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Short Communication

A Scanning Electron Microscopic Study of *Argulus japonicus* (Crustacea: Branchiura) Isolated from Goldfish (*Carassius auratus*) in Korea

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MDZ collected samples, isolated Argulus species and modified the drafted manuscript. SR, HK, BKP performed the SEM analysis and drafted the manuscript. All authors approved the final manuscript.

Key words
Argulus japonicas, Carassius auratus (Goldfish), Fish lice.

ABSTRACT

A freshwater ecto-parasitic crustacean was isolated from the naturally infested goldfish (*Carassius auratus*) purchased from commercial aquarium in Korea. Based on the morphological comparison with all *Argulus* species, isolated fish lice was identified as *Argulus japonicus* Thiele, 1899. *A. japonicus* is native to Asia, which also is a habitat for its typical hosts, such as goldfish and common koi carp (*Cyprinus carpio*). *A. japonicus* has not previously been described from goldfish from the ornamental facilities in Korean. This is the first report of *A. japonicus* morphology with light and scanning electron microscopy.

Argulus, one of the four genera of class Branchiura (Poly, 2008; Martin and Davis, 2001) has a world wide distibution. *Argulus* spp. are commonly known as fish lice cause damages to a host which vary considerably with host species, size, and health status. *Argulus* spp. have also been known to be major parasites in cultured as well as wild fish which further increase the susceptibility of its host to secondary infections (Bandilla *et al*., 2006). Total of 143 species of *Argulus* genus have been described, although they have many synonyms (Anonymous, 2001). Three species have been documented *i.e*. *Argulus coregoni* Thorrell, 1866, *A. foliaceus* Linnaeus, 1758 and *A. japonicus* Thiele, 1900.

A. japonicus, an introduced species from China, is now widespread in coastal rivers and freshwater (Van and Bason, 1984). It is native to Asia, where its typical hosts, goldfish (*Carassius auratus*) and common koi carp (*Cyprinus carpio*) are also located. In Korea *A. japonicus* was harvested from Korean carp (*Cyprinus carpio*) (Han *et al*., 1988), but has not been reported from goldfish so far. We report *A. japonicus* from goldfish purchased from aquarium in Daejeon, Korea.

Materials and methods

In August 2014, the fish lice (7 males) were isolated from a goldfish (*Carassius auratus*) purchased from fish aquarium in Daejeon, Korea. The specimens were preserved in 70% ethanol and photographs were taken under the light microscopy. For scanning electron microscopy (SEM), 2 parasites were washed five times with 0.2 M cacodylate buffer (pH 7.3), fixed in 2.5% glutaraldehyde and post fixed in 1% osmium tetroxide at 4°C.

The specimens were dehydrated in a graded ethyl alcohol series, dried by CO_2 critical point, coated with gold and examined by SEM (S-4800, Hitachi) at 15 kV.

Results

The live parasites are milky white in color, with inverted, brown, oval, melanophores patches scattered over dorsal surface of carapace lobes and abdomen. Total length of body is measured in 1.9-2.3 mm (mean 2.12 mm) and the carapace is comprised within 75-77% of total length. A pair of compound eyes is located on dorsal surface in the center of anterior fourth of carapace (Figs. 1A and 1B). The melanophore patches were scattered over dorsal surface of carapace lobes and abdomen, but no pigmented patches were observed on the swimming legs (Fig. 1A). Two prominent movable compound eyes were visible in the dorsal side of anterior third of carapace (Figs. 1A and 1B). The strong dentate scales were positioned along the edge of the ventral carapace and face towards posterior (Fig. 2A).

First antenna (antennule) (Figs. 2B and 2E) was divided into two segmented parts: proximal part which had no orna-mentation in the form of scales but the basal

