RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 11: 1906-1916 (November 2024)

Exploring the Genetic and Morphological Diversity of *Argulus* Ectoparasite Infecting the Aquaculture and Ornamental Fish in Tripura

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10.18805/IJAR.B-5455

ABSTRACT

Background: The present study screened for isolation of *Argulus* spp. infecting fishes based on the report of causing substantial economic losses both in aquaculture ponds and ornamental shops across several locations in Tripura, India.

Methods: Identification of *Argulus* spp. was performed based on morphological, and molecular techniques. The molecular identification of Argulus spp. was performed using universal eukaryotic 18S rRNA genetic markers. Prevalence analysis was performed to determine the infestation rate of *Argulus* spp. in different hosts.

Result: Seven *A. japonicus*, one *A. coregoni,* and one *A. foliaceus* isolates were identified based on molecular and morphological features. In the present study it was found that *A. japonicus* infected a wide range of host species, including Goldfish, Milky Koi carp, Mrigal, Rohu, Catla, and Subunkin. It was the first instance of *A. japonicus* infecting milky koi carp both in India and globally. On the other hand, *A. foliaceus* and *A. coregoni* infected only tiger oscar and goldfish respectively, indicating a rather narrow range of host diversity.

Key words: Aquaculture, Argulosis, Ornamental fishes, RNA, Small subunit ribosomal.

INTRODUCTION

The increasing global demand for fish force farmers to intensify aquaculture practices creating stressful environments, resulting in an epidemic of infectious diseases (Laltlanmawia *et al*., 2023). Parasitic diseases have emerged as a significant concern within the domain of fish pathogens and are the leading contributors to socio-economic losses in Indian aquaculture (Sahoo *et al*., 2020). Most wild and farmed fish parasites are protozoa, helminths, myxozoans and crustaceans (Thakur *et al*., 2023; Saravanan *et al*., 2021). Among the crustaceans, the most common one affecting Indian major carps (IMCs) is *Argulus*, which is responsible for argulosis and is a significant barrier to the development of the Indian freshwater aquaculture industry (Sahoo *et al.,* 2013; 2020).

Argulosis has several detrimental effects on freshwater fish, including skin ulceration, osmotic imbalance, immune suppression, irregular swimming behaviour, haemorrhages, growth impairment, anaemia and increased susceptibility to secondary infections (Sahoo *et al.,* 2012; Rahman *et al.,* 2019; Datta *et al.,* 2022). There is a global distribution of approximately 129 recognized species of *Argulus*, with 85 species inhabiting freshwater environments and 44 species found in marine waters (Poly, 2008, Kismiyati *et al*., 2023). *Argulus coregoni*, *A. japonicus* and *A. foliaceus* are recognized as the three most extensively investigated species in freshwater ecosystems globally (Steckler and Yanong, 2013). To date, 20 species have been documented in both cultured and wild fish populations in India, as reported by Valarmathi, (2017), Bari (2018) and Sahoo *et al.* (2020). According to Sahoo *et al*. (2013), the prevalence of *A. japonicus* and *A. siamensis* was evident in the genetic composition of *Argulus* species obtained from diverse freshwater aquaculture settings in India.

Taxonomist uses morphological features alone for identification of *Argulus* spp., however, many a time due to lack of expertise, erroneous identification of *Argulus* spp. may occur (Tandel *et al*., 2021). Several researchers have utilized molecular identification techniques to examine *Argulus* spp. and its population (Tandel *et al*., 2021, Wadeh *et al*., 2010). However, according to Nagasawa, (2021), it is conceivable that molecular data pertaining to misidentified congeneric species and may have been erroneously

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How to cite this article: Dekari, D., Saha, H., Chouhan, N., Irungbam, S., Ghosh, L., Saikia, P., Choudhury, T.G. and Saha, R.K. (2024). Exploring the Genetic and Morphological Diversity of *Argulus* Ectoparasite Infecting the Aquaculture and Ornamental Fish in Tripura. Indian Journal of Animal Research. 58(11): 1906-1916. doi: 10.18805/IJAR.B-5455.

Submitted: 23-07-2024 **Accepted:** 13-09-2024 **Online:** 23-10-2024

submitted in GenBank under the classification of *Argulus* spp. Therefore, the identification and characterization of the *Argulus* may be accomplished via the use of both morphological and molecular approaches (Patra *et al*., 2016, Nagasawa, 2021).

Morphological identification and 18S rRNA genetic marker sequencing confirm findings of *Argulus* spp. up to species level in this investigation in a specific geographical region and the spatial distribution of *Argulus* spp. infecting Indian Major carps (IMCs) and ornamental fishes in Tripura, India. The current research additionally evaluated the host ranges of several *Argulus* spp. and the pathological alterations caused by Argulus infestations in hosts.

