, supporting rods each composed of series of sclerites, ventral view; G, second maxilla, ventral view; H, terminal segment of second maxilla, dorsal view; I, serrated scale-like denticle on second segment of second maxilla, ventral view. Abbreviations: a1, first antenna; a2, second antenna; pas, postantennal spine. Scale bars: A, D, F, H, 0.2mm; B, 2mm; C, G, 0.5mm; E, 1mm; I, 0.02mm.

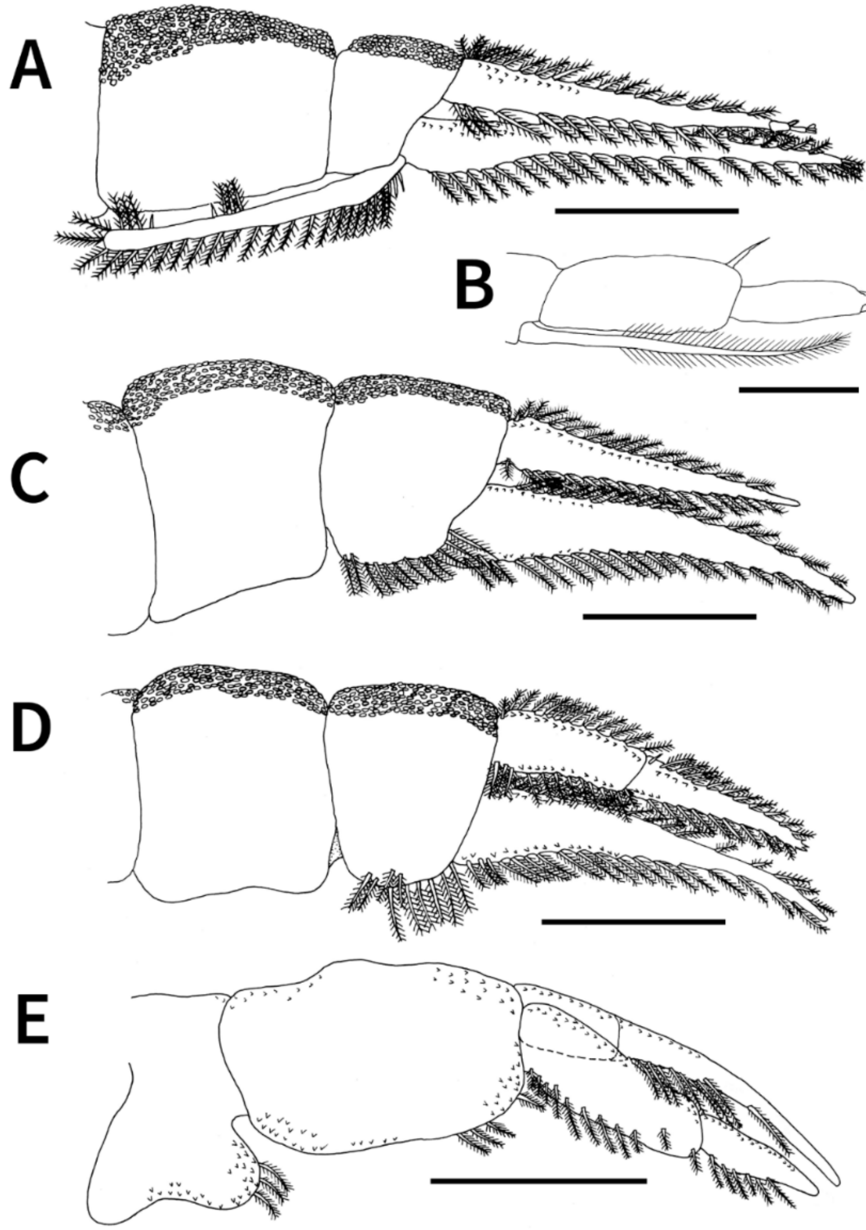

Fig. 2.2.1.3. *Argulus caecus*, ovigerous female, NSMT-Cr 29002. A, first leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view. Scale bars: A, C–E, 1mm; B, 0.1mm.

Fig. 2.2.1.4. *Argulus caecus*, ovigerous female, freshly dead specimen, NSMT-Cr 29002. Dorsal view. Scale bar: 5 mm.

2.2.2 *Argulus matuii* Sikama, 1938

2.2.2.1 *Argulus matuii* from yellowfin seabream, with a note on the body coloration in an ethanol-preserved argulid specimen

[Based on Nagasawa, K. and Mizuno, K. (2022): *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. Crustacean Research, 51: 31–38]

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 thesis 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 sucker membranes of the first maxillae.

2.2.2.1 Introduction

The argulid branchiuran *Argulus matuii* Sikama, 1938 is an ectoparasite of marine fishes in Japan (Nagasawa, 2009, 2011a). 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* infected three fish species held in an aquarium, *i.e.*, white trevally *Pseudocaranx dentex* (Bloch and Schneider, 1801) (reported as "*Caranx delicalissimus*"), gnomefish *Scombrops boops* (Houttuyn, 1782), and red seabream *Pagrus major* (Temminck and Schlegel, 1843) (reported as *Pagrosomus major*). Subsequently, *A. matuii* was collected from bastard halibut *Paralichthys olivaceus* (Temminck and Schlegel, 1846) cultured in Oita Prefecture (Nagasawa and Fukuda, 2009) and white trevally in the Northwestern Pacific off Kanagawa and Chiba prefectures (Saito and Nagasawa, 2010; Nagasawa *et al.*, 2021c). 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.*, 2021c) 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.

2.2.2.1 Materials and methods

A yellowfin seabream (290 mm in total length) was caught using hook and line on 27 May 2017 in a cove 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 and Gooding (1964) and Benz and Otting (1996). The body coloration of the specimen was observed and photographed on both 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 and 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). The scientific and common names of fishes mentioned in this paper follow those in FishBase (Froese and Pauly, 2023).

2.2.2.1 Results

Argulus matuii Sikama, 1938

[Japanese name: Matsui-umichou]

(Figs 2.2.2.1.1–2.2.2.1.3)

Host. Yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782) (Perciformes: Sparidae).

Attachment site. Body surface near the left pectoral fin.

Locality. A cove (33°10'17"N, 132°30'52"E) facing the Bungo Channel off Miura-Higashi, Uwajima, Ehime Prefecture, Japan.

Material examined. An adult female (NSMT-Cr 30676) from a yellowfin seabream *A. latus*.

Adult female (based on one specimen)**.** The argulid collected was an ovigerous female, measuring 7.9 mm in total length (TL, from anterior tip of carapace to posterior tip of abdomen) and 3.8 mm in maximum body width (around 2/5 length of carapace from its anterior tip). *Body* dorsoventrally flattened. *Carapace* elliptical, 6.2 mm long, comprising 78% of TL, almost totally covering first to second pairs of legs (Fig. 2.2.2.1.1A, 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. 2.2.2.1.1C). *Thorax* with four segments; ventral surface covered with numerous minute scales (Fig. 2.2.2.1.1B). *Abdomen* longer than wide, bilobed by anal indentation; each lobe ending in pointed tip (Fig. 2.2.2.1.1A, B). Paired spermathecae oval in anterior region of abdomen (Fig. 2.2.2.1.1B). Caudal rami small and rounded, located at base of anal indentation (no setae visible on each ramus) (Fig. 2.2.2.1.1D).

First antennae with four segments (Fig. 2.2.2.1.1E); 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 damage, no observation possible on setae of third and apical segments). *Second antennae* with five segments (Fig. 2.2.2.1.1E); 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. 2.2.2.1.1E). Preoral sheath on ventral midline of frontal region of carapace, with anterior part of stylet protruding from sheath opening (Fig. 2.2.2.1.1F). *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. 2.2.2.1.1G).

First maxillae forming cup-like suckers (Fig. 2.2.2.1.1B, H), with 90 and 91 supporting rods each in two sucker 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. 2.2.2.1.1I); 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. 2.2.2.1.1B). Postmaxillary spines located just in front of first thoracic segment (Fig. 2.2.2.1.1B).

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. 2.2.2.1.2). 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. 2.2.2.1.2A, B). Sympods of second and third pairs of legs without setae (Fig. 2.2.2.1.2C, D). Endopods of third and fourth pairs of legs two-segmented (Fig. 2.2.2.1.2D, 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. 2.2.2.1.2E).

Color (based on fresh and 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).

Color on 7 February 2018 (Fig. 2.2.2.1.3A, 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.

Color on 4 December 2021 (Fig. 2.2.2.1.3C, 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 and Nagasawa (2009), and the specimen is thus identified as *A. matuii*. As mentioned below, the species is characterized by numerous supporting rods in the sucker 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 congeneric species from Japan. *Argulus matuii* has more supporting rods (70–95 and 75–83 rods, respectively, in the female and male: Sikama, 1938; Saito and Nagasawa, 2010; this paper) than the other species: 66 or 67 rods in the female of *A. scutiformis* Thiele, 1900 (Yamaguti and Yamasu, 1959); 54 or 55 rods in the female of *A. caecus* C. B. Wilson, 1922 (Nagasawa and Hirose, 2021); 54–62 rods in the female of *A. kusafugu* Yamaguti and Yamasu, 1959 (Yamaguti and 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, 1936a).

Several minor morphological differences are found between the present and previously reported specimens of *A. matuii*. For example, the female shown by Saito and Nagasawa (2010, fig. 1) had a "pentagon-shaped" carapace, but the females reported by other scientists (Sikama, 1938; Nagasawa *et al*., 2021c; this paper) had an "elliptical" carapace. The four females reported by Saito and Nagasawa (2010) were as large as 16.2–18.7 mm TL, but the two females reported by Nagasawa *et al.* (2021c) 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 and 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. 2.2.2.1.1I). Further, the endopods of the second and third legs illustrated by Saito and 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. 2.2.2.1.2C, 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 redescription of the species (Saito and Nagasawa, 2010). However, the male and female of *A. matuii* photographed by Nagasawa *et al.* (2021c) showed a clear difference in the shape of the carapace and abdomen. A redescription 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 and Fukuda, 2009; Saito and Nagasawa, 2010; Nagasawa *et al*., 2021c). Yellowfin 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.

2.2.2.1 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.* (2021c), 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. 2.2.2.1.3A, 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. 2.2.2.1.3C, 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 and 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 and Nagasawa, 2010; Nagasawa *et al*., 2021c), 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.

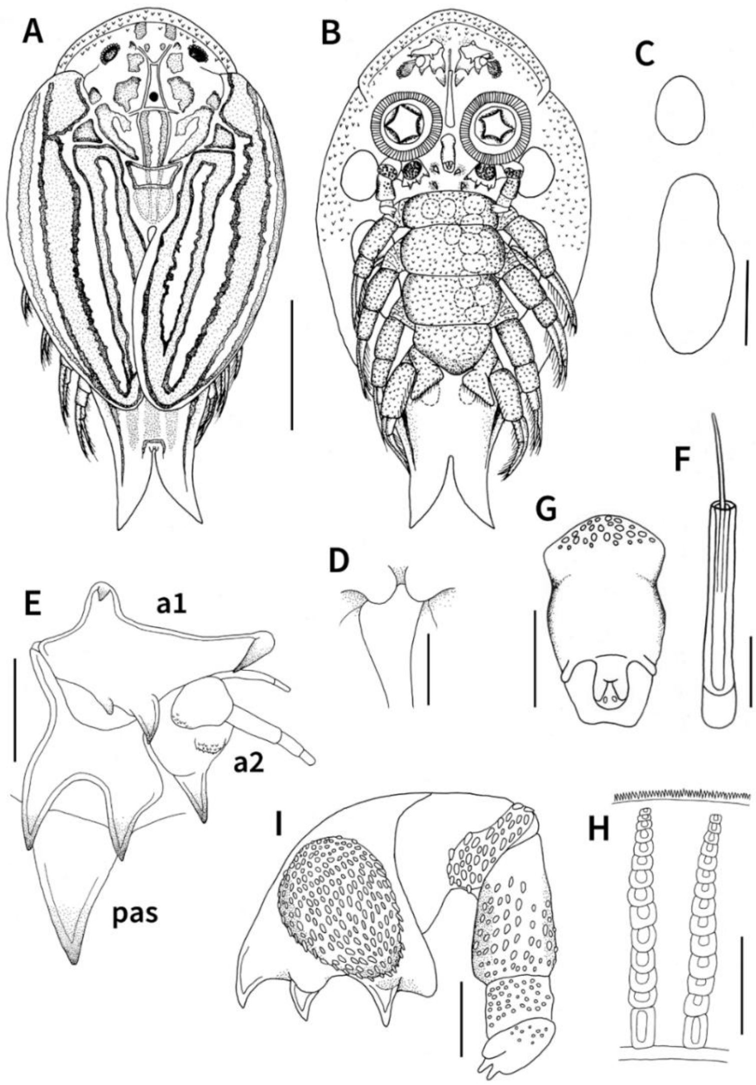

Fig. 2.2.2.1.1. *Argulus matuii*, ovigerous female (7.9 mm total length), NSMT-Cr 30676, from a yellowfin seabream *Acanthopagrus latus* in coastal waters of Ehime Prefecture, Japan. A, habitus, dorsal view; B, habitus, ventral view; C, right respiratory areas, ventral view; D, caudal rami at base of 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 sucker 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.

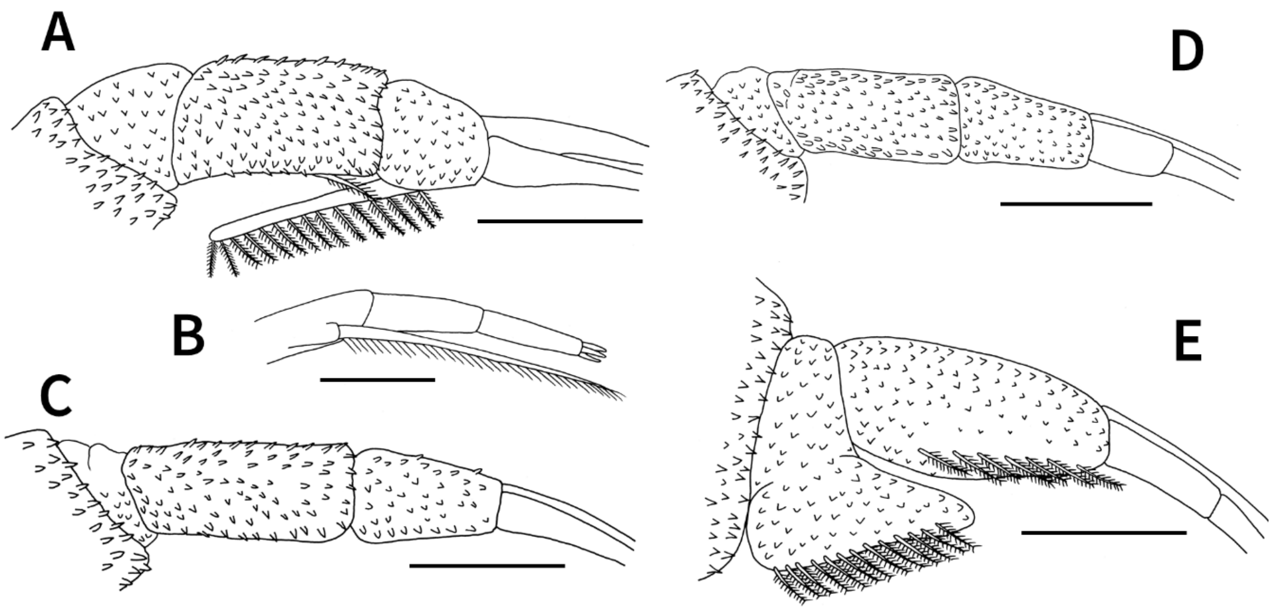

Fig. 2.2.2.1.2. *Argulus matuii*, ovigerous female (7.9 mm total length), NSMT-Cr 30676, from a yellowfin seabream *Acanthopagrus latus* in coastal waters of Ehime Prefecture, Japan. A, left first leg, ventral view; B, second to apical segments of endopod of left first leg, ventral view; C, left second leg, ventral view; D, left third leg, ventral view; E, left fourth leg, ventral view. Distal part of exopods and endopods and setae projecting from these rami are not illustrated in Fig. 2.2.2.1.2A and C–E. Scale bars: A, C–E, 0.5 mm; B, 0.1 mm.

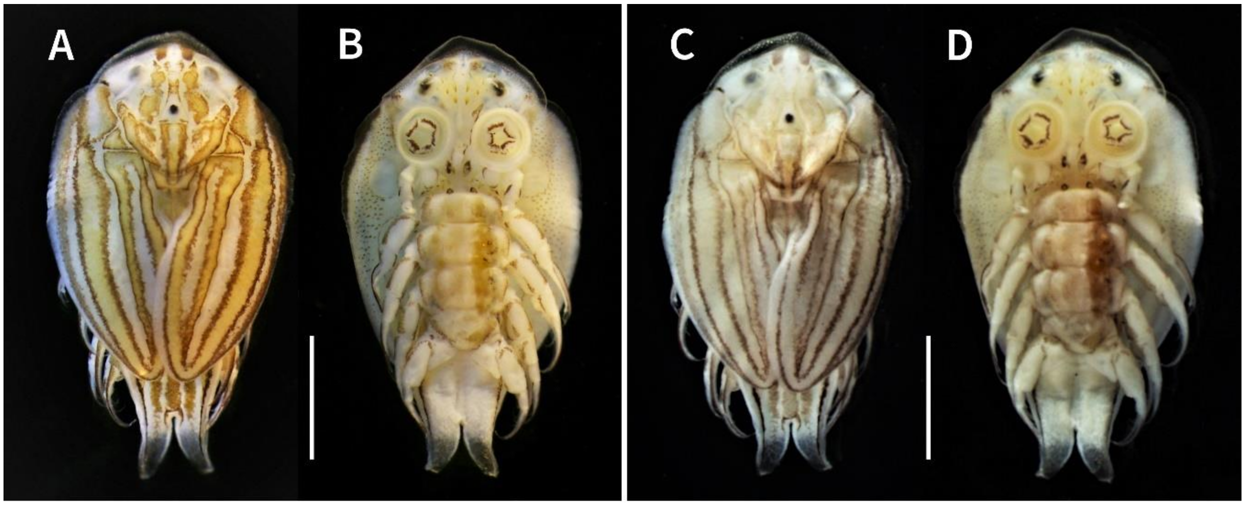

Fig. 2.2.2.1.3. *Argulus matuii*, ovigerous female (7.9 mm total length), NSMT-Cr 30676, from a yellowfin seabream *Acanthopagrus latus* in coastal waters of Ehime Prefecture, Japan. The specimen was fixed in 70% ethanol on 27 May 2017 and photographed on 7 February 2018 (A, B) and 4 December 2021 (C, D). A and C, habitus, dorsal view; B and D, habitus, ventral view. Scale bars: 2 mm.

2.2.2.2 *Argulus matuii* from crescent sweetlips

[Based on Nagasawa, K. and Shirakashi, S. (2022): Crescent sweetlips *Plectorhinchus cinctus* (Teleostei: Haemulidae), a new host for a marine fish parasite *Argulus matuii* (Branchiura: Argulidae). Biogeography, 24: 106–108]

Abstract. An adult female of *Argulus matuii* Sikama, 1938 was collected from the body surface of a crescent sweetlips *Plectorhinchus cinctus* (Temminck and Schlegel, 1843) in coastal waters of the Northwestern Pacific Ocean near Minabe, Wakayama Prefecture, central Japan. This represents a new host record for *A. matuii*. The female is briefly described, and the species is characterized by having numerous supporting rods (n = 90 and 92) in the sucker membranes of the first maxillae. Bright body coloration is known as a distinguished character to differentiate *A. matuii* from the other Japanese marine congeners in fresh specimens, but such coloration faded in the ethanol-preserved female specimen.