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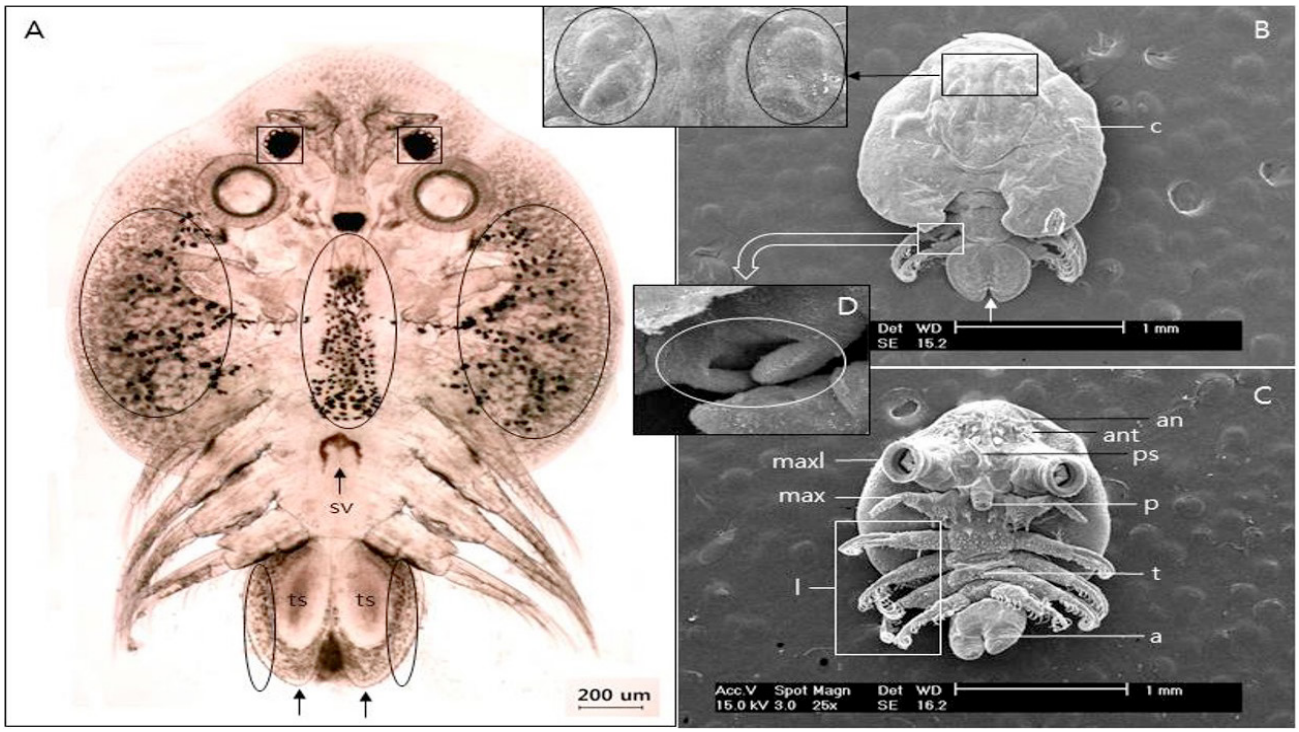

Fig. 1. Whole body of male *Argulus japonicus*. **A,** Light micrograph. Note the melanophores patches (circles) scattered over dorsal surface of carapace lobes and abdomen. Compound eyes (square). Seminal vesicle (sv). Testis (ts). Note the rounded end of abdomen (arrows); **B,** Scanning electron micrograph of dorsal view. Compound eyes (circle in square). Abdomen separated by sinus (arrow); **C,** Scanning electron micrograph of ventral view. Not the pre-oral spine; **D,** Accessory copulatory projections on third leg (circle). *a,* abotomen; *an*, antennule; *ant*, antenna; *c*, carapace; *l*, swimming leg; *max*, maxilla; *maxl,* maxillule; *p*, proboscis; *ps*, preoral spine; *sv*, seminal vesicle; *t*, thorax; *ts*, testis.

podomere (plate) bears a large posterior spine, the second bears a pair of anterior spine with rounded tips, the third bears a pair of median spine, and the last podomere terminates in a hook; proximal part stout, first segment slightly curved, bearing large triangular posterior and anterior spines with rounded tips; second segment with hook-shaped spines bending toward abdomen, slender distal part of antennule bearing anterior blunt knob, extending slightly beyond terminal spine, second segment of distal part half as long as first; terminal segment bearing about five to seven stout apical setae. Two segmented second antenna (antenna) (Figs. 2B and 2E), proximal two segments wider than three terminal slender cylindrical segments; prominent spine on posterior part of first segment; two clusters of setae present on posterior margin of first and second segments, third to fifth segments with apical setae.