MATERIALS AND METHODS

Study site and sample collection

A total of 670 fishes were collected from seven different location of Tripura, India *viz*. farm-fish samples from Udaipur, (23°31′39.7″N,91°30′15.0″E), Amarpur (23°31′ 16.5″N 91°39′17.6″E), Taidu (23°43′53.9″N 91°40′20.9″E); Bamutia (23°57′14.2″N 91°17′10.8″E), Lembucherra (23°54′36.6″N 91°18′55.8″E), Bagabasa (23°33′35.6″N 91°25′18.5″E), Gangachhara (23°27′34.3″N 91°27′ 24.6″E) and ornamental fish samples were collected form aquarium settings of Agartala, Tripura (23°50′16.7"N 91°16′41.3″E) with sampling duration from February 2022 and June 2023. Parasites were manually removed from from different body parts (operculum, dorsal fin, ventral fin, pectoral fin and tail) of infested fish using blunted forceps and a fine brush. The actively swimming argulus parasites were washed three times (10-15 min each) with phosphate buffer saline (PBS, pH 7.2). Morphological and molecular identification were done on Argulus samples for which they were transferred to RNAse/DNAse-free cryotubes and preserved in a diluent for DNA extraction (Himedia, USA) and stored at -20°C for DNA isolation and subsequent analysis. The research was carried out for a duration of two years at College of Fisheries, Central Agricultural University, Lembucherra, Tripura.

Morphological analysis

To facilitate morphological identification, the acquired *Argulus* samples were subjected to an initial washing procedure using PBS (pH 7.2, for 2-5 minutes). Subsequently, the samples were examined at different magnification levels using a Leica S6D stereo zoom microscope and a Zeiss Primostar 3 upright light microscope on the day of collection. To facilitate future morphological investigations, the specimens were preserved in 4% neutral buffered formalin (NBF) and 70% ethanol using the methodology outlined by Sahoo *et al*. (2012) and Tandel *et al.* (2021). The *A. japonicus* specimens were identified morphologically following the key characteristics given by Wadeh *et al.* (2008); Soes *et al.* (2010); Mousavi *et al.* (2011); Sahoo *et al*. (2012) and Nagasawa (2021). *A. foliaceus* specimens were identified according to Tokşen (2006); Møller (2011); Soes *et al.* (2010) and Mousavi *et al.* (2011). However, specimens of *A. coregoni* were confirmed based on the key morphological features described by Shimura (1981); Everts and Avenant-Oldewage (2009); Mousavi *et al.* (2011) and Nagasawa and Yuasa (2019).

Molecular analysis

DNA extraction and PCR amplification

The genomic DNA was isolated from morphologically distinct *Argulus* sp. kept in DNA diluent using the phenol: chloroform isolation procedure as reported by Khan *et al*. (2020) with slight modifications. However, the macerated samples were vortexed and incubated overnight at 54°C in a water bath. The extracted DNA was used as a template for polymerase chain reaction (PCR) and universal primer set-1 amplified the 650 bp fragment of 18S rRNA gene using 18S rRNA F-566:5′- CAG CAG CCG CGG TAA TTC C-3′ (forward) and for 18S rRNA R-1200:5′- CCC GTG TTG AGT CAA ATT AAG C-3′ (reverse) (Hadziavdic *et al*., 2014). PCR amplification was performed in a thermal cycler (Applied biosystem™ Veriti™, Thermofisher Scientific) using the following PCR conditions; 95°C for 15 min, 35 cycles consisting of 95°C for 45 sec, 60°C for 45 sec, 72°C for 1 min and a final extension step of 72°C for 10 min. The other universal eukaryotic primer set-2 for targeting 18S rRNA include, 18S rRNA F-ERIB1, 5′-ACC TGG TTG ATC CTG CCA G-3′ and 18S rRNA R-ERIB10, 5′- CTT CCG CAG GTT CAC CTA CGG-3′ amplified a fragment size of 1800 bp (Patra *et al*., 2016). Initial denaturation step at 95°C for 5 min was followed by 35 cycles of denaturation for 30 sec, annealing at 51°C for 30 sec, extension at 72°C for 60 sec and final extension at 72°C for 5 min. HiPurA® PCR product purification kit (Himedia, India) was used to purify PCR amplicons before bidirectional DNA sequencing using Sanger sequencing (Bioserve Biotechnologies Pvt. Ltd., India).

DNA sequencing and phylogenetic analysis

Based on 18S rRNA sequences from the current isolates and other *Argulus* spp., Molecular Evolutionary Genetics Analysis (MEGA, version 11) was used to align them. The GenBank database was searched for comparable *Argulus* sp. sequences using Nucleotide BLAST. Phylogenetic analysis and species pairwise distances were performed as stated by Tamura *et al*. (2007). The maximum likelihood approach with 1000 replicates bootstrap analysis was used for graphical depiction of 18S rRNA divergences across *Argulus* species. The evolutionary pairwise distances were calculated using the Tamura-Nei substitution model, as described by Tamura *et al*. (2013).

Prevalence analysis

The prevalence study was evaluated using the methodology and formula specified by Margolis *et al.* (1982).

$$\text{Prevalence of infection (\%)} = \frac{\text{No of argulus infested fish}}{\text{Total number of examined fish}} \times 100$$

The Chi-square tests (χ^2) was conducted to compare the difference in parasite prevalence across host species in terms of parasite infection rates.

RESULTS AND DISCUSSION

Host diversity and prevalence analysis

Out of 670 fish specimens examined, 395 individuals were found to be infected with *Argulus* spp. parasites. The most affected species was Goldfish (249 no.), followed by Rohu and Catla species (104 and 21 no., respectively). The remaining fish species had lower rates of infection (Mrigal-13, Subunkin-4, Milky white koi carp-3 and Tiger Oscar-1). This study revealed that *A. japonicus* has a propensity to infect a diverse array of fish hosts, infecting 247 goldfish, 104 rohu, 21 catla, 13 mrigal, 4 subunkin and 3 milky koi carp. Additionally, it was observed that *A. foliaceus* only infected a single tiger oscar (*Astronotus ocellatus*), whereas *A. coregoni* infected two goldfish individuals.