2.2.2.2 Introduction

Argulus matuii Sikama, 1938 is a branchiuran argulid infecting the body surface of marine fishes in Japan (Nagasawa, 2009, 2011a). Six species of marine teleosts have been reported as hosts of *A. matuii*, consisting of three wild species [chicken grunt *Parapristipoma trilineatum* (Thunberg, 1793), white trevally *Pseudocaranx dentex* (Bloch and Schneider, 1801), yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782); Sikama, 1938; Saito and Nagasawa, 2010; Nagasawa *et al*., 2021c; Nagasawa and Mizuno, 2022], three aquarium-held species [white trevally, gnomefish *Scombrops boops* (Houttuyn, 1782), red seabream *Pagrus major* (Temminck and Schlegel, 1843); Sikama, 1938], and one farmed species [bastard halibut *Paralichthys olivaceus* (Temminck and Schlegel, 1846); Nagasawa and Fukuda, 2009]. During a parasitological survey of marine fishes from central Japan, a branchiuran identified as *A. matuii* was collected from a crescent sweetlips *Plectorhinchus cinctus* (Temminck and

Schlegel, 1843). This collection is reported herein as a new host record for *A. matuii*, and the crescent sweetlips is the seventh host of *A. matuii*. This paper also reports on body color fading in the ethanol-preserved branchiuran specimen.

2.2.2.2 Materials and methods

The crescent sweetlips (595 mm long) was collected in coastal waters of the Northwestern Pacific Ocean near Minabe (33°45'31''N, 135°19'23''E), Wakayama Prefecture, on 14 June 2017. The branchiuran was carefully removed from the body surface of the fish by hand, fixed and preserved in 70% ethanol. It was examined for its morphology and body coloration using an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope using the wooden slide technique (Humes and Gooding, 1964: Benz and Otting, 1996) on 22 March 2022 (ca. four years and nine months after fixation). Morphological terminology follows Benz *et al.* (1995) and Benz and Otting (1996). The branchiuran has been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 30789). The scientific and common names of fishes mentioned in this paper follow those in FishBase (Froese and Pauly, 2023).

2.2.2.2 Results and discussion

The branchiuran was an adult female (Fig. 2.2.2.2.1), measuring 9.1 mm in total length (from anterior tip of carapace to posterior tip of abdomen) and 4.5 mm in maximum body width (around midlength of carapace). It is characterized by a dorsoventrally flattened body; an elliptical carapace with a slightly anteriorly protruding frontal region, covering first to third pairs of legs; posterolateral lobes of carapace separated by sinus, ending each in rounded margin; compound eyes visible in frontal region of carapace; naupliar eye present dorsally along midline of carapace; ventral surface of frontal and lateral regions of carapace ornamented with numerous, small sharply pointed spines; first maxillae forming cup-like suckers, with 90 and 92 supporting rods each in two sucker membranes; four-segmented thorax ventrally covered with numerous minute projections; bilobed abdomen ending in pointed tip; and fourth pair of legs each with coxa forming large, ventrally foot-shaped natatory lobe.

The above morphological characters correspond to those of female *A. matuii* reported by Sikama (1938), Saito and Nagasawa (2010), and Nagasawa and Mizuno (2022). Further, the species has been reported to have numerous supporting rods per sucker membrane of the first maxilla (70–95 and 75–83 rods in the female and male, respectively: see Nagasawa and Mizuno, 2022). This is also confirmed in the female examined in this paper (90 and 92 rods). The other Japanese marine *Argulus* species have fewer supporting rods (66 or 67 rods in the female of *A. scutiformis* Thiele, 1900; 54 or 55 rods in the female of *A. caecus* C. B. Wilson, 1922; 54–62 rods in the female of *A. kusafugu* Yamaguti and Yamasu, 1959; and 58–61 rods in the male of *A. quadristriatus* Devaraji and Ameer Hamsa, 1977 (see Nagasawa and Mizuno, 2022 for the literature).

It is known that fresh specimens of *A. matuii* have prominent streaks of brilliant yellow on the dorsal surface of posterolateral lobes of the carapace (Sikama, 1938; Nagasawa and Fukuda, 2009; Nagasawa *et al.*, 2021c). Sikama (1938) says that the bright coloration was "kept for nearly two years in 10% formalin". Nonetheless, in the ethanol-preserved specimen examined herein, such streaks faded, and a total of 14 longitudinal dark brown stripes (seven stripes per lobe) fringed the streaks (Fig. 2.2.2.2.1A). This is a result from fading of the yellow streaks. Recently, Nagasawa and Mizuno (2022) reported a similar body color fading with time in *A. matuii* and emphasized the importance to make a detail examination of morphological characters in indentification of long-term ethanol-preserved argulid specimens.

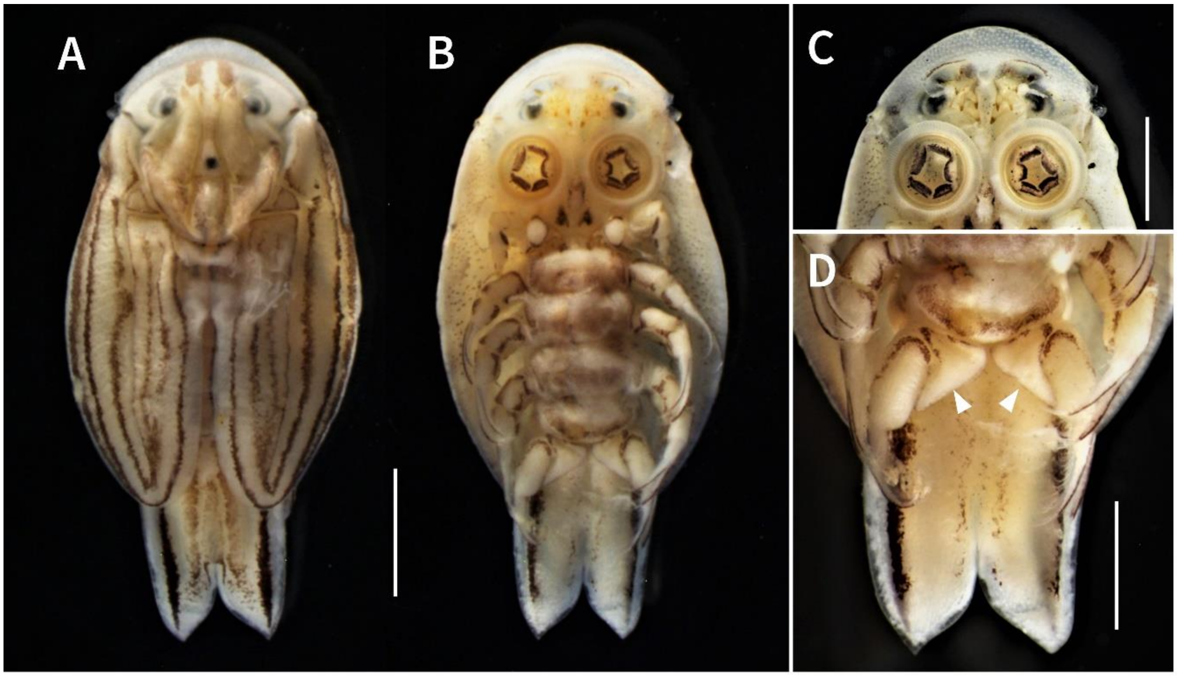

Fig. 2.2.2.2.1. *Argulus matuii*, adult female (9.1 mm total length), NSMT-Cr 30789, rom a crescent sweetlips *Plectorhinchus cinctus* in coastal waters of the Northwestern Pacific Ocean near Minabe, Wakayama Prefecture, central Japan. The specimen was fixed in 70% ethanol on 14 June 2017 and photographed on 22 March 2022. A, habitus, dorsal view; B, habitus, ventral view; C, anterior part of body, ventral view; D, posterior part of body, ventral view. Note a pair of large foot-shaped natatory lobes (arrowheads). Scale bars: A, B, D, 2 mm; C, 1 mm.

Chapter 3. Ecology of *Argulus* spp. parasitic on fishes in Japan

3.1 Occurrence of *Argulus coregoni* on salmonids in mountain streams

[Based on Nagasawa, K, Kishi, D, and Tokukura, T. (2022a): Occurrence of a skin parasite *Argulus coregoni* (Branchiura: Argulidae) on salmonids in mountain streams, central Japan, with discussion on its longitudinal distribution and host utilization in rivers. Species Diversity, 27: 159–166]

Abstract. *Argulus coregoni* Thorell, 1864 was collected from white-spotted char *Salvelinus leucomaenis* (Pallas, 1814), red-spotted masu salmon *Oncorhynchus masou ishikawae* Jordan and McGregor, 1925, masu salmon *O. masou masou* (Brevoort, 1856), and a hybrid between white-spotted char and masu salmon in mountain streams, Gifu Prefecture, central Japan. The host's body surface under and near the pectoral fins was the most common infection site for *A. coregoni*. The infected white-spotted char were caught at high elevations (461–873 m) in the headwater streams. The infected individuals of the two masu salmon subspecies were mostly caught in the upper river reaches, but the elevations where they were caught were lower (237–733 m and 660–707 m, respectively) than those of the white-spotted char. Since *A. coregoni* is also known as a parasite of ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) in the middle and lower river reaches in the prefecture, this parasite utilizes different fish species as its hosts along a river course: white-spotted char and the two masu salmon subspecies serve as the hosts, respectively, in the headwater and the middle to lower sections of the upper reaches, but in the mid- and lower river reaches, ayu is the important host.

3.1 Introduction

Argulus coregoni Thorell, 1864 is a skin parasite of freshwater fishes in northern Europe and eastern Asia (Neethling and Avenant-Oldewage, 2016). In Japan, the species infects wild fishes belonging to five families: Salmonidae, Plecoglossidae (both Salmoniformes), Cyprinidae (Cypriniformes), Amblycipitidae (Siluriformes), and Odontobutidae (Gobiiformes) (*e.g.*, Tokioka, 1936a; Yamaguti, 1937; Nagasawa and Kawai, 2008; Nagasawa, 2009, 2011a; Nagasawa *et al.*, 2014; Nagasawa and Ishikawa, 2015; Nagasawa and Taniguchi, 2021) but is also found on farmed salmonid and plecoglossid fishes (Hoshina, 1950; Nagasawa and Yuasa, 2020). Its larval development, host associations, impacts on host fishes, and treatment have all been studied (Inoue *et al.*, 1980; Shimura and Egusa, 1980; Shimura *et al.*, 1983a, b; Shimura, 1981, 1983a; Shimura and Inoue, 1984; Katahira *et al.*, 2021).

Argulus coregoni is known as a parasite of ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) and white-spotted char *Salvelinus leucomaenis* (Pallas, 1814) in rivers and of salmonids reared at a fisheries research institute in Gifu Prefecture (Nagasawa *et al.*, 2018b, 2020a, b, 2021b; Nagasawa and Morikawa, 2019b). This prefecture lies in a mountainous inland region of central Japan and is home to many freshwater fish populations in numerous rivers and streams (Mukai, 2017). During an ecological study of freshwater fishes in the prefecture, *A. coregoni* was collected from the body surface of salmonids in mountain streams (Fig. 3.1.1). Due to the low population size of the salmonids and difficulty in sampling them, limited information is available on the biology of *A. coregoni* infecting stream-dwelling salmonids, especially those occurring at high elevations. This paper reports on the distribution and habitats of salmonids infected with *A. coregoni* and its occurrence on those salmonids from mountain streams in Gifu Prefecture. The paper also discusses the longitudinal distribution and host utilization of *A. coregoni* in rivers, because this parasite infects ayu in the middle and lower reaches of rivers in the prefecture as well (see above literature).

3.1 Materials and methods

The specimens of *A. coregoni* were carefully removed by hand from the body surface of live salmonids caught by electrofishing from July to October 2019 in seven rivers belonging to the five larger river systems in Gifu Prefecture (Fig. 3.1.2, Table 3.1.1). No data on prevalence of infection were taken. Soon after capture, infected fish were individually identified to species, anesthetized with FA100 (containing mainly eugenol), measured for fork length (FL) to the nearest mm, and then photographed for recording the attachment site of each *A. coregoni* specimen. One fish with an irregular body color pattern (Fig. 3.1.1F) was identified as a hybrid between a white-spotted char and a masu salmon, *Oncorhynchus masou masou* (Brevoort, 1856) (see Kato, 1977; Kishi and Tokuhara, 2012; Mukai *et al.*, 2015). The specimens of *A. coregoni* were fixed and preserved in 99% ethanol. Their identification was later confirmed at the Aquaparasitology Laboratory, Shizuoka, based on Tokioka (1936a), Yamaguti (1937), and Nagasawa and Taniguchi (2021). The specimens of *A. coregoni* were examined for their sex and total length (TL) measured to the nearest 0.1 mm. Voucher specimens of *A. coregoni* have been deposited in the Crustacea (Cr) collection of the National Science Museum of Nature and Science, Tsukuba, Ibaraki Prefecture [NSMT-Cr 30777 (Fig. 3.1.1A, B), one male from white-spotted char from the Gamada River; NSMT-Cr 30778, two males and one female from red-spotted masu salmon from the Maze River; NSMT-Cr 30779, one male and one female from masu salmon from the Itoshiro River). Data on the elevation (m) at each collection site were obtained from a GSI map (https://maps.gsi.go.jp/) provided by the Geospatial Information Authority of Japan.

Previously, two subspecies of white-spotted char [*Salvelinus leucomaenis pluvius* (Hilgendorf, 1876) and *S. leucomaenis japonicus* Oshima, 1961] were identified from Gifu Prefecture (*e.g.*, Kawanabe, 1989; Hosoya, 2015). However, a recent molecular work has not supported the recognition of these subspecies in white-spotted char (Yamamoto *et al.*, 2004), thus, *S. leucomaenis* is used in this paper. The common name of *S. leucomaenis* follows Dunham *et al.* (2008), and those of two subspecies of *Oncorhynchus masou* (*O. masou masou* and *O. masou ishikawae* Jordan and McGregor, 1925) are based on Kishi *et al.* (2016).

3.1 Results

Distribution and habitats of infected salmonids. *Argulus coregoni* was found infecting 20 individual salmonids, which were identified as white-spotted char (*S. leucomaenis*) (n = 11), red-spotted masu salmon (*O. masou ishikawae*) (n = 5), masu salmon (*O. m. masou*) (n = 3), and a hybrid between white-spotted char and masu salmon (n = 1) (Fig. 3.1.1, Table 3.1.1). The infected white-spotted char were caught at seven sites in six rivers at high elevations (461–873 m) (Table 3.1.1). The six rivers belong to the five large river systems: three

(Kuzuryu, Sho, and Jinzu) and two (Kiso and Nagara) of these river systems flow into the Sea of Japan and the Northwestern Pacific through Ise Bay, respectively (Fig. 3.1.2). The infected red-spotted masu salmon were caught at four sites in three rivers (Table 3.1.1), which are major tributaries to the Kiso and Nagara river systems (Fig. 3.1.2). The elevations at which the red-spotted masu salmon were caught (237–733 m) were lower than those of the white-spotted char catch. The infected masu salmon were caught at two sites in the Itoshiro River at 660–707 m elevation, and the infected hybrid was also caught at a site in the same river at 715 m elevation (Table 3.1.1). The Itoshiro River is an upstream tributary in the Kuzuryu River system (Fig. 3.1.2).

All infected salmonids were collected from mountain streams, including the main streams of the rivers (Fig. 3.1.3A, E, H) and small tributaries flowing into the main streams (Fig. 3.1.3B–D, F, G). These sites showed a wide range of conditions in space, width, water volume, and gradient, but the water was constantly clear, the water depth was usually less than 1 m, and the substratum consisted of a mixture of gravel and cobble, often with boulders.

Occurrence on hosts, attachment sites, and body size. In total, 32 specimens of *A. coregoni* were collected from 20 infected salmonids [103–257 (mean = 174) mm FL]: one specimen per host was most common (60%), followed by two (25%) and three (10%) specimens. Only a single fish (white-spotted char, 195 mm FL, from the Tsukechi River) harbored four specimens of *A. coregoni* (Table 3.1.1).

The lateral body surface under and near the pectoral fins of salmonids was the most common attachment site for *A. coregoni*, followed by the lateral body surface below the dorsal fin and the dorsal body surface of the anterior trunk (Figs 3.1.1, 3.1.4). Only a few specimens of *A. coregoni* were found on the posterior body surface of the host.

The 32 specimens of *A. coregoni* included 14 males (44%) and 18 females (56%). Excluding a single small male specimen (3.1 mm TL) collected in early September 2019, the other 13 males were stouter and bigger [5.3–9.6 (mean = 7.5) mm TL]. The females were composed of 15 ovigerous specimens [7.5–11.0 (mean = 8.7) mm TL] and three smaller, non-ovigerous specimens [6.0–7.0 (mean = 6.5) mm TL]. These non-ovigerous females were collected from early to late September 2019, but the ovigerous females were collected during the whole survey period (from late July to mid-October 2019).

3.1 Discussion

Following a recently published record of *A. coregoni* from a white-spotted char in the Maze River (Nagasawa *et al.*, 2021b), this paper represents the second record of the species from salmonids in the mountainous region of Gifu Prefecture and reveals that it also parasitizes red-spotted masu salmon, masu salmon, and the hybrid between white-spotted char and masu salmon. The infected whitespotted char were caught in the streams in all five river systems investigated. In contrast to this, as the two masu salmon subspecies have separated natural distribution ranges in central Japan (Kawanabe, 1989; Kato, 1991), the infected individuals of masu salmon and red-spotted masu salmon occurred in the river systems emptying into the Sea of Japan and the Northwestern Pacific, respectively (Table 3.1.1).

Among stream-dwelling salmonids, white-spotted char occurs in the uppermost reaches of rivers in central Japan (*e.g.*, Kato, 1992; Kitano and Kubota, 1999; Kishi and Tokuhara, 2012; Kishi *et al.*, 2016). During this study, one infected white-spotted char (145 mm FL) was caught in a tributary of the Sho River at 873 m elevation, which represents the second highest elevation record of the natural distribution of *A. coregoni* in Japan. The highest elevation collection record (1075 m) of the species was taken from a white-spotted char (reported as *S. leucomaenis japonicus*) in a mountain stream, Nagano Prefecture, east of Gifu Prefecture (Nagasawa and Kawai, 2015). *Argulus coregoni* was also reported from brown trout, *Salmo trutta* Linnaeus, 1758, in a high elevation lake (1269 m), central Japan, but it had not been naturally distributed in the lake but was introduced there (Nagasawa, 2009; Nagasawa and Kawai, 2015). Based on these collection records, *A. coregoni* has been regarded as one of the fish parasites found in high elevation mountain streams of Japan (Nagasawa and Kawai, 2015).

Both red-spotted masu salmon and white-spotted char often co-occur in the upper reaches of rivers in central Japan, but the former is found in slightly lower streams than the latter (Kitano and Kubota, 1999, 2007; Kishi and Tokuhara, 2012). Similar distribution differences are also known for masu salmon and white-spotted char in the upper reaches in central Japan (Miyasaka *et al.*, 2003). The highest elevations where the infected masu salmon and red-spotted masu salmon were caught in this study are, respectively, 707 and 733 m, both of which are lower than that (873 m) of the white-spotted char catch (Table 3.1.1). One extraordinarily collection was an infected red-spotted masu salmon was at 237 m elevation in the Yoshida River (Table 3.1.1, location 4 in Fig. 3.1.2), but this fish occurred in a small tributary flowing into the river (Fig. 3.1.3D). This tributary had clean water and gravel and cobble substratum conditions which were similar to those in the upper reaches. White-spotted char and the two masu salmon subspecies are predominant in the upper reaches of rivers in Gifu Prefecture (Kishi and Tokuhara, 2012; Kishi *et al.*, 2016), and these salmonids most probably serve as the major hosts of *A. coregoni* there. Further, based on the observed difference in their distribution within the upper reaches, it is likely that white-spotted char and the two masu salmon subspecies are important hosts in the headwater and the middle to lower sections of the upper reaches, respectively.