Pre-oral spine was located at the median line between first maxilla suckers and is consisting of tapering spine carried on long eversible sheath, sleeve-like in shape, without scales, and the proximal part of pre-oral spine was somewhat thicker and longer than distal part (Figs. 1C, 2D and 2E). Proboscis and mouth tube lacking scales on ventral surface was three times longer than wide (Figs. 1C and 2G). Mouth tube was located at the terminal of proboscis and somewhat depressed. Also, small scales were positioned only on labium, two labial tubules present within mouth tube (Fig. 2G), as well as paired mandibles, each consisting of two sections.

First maxillae are modified into sucking disks and the rim was supported with 41-42 rods which was overlapping small sclerites. The basal rod was elongated and rods were decreasing in size toward periphery (Figs. 1C and 2C). The five-segmented second maxillae had a large basal segment and armed with simple plate of scales which bore three elongate, sharp, finger-like teeth at posterior end (Figs. 2E, 2F and 2H). The basal plate had blunt triangular scales (Fig. 2H). The ventral surface of second (anterior part), third and fourth segments were adorned with scales (Fig. 2F). Terminal segment of maxilla in the form of a blunt fused extension had two hook-like sharp claws (Fig. 2I). A pair of respiratory areas was located on ventral surface

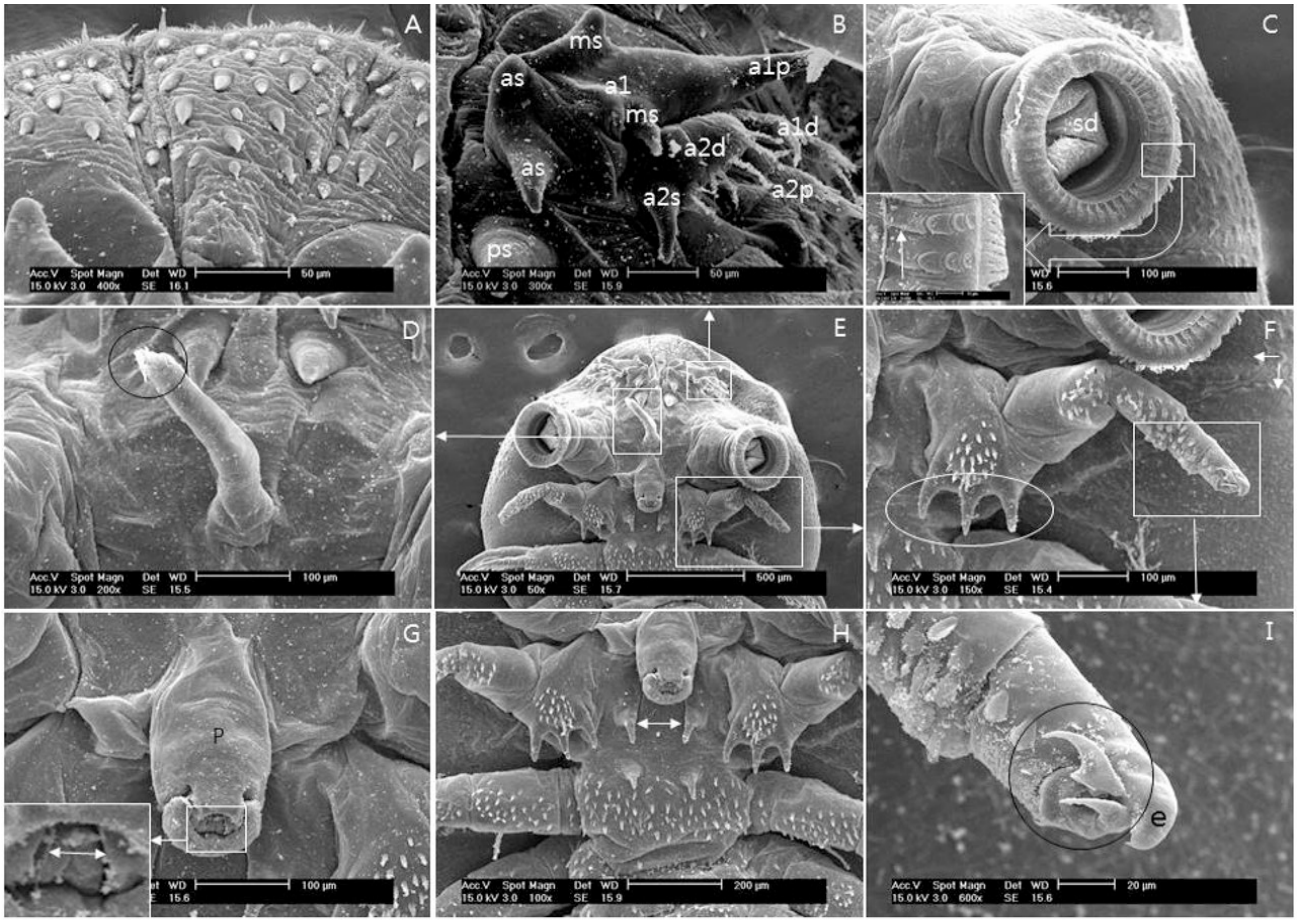

Fig. 2. Scanning electron micrographs of anterio-ventral view of male *A. japonicus*. **A,** Dentate scales that face towards posterior that occur along the edge of the ventral carapace; **B,** Antennule and antennae; **C,** First maxilla. Rim of sucker with supporting rods and sclerites. Basis elongated plate (arrow in square); **D,** Pre-oral spine, Note the terminal (circle); **E,** Anterio- ventral part; **F,** Maxilla with five segments, basal podomere (plate) with three tooth (circle). Two lobes of respiratory area (arrows); **G,** Proboscis without any dorsal scales. Mouse tube (arrow in square); **H,** Basement of maxillae and