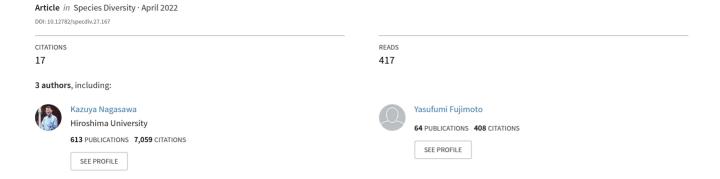
## Redescription of Argulus mongolianus (Crustacea: Branchiura: Argulidae), an ectoparasite of freshwater fishes in East Asia, with its first record from Japan



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# Redescription of *Argulus mongolianus* (Crustacea: Branchiura: Argulidae), an Ectoparasite of Freshwater Fishes in East Asia, with Its First Record from Japan

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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.

Key Words: crustacean parasite, fish louse, new country record, new host records.

#### 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 (1936a), Kimura (1970), and Ikuta and Makioka (1997) for *A. japonicus*; Hoshina (1950), Shimura (1981, 1983), Nagasawa and Yuasa (2020), and 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 1936b; Yamaguti 1937; Nagasawa 2009, 2011a, 2021; Nagasawa et al. 2012, 2018, 2021). 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. mon-*

*golisnus* 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. The prevalence and intensity of infection with *A. mongolianus* and *A. japonicus* on the fishes from the two lakes will be reported in a separate paper.

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 (M. Shimomura and T. Nakano, 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) (H. Komatsu, personal communication). These facts indicate that the specimen used in the original description has been lost.

#### **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 km², maximum depth: 1.6 m) and Lake Uchinuma (0.98 km², 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).

#### **Results**

*Argulus mongolianus* Tokioka, 1939 [Japanese name: Mouko-chou, based on Tokioka (1940)] (Figs 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: 70.

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, body surface.

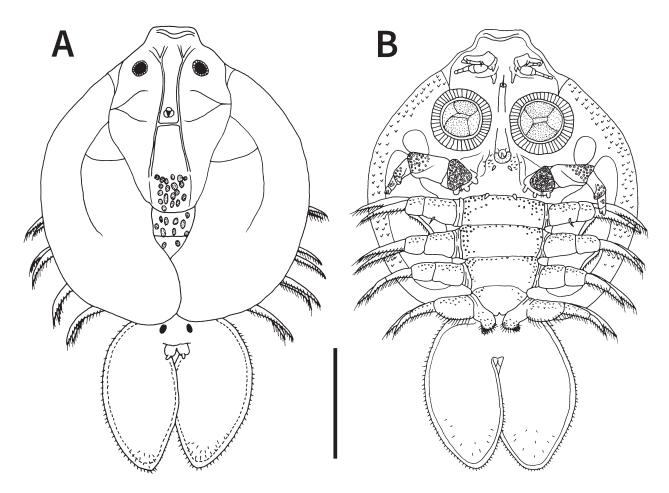


Fig. 1. Argulus mongolianus, adult female, NSMT-Cr 29369, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. A, Habitus, dorsal view; B, habitus, ventral view. Scale bar: 2 mm.

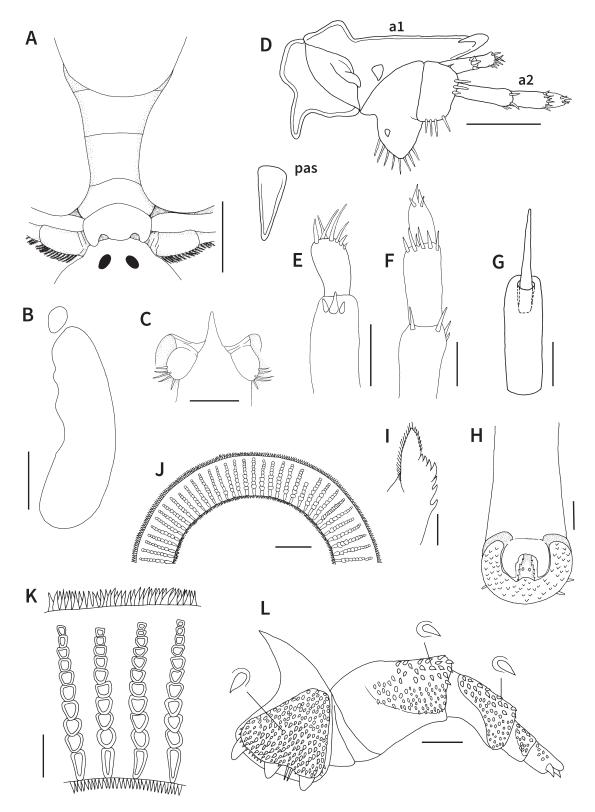


Fig. 2. Argulus mongolianus, adult female (different specimen shown in Fig. 1), NSMT-Cr 29371, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. A, Thorax partially covered with carapace, anterior portion of abdomen, and sympods of fourth pair of legs, dorsal view; B, respiratory 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; 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.