There are six records of *A. coregoni* collected from ayu (*P. a. altivelis*) in the Kiso and Nagara river systems (Nagasawa *et al.*, 2018b, 2020a; Nagasawa and Morikawa, 2019b) (Fig. 3.1.2, Table 3.1.2): the infected ayu were caught in the middle reaches at 269–557 m elevation and in the lower reaches at 65 m elevation. As stated in the Introduction, *A. coregoni* is not a strictly host-specific parasite, and many species of freshwater fishes are present in such reaches: for example, 36 fish species have been recorded from the lower reaches of the Nagara River (Goto and Goto, 1971). Thus, freshwater fishes other than ayu possibly serve as the hosts of *A. coregoni* in the middle and lower reaches of rivers, but the presence of the six collection records of this parasite from ayu indicates that this fish is one of the important hosts in these reaches. Recently, Nagasawa and Morikawa (2019a) have suggested the importance of ayu as the host of *A. coregoni* in the middle reaches of rivers in Mie Prefecture, central Japan.

Based on the above discussion on the distribution and host utilization of *A. coregoni* in rivers, it can be summarized that 1) the species occurs from the upper through the middle to the lower reaches; 2) it utilizes different fish species as its hosts along a river course; 3) white-spotted char and the two masu salmon subspecies (masu salmon and red-spotted masu salmon) serve as the hosts, respectively, in the headwater and the middle to lower sections of the upper reaches; and 4) ayu is the important host in the middle to lower reaches.

The number of specimens of *A. coregoni* found on a single host was low, ranging from one to four, and, most commonly (60%), only one specimen was found per host. The smallest specimen was a male of 3.1 mm TL (collected in early September 2019) and is herein regarded as a larva at the eighth or ninth stage (see Shimura, 1981). In this survey, the author recognized the infected salmonids by naked eye, and there is the possibility that we overlooked smaller larvae at earlier stages in the field. In other words, the highest number of individuals of *A. coregoni* per host may be more than four.

The most common attachment site of *A. coregoni* on the examined salmonids was the body surface under and near the pectoral fins (Fig. 3.1.4). In this study, all of the infected salmonids were caught in mountain streams (Fig. 3.1.3), and it is inferred that *A. coregoni* utilizes that site not to detach from the host's skin under the fast-flow stream conditions. A similar suggestion was made by Shimura (1983) regarding the distribution of the same parasite on masu salmon reared at a governmental fish hatchery in Tokyo. He stated that, as masu salmon are a swift swimmer, *A. coregoni* selects the sites sheltered from water flow.

The specimens of *A. coregoni* collected during the survey period (from late July to mid-October 2019) consisted of 14 males and 18 females. Based on their TL range (male, 3.1–9.6 mm; female, 6.0–11.0 mm) and the information on the hatching season and growth of the species at the fish hatchery in Tokyo (Shimura, 1983a), the specimens collected in this study are inferred to have hatched during the spring to summer of 2019 from the overwintered eggs that were deposited in the early to late fall of 2018. The presence of the above-mentioned male larva (3.1 mm TL) in early September 2019 implies that this specimen hatched in July or August 2019. Further, ovigerous females of *A. coregoni* were collected throughout the whole survey period, which indicates that eggs are deposited at least from late July to mid-October in the mountain streams.

During this study, we collected *A. coregoni* from salmonids without recording prevalence of infection data, and the infected salmonids were caught at 10 sites in the seven rivers in Gifu Prefecture (Table 3.1.1). In this prefecture, due to intensive studies on the ecology of freshwater fishes, sufficient information has been accumulated on the distribution and abundance of salmonids in high elevation mountain streams (Kishi and Tokuhara, 2012; Kishi *et al.*, 2016). It is thus desirable to examine salmonids from many streams in various regions in order to clarify the host utilization, prevalence of infection, and life cycle of *A. coregoni* occurring in those mountain streams.

White-spotted char is distributed in Far East Asia, ranging from around the Kamchatka Peninsula, Russia, to central Honshu, Japan (Dunham *et al.*, 2008). Gifu Prefecture is located near the southern limit of the distribution of the species, and its range and habitats in central Japan including the prefecture have been predicted to be limited by global warming (Takegawa *et al.*, 2017), which suggests that *A. coregoni* will lose their local host populations. The geographical distribution of masu salmon is similar to that of white-spotted char, and red-spotted masu salmon occurs only in central and western Japan (Kato, 1991). Gifu Prefecture also lies near the southern limit of the distribution of these two masu salmon subspecies, especially masu salmon (Kawanabe, 1989), and they may also be negatively affected by global warming. It is, therefore, important to study and record the present state of the distribution of the salmonids and *A. coregoni* in high elevation mountain streams for a future evaluation of the impact of global warming on the populations of these animals in central Japan.

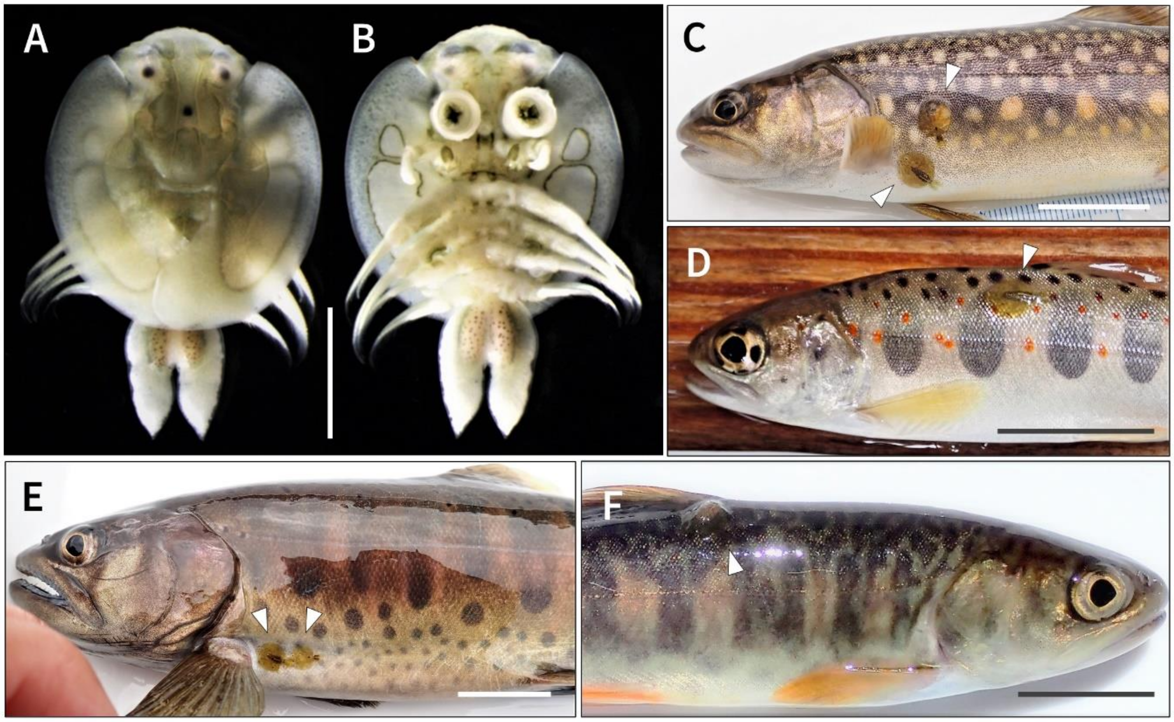

Fig. 3.1.1. *Argulus coregoni*, male, NSMT-Cr 30777, from a white-spotted char *Salvelinus leucomaenis*, from the Gamada River, Gifu Prefecture, ethanol-preserved specimen, A, dorsal view; B, ventral view; C, two females infecting a white-spotted char (180 mm FL) near the left pectoral fin (from the Maze River); D, one female infecting a red-spotted masu salmon *Oncorhynchus masou ishikawae* (103 mm FL), near the base of the dorsal fin (from a tributary of the Hida River); E, one female (left) and one male (right) infecting a masu salmon *O. masou masou* (257 mm FL), near the left pectoral fin (from the Itoshiro River); F, one female infecting a hybrid between white-spotted char and masu salmon (165 mm FL) near the dorsal fin (from the Itoshiro River). Arrowheads indicate individuals of *A. coregoni*. See Fig. 3.1.2 for the locations of the rivers. Scale bars: A, B, 2 mm; C–F, 20 mm.

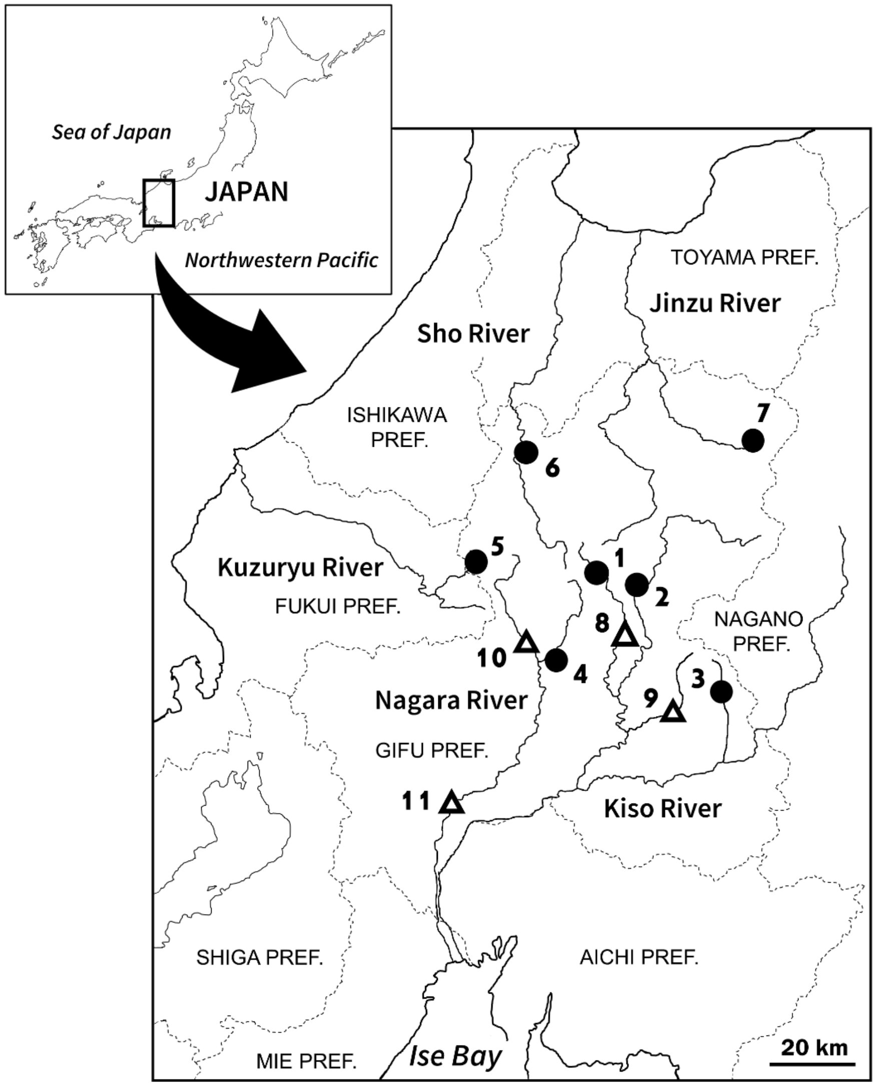

Fig. 3.1.2. Map showing the collection localities of salmonids infected with *Argulus coregoni* (closed circles 1–7) in rivers of Gifu Prefecture, central Japan. The collection localities of ayu *Plecoglossus altivelis altivelis* infected with *A. coregoni*, are also shown (open triangles 8–11). 1, upper reaches of the Maze River; 2, tributary of the Hida River; 3, tributary of the Tsukechi River; 4, tributary of the Yoshida River; 5, the Itoshiro River; 6, tributary of the Sho River; 7, the Gamada River; 8, middle reaches of the Maze River; 9, middle reaches of the Shira River; 10, middle reaches of the Nagara River; 11, lower reaches of the Nagara River.

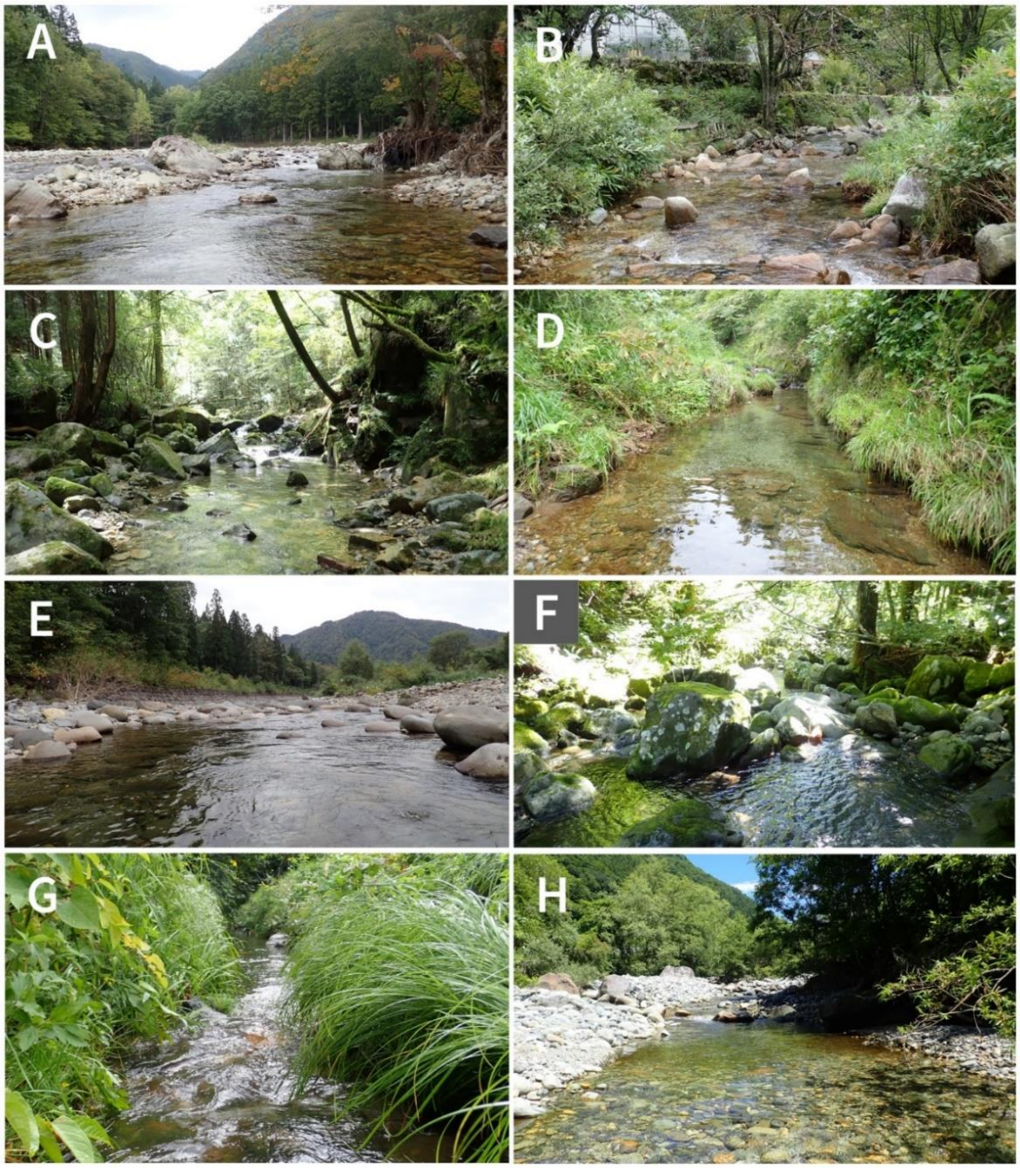

Fig. 3.1.3. Mountain streams where the salmonids infected with *Argulus coregoni* were caught in Gifu Prefecture, central Japan. A, main stream of the upper Maze River (locality 1 in Fig. 3.1.2); B, tributary of the Hida River (locality 2); C, tributary of the Tsukechi River (locality 3); D, tributary of the Yoshida River (locality 4); E, main stream of the Itoshiro River (locality 5); F, tributary of the Itoshiro River (locality 5); G, tributary of the Sho River (locality 6); H, main stream of the Gamada River (locality 7).

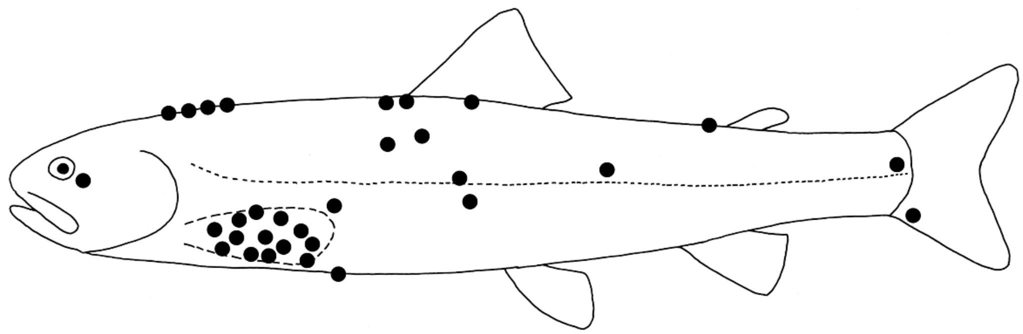

Fig. 3.1.4. Distribution of 31 specimens of *Argulus coregoni* (closed circles) on the host's body surface. A total of 32 specimens of *A. coregoni* were collected, but the attachment site for one individual was not recorded.

Table 3.1.1. *Argulus coregoni* collected from salmonids in mountain streams of Gifu Prefecture, central Japan.