first leg. Basal plate scales of maxillae (arrow); **I,** Terminal segment of maxilla in a blunt fused extension has two recurved sharp claws (Circle). *e,* extension; *a,* antenna; *as,* anterior spine; *a1*, first antenna; *a1d,* first antenna distal part; *a2d*, second antenna distal part; *a2p*, second antenna proximal part; *a2s,* spine of second antenna; *ms*, median spine; *p,* proboscis; *ps* posterior spine; *a1p,* first antenna proximal hook; *sd*, suckiong disc.

of lateral lobes and the smaller anterior area was less than half the size of larger posterior region (Figs. 2F and 3D). Thorax had the distinct four-segmented leg (Figs. 1A, 1C and 3A). The terminal of all swimming legs was biramous (Figs. 1A and 3A) and nearly of same size except the fourth pair which was relatively shorter. The segments of swimming legs were densely ornamented with blunt elongate scales scattered on ventral surface, and dorsal side was smooth without scales (Figs. 2F and 2I). Exopods were slightly longer than endopods in all legs (Figs. 3A and 3D). In dorsal view, a pair of accessory copulatory projections located on third legs, elongated and positioned on posterior margin of basis (Fig. 1D). In ventral view, third legs had a socket on posterior side (Fig. 3F).

Male's genital aperture was located on the posterior margin of fourth thorax (or fourth leg, fourth thoracic leg) and spermatophore opened towards abdomen (Fig. 3E). The dorsal and ventral surfaces of abdomen were smooth (Figs. 1A and 1B). The bilobed abdominal segment was simple and unsegmented. Abdomen length was measured as 0.44-0.47 mm (mean 0.46 mm), comprising 21% of total length, more than two times longer than wide posterior lobes rounded to relatively tapering points, separated by sinus (0.12 mm), comprising 25-26% of total abdomen length (Figs. 1A, 1B and 1C). A pair of caudal ramus in anal sinus are positioned behind anus and had 4 setae on terminates (Fig. 3B). The parasites had the characteristic abdominal lobes which had round terminal ends and were

not covered by the carapace (Fig. 1B).