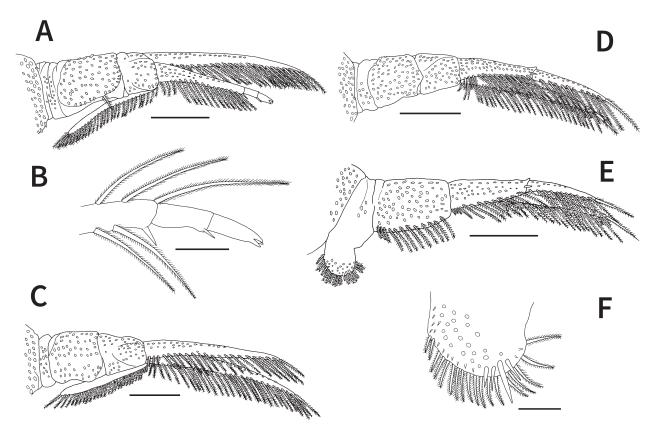


Fig. 3. Argulus mongolianus, adult female (different specimen shown in Fig. 1), NSMT-Cr 29371, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. A, First leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view; F, natatory lobe, ventral view. Scale bars: A, C–E, 0.5 mm; B, F, 0.1 mm.

**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 *Cy. carpio* from Lake Uchinuma on 12 May 2020; two adult females and one adult male, NSMT-Cr 29374, from the body surface of *Ca. 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.

**Redescription.** Based on six adult female 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 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 1A, B, 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. 1B). Posterolateral lobes of carapace partially overlapped (Fig. 1B) or not overlapped (Fig. 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 1B, 2B). Thorax with four segments, bearing small spiniform projections on ventral surface; third segment narrower than first and second segments (Fig. 1A, B); fourth segment narrowest, with two posteriorly directed lateral protrusions and weakly convex posterior margin in dorsal view (Fig. 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. 1A, B). Paired spermathecae each oval, located anteriorly in abdomen (Figs 1A, B, 2A). Caudal rami located at base of anal indentation, each with five naked setae on posterior margin (Figs 1A, 2C).

First antennae with four segments (Fig. 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. 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 1B, 2D). Preoral sheath cylindrical and visible near anterior regions of first maxillae on ventral midline of carapace (Figs 1B, 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 1B, 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. 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. 2I); small paired buccal spines present on ventral surface of labium (Fig. 2H).

First maxillae forming well developed cup-like suckers (Fig. 1B), with 64-71 (mean=68, n=10) supporting rods in sucker membrane (Fig. 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. 2K). Both outer and inner margins of rim of sucker membrane with numerous apically pointed projections (Fig. 2J, K). Second maxillae with five segments (Fig. 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. 1B). Post-maxillary spines small and located just anterior to first segment of thorax (Fig. 1B).

First to fourth pairs of legs (Fig. 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. 3A, 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 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. 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. 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. 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 twosegmented, 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.

**Description of 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 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 4A, B, 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

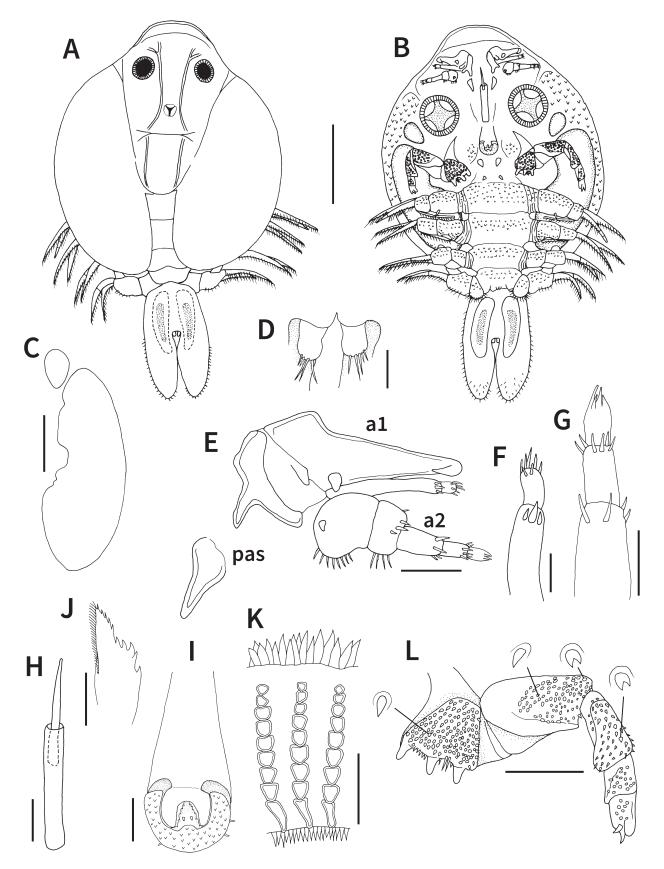


Fig. 4. Argulus mongolianus, adult male, NSMT-Cr 29372, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. A, Habitus, dorsal view; B, habitus, ventral view; C, respiratory areas, ventral view; D, caudal rami, dorsal view; E, first antenna (a1), second antenna (a2), and postantennal 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.