Collection locality						Host		*A. coregoni* collected (total length, mm)	Collection date
River system	River	Site	Elevation (m)	Location	Number in Fig. 3.1.2	Species	Fork length (mm)		
Kiso River	Maze River	Main stream	733	Oppara, Takayama	1	*Salvelinus leucomaenis*	162	One female (9.5)	1 October 2019
							180	One male (8.3), one female (9.0)	2 October 2019
						Oncorhynchus masou ishikawae	128	One male (8.0)	23 July 2019
							255	Two males (7.7, 7.0), one female (7.5)	1 October 2019
	Hida River	Inflowing tributary -1	519	Shimi, Gero	2	*Salvelinus leucomaenis*	159	Two females (8.2, 6.4)	24 September 2019
							147	One male (7.0)	
							152	One female (8.0)	
						Oncorhynchus masou ishikawae	212	One female (8.0)	
		Inflowing tributary -2	458	Shimi, Gero	2	*Oncorhynchus masou ishikawae*	103	One female (7.9)	24 September 2019
	Tsukechi River	Inflowing tributary	461	Inari, Nakatsugawa	3	*Salvelinus leucomaenis*	195	One male (9.2, three females (11.0, 9.8, 6.0)	10 September 2019
Nagara River	Yoshida River	Inflowing tributary	237	Asahi, Gujo	4	*Oncorhynchus masou ishikawae*	200	One male (7.6)	13 September 2019
Kuzuryu River	Itoshiro River	Main stream -1	707	Itoshiro, Gujo	5	*Oncorhynchus masou masou*	117	One male (7.0), one female (9.0)	30 September 2019
							112	One female (7.0)	
		Main stream -2	660	Itoshiro, Gujo	5	*Salvelinus leucomaenis*	197	Two females (10.0, 9.0)	17 October 2019
						Oncorhynchus masou masou	257	Two males (7.5, 6.9), one female (8.2)	
		Inflowing tributary	715	Itoshiro, Gujo	5	*Salvelinus leucomaenis*	204	One male (8.8)	7 September 2019
							152	One male (9.6)	
						Hybrid (*S. leucomaenis* × *O. masou masou*)	165	One female (10.0)	
Sho River	Sho River	Inflowing tributary	873	Kurodani, Takayama	6	*Salvelinus leucomaenis*	145	Two males (5.3, 3.1)	4 September 2019
Jinzu River	Gamada River	Main stream	836	Kansaka, Takayama	7	*Salvelinus leucomaenis*	240	One male (6.0)	8 August 2019

Table 3.1.2. Previous records of *Argulus coregoni* collected from ayu, *Plecoglossus altivelis altivelis*, in rivers of Gifu Prefecture, central Japan.

Collection locality						Reference
River system	River	Reaches	Elevation (m)	Location	Number in Fig. 3.1.2	
Kiso River	Maze River	Middle reaches	557	Maze-Nakagiri, Gero	8	Nagasawa *et al.* (2018)
	Shira River	Middle reaches	329	Kando, Higashi-Shirakawa	9	Nagasawa *et al.* (2018)
Nagara River	Nagara River	Middle reaches	430	Nagataki, Gujo	10	Nagasawa *et al.* (2020a)
			367	Shirotori, Gujo	10	Nagasawa *et al.* (2020a)
			269	Tokunaga, Gujo	10	Nagasawa *et al.* (2018), Nagasawa and Morikawa (2019)
		Lower reaches	65	Sodai (reported incorrectly as "Soda"), Mino	11	Nagasawa *et al.* (2018)

3.2 Distribution of *Argulus japonicus* and *A. coregoni* in the Lake Biwa Basin

[Based on Nagasawa, K. (2023c): Distribution of fish parasites *Argulus japonicus* and *Argulus coregoni* (Crustacea: Branchiura: Argulidae) in the Lake Biwa Basin, central Japan. Species Diversity, 28: 217–223]

Abstract. *Argulus japonicus* Thiele, 1900 and *A. coregoni* Thorell, 1864 are ectoparasites of freshwater fishes and are known to occur in Lake Biwa and its adjacent inland waters in Shiga Prefecture, central Japan. Since these parasites are not common in the wild in Japan, this study examined the specimens loaned from the Lake Biwa Museum and those previously reported from this prefecture in order to clarify their distributional patterns. The specimens of *A. japonicus* were obtained in Lake Biwa and a nearby pond, whereas those of *A. coregoni* were almost exclusively collected in the large rivers flowing into the lake and most of them were from mountain streams which are higher-elevated than the lake or pond. These results indicate that each of *A. japonicus* and *A. coregoni* has its own preference dependent on different environmental conditions: *A. japonicus* inhabits the still or slow-flowing waters, but *A. coregoni* occurs in the running waters, especially in the colder, well oxygenated mountain streams.

3.2 Introduction

Argulid branchiurans of the genus *Argulus* Müller, 1785 represent one of the major fish parasite taxa/groups in fresh and marine waters (Yamaguti, 1963; Neethling and Avenant-Oldewage, 2016). To date, a vast amount of knowledge has been accumulated on these parasites, but most of the previous works have focused on their taxonomy, and little information is available on their ecology. This is because argulid branchiurans are not common parasites in the wild (Tokioka, 1936c), and it is almost impossible to constantly collect them from wild fishes in Japan. This is particularly true for two species, *Argulus* *japonicus* Thiele, 1900 and *A. coregoni* Thorell, 1864, infecting freshwater fishes in Japan. While a few papers have reported on the ecology of these species in fish farms or hatcheries (Kimura, 1970 for *A. japonicus*; Shimura, 1983a for *A. coregoni*), there is limited information on their geographical distribution, utilization of wild hosts, and life history in the rivers and lakes of Japan.

Recently, several studies have been conducted on the distribution and host utilization of *A. coregoni* in four prefectures (Gifu, Aichi, Mie, and Shiga), central Japan (*e.g*., Nagasawa *et al*., 2018b, 2021b, 2022a; Nagasawa and Kawai, 2019). In particular, one of these studies (Nagasawa *et al*., 2022a) has revealed that *A. coregoni* occurs from the upper to the lower reaches of rivers in Gifu Prefecture and utilizes various salmonids and ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) in the upper and the middle-lower reaches, respectively. However, the distributional pattern of *A. japonicus* in various water bodies of central Japan is still poorly understood.

Lake Biwa is in Shiga Prefecture, and is the largest lake (674 km^2) in Japan. More than 400 rivers, streams, and irrigation canals flow into the lake, and most of the large inflowing rivers originate from the mountain areas near the borders of Shiga and neighboring prefectures (Fig. 3.2.1). The Seta River drains the lake. Many studies on the biology of aquatic animals, including their parasites, have been conducted in the Lake Biwa Basin (Kawanabe *et al*., 2020; Nishino, 2020), where both *A. coregoni* and *A. japonicus* are found (Nagasawa, 2020). Specimens of the two species collected from the Lake Biwa Basin have been deposited in the Lake Biwa Museum (Grygier, 2004; Nagasawa, 2009, 2011b).

In order to clarify the distributional patterns of *A. japonicus* and *A. coregoni* in the inland waters of central Japan, this paper reports on their occurrence in the Lake Biwa Basin, based on an examination of the specimens from the LBM and those of both species previously reported from the prefecture.

3.2 Materials and methods

Most of the specimens of *A. japonicus* and *A. coregoni* examined in this study were received on loan from the Lake Biwa Museum , Kusatsu, Shiga Prefecture (LBM). These specimens had been collected from both wild and captive fishes in Shiga and adjacent prefectures and also found as detached individuals in small fixed nets (called "eri") installed in coastal waters of Lake Biwa but, in this study, only the specimens taken from the wild in Shiga Prefecture were examined (LBM1430001719, 1430001720, 1430003568–1430003572 for *A. japonicus*; LBM1430000945, 1430001942, 1430002322, 1430002522–1430002554, 1430003574 for *A. coregoni*). They were collected between 1985 and 2009. Furthermore, some specimens of *A. japonicus* and *A. coregoni* previously reported from Shiga Prefecture (Goda *et al*., 2017; Nagasawa *et al.*, 2018b, 2021b; Nagasawa and Kawai, 2019) were also re-examined. As the specimen of *A. coregoni* reported by Tokioka (1936a) and those of *A. japonicus* reported by Okano (1996) could not be located (perhaps they were lost), their published data are used herein. Collection locality and date of collection of individual LBM specimens were obtained from their specimen labels, and data on the elevation (m) at each collection locality were taken from a GSI map (https://maps.gsi.go.jp/) provided by the Geospatial Information Authority of Japan.

The specimens were observed using an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope at the Aquaparasitology Laboratory, Shizuoka Prefecture. Identification was done based on Tokioka (1936a), Yamaguti (1937), Hoshina (1950), Nagasawa (2021), and Nagasawa and Taniguchi (2021). In particular, since *A. japonicus* and *A. coregoni* bear a close morphological resemblance, the specimens were carefully examined for the number of plumose setae on the posterior margin of the coxa of the first leg, using the wooden slide procedure (without dissection) recommended by Benz and Otting (1996): *A. japonicus* and *A. coregoni* has a single plumose seta and four to nine plumose setae on the posterior margin of the coxa, respectively (Nagasawa, 2021 for *A. japonicus*; Hoshina, 1950; Nagasawa and Taniguchi, 2021 for *A. coregoni*). After the specimens were identified, their sex and total length (TL, to the nearest 0.1 mm, from the anterior tip of the carapace to the posterior tip of the abdomen) were recorded. The scientific names of fishes mentioned in this paper follow Hosoya (2015), except for white-spotted char *Salvelinus leucomaenis* (Pallas, 1814), which is based on Kikko *et al*. (2008a, b, 2009).

3.2 Results

The *A. japonicus* specimens were mostly collected in Lake Biwa (Table 3.1.1, localities 1–8 in Fig. 3.2.1, but several specimens were taken from a pond near the lake (locality 9 in Fig. 3.2.1). The specimens from Lake Biwa consisted of four males (2.0–4.2 mm TL) and six females (1.8–5.6 mm TL). Two of these specimens were collected from a three-lips *Opsariichthys uncirostris uncirostris* (Temminck and Schlegel, 1846) (Cypriniformes: Cyprinidae), but there is no host information for the others because seven specimens were found detached in coastal fixed nets and one specimen was collected with a plankton net hauled vertically in offshore waters. The pond specimens (3–5 mm TL) were also sampled by a plankton net. Lake Biwa and the pond lie at ca. 85 m elevation.

For *A. coregoni*, one male specimen (4.1 mm TL) was collected at Otsu on the coast of Lake Biwa (locality 10 in Fig. 3.2.1), but all other specimens were collected in the large rivers flowing into Lake Biwa, consisting of seven males (4.2–8.0 mm TL) and eight females (2.2–11.3 mm TL) (Table 3.2.2). Although two specimens were sampled in the lower reaches of the Ado River (locality 12 in Fig. 3.2.1), 13 specimens were collected from the mountain streams, including tributaries of the upper Ado, Ishida, and Echi rivers (n = 12, localities 11, 13, 15, 16 in Fig. 3.2.1) and the mainstream of the upper Ane River (n = 1, locality 14 in Fig. 3.2.1). In these mountain streams, 10 specimens were taken from red-spotted masu salmon *Oncorhynchus masou ishikawae* Jordan and McGregor, 1925 (Salmoniformes: Salmonidae) caught at higher elevations (ca. 380–650 m) and three specimens were also collected at higher elevations (215–ca. 550 m) without host information. The host fish in the lower Ado River was ayu *P. a. altivelis* (Salmoniformes: Plecoglossidae) caught at 92 m elevation.

3.2 Discussion

As stated in the Introduction, both *A. japonicus* and *A. coregoni* are infrequently encountered in Japan. Because of such rare occurrence of the two species in the wild, this study has examined both the LBM specimens of *Argulus* collected in Shiga Prefecture from 1985–2009 and also re-examined the specimens of the genus previously reported from the prefecture. Based on these examinations, this study has shown that there is a marked difference between *A. japonicus* and *A. coregoni* in their distributional patterns in the Lake Biwa Basin: *A. japonicus* occurred in Lake Biwa and a nearby pond, but *A. coregoni* was almost exclusively found in the rivers flowing into the lake. Some specimens of *A. japonicus* were collected by plankton nets, and they are regarded as detached, free-swimming individuals, searching to find a new host or a substrate for egg deposition.

The distributional patterns and host utilization of *A. coregoni* found in this study are similar to its behavior in rivers of Gifu Prefecture east of Shiga Prefecture (Nagasawa *et al*., 2022a). In Gifu Prefecture, this species is found from the upper to the lower reaches of rivers and infects salmonids and ayu in the upper and the middle-lower reaches, respectively. In addition to red-spotted masu salmon, other salmonids, *i.e*., white-spotted char, masu salmon *Oncorhynchus masou masou* (Brevoort, 1856) and their hybrid, serve as hosts for *A. coregoni*. In the present study, 13 of the 15 *A. coregoni* specimens were collected from the mountain streams, where 10 and three specimens were taken from red-spotted masu salmon and unknown hosts, respectively. In these streams, especially in the headwater tributaries, white-spotted char occurs (Kikko *et al*., 2008a, b, 2009), suggesting that *A. coregoni* also infects this salmonid. Moreover, juveniles and adult mature (precocious) males of Biwa salmon *Oncorhynchus* sp. are found in the rivers flowing into Lake Biwa (Fujioka, 1991; Kuwabara and Iguchi, 1994) and may serve as a host for *A. coregoni* as well.

It is challenging to explain Tokioka's (1936a) report of one specimen of *A. coregoni* at Otsu. He collected this specimen from a bitterling *Acheilognathus* sp. (Cypriniformes: Cyprinidae) (reported as *A. moriokae* Jordan and Thompson, 1914, see the footnote of Table 3.2.2 for the scientific name of this species). The record lacks detail of the collection locality, but the specimen was most probably sampled in the lake because Otsu is located on the coast of the lake and Kyoto Imperial University (now Kyoto University), at which he worked, had the Otsu Hydrobiological Station on the lake side. Recently, Nagasawa and Taniguchi (2021) have suggested that Tokioka's specimen might have detached from an ayu in the lower reaches of an inflowing river and infected the bitterling in Lake Biwa after its downstream drift to the lake. These authors also have indicated a low possibility of a long-distance downstream drift of Tokioka's specimen from an upper stream to Lake Biwa because it might have died or been unsuccessful in finding a new host (salmonid or ayu) during its drifting or swimming to the lake.

As stated above, detached individuals of *A. coregoni* can drift from the lower reaches of the rivers to Lake Biwa, but there is a very low possibility that they can establish their population in the lake. This is supported by the fact that since 1936 the species has not been reported from the lake and the *Argulus* specimens collected in the lake are all identified as *A. japonicus*. In other words, Lake Biwa is not a suitable habitat of *A. coregoni* (see below for further discussion).

Most of the *A. japonicus* specimens were collected in Lake Biwa, and several specimens were also sampled from in a pond near the lake. Since the Lake Biwa specimens were mostly found detached in coastal fixed nets or a plankton net, their fish hosts are unknown. At present, three-lips is the only known host of *A. japonicus* in Lake Biwa. To date, more than 70 species of fishes have been reported from Lake Biwa (Yuma *et al*., 1998), and *A. japonicus* is highly likely to parasitize some of these fishes. In particular, various cyprinid species are utilized by *A. japonicus* in Japan (*e.g.*, Takeda *et al*., 2000; Nagasawa, 2011a; Nagasawa *et al*., 2012, 2018a, 2021a; Yamauchi and Shimizu, 2013; Nagasawa and Sato, 2014) and it is speculated that this parasite will be found on many of the more than 35 cyprinid species recorded by Yuma *et al*. (1998) in Lake Biwa.

Many small rivers and irrigation canals flow into Lake Biwa. While no argulid specimen was collected from those waters, *A. japonicus* may occur there because this species has been collected from slow-flowing brooks and irrigation canals in other regions of Japan (Nagasawa and Sato, 2014; Nagasawa, 2017b; Nagasawa *et al.*, 2018a). Moreover, the species may be present in ponds and reservoirs near the lake as there are several records of the species from such still water bodies in western Japan (Nagasawa *et al.*, 2012; Nagasawa, 2021).

In Japan, cyprinids and salmonids have generally been regarded as the main hosts for *A. japonicus* and *A. coregoni*, respectively (Nagasawa, 2009, 2011a). Nevertheless, *A. japonicus* is known to infect fishes of three other families, including the Siluridae (Nagasawa *et al*., 2010; Yamauchi *et al.*, 2011), Adrianichthyidae (Egami *et al*., 1988) and Centrarchidae (Nagasawa, 2021) in Japan. Furthermore, *A. coregoni* also has been reported from non-salmonid fishes, including the Cyprinidae (Tokioka, 1936a; Nagasawa and Taniguchi, 2021), Odontobutidae (Nagasawa *et al*., 2014), Amblycipitidae (Nagasawa and Ishikawa, 2015) and Plecoglossidae (*e.g*., Nagasawa *et al*., 2018b) in Japan. These facts show that both *A. japonicus* and *A. coregoni* show some preference for cyprinids and salmonids, respectively, but are non-host-specific: that is, these parasites are able to infect any freshwater fish species. It is thus important to explain the observed difference in the distributional patterns of the two species, based on the environmental conditions required from their habitats, rather than based on their host preference.

Except the one specimen reported in 1936 from Otsu, all of the *A. coregoni* specimens examined in this study were collected in the large rivers flowing into Lake Biwa, and the majority of them were obtained from the mountain streams at higher elevations (215–ca. 650 m). Japanese mountain streams are characterized by strong currents, low water temperatures, high oxygen concentrations, and stony riverbeds (Mizuno and Gose, 1972), and the result of this study shows that *A. coregoni* mainly inhabits the lotic, cold, well oxygenated, higher-elevated mountain streams. This species has been similarly reported to occur in mountain streams of neighboring Gifu Prefecture (Nagasawa *et al*., 2022a). While no observation was made on the reproductive behavior of wild *A. coregoni* in Japan, stones on the riverbed are probably used as a substrate for the egg deposition of the species. In this study, two specimens of *A. coregoni* were also collected from ayu in the lower reaches of the Ado River. In central Japan, ayu is commonly found in the middle and lowers reaches of rivers, where *A. coregoni* utilizes this fish as its preferred host (Nagasawa *et al*., 2022a).

The distributional patterns of *A. japonicus* markedly differ from those of *A. coregoni* in the Lake Biwa Basin: the species was collected only in Lake Biwa and a nearby pond (Fig. 3.2.1). Both the lake and the pond hold almost still waters, and the water temperature and the oxygen level are higher and lower, respectively, than those in the stream habitat of *A. coregoni*, which indicates that *A. japonicus* inhabits the still or slow-flowing water bodies and is more tolerant to higher water temperatures and lower oxygen concentrations than *A. coregoni*. Thus, it is likely that the environmental conditions in Lake Biwa do not allow the survival of *A. coregoni* in this lake because the habitat of the species is the lotic, cold, highly oxygenated streams.

Two species of *Argulus, A. coregoni* and *A. foliaceus* (Linnaeus, 1758), natively occur in inland waters of Europe, where these species show their unique own distributional patterns and host preference (*e.g*., Campbell, 1971; Mikheev *et al*., 2015). For example, in central Finland, *A. coregoni* and *A. foliaceus* co-exist in the lakes and rivers, but their host and habitat utilizations are different: *A. coregoni* is found on salmonids occurring in running waters with higher oxygen concentrations, whereas *A. foliaceus* infects any fish species in shallow, slow-flowing waters. These species use different substrates for their egg deposition: *A. coregoni* lays eggs on stones but *A. foliaceus* on vegetation (Mikheev *et al*., 2015). As reported above, the habitats of the two Japanese species (*A. japonicus* and *A. coregoni*) similarly do not overlap in the Lake Biwa Basin (Fig. 3.2.1): *A. japonicus* and *A. coregoni* occur in the lentic and lotic waters, respectively.

Despite the previous and present studies, our knowledge on the ecology of *A. japonicus* and *A. coregoni* is still very limited. As these species are not common parasites, we need continuous field studies on the host range of *A. japonicus* in Lake Biwa and adjacent waters and their life cycles in their habitats. It is desirable to conduct a longitudinal survey in the upper, middle, and lower reaches of the rivers in order to clarify the host utilization of *A. coregoni* in respective reaches.