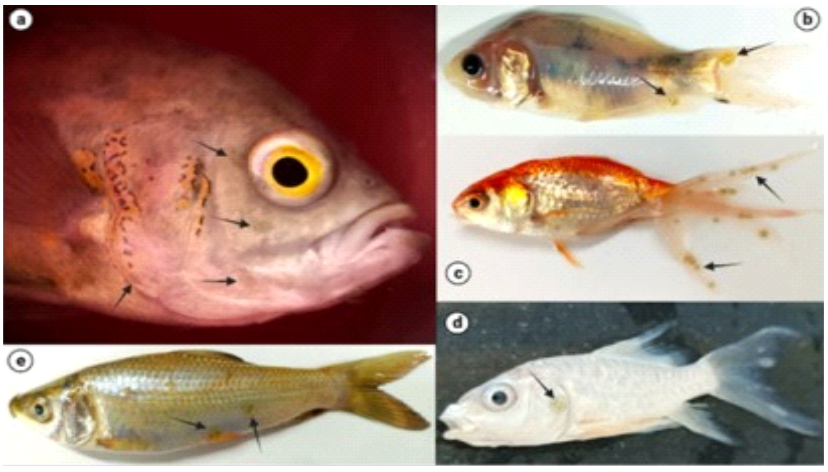
In the Indian context, *A. japonicus* has been documented in many fish species, including *Cyprinus carpio* (Sahoo *et al*., 2012), goldfish/Prussian carp (Kumari *et al*., 2019) and Himalayan snow trout, *Schizothorax richardsonii* (Tandel *et al*., 2021). Saha and Bandyopadhyay (2015), were the first to report the infestation of *A. coregoni*, *A. japonicus*, and *A. foliaceus*, in Red Can Oranda Gold Fish (*Carassius auratus auratus*) in West Bengal. Similarly, in our present study, *A. japonicus* has been found to infect a wide range of host species with various infestation rate including goldfish (90.20%), milky koi carp (50%), mrigal (46.43%), rohu (38.24%), Catla (36.21%) and subunkin (27%), whereas, *A. foliaceus* and *A. coregoni* infected only tiger oscar (50%) and goldfish (13.33%), respectively (Fig 1 and 2), showing low host diversity than *A. japonicus*. However, there was no significant difference in terms of prevalence rates between the host species and *Argulus* spp. (P>0.05) (Fig 2). This is the first record of *A. japonicus* infecting milky koi carp and *A. foliaceus* infecting tiger oscar in India. This phenomenon could potentially be attributed to the transmission of *Argulus* spp. infections among fish hosts in the same aquatic system or to the introduction of infected fish into a culture environment.

Pathological changes

Acute hemorrhagic inflammation of the epidermis, increased mucosal production, scale spillage and fin corrosion were identified at the sites of *Argulus* infestation (Alas *et al*., 2010). These were consistent with the pathological findings in the present study (Fig 3).

Morphological description of *Argulus* spp.

The morphological characteristics previously described were used to differentiate *A. coregoni, A. foliaceus* and *A. japonicus* based on the morphology of the abdominal lobes, respiratory regions and length of the cephalothoracic carapace as described by Noaman *et al*. (2010) and Wafer *et al*. (2015). As per Noaman *et al*. (2010), *A. coregoni* exhibits pointed abdominal lobes, posterior lobes of the cephalothoracic carapace do not extend beyond the commencement of the abdomen, similar to *A. foliaceus*. However, in *A. foliaceus* it has rounded abdominal lobes with the posterior emargination not extending to the midline. Additionally, adults of *A. coregoni* has a body length of 12 mm. In this context, it is observed that the fully developed female specimen of *A. coregoni* displays a greater magnitude in terms of physical dimensions and spermathecae are filled with sperms when compared with both *A. japonicus* and *A. foliaceus* as per Shimura (1981) (Fig 4-IIA, B). Conversely, the posterior lobes of the cephalothoracic carapace in *A. japonicus* extend beyond the commencement of the abdomen. Furthermore, it has been observed that the abdominal lobes in *A. japonicus*