Discussion

Argulus and *Dolops* share some morphological similarities which are not found in *Chonopeltis*. Møller *et al*. (2008) and Gresty *et al*. (1993) reported the similarities between *Argulus* and *Dolops*: the first and second antennae and the second maxillae (Fig. 2B). In adult members of both genera, the first segment of the first antenna is a characteristic hook, bearing two smaller distal segments. The small second antennae are situated just below the first and have the same number and proportions of the segments in both genera. Similarly, the relative size and proportions of the second maxilla segments are the same, and the most basal segment has three characteristic teeth (Fig. 2F) along the posterior margin. These morphological characters are shared by our samples. But, the maxillules (first maxillae) of adult *Dolops* lack suction discs and have segmented first maxillae, each ending in a distal hook, whereas the first maxilla are transformed into large, strong, cup-shaped sucker (Fig. 2C) in the other three genera (Fryer, 1961). In *Chonopeltis*, morphological differences include the absence of a pre-oral spine, trifoliate carpace and the absence of antennules (Tam *et al*., 2005). The pre-oral spine (Fig. 2D) is found in *Argulus* and *Dipteropeltis* (Ider *et al*., 2014) and supports a close relationship between these genera. Species of *Argulus* are the only ones to possess an oral spine (Tam *et al*., 2005). In our specimen, the first maxilla are cup-shaped (Fig. 2C); a perioral spine (Fig. 2D) and antennules (Fig. 2B) are present; three characteristic teeth of second maxilla (Fig. 2F); oral papilla is absent.

Poly (2003) discussed that the number of setae (spine) on the endopod of the first leg and number of setae on the caudal rami may be useful characters for taxonomy or systematics: three setae on the endopod of the first leg possess species: *A. matuii*. *A. foliaceus* (two in the first developmental stage), *A. japonicas, A. stizostethii*, *A. flavescens, A. rhipidiophorus, A. major*, *A. ambystoma, A. catostomi*. But, *A. melanostictus* has two setae. Also, it was described the species according to numbers of setae on the caudal rami: *A. ambystoma, A. americanus, A. maculosus, A.versicolor, A. diversus, A. stizostethii, A. flavescens, A. foliaceus, A. meehani* and *A. chesapeakensis* have five setae on the caudal rami whereas *A. ellipticaudatus* and *A. melanostictus* apparently have only four, *A. foliaceus* (3rd stage) has four and *A. japonicas* (1st stage) has three. In our study, the number of setae on the endopod of the first leg is three (Fig. 3D) and the number of setae on the caudal rami is four (Fig. 3D).