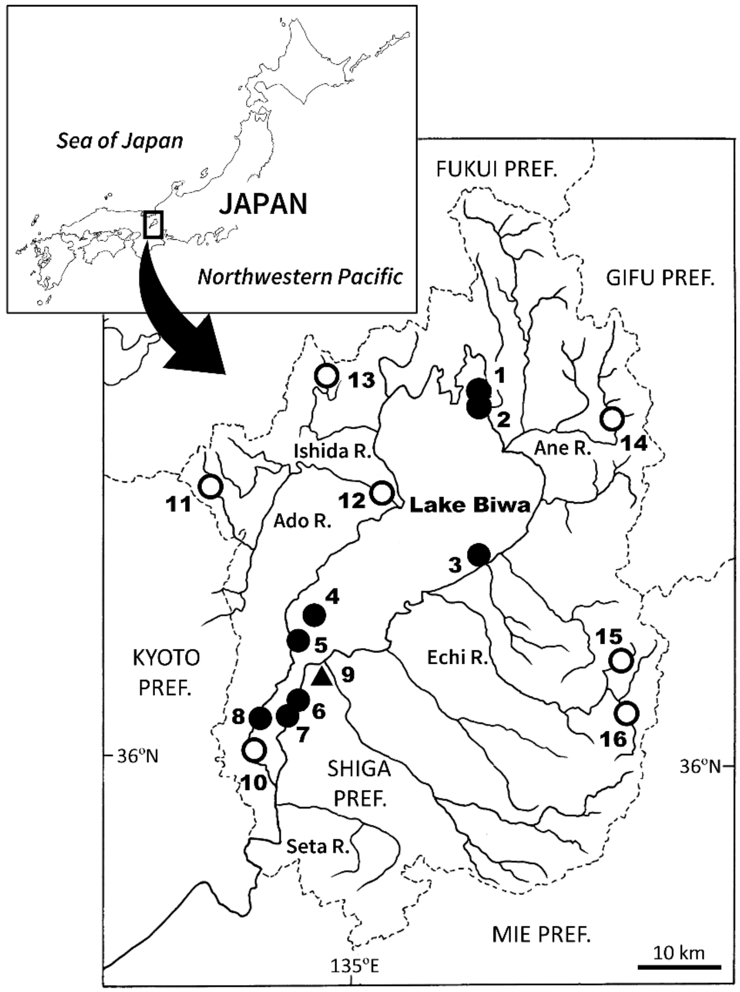

Fig. 3.2.1. A map of Shiga Prefecture, central Japan, to show the distribution of *Argulus japonicus* (closed circles, in Lake Biwa; closed triangle, in Chirinsan-no-ike Pond) and *A. coregoni* (open circles) in the Lake Biwa Basin. Only large rivers are shown. Dashed lines indicate the borders of Shiga and neighboring prefectures. 1, Katayama; 2, Onoe; 3, near the mouth of the Uso River; 4, off Omi-maiko; 5, Wani-Imajuku; 6, Akanoi; 7, Shina; 8, Hiei-tsuji; 9, Chirinsan-no-ike Pond ; 10, Otsu; 11, Harihata River; 12, lower Ado River; 13, Kawachidani Stream; 14, upper Ane River; 15, Oike River; 16, Kanzaki River. See Tables 3.2.1 and 3.2.2 for detailed information on the collection localities of *A. japonicus* and *A. coregoni*, respectively.

Table 3.2.1. *Argulus japonicus* collected in the Lake Biwa Basin, Shiga Prefecture, central Japan.

No.	Collection locality				Collection date	Host	*A. japonicus* collected (total length, mm)	Reference
	Site	Location	Number in Fig. 1	Elevation (m)				
1	Lake Biwa	Katayama, Nagahama	1	85	2019.07.10	*Opsariichthys uncirostris uncirostris*	Two males (2.9, 3.3)	cf. Nagasawa *et al.* (2021a)
2		Onoe, Nagahama	2	85	1985.06.15	—*	One female (4.3)	This paper
3		Onoe, Nagahama	2	85	1985.06.30	—	One female (4.7)	This paper
4		Near the mouth of the Uso River, Sugoshi, Hikone	3	85	1991.02.01	—	One female (5.6)	This paper
5		Off Omi-maiko, Otsu	4	85	2016.09.07	—**	One male (4.2)	cf. Goda *et al.* (2017)
6		Wani-Imajuku, Otsu	5	85	1993.06.05	—	One female (5.5)	This paper
7		Akanoi, Kusatsu	6	85	1991.06.26	—	One female (3.5)	This paper
8		Shina, Kusatsu	7	85	1993.06.11	—	One male (2.0)	This paper
9		Hiei-tsuji, Otsu	8	85	Unknown date in 1992	—	One female (1.8)	This paper
10	Chirinsan-no-ike Pond	Tatsuta, Moriyama	9	85	Unknown date in 1992–1993	—***	Unsexed specimens (3–5)	Okano (1996)

* Seven specimens (No. 2–4, 6–9) were found as detached individuals in fixed nets (called "eri") installed in coastal waters of Lake Biwa.

** This speciemen (No. 5) was collected by a vertical haul of a plankton net from near the bottom (75.4 m) to the depth of 20 m in Lake Biwa (Goda *et al* . 2017).

*** The specimens (No. 10) were collected by a plankton net in this pond (Okano 1996).

Table 3.2.2. *Argulus coregoni* collected in the Lake Biwa Basin, Shiga Prefecture, central Japan.

No.	Collection locality				Collection date	Host	*A. coregoni* collected (total length, mm)	Reference
	Site	Location	Number in Fig. 1	Elevation (m)				
1	Lake Biwa*	Otsu	10	85	Not reported	*Acheilognathus* sp.**	One male (4.1)	Tokioka (1936a)
2	Harihata River (triburay of the upper Ado River)	Between Kuwabara and Furuya, Kutsuki, Takashima	11	405	1991.08.16	—***	One male (4.2)	This paper
3	Lower Ado River (mainstream)	Tokiwagi, Takashima	12	92	2012.08.15	*Plecoglossus altivelis altivelis*	One female (2.2), one male (7.8)	ch. Nagasawa *et al*. (2018)
4	Kawachidani Stream (tributary of the upper Ishida River)	Hiokimae, Imazu, Takashima	13	ca. 380–500	2007.09.05 or 2007.09.19	*Oncorhynchus masou ishikawae*	Three females (6.0, 7.5, 9.6)	This paper
5	Upper Ane River (mainstream)	Okubo, Maibara	14	215	1993.07.29	—	One male (6.0)	This paper
6	Oike River (tributary of the upper Echi River)	Kimigahata, Higashi-Omi	15	400	2018.09.14	*Oncorhynchus masou ishikawae*	One female (11.3)	cf. Nagasawa and Kawai (2019)
7		Kimigahata, Higashi-Omi	15	ca. 450–550	2009.08.15	—	One male (5.0)	This paper
8	Kanzaki River (tributary of the upper Echi River)	Yuzurio, Higashi-Omi	16	ca. 350–650	2001.09.23	*Oncorhynchus masou ishikawae*	One female (9.7)	This paper
9		Yuzurio, Higashi-Omi	16	ca. 350–650	2001(?).09.29	*Oncorhynchus masou ishikawae*	One female (10.0), three males (7.0. 7.6, 8.0)	This paper
10		Near hydroelectric power plant, Yuzurio, Higashi-Omi	16	420	2002.09.30	*Oncorhynchus masou ishikawae*	One female (10.0)	This paper

* Although Tokioka (1936) reported that the specimen was collected at Otsu, this locality is on the coast of Lake Biwa and there was the Otsu Hydrobiologica Station of Kyoto Imperial University.

** Tokioka (1936) reported that the specimen was taken from a bitterling "*Acheilognathus moriokae*", but this cyprinid (current scientific name, *A. melanogaster*) does not occur in the Lake Biwa basin. Thus, Goda *et al*. (2017) regarded it as an unidentified species of the genus.

*** No host information was found on the specimen labels.

3.3 Occurrence of *Argulus japonicus* on cyprinids in unusual water environments

3.3.1 Survival of *Argulus japonicus* parasitic on a cyprinid fish in a brackish water lake

[Based on Nagasawa, K. (2019): Survival of *Argulus japonicus* (Branchiura: Argulidae) on a cyprinid in drought-induced brackish waters in a Japanese lake. Crustacean Research, 48: 99–103]

Abstract. Two specimens of a freshwater fish parasite *Argulus japonicus* Thiele, 1900 were found to be mixed in a sample of over 600 specimens of a brackish-water fish parasite *Caligus orientalis* Gusev, 1951 in a glass vial kept at Hiroshima University. These specimens were all collected in September 1973 from the body surface of a moribund Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846 in drought-induced brackish waters of Lake Shinji, Shimane Prefecture, Japan. This collection indicates that *A. japonicus* can survive in brackish waters under unusual climate conditions.

3.3.1 Introduction

Argulus japonicus Thiele, 1900 is an ectoparasite of freshwater fishes (Poly, 2008; Neethuling and Avenant-Oldewage, 2016). This species was originally described using material from Japan (Thiele, 1900), where it has been reported from cyprinids in inland waters and almost exclusively those fishes reared at fish ponds and aquaria (Nagasawa, 2011a, b, 2017b, 2018; Nagasawa *et al.*, 2010, 2012, 2013, 2018a; Yamauchi and Shimizu, 2013; Nagasawa and Sato, 2014; Nagasawa and Miyajima, 2018; see Nagasawa, 2009 for the earlier literature). Nevertheless, Yamauchi *et al.* (2011) collected *A. japonicus* from an aquarium-held fish reared in brackish waters (salinity: 1–7‰). This collection is interesting because *A. japonicus* was regarded as a freshwater species and had never been recorded from salt waters.

This paper reports on the author's observation to support Yamauchi *et al.*'s finding on the survival of *A. japonicus* in brackish waters.

3.3.1 Materials and methods

Two specimens of *A. japonicus* and 605 specimens of copepod *Caligus orientalis* Gusev, 1951 (Siphonostomatoida: Caligidae) were found in a glass vial kept at the Laboratory of Aquatic Pathology, Hiroshima University, Higashi-Hiroshima, Japan. The latter species is known as a brackish-water fish parasite (Nagasawa, 2004). Based on a specimen label, all of these specimens were collected from the body surface of a moribund Japanese white crucian carp *Carassius cuvieri* Temminck and Schlegel, 1846 (written as "kawachi-buna" in Japanese) (body size unknown) by Hiroya Suzumoto (Mitoya Inland Branch, Shimane Prefectural Fisheries Experimental Station) on 11 September 1973 in brackish waters of Lake Shinji (79.1 km^2, 6.4 m in maximum depth, 35°27′01″N, 132°56′58″E at its center), Shimane Prefecture, Japan. The lake is connected to the Sea of Japan through a brackish-water lake (Lake Naka-umi) and two short channels. The specimens of *A. japonicus* are deposited in the Crustacea collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture, Japan (NSMT-Cr 26691).

3.3.1 Results and discussion

The specimens of *A. japonicus* consisted of one male and one female, measuring 4.0 × 2.5 mm and 4.8 × 3.1 mm, respectively (Fig. 3.3.1.1). They were damaged, probably due to rough removal from the host fish and/or infection in brackish waters. Thus, their detailed morphological features were not observed well, but they correspond to *A. japonicus* described by Tokioka (1936a) and Yamaguti (1937). The male specimen somewhat resembles that of *Argulus coregoni* Thorell, 1864 but has not a digitiform process on the dorso-posterior margin of the second leg, which is one of the reliable features to characterize *A. coregoni* (Tokioka, 1936a; Yamaguti, 1937). In Lake Shinji, *A. japonicus* was previously collected from common carp *Cyprinus carpio* Linnaeus, 1758 (Nagasawa *et al.*, 2009).

In the summer of 1973, there was a serious drought around the lake, which caused a marked increase in lake water salinity up to 12.7‰ at the surface and 16.3‰ near the bottom [Suzumoto, 1974; chlorinity values (C) reported by this author are here transferred to salinity values (S, ‰) using an equation: $S = 0.030 + 1.8050 \times C$]. Under such unusual conditions, marine and brackish-water fishes from the nearby brackish-water lake and the Sea of Japan invaded Lake Shinji, where the co-invading parasite *C. orientalis* reproduced and heavily parasitized *Carassius cuvieri* (reported as *Carassius auratus*) in the brackish-water region (Suzumoto, 1974). Suzumoto (1974) examined those fish and reported on the heavy infection of *C. orientalis* but did not state the concurrent infection with *A. japonicus*. He probably overlooked the two specimens of *A. japonicus* mixed in the sample of over 600 specimens of *C. orientalis*.

The present finding of *A. japonicus* from the fish in the drought-induced brackish waters of Lake Shinji indicates that *A. japonicus* can survive in such salt waters, as previously observed in an aquarium (Yamauchi *et al.*, 2011). The salinity of Lake Shinji strikingly increased in July 1973 (Suzumoto, 1974), and the parasite specimens were sampled on 11 September 1973. Thus, *A. japonicus* most likely could survive for about two months in brackish waters. In other words, the species is able to tolerate salinity change for a certain period.

Carassius cuvieri is endemic to Lake Biwa and the Yodo River system, central Japan, and has been introduced to many Japanese rivers and lakes, including Lake Shinji. The Hii River flows into the western end of Lake Shinji, and the salinity of the lake increases from the western coast towards the eastern area (Date *et al.*, 1989). While *C. cuvieri* is generally regarded as a freshwater species, it is actually euryhaline as it is known to occur even in the brackish lower reaches of the Nagara River, a big river in central Japan (Suzuki and Kimura, 1977, 1978). In Lake Shinji and its adjacent region, *C. cuvieri* usually occurs in the lower reaches of the Hii River and the western (freshwater) area of the lake (Koshikawa, 1985), where the individual of *C. cuvieri* examined by Suzumoto (1974) perhaps became infected by *A. japonicus*. Moreover, because of its salt tolerance, this individual is considered to have survived along with *A. japonicus* in the drought-induced brackish waters.

Argulus flavescens Wilson, 1916 and *A. kosus* Avenant-Oldewage, 1994 are known to occur in both fresh and salt waters in North America (Causey, 1960; Cressey, 1972; Suárez-Morales *et al.*, 1998) and South Africa (Van As *et al.*, 1999), respectively. These species are completely separated from *A. japonicus* in their geographical distribution, and, most probably, their euryhaline characteristic has evolved independently. For understanding their occurrence and host utilization within their distributional range, it is desirable to experimentally access their tolerance to various salinities.

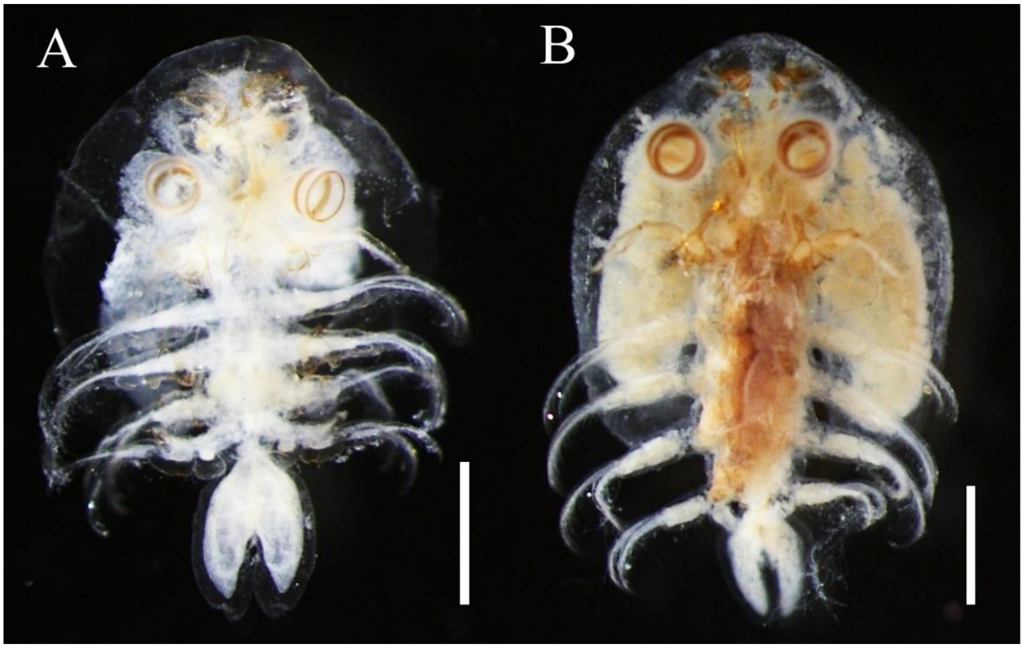

Fig. 3.3.1.1. *Argulus japonicus*, male (A) and female (B), NSMT-Cr 26691, ventral view, from the body surface of *Carassius cuvieri* in brackish waters of Lake Shinji, Shimane Prefecture, Japan, on 11 September 1973. Ethanol-preserved specimens. Scale bars: A and B, 1 mm.

3.3.2 New records of a freshwater fish parasite *Argulus japonicus* (Branchiura: Argulidae) from northern Honshu, Japan, with a note on its occurrence in a brackish water lake

[Based on Nagasawa, K., Nitta, M., and Azuma, N. 2024. New records of a freshwater fish parasite *Argulus japonicus* (Branchiura: Argulidae) from northern Honshu, Japan, with a note on its occurrence in a brackish water lake. Crustacean Research, 53 (in press)]

Abstract. One and two specimens of *Argulus japonicus* Thiele, 1900 were collected from the body surface of big-scaled redfin *Pseudaspius hakonensis* (Günther, 1877) (Cypriniformes: Leuciscidae) in the Babame River, Akita Prefecture, and Lake Jusan, Aomori Prefecture, northern Honshu, Japan, respectively. This is two new prefecture records in Japan and a new host record for *A. japonicus*. Lake Jusan is the northernmost collection locality of this parasite recorded from wild freshwater fishes in Japan. As Lake Jusan is a brackish water lake and big-scaled redfin is known to migrate from fresh to salt waters, it is inferred that the specimens of *A. japonicus* survived in brackish waters attaching on big-scaled redfin after the fish became infected in an inflowing river and migrated to the lake. The collection of *A. japonicus* in Lake Jusan represents the second record for the species from brackish water lakes in Japan.

3.3.2 Introduction

Argulus japonicus Thiele, 1900 is an ectoparasite of freshwater fishes (Yamaguti, 1963; Neethling and Avenant-Oldewage, 2016). This species was originally described by Thiele (1900) from a female specimen collected in Japan, where it has since been studied for various aspects of its biology (Nagasawa, 2009, 2011a). Nonetheless, the geographical distribution of the species is still poorly understood in this country, especially in the northern region of Honshu, the largest major island of Japan. Although there are six prefectures (Aomori, Iwate, Miyagi, Akita, Yamagata, and Fukushima) in this region, *A. japonicus* has been reported only from Miyagi Prefecture (Nagasawa *et al*., 2022b, 2023b).

During a parasitological survey of freshwater fishes in northern Honshu, we collected specimens of *A. japonicus* from big-scaled redfin *Pseudaspius hakonensis* (Günther, 1877) (Cypriniformes: Leuciscidae) from Akita and Aomori prefectures. This represents two new prefecture records in Japan and a new host record for *A. japonicus*.