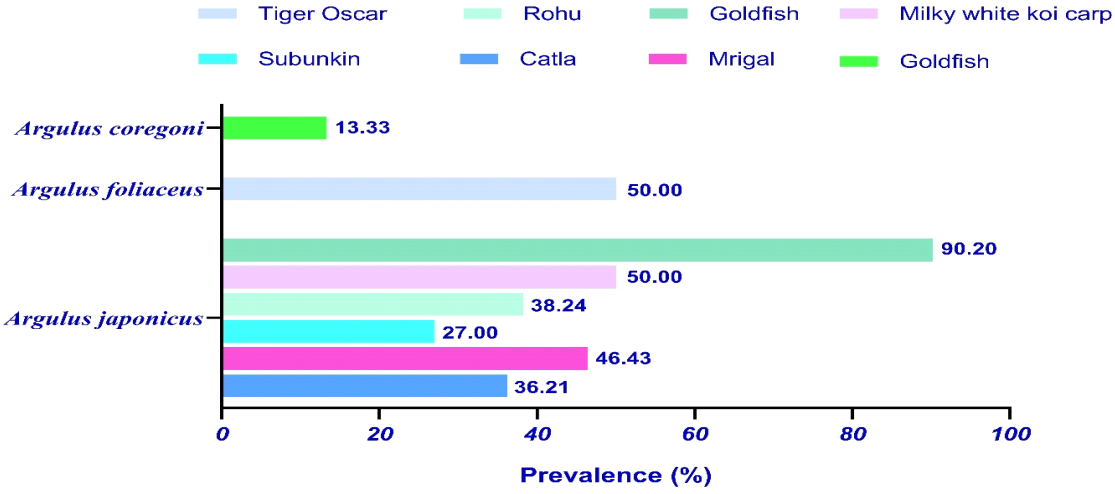
(a) Tiger oscar (*Astronotus ocellatus*) (b) London Shubunkin (*Carassius auratus auratus*) (c) Goldfish (*Carassius auratus*) (d) Platinum ogon butterfly koi/Milky white koi carp (*Cyprinus carpio carpio*) (e) Rohu (*Labeo rohita*).

Fig 1: *Argulus* infested fish host.

have a more pointed shape compared to those in *A. foliaceus*, as reported by Noaman *et al*. (2010) and Wafer *et al*. (2015). These were the best visual cues used for the distinction among *A. coregoni, A. foliaceus* and *A. japonicus* in the current study (Fig 4-I and II). The female isolates of *A. japonicus, A. foliaceus and A. coregoni* reported in the present study have a body length and width of 6-8 and 3-5 mm, 4-8 and 3-5 mm and 7-12 and 4-6 mm respectively.

In the adult gravid female specimens of *A. japonicus*, several morphological variations were observed in the body length, width, carapace appearance, shape and appearance of the abdominal lobes (Table 1 and Fig 4-I). Moreover, a shovel shaped peg on the posterior distal end of basis located on the coxa of the 4th swimming legs is characteristics feature of male specimen in *A. japonicus* (Sahoo *et al*., 2012), has also been reported in the present study (Fig 5a-c). All the male isolates of *A. japonicus* in the present study were in the size of 4-5 (body length) and 3-4 mm (width) in size.

Based on the morphological variations and their sampling sites, three female isolates of *A. japonicus* were designated as COF_AHE_P (01) (Udaipur), COF_AHE_P (03) (Lembucherra), and COF_AHE_P (06) (Agartala); and one female isolate each of *A. coregoni* (COF_AHE_P (07)) (Agartala) and *A. foliaceus* (COF_AHE_P (08)) (Agartala) (Table 2). However, there was no morphological variationsin collected male specimens of *A. japonicus* unlike the female counterparts. But, based on their sampling sites the male specimens have been designated as *A. japonicus* COF_AHE_P (02) (Udaipur), COF_AHE_P (04) (Lembucherra),



Chi (χ^2) (*P*-value): P (%) (30.50ns (0.906); 'ns' represent statistically non-significant differences at significant levels $p<0.05$.

Fig 2: Prevalence value of *Argulus* spp. wise variation in host fishes.



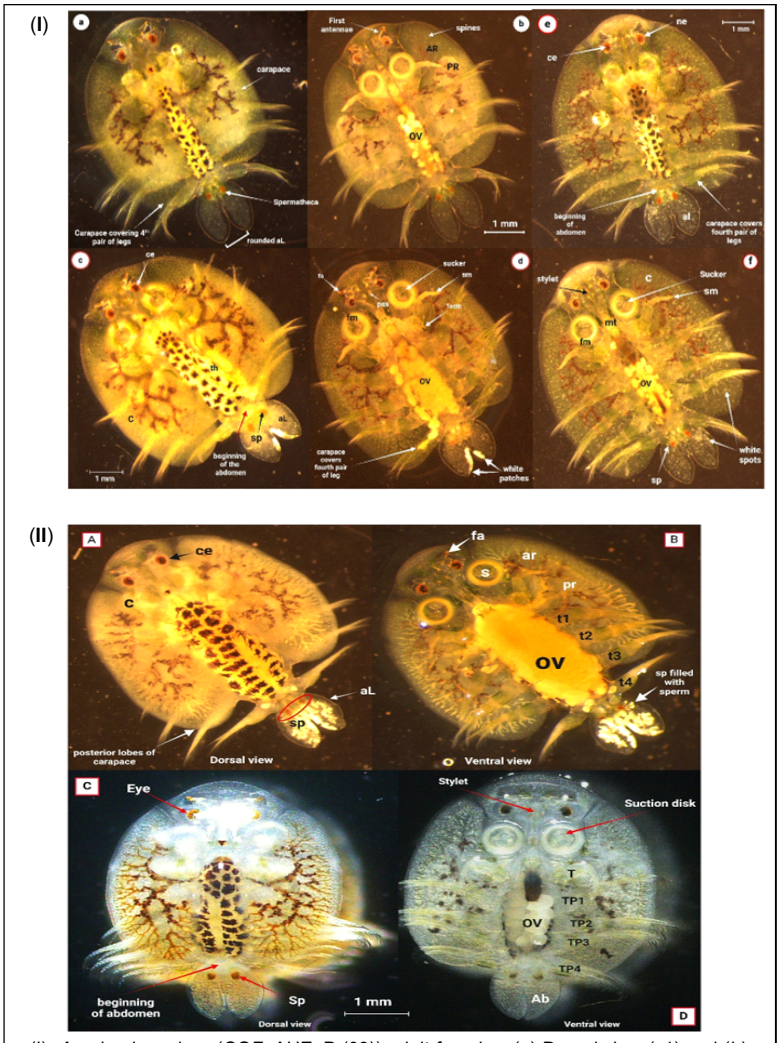
a, b, c, d: acute haemorrhagic septicaemia (skin, fin, operculum and ventral side of the body), e: erosion of the caudal fin, f: corneal opacity.