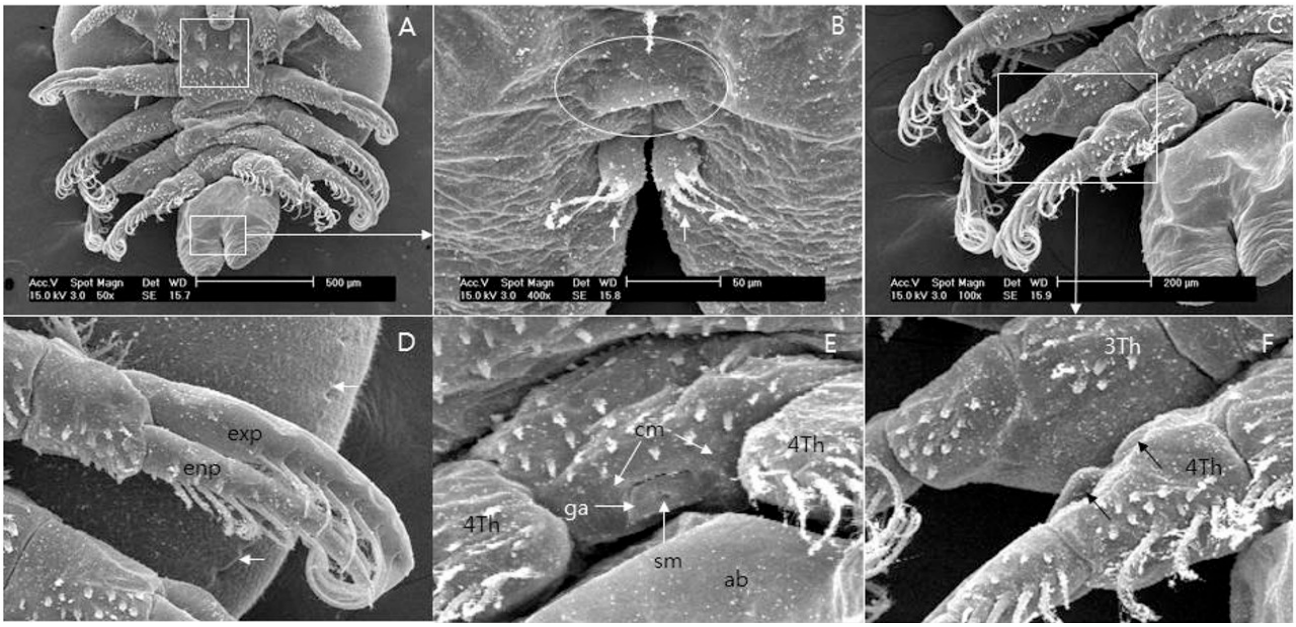

Fig. 3. Scanning electron micrographs of postero-ventral view of male *A. japonicus*. **A,** Thorax and abdomen. Spines of each post maxillary spine (thoracic spine?) (square); **B,** Anus (circle). Caudal rami (arrow). Note the four setae; **C,** Third and fourth leg; **D,** First swimming leg; Exopodium and endopodium with plumose setae. Posterior region of respiratory area (arrows); **E,** Male's genital aperture to show spermatophore secretion; **F,** Peg (arrow) on the right fourth leg and the socket of right third leg. Note the socket (indentation) on the ventral side of forth leg. *ab*, abdomen; *cm*, contracted muscle; *exp,* exopodium; *enp,* endopodium; *ga,* genital aperture; *sm*, spermatophore; *3Th*, third leg; *4Th*, fourth leg.

The number of suction cup rods is various in species and sexes: *A. foliaceus*, 40 to 51 (16); *A japonicas,* 38-50 (Hsiao, 1950); *A, japonicus* 44-50 (Pilgrim, 1967); *A. matuii,* 75-83 males and 81-95 females (Sikama, 1938); *A. ambystoma,* 43-56 (males) and 45-54 (females) (Poly, 2003). In our case, the number of male' sucker cup rods is counted in 41-42. The proboscis is adorned with pectinate scales in *A. personatus* (Tam et al., 2005), whereas, without scales in *A. japonicus* (this study).

Three species among about 15 species of freshwater lice have been frequently found; *A. foliaceus, A. coregoni* and *A. japonicus.* They have been found throughout much of Europe and many other parts of the world (Post, 1987). *Argulus* spp. are louse-like in appearance, and are semitransparent, with a pale green or brown cryptic colouration. They are broadly ovoid in shape, dorso-ventrally flattened and convex dorsally. *A. foliaceus* and *A. japonicus* are of similar size, adults being around 6-8mm in length (Rushton-Mellor and Boxshall, 1994). They differ from *A. coregoni* in size, and are smaller than *A. coregoni* the adult of which can reach 12 mm in length (Gurney, 1948). *A. japonicus* body average length is 6.5 mm for females, and 3.6 mm for males. *A. foliaceus* reaches 4.7 mm in length it is considered to be adult and capable of laying eggs. *A. coregoni* is recognizable by the abdomen lobes which have sharply pointed terminal ends and are not covered by the carapace whereas *A. japonicus* have rounded terminal end of abdomen (Fig. 1). The carapace of *A. foliaceus* has a band of sharp triangular scales along the outer edges and it tapers towards the abdomen on either side. The scales face towards the posterior. In *A. japonicus,* the characters of body shape, abdomen, carapace, first and second antenna, first and second maxillae, thorax segments, respiratory area, and swimming leg structure show a significant convergent between male and female in morphology with the exception of the total body size, second swimming leg structure, position and structure of the copulatory accessories, number and structure of overlapping sclerites of sucking disk rim, as well as melanophores patches of male (Fig. 1A). The *Argulus*-infected fish may show behavioral changes that develop in outbreak increases. In early outbreak stage, the fishes in lakes are reported to jump, flash and swim erratically, possibly in an attempt to rid themselves of lice.

Thereafter, fishes are reduced feeding and may show a significantly reduced growth rate, and loss of physical condition makes them susceptible to stress and secondary infection (Rahman, 1996). *Argulus* spp. have often been associated with secondary infections; virus, bacteria and fungus (Rahman, 1996). Mortality may also have significantly increased on wild fish populations. Menezes *et al*. (1990) and Northcott (1997) describe cases of massive mortalities in still waters where lice numbered up to 1500 lice/fish. Jafri and Ahmed (1994) reported mortalities of 30-40 fish/day caused by infections of 70-100 lice/15-25cm carp, in an Indian fish farm.

We concluded that the ectoparasite isolated from goldfish form commercial aquarium in Korea is *A. japonicus* which is distinguishes from other species of *Argulus*.

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Conflict of Interest Statement

The authors declare that there is no conflict of interests regarding the publication of the manuscript.

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