3.3.2 Materials and methods

Ten individuals of big-scaled redfin were caught with a scoop net in the middle-reaches of the Babame River (39°56'36"N, 140°07'53"E) at Takasaki-Hirogano in Gojome, Akita Prefecture, on 30 July 2015 (locality 2 in Fig. 3.3.2.1). These fish were transported frozen to the laboratory of Hiroshima University, where they were thawed, measured for their body length (BL, mm), and examined for metazoan parasites. Seven individuals of big-scaled redfin were also caught with a small fixed net installed in coastal waters of a brackish water lake, Lake Jusan (41°00'17"N, 140°21'32"E), at Jusan in Goshogawa, Aomori Prefecture, on 26 September 2016 (locality 1 in Fig. 3.3.2.1). They were transported alive to the laboratory of Hirosaki University and similarly examined for their BL and metazoan parasites. When crustacean parasites were found at both laboratories, the parasites were carefully taken from the hosts using forceps and fixed in 70% ethanol. Later, these specimens were observed under an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope at the Aquaparasitology Laboratory. Identification was made using the wooden slide procedure recommended by Humes and Gooding (1964) and Benz and Otting (1996). The specimens were soaked in lactophenol for 2 hours before observation under the compound microscope, and drawings were made with the aid of a drawing tube attached to this microscope. After the specimens were identified, they were recorded for their sex and total length (TL, from the anterior tip of the carapace to the posterior tip of the abdomen). They have been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 31502, one specimen from the Babame

River; NSMT-Cr 31503, two specimens from Lake Jusan). The scientific and common names of fishes mentioned in this paper follow Hosoya (2015), except for those of *P. hakonensi* and the common names of *Carassius cuvieri* Temminck and Schlegel, 1846 and *Cyprinus carpio* Linnaeus, 1758, which are based on Froese and Pauly (2023).

3.3.2 Results

Argulus japonicus was collected from one (122 mm BL) of the 10 big-scaled redfin [86–122 (mean = 97) mm BL] in the Babame River, Akita Prefecture, and from two (216 and 230 mm BL) of the seven big-scaled redfin [190–230 (215) mm BL] in Lake Jusan, Aomori Prefecture. The number of *A. japonicus* per infected fish was one in both localities, and the species was found on the host's operculum (in the Babame River) and body surface (in Lake Jusan).

The collected specimens of *A. japonicus* are all females, being 3.1 (from the Babame River), 3.9, and 6.0 mm TL (from Lake Jusan). They is characterized by a dorsoventrally flattened body; a nearly circular carapace, covering the first to third pairs of legs; compound eyes visible in the frontal region of the carapace; a naupliar eye present dorsally along midline of the carapace; the ventral surface of the frontal and lateral regions of the carapace ornamented with numerous, small sharply pointed spines; posterolateral lobes of the carapace separated by a sinus, ending each in a rounded margin; paired respiratory areas, each comprising a small anterior area and a large posterior area; the thorax consisting of four segments, each bearing a pair of legs; a bilobed abdomen ending in a rounded tip; the first and second antennae found beneath the frontal region of the carapace; the preoral sheath and stylet located along the midline posterior to the second antennae; the mouth tube longer than wide, becoming wider posteriorly; the first maxillae forming cup-like suckers; the second maxillae each with a robust first segment bearing three large projections; the first to fourth pairs of legs biramous, each comprising the coxa, basis, exopod, and endopod; and the fourth pair of legs each with the coxa forming a natatory lobe (Fig. 3.3.2.2). In addition, the number of plumose setae on the posterior margin of the coxa in each of the first legs is constantly one (Fig. 3.3.2.3). The number of supporting rods per first maxilla ranges from 45 to 54 (52 and 54 in a specimen from the Babame River; 46 and 46, and 45 and 49, each in two specimens from Lake Jusan).

The body of the fresh specimen is nearly transparent and its respiratory areas are pale brown (Fig. 3.3.2.2A, B), whereas the body of the ethanol-preserved specimen is white and its carapace and thorax have dark brown dorsal spots (Fig. 3.3.2.2C, D).

Remarks. The morphological characters of the specimens collected in this study are almost identical to those of female *A. japonicus* reported by Tokioka (1936a), Yamaguti (1937), and Nagasawa (2021), and they are herein identified as this species.

In Japan, three species of the genus *Argulus*, *i.e*., *A. japonicus*, *A. coregoni* Thorell, 1864, and *A. mongolianus* Tokioka, 1939, have been reported from wild freshwater fishes (Nagasawa *et al*., 2022b). Of these species, *A. japonicus* and *A. coregoni* bear a morphological resemblance to each other (Tokioka, 1936a; Yamaguti, 1937) and show no strict host specificity (Nagasawa, 2011a, 2021), and the latter species also occurs in northern Honshu (Nagasawa and Ishikawa, 2015; Nagasawa *et al*., 2019, 2020c, 2023a). Thus, it is necessary to distinguish *A. japonicus* from *A. coregoni* using reliable morphological characters. As reported above, the specimens collected in this study have the first pair of legs each with a single plumose seta on the posterior margin of the coxa (Fig. 3.3.2.3), which can be used as one of the features to differentiate *A. japonicus* from *A. coregoni* (Nagasawa, 2021; Nagasawa *et al*., 2022b): the latter species has more than four plumose setae on the same posterior margin (4–7 in Yamaguti, 1937; 4–9 in Hoshina, 1950; 6 in Nagasawa and Taniguchi, 2021). Moreover, the present specimens have 45–54 supporting rods per first maxilla. This number is almost similar to those reported from *A. japonicus* (ca. 50 in Tokioka, 1936a; 40–50 in Yamaguti, 1937; 50 and 52 in Nagasawa, 2021; 46–51 in Nagasawa *et al*., 2023b; 47 and 48 in Nagasawa *et al.*, 2023c). In contrast to this, *A. coregoni* has usually more than 60 supporting rods (ca. 60 in Tokioka, 1936a; 60–70 in Yamaguti, 1937; 54–73 in Hoshina, 1950; 67 and 72 in Nagasawa and Taniguchi, 2021).

While there are some records of disease-causing argulid branchiurans or fish lice in northern Honshu, their identification has not been made based on morphological and/or molecular features (Nagasawa *et al*., 2023b). In this region, *A. japonicus* is known to occur only in two small lakes (Lake Izunuma and Lake Uchinuma), Miyagi Prefecture (Nagasawa *et al*., 2022b, 2023b), and the present collection of the species extends its distribution range from Miyagi Prefecture northward to Akita and Aomori prefectures (Fig. 3.3.2.1). Moreover, *A. japonicus* was reported from reared common carp *Cyprinus carpio* from Hokkaido Island north of Honshu (Nagasawa *et al.*, 1989; Nagasawa, 1994, 2018) but there is no record of this parasite from wild freshwater fishes of the island. Thus, Lake Jusan represents the northernmost collection locality in Japan for *A. japonicus* recorded from wild freshwater fishes.

Big-scaled redfin, from which we collected the specimens of *A. japonicus*, is distributed in East Asia surrounding the Sea of Japan, including the Japanese Archipelago, the eastern Korean Peninsula, Primorsky Kray, and Sakhalin Island (Russia) (Sakai *et al.*, 2002; Watanabe *et al*., 2018). To date, *A. japonicus* has not been reported from this fish species in Japan (Nagasawa and Katahira, 2013) and the above adjacent regions (*e.g*., Smirnova, 1971; Sokolov *et al.*, 2012). Here, big-scaled redfin is regarded as a new host of *A. japonicus*.

3.3.2 Discussion

Since *A. japonicus* is the fresh water parasite that completes its life cycle in fresh waters (Tokioka, 1936b), it is interesting to note that this parasite was collected in Lake Jusan, which is connected to the Sea of Japan and its salinity often exceeds 20 psu in coastal waters near the mouth of the Iwaki River, one of the rivers flowing into the lake (Umeda *et al.*, 2008). A similar occurrence in both freshwater and salt waters has been reported in three *Argulus* species in other parts of the world: *A. dartevellei* Braian, 1940 in Congo (Fryer, 1960), *A. kosus* Avenant-Oldewage, 1994 in South Africa (Van As *et al.*, 1999), and *A. flavescens* Wilson, 1916 in North America (Causey, 1960; Cressey, 1972; Suárez-Morales *et al.*, 1998). In Japan, *A. japonicus* was collected in drought-induced brackish waters in Lake Shinji as well, and Nagasawa (2019) has suggested that this parasite could survive in those lake waters for a certain period along with its euryhaline host, Japanese white crucian carp *Carassius cuvieri* (Cypriniformes: Cyprinidae). This fish usually occurs in fresh waters but can stay even in brackish waters (Suzuki and Kimura, 1977, 1978). As big-scaled redfin is also a euryhaline species and some individuals migrate from fresh to salt waters for feeding and growth

(Nakamura, 1969; Sakai, 1995), *A. japonicus* is inferred to be able to survive in brackish waters of Lake Jusan when this parasite is attached on such lake-migrating big-scaled redfin.

Based on the above consideration, Lake Jusan is not regarded as the habitat constantly utilized by *A. japonicus*, and this parasite is very likely to maintain its populations in the rivers flowing into the lake. The Iwaki River is the largest among those rivers, and at least 20 species of freshwater fishes are found in its middle-reaches, where big-scaled redfin is the most abundant, followed by fat minnow *Phoxinus lagowskii steindachneri* Sauvage, 1883, pale chub *Opsariichthys platypus* (Temminck and Schlegel, 1846), and ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) (Izumi *et al.*, 2006). It is thus reasonable to infer that big-scaled redfin serves as a host for *A. japonicus* in the Iwaki River and some of its infected individuals migrate to Lake Jusan. In addition, pale chub has been reported as a host of *A. japonicus* in other localities of Japan (Nagasawa and Sato, 2014; Nagasawa, 2017b; Nagasawa *et al*., 2023c) and this fish may also harbor the parasite in the Iwaki River.

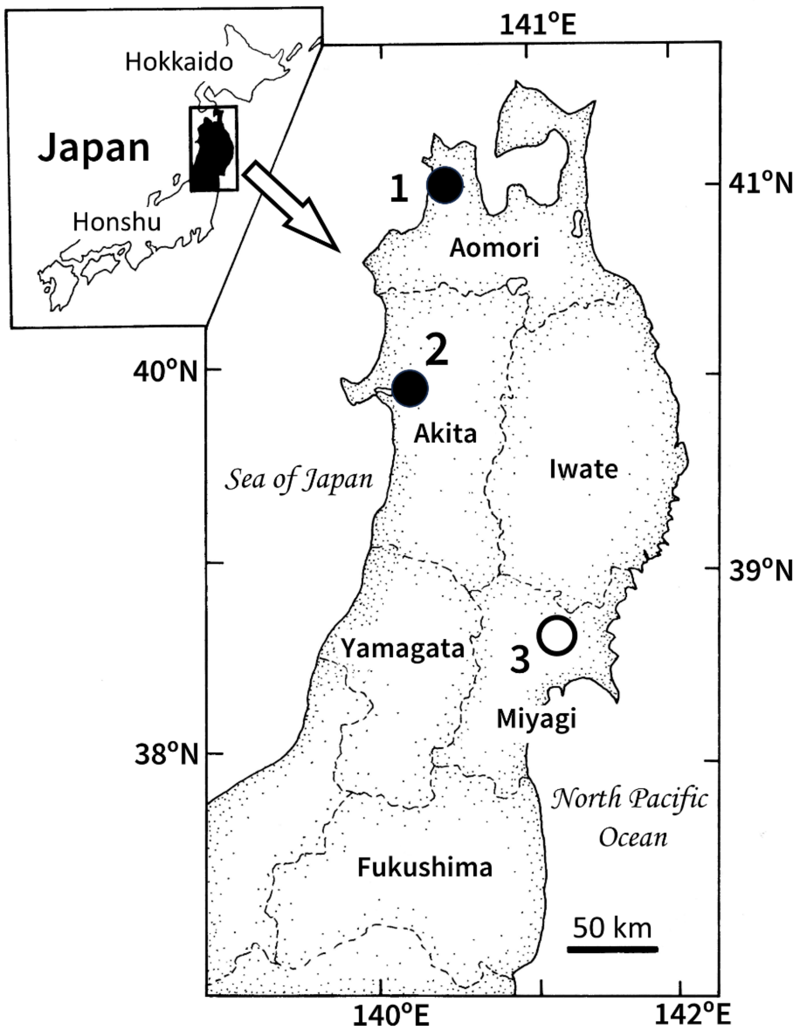

Fig. 3.3.2.1. Map of northern Honshu, Japan, showing the collection localities of *Argulus japonicus* in the previous (open circle) and present (closed circles) studies. 1, Lake Jusan, Aomori Prefecture (this paper); 2, Babame River, Akita Prefecture (this paper); 3. Lake Izunuma and Lake Uchinuma, Miyagi Prefecture (Nagasawa *et al.*, 2022b, 2023b).

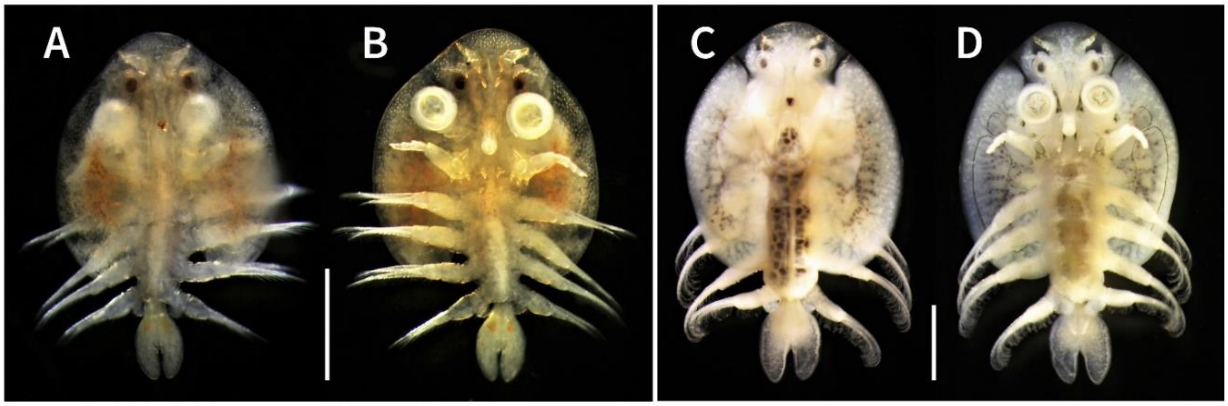

Fig. 3.3.2.2. *Argulus japonicus*, female (A and B, 3.1 mm total length) and female (C and D, 6.0 mm total length), NSMT-Cr 31502 and 31503, from the body surface of big-scaled redfin *Pseudaspius hakonensis* in the Babame River, Akita Prefecture, and Lake Jusan, Aomori Prefecture, northern Honshu, Japan, respectively. The frozen-thawed (A and B) and ethanol-preserved specimens (C and D) of *A. japonicus* were photographed. A and C, habitus, dorsal view; B and D, habitus, ventral view. Scale bars: A and B, 1 mm; C and D, 1 mm.

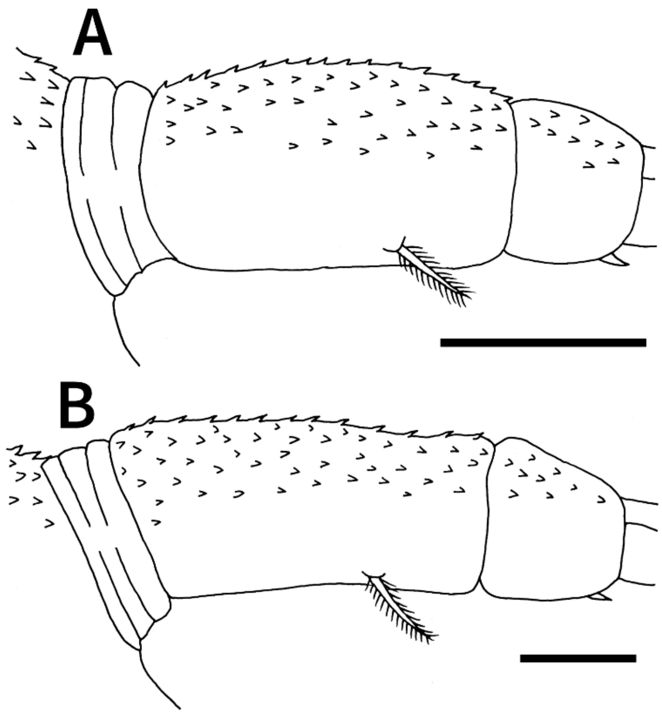

Fig. 3.3.2.3. Coxa (with a plumose seta) and basis of the first leg in the female (A, 3.1 mm total length) and female (B, 6.0 mm total length) of *Argulus japonicus*, NSMT-Cr 31502 and 31503, from big-scaled redfin *Pseudaspius hakonensis* in the Babame River, Akita Prefecture, and Lake Jusan, Aomori Prefecture, northern Honshu, Japan, respectively. Ventral view. Scale bars: A and B, 0.2 mm.

3.3.3 Occurrence of *Argulus japonicus* on a cyprinid in a river flowing through a residential area

[Based on Nagasawa, K., Nitta, and Kawai, K. (2023c): First specimen-based record of *Argulus japonicus* (Branchiura: Argulidae), an ectoparasite of freshwater fishes, from Okayama Prefecture, western Japan. Biogeography, 25: 19–21]

Abstract. An argulid branchiran is known to infect carp *Cyprinus carpio* Linnaeus, 1758 farmed in Okayama Prefecture, western Japan, but no species identifcation has been made to date. A male of *Argulus japonicus* Thiele, 1900 was collected from the body surface of a pale chub *Opsariichthys platypus* (Temminck and Schlegel, 1846) (Cyprinidae) in the Seno-o River, a tributary of the Kurashiki River, Okayama Prefecture. The specimen collected is briefly described and reported herein as the first specimen-based record for *A. japonicus* from this prefecture. The collection site was the middle-reaches of the Seno-o River flowing through a residential area, where the current was very slow due to a sluice gate installed downstream and only cyprinids including pale chub were collected. This indicates that *A. japonicus* can maintain its population in such lentic waters utilizing cyprinids as its hosts.

3.3.3 Introduction

Argulus japonicus Thiele, 1900 is one of the parasites found on Japanese freshwater fishes (Nagasawa, 2009, 2011a). There are some records of an argulid branchiuran from farmed carp *Cyprinus carpio* Linnaeus, 1758 in Okayama Prefecture, western Japan, but no species identification has been made to date based on its morphological and/or molecular features (Sugiyama and Ueki., 1977, 1978, 1980; Ueki and Ishida, 1986; Yamanoi *et al.*, 1992, 1995; Masunari *et al*., 1999, 2001; Mito and Murata, 2004). During a parasitological survey of freshwater fishes in this prefecture, we collected a specimen of *A. japonicus* from a pale chub *Opsariichthys platypus* (Temminck and Schlegel, 1846) (Cyprinidae). Here, the specimen of *A. japonicus* collected is briefly described and reported as the first specimen-based record for the species from Okayama Prefecture.

3.3.3 Materials and methods

In total, 14 individuals of three cyprinid species, representing pale chub (n = 6), Korean moroko gudgeon *Squalidus chankaensis tsuchigae* (Jordan and Hubbs, 1925) (n = 5) and silver crucian carp *Carassius* sp. (n = 3), were collected using hook and line in the Seno-o River (34°36'16"N, 133°51'52"E), a tributary of the Kurashiki River at Uchio in Okayama City, Okayama Prefecture, on 2 August 2014. The collection site was the middle-reaches of the former river (ca. 5.0 m width, ca. 0.5 m depth) flowing through a residential area (Fig. 3.3.3.1A). Fishes were transported alive to the laboratory of Hiroshima University, Hiroshima Prefecture, where they were identified, measured for body length (BL, mm), and examined for ecto- and endoparasites. One argulid branchiuran was taken from a pale chub using forceps and fixed in 70% ethanol. Later, this specimen was observed under an Olympus SZX10 stereo microscope and an Olympus BX51 phase-contrast compound microscope at the Aquaparasitology Laboratory, Shizuoka Prefecture. Identification was made using the wooden slide procedure recommended by Benz and Otting (1996). After the specimen was identified, it was recorded for its sex, total length (TL, from anterior tip of carapace to posterior tip of abdomen), carapace length (from anterior tip to posterior tip of carapace), and body width (around midlength of carapace). It has been deposited in the Crustacea (Cr) collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture (NSMT-Cr 31492). The scientific and common names of fishes mentioned in this paper follow Hosoya (2015).