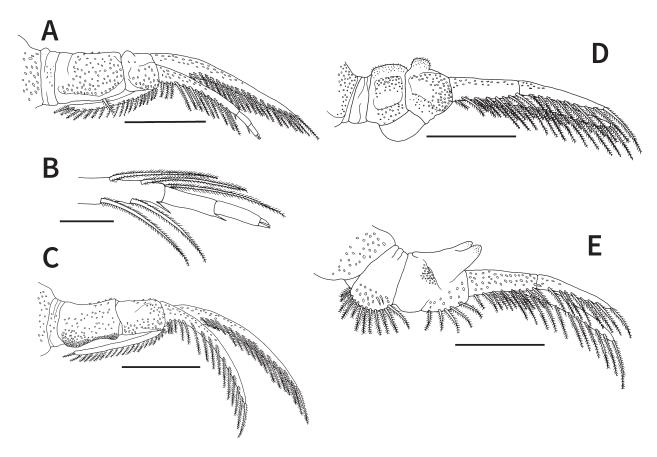


Fig. 5. Argulus mongolianus, adult male, NSMT-Cr 29372, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. A, First leg, ventral view; B, distal part of endopod of first leg, ventral view; C, second leg, ventral view; D, third leg, ventral view; E, fourth leg, ventral view. Scale bars: A, C–E, 0.5 mm; B, 0.1 mm.

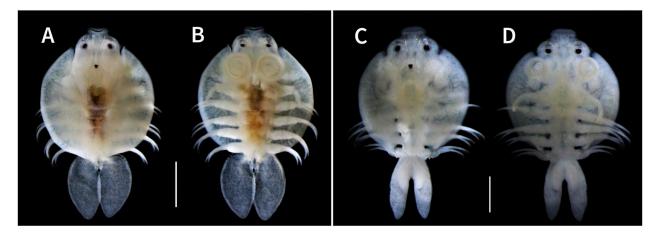


Fig. 6. Argulus mongolianus, adult female (A and B), NSMT-Cr 29369, and adult male (C and D), NSMT-Cr 29370, from Micropterus salmoides in Lake Izunuma, Miyagi Prefecture, Japan. Ethanol-preserved specimens. A, C, Habitus, dorsal view; B, D, habitus, ventral view. The adult female and male were both collected on 2 June 2020 and photographed on 2 October 2021. Scale bars: A, 2 mm; B, 1 mm.

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. 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. 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. 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. 4A, B). Testes elliptical, located in anterior to middle portion of abdomen (Fig. 4A, B). Caudal rami located at base of anal indentation, each with five naked setae on posterior margin (Fig. 4D).

First antennae with four segments (Fig. 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. 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. 4E). Preoral sheath cylindrical, located between first maxillae on ventral midline of carapace (Fig. 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. 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. 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. 4J); small buccal paired spines present on ventral surface of labium (Fig. 4I).

First maxillae forming cup-like suckers (Fig. 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. 4K). Both outer and inner margins of rim of sucker membrane with numerous apically pointed projections (Fig. 4K). Second maxillae with five segments (Fig. 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. 4B). Postmaxillary spines small and located anterior to first segment of thorax (Fig. 4B).

First to fourth pairs of legs (Fig. 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. 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. 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. 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 twosegmented, proximal and terminal segments with eight and nine plumose setae, respectively, near ventroposterior margin. Fourth leg (Fig. 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 centro-posterior 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. 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 1A, B, 4A, B, 6A–D), a circular carapace and oval abdominal lobes in the female (Figs 1A, B, 6A, B), oblong abdominal lobes in the male (Figs 4A, B, 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 3A, 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 1A, B, 4A, B, 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. 5D, E). The male possesses both posteriorly directed and apically bifurcated scales on the second and third segments of the second maxilla (Fig. 4K), but the female has only posteriorly directed scales on these segments (Fig. 2K). The posterior margin of the coxa of the male's second leg is densely armed with small projections (Fig. 5C), but the female has no such ornamentation on the same margin (Fig. 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 3A, B, 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 2019; 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 *Ca. auratus langsdorfii*; Nagasawa 2011b, reported as *Ca. 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 study):

1.	Centrofrontal	region	of carapace	protruding	anteriorly
				A. m	ongolianu

#### 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, 2017, 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, H. 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, largescale 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, unpublished data), 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.

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