Fig 3: Clinical signs of *Argulus* infested goldfish.

COF_AHE_P (05) (Agartala) and COF_AHE_P (09) (Agartala) (Table 2).

Molecular and phylogenetic analyses

The *Argulus* samples obtained in this investigation was subjected to molecular characterization using the 18S rRNA genetic marker. The sequences of PCR product (650 bp and 1800 bp) of Argulus samples acquired in this study were subsequently analyzed using NCBI Gene Bank BLAST analysis demonstrated a high level of similarity, with six *A. japonicus* isolates, namely COF_AHE_P(02) (OR418123.1), COF_AHE_P(03) (OR418124.1), COF_AHE_P(04) (OR418125.1), COF_AHE_P(05) (OR418126.1), COF_AHE_P(06) (OR418127.1) and COF_AHE_P(09) (OR418130.1), exhibited sequence similarities of 99.33%, 99.32%. 99.49%, 99.83%, 97.77% and 99.49% respectively, when compared to *A. japonicus* isolate A850 (OR687243.1) from Uttarakhand India. *A. coregoni* isolate COF_AHE_P(07) (OR418128.1) and *A. foliaceus* COF_AHE_P(08) (OR418129.1) exhibited a 100% and 99.66% sequence identity, respectively, when compared

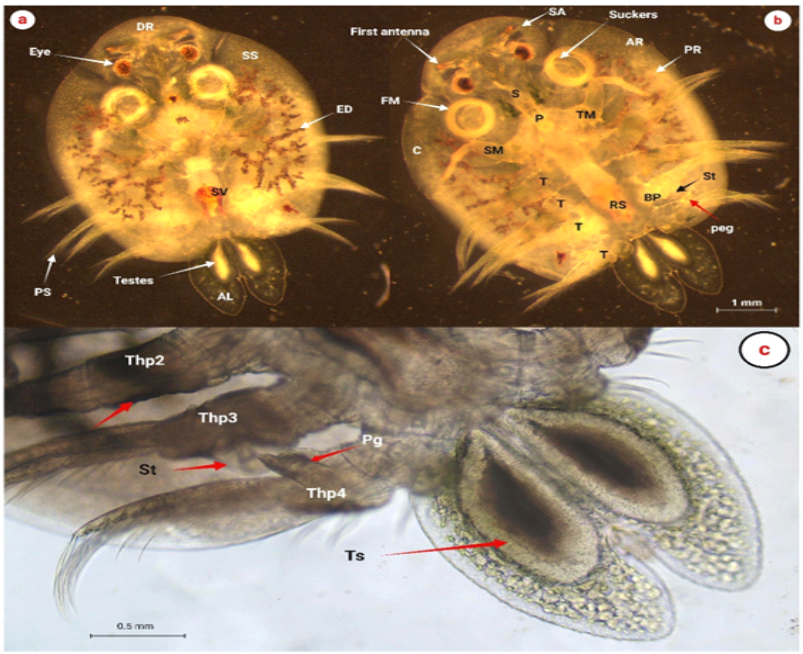


(I): *Argulus japonicus* (COF_AHE_P (03)) adult female - (a) Dorsal view (x1) and (b) Ventral view (x1); *Argulus japonicus* (COF_AHE_P (06)) adult female - (c) Dorsal view (x10) and (d) Ventral view (x10); *Argulus japonicus* (COF_AHE_P (01)) adult female - (e) Dorsal view (x1), (f) Ventral view (x1). (II): (A) Dorsal view (x1) and (B) Ventral view (x1) of *Argulus coregoni* (COF_AHE_P (07)) adult female; (C) Dorsal view (x1) and (D) Ventral view (x1) of *Argulus foliaceus* (COF_AHE_P (08)). aL: Abdominal lobe; sp, spermathecae; th: Thorax, ce, compound eye; ne: Naupliur eye; sm: Second maxilla; mt: Mouth tube; pas: Postantenal spine; ov: Ovary, fa: First antennae; AR: Anterior respiratory area; PR: Posterior respiratory area; pss: Pre-oral sheath and stylet; c: Carapace; fm: First maxillae; sm: Second maxillae; ab: Abdomen; t1-4: Thoracopods; fa: First antennae; T: Teeth.

Fig 4: Stereo zoom microscopic image with key morphological characters.

to the *A. coregoni* (JQ740820.1) and *A. foliaceus* (JQ740819.1) isolates from Iran. The level of similarity between *A. japonicus* isolates COF_AHE_P(01) (OR237569.1) and *A. japonicus* isolate MWS2 (KF747859)from China was found to be 96.70%. Using multiple sequence alignment, the nucleotide sequences were then aligned with the inclusion of 18S rRNA sequences from fifteenth and five different *Argulus* species and one additional outgroup species.The phylogenetic tree illustrating the evolutionary relationship of the *Argulus* spp. was depicted in Fig 6A and 6B.