3.3.3 Results and Discussion

One (82.4 mm BL) of the six pale chub examined (61.9–89.9 mm BL) was found to be infected by a male of *A. japonicus* on the body surface. No argulid branchiuran was collected from the two other cyprinids: Korean moroko gudgeon (61.9–72.4 mm BL, n = 5) and silver crucian carp (31.3–39.6 mm BL, n = 3).

The male specimen of *A. japonicus* collected (Fig. 3.3.3.1B, C) was small, measuring 2.5 mm TL, 1.7 mm carapace length (68.0% of TL), and 1.6 mm body width (64.0% of TL). In Japan, three species of *Argulus* (*A. japonicus*, *A. coregoni* Thorell, 1864, and *A. mongolianus* Tokioka, 1939) are known to infect wild freshwater fishes (Nagasawa *et al.*, 2022b). Of these species, *A. japonicus* is morphologically similar to *A. coregoni* but can be differentiated from the latter species by having the first pair of legs each with a single plumose seta on the posterior margin of the coxa (Yamaguti, 1937; Nagasawa, 2021), which is confirmed in this study as well (Fig. 3.3.3.1D). Furthermore, the present specimen has 47 and 48 supporting rods per first maxilla, which correspond to the number of supporting rods previously recorded from *A. japonicus* (ca. 50 in Tokioka, 1936a; 40–50 in Yamaguti, 1937; 50 and 52 in Nagasawa, 2021). It is important to distinguish *A. japonicus* from *A. coregoni* because the latter species was recently reported from a cyprinid in the lower reaches of the Asahi River, Okayama Prefecture (Nagasawa and Taniguchi, 2021). The collection site in the Asahi River is located only ca. 17 km northeast of that in the Seno-o River.

The present study has shown that *A. japonicus* occurs in Okayama Prefecture. This parasite has been recorded from adjacent prefectures of western Honshu, including Hyogo, Shimane, Hiroshima, and Yamaguchi prefectures (Nagasawa *et al.*, 2009; Nagasawa, 2019, 2021, 2023a), and pale chub is known to serve as a host for the parasite in other localities of Japan (Nagasawa and Sato, 2014; Nagasawa, 2017b). Since *A. japonicus* shows no strict host specificity (Nagasawa, 2021), it may also parasitize the two other species (Korean moroko gudgeon and silver crucian carp) in the Seno-o River. In this study, fish collection was made at the middle-reaches of the river flowing through a residential area (Fig. 3.3.3.1A), where the current was very slow due to a sluice gate installed downstream and only cyprinids were collected. This indicates that *A. japonicus* can maintain its population in such lentic waters utilizing cyprinids as its hosts.

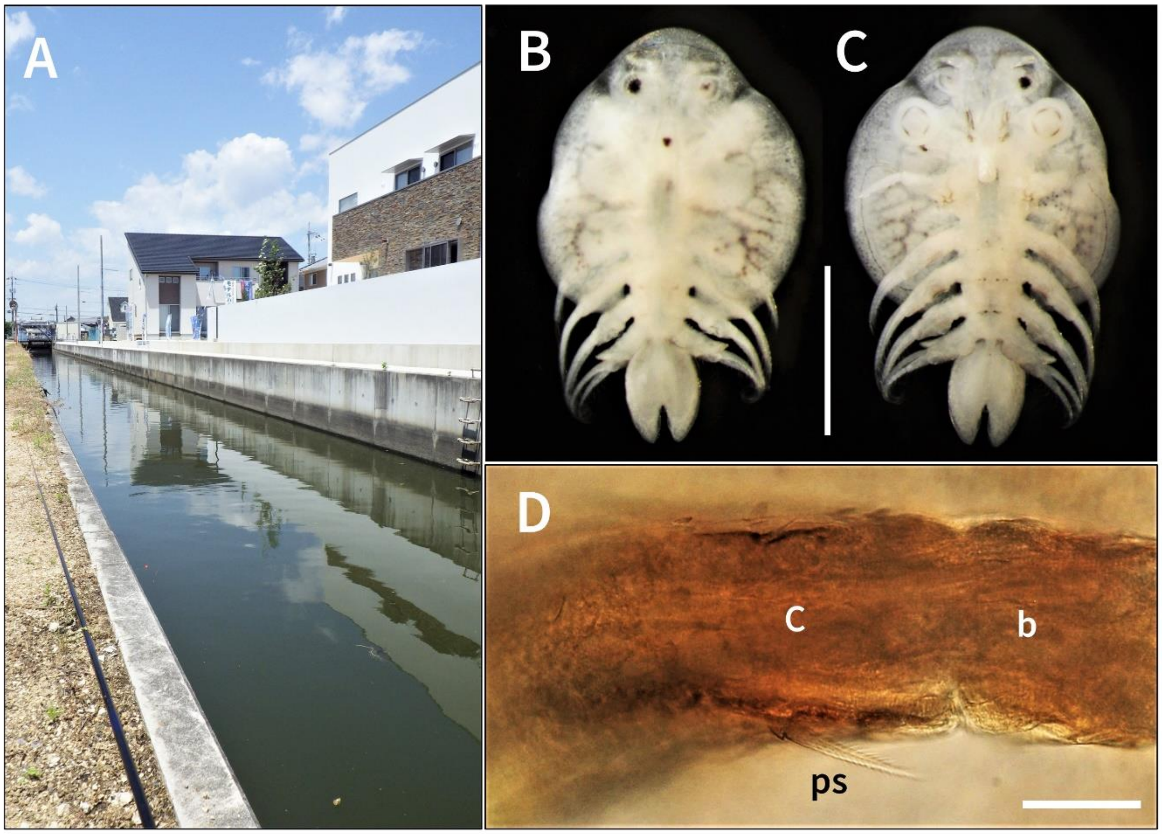

Fig. 3.3.3.1. Collection site in the Seno-o River, Okayama Prefecture (A, photo taken on 21 May 2015) and *Argulus japonicus*, male (2.5 mm total length), NSMT-Cr 31492, from pale chub *Opsariichthys platypus* (B–D). The ethanol-preserved specimen of *A. japonicus* was photographed. B, habitus, dorsal view; C, habitus, ventral view; D, micrograph of coxa (with a single plumose seta) and basis of first leg, ventral view. Abbrevations: b, basis; c, coxa; ps, plumose seta. Scale bars: B, C, 1 mm; D, 0.05 mm.

3.4 Infection of *Argulus coregoni* on farmed freshwater fishes

[Based on Nagasawa, K. and Yuasa, A. (2020): The fish louse *Argulus coregoni* from *Oncorhynchus masou ishikawae* (Salmonidae) cultured in Shikoku, western Japan, with a list of occurrence records of *A. coregoni* from fishes reared in Japan. Crustacean Research, 49: 1–8]

Abstract. *Argulus coregoni* Thorell, 1864 is briefly described based on specimens from the body surface of red-spotted masu salmon *Oncorhynchus masou ishikawae* Jordan and McGregor, 1925 cultured at a trout farm in Tokushima Prefecture, Shikoku, western Japan. This represents the first confirmed record of *A. coregoni* from farmed salmonids in Shikoku. A list of occurrence records of *A. coregoni* from fishes reared in Japan is also provided based on the literature published between 1950 and 2019. The species has so far been reported from three species and two subspecies of salmonids and one species of plecoglossid reared at 14 sites in 10 prefectures (Tochigi, Tokyo, Nagano, Ishikawa, Gifu, Aichi, Wakayama, Hyogo, Tokushima, and Yamaguchi). Most of these sites were governmental or research facilities, and it is evident that little attention has been paid to *A. coregoni* at commercial tout farms. It is likely that the species occurs at more trout farms in Japan.

3.4 Introduction

In Japan, freshwater salmonid culture is conducted mainly in mountain areas, where cold waters are abundantly available. To date, four species of crustacean parasites have been reported from salmonids farmed in Japan: three of them are lernaeopodid copepods, *Salmincola californiensis* (Dana, 1852) (Hoshina and Nishimura, 1976, 1977), *S. carpionis* (Krøyer, 1837) (Wakabayashi, 1997), and *S. stellata* Markevich, 1936 (Nagasawa and Urawa, 1991), while the remaining species is the argulid branchiuran, *Argulus coregoni* Thorell, 1864. Most of the Japanese records of *A. coregoni* from cultured salmonids are based on the collections in central Honshu (sites 1–12 in Fig. 3.4.3), and only a single record is present of the species infecting farmed salmonids from western Japan, where it parasitizes red-spotted masu salmon *Oncorhynchus masou ishikawae* Jordan and McGregor, 1925 in Yamaguchi Prefecture, the westernmost prefecture of Honshu (site 14 in Fig. 3.4.3; Nagasawa *et al*., 2017). In a newsletter from the Fisheries Research Institute, Yuasa (2014) briefly reviewed infectious diseases of red-spotted masu salmon farmed in Tokushima Prefecture, Shikoku, western Japan, and showed a photograph of fish louse from a trout farm at Naka (site 13 in Fig. 3.4.3). The photograph was of low quality, and the fish louse was provisionally reported as *A. coregoni*. However, no morphological information was given for this identification. Recently, the present author collected argulid specimens from red-spotted masu salmon at the same trout farm and identified them as *A. coregoni*. This paper briefly describes the male and female of *A. coregoni* as the first confirmed record of the species from farmed salmonids in Shikoku. The paper also provides a list of records of *A. coregoni* from fishes reared in Japan, based on the literature published between 1950 and 2019. Hoshina (1950) reported *A. coregoni* for the first time from fish-rearing facilities in Japan.

3.4 Materials and methods

Argulid specimens were collected from the anterior body surface of red-spotted masu salmon at a trout farm, Naka, Tokushima Prefecture, on 23 August 2019. The specimens were fixed in 70% ethanol in the field and later sent to the Laboratory of Aquaparasitology, Shizuoka, where they were individually identified, sexed, and measured for total length (from the anterior tip of the carapace to the posterior end of the abdomen) and body width (maximum width of carapace). They are retained by the first author for a taxonomic study of *Argulus* spp. from Japanese freshwater fishes but will be deposited in the Crustacea collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture, Japan. The scientific names of fishes mentioned herein follow Nakabo (2013).

3.4 Results

In total, 21 specimens of *A. coregoni* were collected from seven of the 20 age-1 red-spotted masu salmon examined (ca. 20 cm in total length; prevalence, 35.0%; mean intensity, 3.0).

The specimens consisted of seven adult males and 14 adult females. A brief description of the specimens is given here.

Adult male (Fig. 3.4.1A, B): Body dorsoventrally flattened, 5.3–8.1 (mean, 6.7) mm in total length (TL) and 3.0–7.3 (5.5) mm in body width (n = 7). Carapace comprising 68.7–74.5% (mean, 72.6%, n = 7) of TL, circular with frontal region delimited by pronounced anterolateral depressions, and usually covering all portions of thorax. Paired compound eyes located dorsally in front of joint of frontal region and main portion of carapace. Naupliar eye present at midline of anterior dorsal surface of main portion of carapace. Ventral surface of marginal portions of frontal and lateral regions of carapace ornamented with numerous small spines. Posterolateral lobes of carapace bluntly rounded and slightly overlapped. Paired respiratory areas, each consisting of anterior small and posterior large portions, on ventral surface of lateral regions of carapace. Thorax four-segmented. Abdomen bilobed, longer than wide, and slightly pointed. Paired testes with small black spots on both dorsal and ventral surfaces. Digitiform process on dorsoposterior margin of second leg present only in male.

Adult female (Fig. 3.4.1C, D): Body 4.8–10.6 (mean, 8.4) mm in TL and 3.2–5.0 (4.4) mm in body width (n = 14). Carapace comprising 65.3–79.7% (mean, 72.5%, n = 14) of TL. Similar to adult male, but differences as follows: posterolateral lobes of carapace not overlapped; thorax quite visible in interspace between lobes and speckled dorsally with large pigment spots; abdomen less pointed; and paired spermathecae without any pigmentation present in anterior region of abdomen.

3.4 Discussion

The specimens of *A. coregoni* collected correspond to morphological features of the species previously reported from Japan (Tokioka, 1936a; Yamaguti, 1937; Hoshina, 1950). As figured by Tokioka (1936a: fig. 3) and Yamaguti (1937: fig. 17), a digitiform process on the dorsoposterior margin of the male’s second leg is the feature to characterize the species, and this study has confirmed that the process is present in the male specimens. The present collection of the species represents its second record from Shikoku, where the species was previously found from wild ayu *Plecoglossus altivelis altivelis* (Temminck and Schlegel, 1846) in the neighboring Kochi Prefecture (Nagasawa and Ikeda, 2011).

Two species of *Argulus* are known to infect freshwater fishes farmed in Japan: *A. coregoni* and *A. japonicus* Thiele, 1900 (Nagasawa, 2009, 2011a). The former species is distinguished from the latter by the presence of a digitiform process on the dorsoposterior margin of the second leg in male (vs. absence of such a process in male *A. japonicus*, see Tokioka, 1936a; Yamaguti, 1937); a slightly pointed abdomen in male (Fig. 3.4.1A, B) (vs. non-pointed abdomen in male *A. japonicus*, see Nagasawa and Ishiyama, 2019b: fig. 1); testes with small black spots on both the dorsal and ventral surfaces (Fig. 3.4.1A, B) (vs. those without any black dot in male *A. japonicus*, see Nagasawa and Ishiyama, 2019b: fig. 1); and thorax speckled dorsally with large pigment spots in female (Fig. 3.4.1C, D) (vs. non-speckled thorax in female *A. japonicus*, see Nagasawa and Ishiyama, 2019b: fig. 1).

Based on the life cycle of *A. coregoni* studied at a trout hatchery in central Japan (Shimura, 1983a), the specimens of the species collected in this study correspond to individuals of the first generation that hatch in spring and summer from overwintered eggs laid in the preceding autumn. In particular, the female specimens collected consist of two size groups (Fig. 3.4.2), and large females (9.8–10.6 mm TL length, n = 8) were all ovigerous (see Fig. 3.4.1D). Thus, these mature females were most likely to soon lay eggs, which become individuals of the second generation and the overwinters.

According to the manager of the trout farm surveyed in this study, no large skin parasites had infected salmonids before the mid-1980’s, when such parasites were introduced along with live salmonids from a trout farm using waters from a different river in Shikoku. Argulids were actually recognized there in 2013 (Yuasa, 2014) and the newly-collected specimens in this study are identified as *A. coregoni*. These indicate that *A. coregoni* has been established at the trout farm since its introduction in the mid-1980’s.

As reported by Shimura (1983a), the presence of two size groups of the female specimens of *A. coregoni* in this study (Fig. 3.4.2) is inferred to have resulted from a difference in month of hatching in each size group: the large and small individuals might have hatched in May to June and July, respectively. Moreover, the male specimens consisted of individuals of 5.3–8.1 mm TL (Fig. 3.4.2), and the absence of larger male specimens can be explained by Shimura’s

(1983a) observation of the male of *A. coregoni* stopping growth when it reaches to about 8 mm.

While *A. coregoni* is known to occur in 21 prefectures of Japan (see Nagasawa, 2009; Nagasawa *et al.*, 2019; Nagasawa and Ishiyama, 2019a for the earlier literature), the species has been recorded from salmonids and/or ayu reared at 14 sites in 10 prefectures (Tochigi, Tokyo, Nagano, Ishikawa, Gifu, Aichi, Wakayama, Hyogo, Tokushima, and Yamaguchi) (Fig. 3.4.3, Table 3.4.1). Salmonids were recorded from all of the 14 sites, while ayu was only from two sites. The majority of these 14 collection sites (71.4%) were governmental or research facilities, and it is evident that little attention has been paid to *A. coregoni* at commercial tout farms. The species is thus expected to occur at more trout farms, and we need to investigate its occurrence at trout farms in various regions of Japan. One of the reasons for such inactive research on *A. coregoni* infecting commercially farmed fishes is because only a few researchers worked on argulid branchiurans in fisheries science in Japan.

The fishes reported as the hosts of *A. coregoni* are three species and two subspecies of the family Salmonidae [rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792); masu salmon *O. masou masou* (Brevoort, 1856); red-spotted masu salmon *O. masou ishikawae*; brook trout *Salvelinus fontinalis* (Mitchill, 1814); yamato charr *S. leucomaenis japonicus* Oshima, 1961] and one species of the family Plecoglossidae (ayu *P. a. altivelis*) (Table 3.4.1). In Japan, two more subspecies of charrs (*Salvelinus*), white-spotted char *S. l. leucomaenis* (Pallas, 1814) and Nikko char *S. leucomaenis pluvius* (Hilgendorf, 1876) are also farmed, and *A. coregoni* may be found on these chars in future parasitological surveys at trout farms. Both rainbow trout and brook trout are the species introduced from North America, while the others are native to Japan. As reported from fishes of other families (Tokioka, 1936a; Nagasawa *et al.*, 2014; Nagasawa and Ishikawa, 2015), *A. coregoni* is not strictly host-specific.

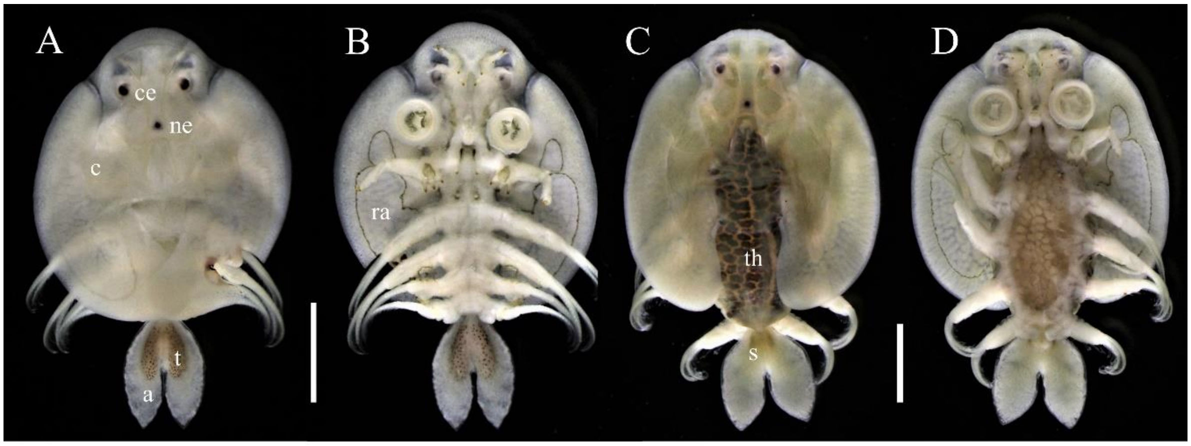

Fig. 3.4.1. *Argulus coregoni*, adult male (A, B, 8.1 mm total length) and adult female (C, D, 10.2 mm total length), from the body surface of red-spotted masu salmon *Oncorhynchus masou ishikawae* farmed at a trout farm, Naka, Tokushima Prefecture, Shikoku, western Japan. Ethanol-fixed specimens, dorsal (A, C) and ventral (B, D) views. The posterior margin of the right posterolateral lobe of the carapace is slightly damaged in the male specimen. Abbreviation: a, abdomen; c, carapace; ce: compound eye; ne, nauplius eye; ra, respiratory area; s, spermatheca; t, testis; th, thorax. Scale bars: A, B, 2 mm; C, D, 2 mm.