The evolutionary pair-wise distances among the collected specimens of *A. japonicus* (OR418123, OR418124, OR418125, OR418126, OR418127, OR418130, OR237569) in comparison with specimens from other regions of India

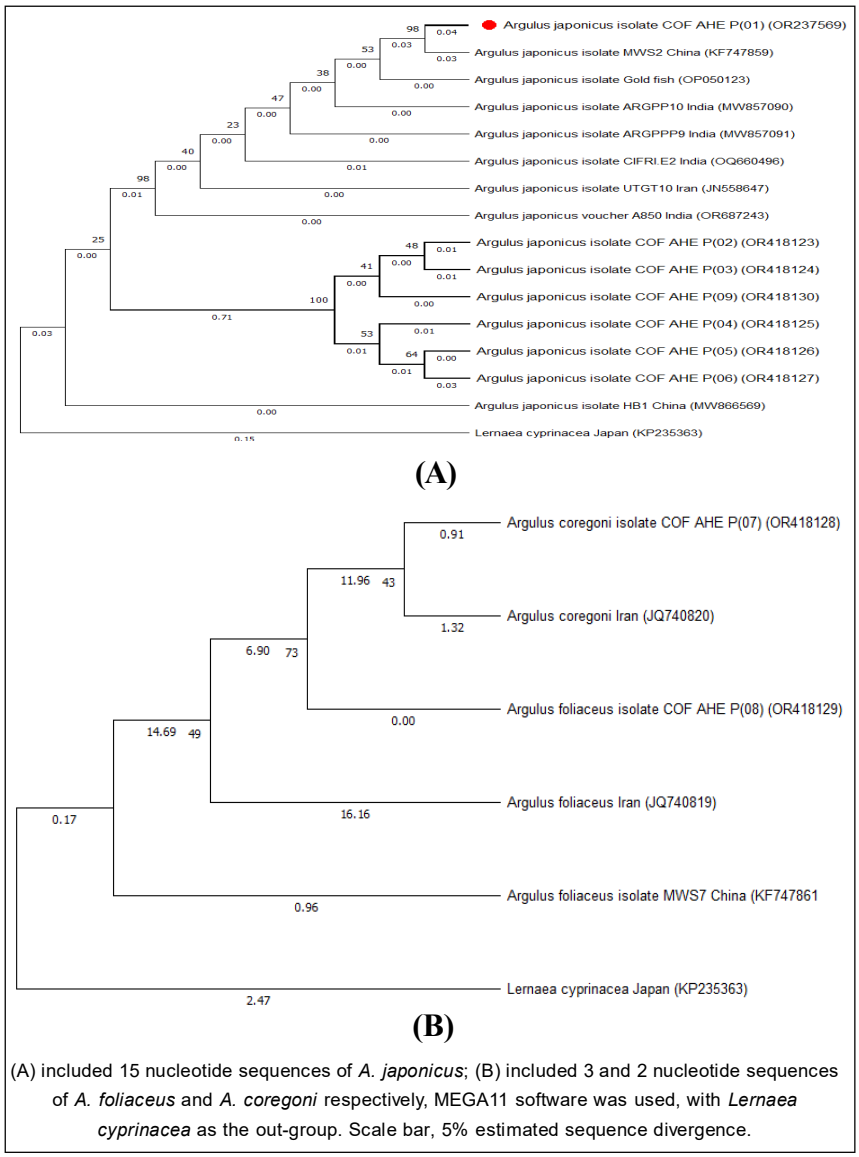


(a) Dorsal view and (b) Ventral view. AR: Anterior respiratory area; PR: Posterior respiratory area; BP: Bilobed protuberance; DR: Dorsal ridge; AL: Abdominal lobe; S: Pre-oral stylet; C: Carapace; PS: Pinnate setae; P: Feeding proboscis; ED: Enteral diverticula; RS: Reproductive system; SV: Seminal vesicle; T: Thoracopods; FM: First maxillae; SM: Second maxillae; TM: Teeth on second maxillae; SS: Small spines with hook-like projections; SA: Second antenna; St: Socket; Pg: Peg; Ts: testes; Thp2: Thp3 and Thp4, 2nd , 3rd and 4th pair of thoracopods.

Fig 5: Stereo zoom and light microscopic image of *Argulus japonicus* adult male Thiele, 1900 (x1).

Table 1: Differences in the morphological characters within the female isolates of *Argulus japonicus* (COF_AHE_P (01)), *Argulus japonicus* (COF_AHE_P (03)) and *Argulus japonicus* (COF_AHE_P (06).

Morphological characteristics	*Argulus japonicus* (COF_AHE_P (01))	*Argulus japonicus* COF_AHE_P (03)	*Argulus japonicus* (COF_AHE_P (06))
Body length (mm) and width (mm)	6-8, 3-6	6-8, 3-5	6-8, 3-5
Abdominal lobes	Rounded with slightly pointed edges; white spots scattered all over the abdomen (except testes)	Rounded with blunt edges; without white spots and patches	Rounded with slightly pointed edges; white patches at the posterior region of the abdomen but without any white spots
Carapace	Horseshoe shaped; white spots scattered all over the carapace [Fig 4 (I - e, f)].	Horseshoe shaped; without white spots [Fig 4 (I - a, b)].	Horseshoe shaped; without white spots [Fig 4 (I - c, d)].
Posterior lobes of the carapace	Covers the fourth pair of legs and extends beyond the beginning of the abdomen (posterior lobes filled with white spots) [Fig 4 (I - e, f)].	Covers the fourth pair of legs and extends beyond the beginning of the abdomen [Fig 4 (I - a, b)].	Covers the fourth pair of legs and extends beyond the beginning of the abdomen [Fig 4 (I - c, d)]



(A) included 15 nucleotide sequences of *A. japonicus*; (B) included 3 and 2 nucleotide sequences of *A. foliaceus* and *A. coregoni* respectively, MEGA11 software was used, with *Lernaea cyprinacea* as the out-group. Scale bar, 5% estimated sequence divergence.

Fig 6: A phylogenetic tree of present *Argulus* spp. constructed from 18S rRNA sequences using the Neighbor-Joining method with bootstrap (1000 replicates): Evolutionary distances were calculated using maximum composite likelihood.

Table 2: Geographical locations, sexes and host ranges of the collected *Argulus* spp. samples with GenBank™ accession numbers.

Argulus spp. with codes	Geographical origin	Sex	Host Fish species	GenBank™ accession number
Argulus japonicus COF_AHE_P (01)	India, Udaipur, Fish farms	Female	Rohu (*Labeo rohita*)	OR237569.1
Argulus japonicus COF_AHE_P (02)	India, Udaipur, Fish farms	Male	Catla (*Labeo catla*)	OR418123.1
Argulus japonicus COF_AHE_P (03)	India, Lembucherra, Fish farms	Female	Rohu (*Labeo rohita*)	OR418124.1
Argulus japonicus COF_AHE_P (04)	India, Lembucherra, Fish farms	Male	Rohu (*Labeo rohita*)	OR418125.1
Argulus japonicus COF_AHE_P (05)	India, Agartala, Aquarium shops	Male	London Shubunkin (*Carassius auratus auratus*)	OR418126.1
Argulus japonicus COF_AHE_P (06)	India, Agartala, Aquarium shops	Female	Milky white koi carp (*Cyprinus carpio carpio*)	OR418127.1
Argulus japonicus COF_AHE_P (09)	India, Agartala, Aquarium shops	Male	Goldfish (*Carassius auratus*)	OR418130.1
Argulus coregoni COF_AHE_P (07)	India, Agartala, Aquarium shops	Female	Goldfish (*Carassius auratus*)	OR418128.1
Argulus foliaceus COF_AHE_P (08)	India, Agartala, Aquarium shops	Female	Tiger oscar (*Astronotus ocellatus*)	OR418129.1

Table 3: Pairwise analysis of sequence dissimilarities (in %) between sequences of *Argulus japonicus* and out group species accessible in the GenBank database.

NCBI accession number	OR418123	OR418124	OR418125	OR418126	OR418127	OR418130	MW866569	JN558647	OR237569	KF747859	OP050123	OR687243	OQ660496	MW857091	KP235363
OR418123															
OR418124	0.01														
OR418125	0.02	0.02													
OR418126	0.02	0.02	0.01												
OR418127	0.04	0.03	0.03	0.03											
OR418130	0.01	0.01	0.01	0.005	0.03										
MW866569	0.77	0.77	0.78	0.76	0.76	0.76									
JN558647	0.77	0.77	0.78	0.76	0.76	0.76	0.001								
OR237569	0.77	0.78	0.80	0.77	0.75	0.77	0.07	0.07							
KF747859	0.78	0.78	0.80	0.77	0.75	0.77	0.06	0.05	0.06						
OP050123	0.76	0.77	0.77	0.76	0.75	0.76	0.003	0.00	0.02	0.01					
OR687243	0.77	0.77	0.79	0.77	0.76	0.77	0.003	0.00	0.02	0.01	0.003				
OQ660496	0.77	0.77	0.78	0.76	0.76	0.76	0.002	0.00	0.07	0.05	0.002	0.003			
MW857091	0.75	0.76	0.78	0.76	0.75	0.76	0.00	0.00	0.02	0.01	0.000	0.001	0.002		
KP235363	0.89	0.90	0.91	0.90	0.87	0.89	0.20	0.17	0.24	0.21	0.20	0.200	0.120	0.167	

*OR418123, *Argulus japonicus* COF_AHE_P (02); OR418124, *Argulus japonicus* COF_AHE_P (03); OR418125, *Argulus japonicus* COF_AHE_P (04); OR418126, *Argulus japonicus* COF_AHE_P (05); OR418127, *Argulus japonicus* COF_AHE_P (06); OR418130, *Argulus japonicus* isolate COF_AHE_P(09); MW866569, *Argulus japonicus* isolate HB1 China; JN558647, *Argulus japonicus* strain UTGT10 Iran; OR237569, *Argulus japonicus* COF_AHE_P (01); KF747859, *Argulus japonicus* isolate MWS2 China; OP050123, *Argulus japonicus* isolate Gold fish India; OR687243, *Argulus japonicus* voucher A850 India; OQ660496, *Argulus japonicus* isolate CIFRI.E2 India; MW857091, *Argulus japonicus* isolate ARGPPP9 India; KP235363, *Lernaea cyprinacea* Japan.