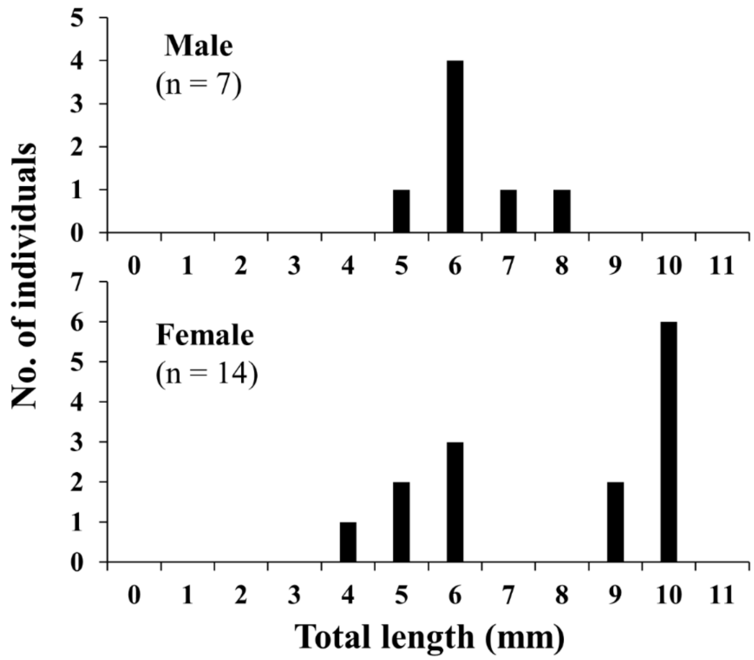

Fig. 3.4.2. Total length distributions of male (top) and female (bottom) *Argulus coregoni* from the body surface of red-spotted masu salmon *Oncorhynchus masou ishikawae* farmed at a trout farm, Naka, Tokushima Prefecture, Shikoku, western Japan, on 23 August 2019.

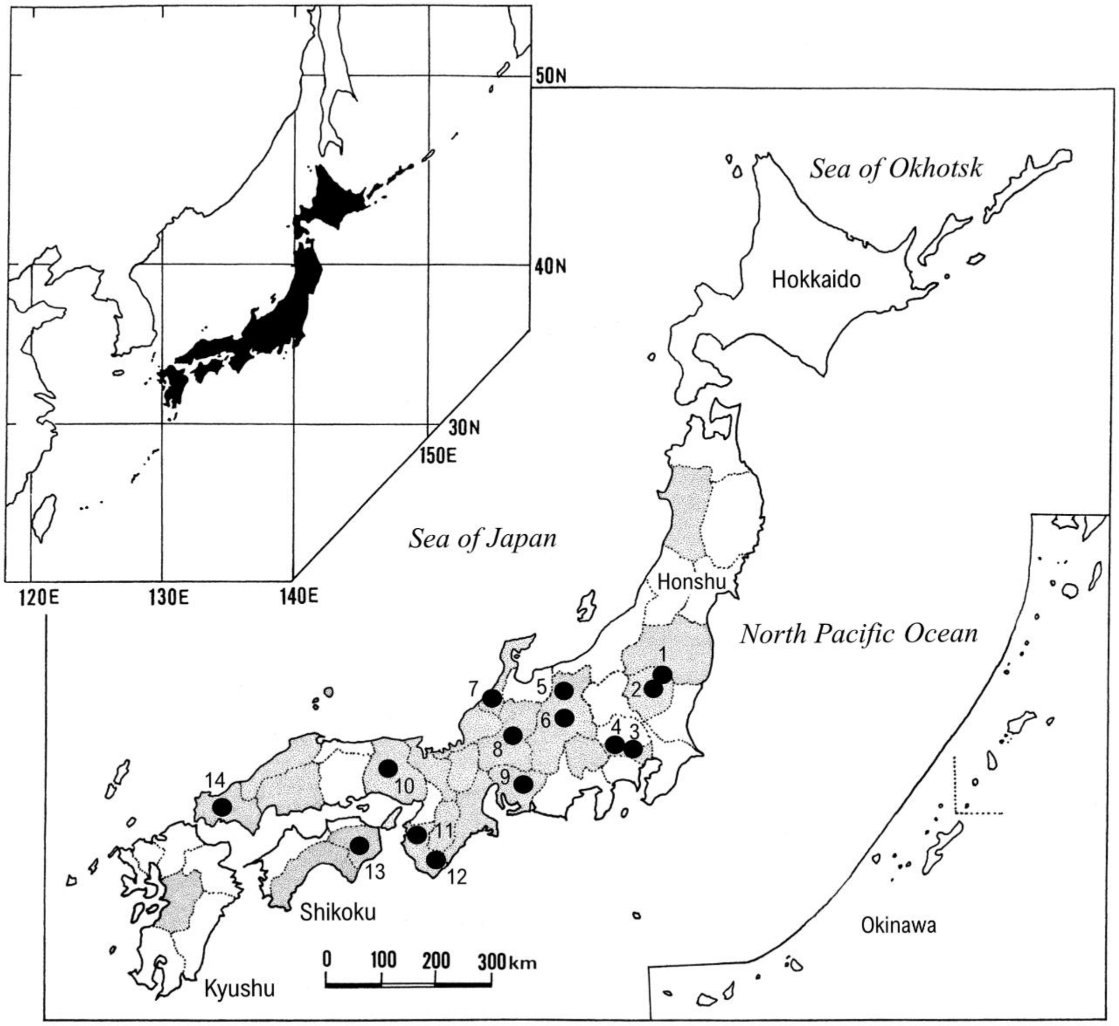

Fig. 3.4.3. A map of the Japanese Archipelago, showing the collection sites (closed circles) of *Argulus coregoni* infecting fishes in captivity. Information on these sites (1–14) is given in Table 3.4.1. Twenty-one prefectures, from which *A. coregoni* has been recorded, are as follows (shown as tinted areas, from the northeast to the southwest): Akita, Fukushima, Tochigi, Tokyo, Yamanashi, Nagano, Ishikawa, Gifu, Aichi, Shiga, Mie, Kyoto, Nara, Wakayama, Okayama, Tokushima, Kochi, Shimane, Hiroshima, Yamaguchi, and Kumamoto) (see Nagasawa, 2009; Nagasawa *et al.*, 2019; Nagasawa and Ishiyama, 2019a for the literature). Prefectural boundaries are indicated by dotted lines.

Table 3.4.1. Records of *Argulus coregoni* from fishes reared in Japan.

Prefecture	Collection site	Number in Fig. 3.4.3	Host		Reference
			Family	Species	
Tochigi	Fish ponds in Ohtawara	1	Salmonidae	*Oncorhynchus mykiss*	Nagasawa *et al.* (2015)
	Fish farm in Sakura	2		*Oncorhynchus mykiss*	Nagasawa *et al.* (2015)
		2		*Salvelinus fontinalis*	Nagasawa *et al.* (2015)
Tokyo	Yoshino Fish Farm, Tokyo Fisheries Research Institute, in Ome	3		*Oncorhynchus mykiss*	Hoshina (1950, reported as *Salmo irideus*)
		3		*Salvelinus fontinalis*	Hoshina (1950)
	Okutama Branch, Tokyo Metropolitan Fisheries Experimental Station, in Okutama	4		*Oncorhynchus masou masou*	Inoue *at al.* (1980, reported as *O. masou*), Shimura and Egusa (1980, reported as *O. masou*), Shimura (1981, 1983a, 1983b, reported as *O. masou*), Shimura *et al.* (1983a, 1983b, reported as *O. masou*), Shimura and Inoue (1984, reported as *O. masou*)
		4		*Oncorhynchus mykiss*	Shimura and Egusa (1980, reported as *Salmo gairdneri*), Shimura (1981, 1983a, reported as *S. gairdneri*), Shimura *et al.* (1983b, reported as *S. gairdneri*), Shimura and Inoue (1984, reported as *S. gairdneri*)
		4		*Salvelinus fontinalis*	Inoue *at al.* (1980), Shimura and Egusa (1980)
Nagano	Kizaki Branch, National Fisheries Research Institute, in Omachi	5		*Oncorhynchus mykiss*	Hoshina (1950, reported as *Salmo irideus*)
	Akashina Fisheries Guidance Center in Azumi	6		*Salvelinus fontinalis*	Hoshina (1950)
Ishikawa	Ishikawa Prefecture Fisheries Research Center in Kaga	7		*Oncorhynchus masou masou*	Nagasawa and Ishiyama (2019)
Gifu	Gero Branch, Gifu Prefectural Research Institute for Fisheries and Aquatic Environments, in Gero*	8		*Oncorhynchus mykiss*	Hosoe *et al.* (1975)
		8		*Oncorhynchus masou ishikawae*	Hosoe *et al.* (1975), Tokuhara *et al.* (2010, 2019)
Aichi	Horai Fish Farm, Aichi Prefectural Fisheries Experimental Station, in Shinshiro	9		*Oncorhynchus mykiss*	Uno *et al*. (1975), Ishii *et al.* (1978)
		9		*Oncorhynchus masou ishikawae*	Uno *et al.* (1975), Ishii *et al.* (1978)
		9		*Salvelinus leucomaenis japonicus*	Ishii *et al.* (1978, reported as "iwana" in Japanese)***
Hyogo	Institute of Hanzaki in Asago	10		*Oncorhynchus masou ishikawae*	Nagasawa *et al.* (2009)
Wakayama	Trout farm in Yamaji-Gôri**	11		*Oncorhynchus mykiss*	Hoshina (1950, reported as *Salmo irideus*)
	Fisheries Laboratory, Kinki University, in Shingu	12		*Oncorhynchus masou ishikawae*	Nagasawa and Ohya (1991a, reported as *O. rhodurus*), Kaji *et al.* (2011, reported as *O. masou ishikawai*)
Tokushima	Trout farm in Naka	13		*Oncorhynchus masou ishikawae*	Yuasa (2014), This paper
Yamaguchi	Trout farm in Tokuji-Fukadani	14		*Oncorhynchus masou ishikawae*	Nagasawa *et al.* (2017)
Nagano	Akashina Fisheries Guidance Center in Azumi	6	Plecoglossidae	*Plecoglossus altivelis altivelis*	Hoshina (1950, reported as *P. altivelis*)
Wakayama	Fisheries Laboratory, Kinki University, in Shingu	12		*Plecoglossus altivelis altivelis*	Nagasawa and Ohya (1991b, reported as *P. altivelis*)

* This institute was reported as the Gifu Prefectural Fisheries Experimental Station by Hosoe *et al.* (1975).

** The location of "Yamagi-Gôri" was discussed by Nagasawa and Ohya (1991a: 87, footnote 1).

*** Nagasawa (2009) regarded "iwana" reported by Ishii *et al.* (1978) as *Salvelinus leucomaenis japonicus*.

Chapter 4. General discussion

As mentioned in Chapter 1 (Introduction), 12 nominal species of the branchiuran genus *Argulus* have been reported from fishes in Japan, and they comprise six freshwater species (*A. americanus*, *A. coregoni*, *A. japonicus*, *A. lepidostei*, *A. mongolianus*, and *A. nobilis*) and six marine species (*A. caecus*, *A. kusafugu*, *A. matuii*, *A. onodai*, *A. quadristriatus*, and *A. scutiformis*). Of these species, four freshwater species (*A. japonicus*, *A. coregoni*, *A. mongolianus*, and *A. nobilis*) and two marine species (*A. caecus* and *A. matuii*) are redescribed in Chapter 2 based on a detailed examination of newly collected specimens from Japan.

Two freshwater species, *A. mongolianus* and *A. nobilis*, are reported for the first time in Japan: the former species is most probably of Chinese origin, and the latter species of North American origin. In particular, *A. mongolianus* has already established its populations in Japan and the specimens of the species were actually collected from two lakes in Miyagi Prefecture, northern Honshu, and a reservoir in Fukuoka Prefecture, northern Kyushu. The fish hosts reported from these localities include largemouth bass *Micropterus salmoides* (Centrarchidae); common carp *Cyprinus carpio*; Japanese white crucian carp *Carassius cuvieri*; silver crucian carp *Carassius* sp. (Cyprinidae); and Japanese eel *Anguilla japonica* (Anguillidae), which indicates that *A. mongolianus* shows no host specificity. Since fish translocations have been widely conducted and played a significant role in spreading *A. mongolianus* in Japan, it is very likely that this parasite occurs on more fish species in various regions of the country.

Argulus nobilis is specific to gars (Lepisosteidae) in the U.S.A. (Hoffman, 1967, 1999; Cressey, 1972). In this study, four specimens of this species were found in the collection of the Lake Biwa Museum, Kusatsu, Shiga Prefecture. Based on their labels, the specimens were collected from an unidentified gar and shortnose gar *Lepisosteus platostomus* in 1992, but it is unknown whether they were taken from gars soon after imported from the U.S.A. or those that had been held in Japan. The Lepisosteidae consists of seven species, all of which are reared by hobbyists and at public aquaria in Japan (Taki *et al.*, 1995). Except *A. lepidostei* (Tsutsumi, 1968) and *A. nobilis* (this dissertation), there is no information on the parasites of gars held in Japan, and it is important to study the parasite fauna of those gars.

Despite the fact that *A. japonicus* and *A. coregoni* have been regarded as one of the important parasites in the culture of cyprinids and salmonids in Japan, respectively (*e.g*., Egusa, 1978), the past identification of these parasites seems to be not strict because there are only two papers reporting the morphology of both species taken from farmed fishes (Nakazawa, 1914 for *A. japonicus*; Hoshina, 1950 for *A. coregoni*). The two species are not host-specific and morphologically similar, and it is desirable to strictly identify them based on their morphological features. In such a situation, the present study has shown that the two species can be readily differentiated by counting the number of plumose setae on the posterior margin of the coxa of the first pair of legs (one in *A. japonicus* vs. four to nine in *A. coregoni*) and the number of supporting rods in two sucker membranes of the first maxillae (ca. 40–50 rods in *A. japonicus* vs. more than 60 rods in *A. coregoni*). This is one of the important findings in this study.

As stated above, six species of *Argulus* have been reported from marine fishes in Japan, but in this study, only two species (*A. caecus* and *A. matuii*) were collected for redescription. This is because argulid branchiurans are rare fish parasites in the wild and no specimen of the other four species was obtained for this study. Nevertheless, it is highly necessary to collect and redescribe *A. onodai* because its morphological information is very limited: this species was briefly described in 1936 but since has not been found or redescribed from Japan, although it was found in China (Wang, 1964). The species is morphologically similar to *A. caecus* and both species are found on puffers of the tetraodontid genus *Takifugu* (reported as "*Spheroides*") in coastal Pacific waters of Japan (Tokioka, 1936a). Moreover, *A. onodai* was described from only a specimen (8.4 mm long) but *A. caecus* has been described based on much larger specimens (19 mm in Wilson, 1922; up to 16 mm long in Tokioka, 1936a; 18.9

mm long in this dissertation). A study is necessary on morphological variations of the two species with an increase in their body size.

A taxonomic work is also needed on unidentified argulid branchiurans found in fresh and marine waters of Japan. The unidentified freshwater species is *Argulus* sp. collected from dark chub *Candidia temminckii*, pale chub *Opsariichthys platypus*, and big-scaled redfin *Pseudaspius hakonensis* (all Cyprinidae) in the Takami River, a tributary of the Yoshino River, Nara Prefecture, central Japan (Nakamura *et al.*, 2000). The marine species *Argulus* sp. was found on dotted gizzard shad *Konosirus punctatus* (Temminck and Schlegel, 1846) (Dorosomatidae) in coastal waters of Shimonoseki City, Yamaguti Prefecture, western Japan (Kondo *et al.*, 2014: fig. 2). The specimen of this unidentified species does not match the general morphology of the six nominal species of the genus reported from Japan. Moreover, a planktonic specimen of *Argulus* sp. was collected in the Sea of Japan off Yamagata Prefecture, northern Japan (Suzuki, 1979: 264) and there is a record of "Argulidae sp." collected from the Pacific coast of Wakayama Prefecture, central Japan (Imahara, 1996: 64).

Ecological aspects of *A. coregoni* and *A. japonicus* infecting freshwater fishes in Japan are reported in Chapter 3, which consists of three sections, each reporting the distribution patterns of *A. coregoni* in mountain streams, those of both *A. coregoni* and *A. japonicus* in the Lake Biwa Basin, and the occurrence of *A. japonicus* in unusual water bodies (*i.e.*, two brackish water lakes and a river through a residential area). In the first section, *A. coregoni* is shown to infect salmonids (white-spotted char *Salvelinus leucomaenis*, red-spotted masu salmon *Oncorhynchus masou ishikawae*, and masu salmon *O. masou masou*) in the headwater and the middle to lower sections of the upper reaches of rivers at high elevations (237–873 m) but to parasitize ayu *Plecoglossus altivelis altivelis* (Plecoglossidae) in the middle and lower reaches of rivers at lower elevations (65–557 m). This result demonstrates that *A. coregoni* utilizes different fish species as its hosts along a river, and the present dissertation has clarified the longitudinal distribution patterns of *A. coregoni* in the Japanese mountain streams. The field work was successfully conducted with the assistance of local fish biologists who are very aware of the ecology of salmonids and can use an electric shocker to catch them without giving damages in the high-elevated mountain streams.

In the second section of Chapter 3, the distribution patterns of *A. coregoni* and *A. japonicus* in the Lake Biwa Basin are studied using the specimens loaned from the Lake Biwa Museum, Kusatsu, Shiga Prefecture, and those previously reported from this prefecture. Lake Biwa is the largest lake in Japan, and some large rivers, which originate from the mountain areas near the borders of Shiga and neighboring prefectures, flow into the lake. Interestingly, there is a marked difference in the distribution patterns of *A. coregoni* and *A. japonicus* in the basin: the specimens of the former species were mostly collected from red-spotted masu salmon in the upper reaches of large rivers flowing into the lake at higher elevations (215–650 m), but those of the latter species were taken in the lake and a nearby pond at about 85 m elevation. This difference in the distribution patterns of *A. coregoni* and *A. japonicus* in the Lake Biwa Basin is most probably due to a difference in their preference for the habitats affected by various environmental conditions. That is, *A. coregoni* inhabits the lotic, cold, highly oxygenated streams, whereas *A. japonicus* occurs in the lentic, warm, less oxygenated waters. An experimental work is necessary to compare the survival of these species under different environmental conditions (*e.g.*, water temperature, dissolved oxygen, and water current).

In the third section of Chapter 3, *A. japonicus* is recorded from two brackish water lakes, Lake Shinji, Shimane Prefecture, and Lake Jusan, Aomori Prefecture, where the parasite was found infecting euryhaline fishes, Japanese white crucian carp and big-scaled redfin *Pseudaspius hakonensis*, respectively. Although this parasite completes its life cycle in fresh waters, such collections in the brackish water lakes indicate that it can survive in salt waters for a certain period. Furthermore, *A. japonicus* was found on pale chub *Opsariichthys platypus* (Cyprinidae) in a river flowing through a residential area in Okayama Prefecture. As the river was blocked by a sluice gate installed downstream, the parasite is inferred to maintain its population in the still or slow-flowing (lentic) environments.

As stated above, in the present work, much information has been obtained on the morphology, taxonomy, and ecology of the Japanese *Argulus* species. Nevertheless, due to their rare occurrence in wild fish populations, it was impossible to collect their additional specimens, especially those of the marine species, for this study. A continuous field sampling and collaboration with local scientists are essential for collecting more specimens of *Argulus* spp. in Japan. For the two freshwater native species (*A. japonicus* and *A. coregoni*), it is desirable to conduct samplings in many locations to clarify their geographical distribution and host utilization in Japan. In particular, since there is no record of these species from wild fishes in Hokkaido, it is important to study whether they occur in this subarctic region from the viewpoint of their biogeography.

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