Table 4: Pairwise analysis of sequence dissimilarities (in %) between sequences of *Argulus foliaceus*, *Argulus coregoni* and out group species accessible in the GenBank database.

NCBI accession number	OR418128	JQ740820	OR418129	JQ740819	KF747861	KP235363
Argulus_coregoni_isolate_COF_AHE_P(07)_(OR418128)						
Argulus_coregoni_18S_Iran_(JQ740820)	0.01					
Argulus_foliaceus_isolate_COF_AHE_P(08) (OR418129)	0.00	0.01				
Argulus_foliaceus_18S_ Iran (JQ740819)	0.01	0.00	0.01			
Argulus_foliaceus_isolate_MWS7_18S_Iran (KF747861)	0.01	0.03	0.01	0.03		
Lernaea_cyprinacea_18S_(KP235363)	0.21	0.20	0.21	0.20	0.19	

(Uttarakhand) (OR687243) and China (KF747859), exhibited a range of 0.01% to 0.78% (Table 3). Similarly, for *A. coregoni* it ranged from 0.0% (*A. coregoni* COF_AHE_P (07) OR418128) to 0.01% (*A. coregoni* Iran JQ740820) (Table 4). In case of *A. foliaceus* the evolutionary pair-wise distances varied from 0.0% (*A. foliaceus* COF_AHE_P (08) OR418129) to 0.03 % (*Argulus foliaceus* isolate MWS7 18S Iran (KF747861) (Table 4) and between *Argulus* spp. and the outgroup (*Lernaea cyprinacea* Japan KP235363) exhibited a range of 0.01% to 0.91% (Table 3) and 0.01% to 0.21% (Table 4). The present findings indicate that the morphological and phylogenetic characteristics of the *Argulus* specimens under investigation corresponded to those of *A. japonicus*, *A. foliaceus* and *A. coregoni*. Based on genetic diversity studies, *A. japonicus* specimens showed variations in genetic makeup within individuals and across species, suggesting genetic drift due to stable or unstable environmental factors in each region. These parasites may have come from various nations or locations with different sampling circumstances. Another possible explanation for the variations could be mutations in the mitochondrial DNA of the isolates, as suggested by Wadeh *et al*. (2010). Genomic and morphological variation among *A. japonicus* (Fig 4-I) species may be influenced by environmental variables during climatic adversity. However, more research is needed to grasp these issues.

CONCLUSION

The comprehensive examination of *Argulus* spp. in this study yielded valuable insights into their morphological features, genetic composition, diverse fish hosts and pathological changes. The morphological characterization revealed distinct traits among the species for precise identification. Molecular analysis affirmed these identifications, showcasing high sequence similarities among isolates and correlations with known sequences from different geographical locations, establishing the presence of *A. japonicus, A. foliaceus* and *A. coregoni*. The prevalence study delineated host preferences, with *A. japonicus* exhibiting a broader host range compared to *A. foliaceus* and *A. coregoni*, highlighting the varied impact on different fish species. However, future study is necessary to get a more comprehensive knowledge of the morphological and molecular variations of Argulus within and between species, as well as across various geographical regions.

ACKNOWLEDGEMENT

The research was conducted with facilities developed under the project “National Surveillance Programme for Aquatic Animal Diseases - Phase II,” Department of Fisheries, Ministry of Fisheries, Animal Husbandry, and Dairying, Government of India. The first author expresses gratitude to the Ministry of Tribal Affairs, Government of India, for the “National Fellowship and Scholarship for Higher Education of ST Students” scholarship. The authors appreciate Dr. Anupam Mishra, Vice-chancellor of CAU, Imphal, for kindly providing all research facilities.

Ethical concern

The Institutional Animal Ethics Committee (IAEC) of College of Fisheries, CAU (I), Tripura, dated March 1, 2022, conducted a thorough evaluation and granted approval for this work vide approval number CAU-CF/48/IAEC/2018/02 where live fishes were treated in a manner consistent with ethical standards.

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

Alas, A., Oktener, A. and Solak, K. (2010). A study on the morphology of *Argulus foliaceus* Lin., 1758 (Crustacea; Branchiura) procured from Cavuscu Lake (Central Anatolia-Turkey) with scanning electron microscopy. Turkish Journal of Biology. 34(2): 147-151. https://doi.org/10.3906/biy-0811-27.

Bari, I.P.M. (2018). A new ectoparasitic crustacean, *Argulus maharashtrians* sp. from Parbhani district (MS). International Journal of Fauna and Biological Studies. 5: 05-09.

Datta, N., Kar, P.K. and Saha, S.K. (2022). Primary stress response and biochemical profile of *Labeo rohita* (Hamilton, 1822) experimentally parasitized with *Argulus bengalensis* (Ramakrishna, 1951). Journal of Fish Biology. 100(6): 1375-1387. https://doi.org/10.1111/jfb.15048.

Everts, L. and Avenant-Oldewage, A. (2009). First record of *Argulus coregoni*: A fish ectoparasitic crustacean from Malaysia and additional notes on the morphology. Malaysian Applied Biology. 38(2):61-71.

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E.M. and Troedsson, C. (2014). Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PloS one. 9(2): p.e87624. https://doi.org/10.1371/journal.pone.0087624.

Khan, W., Safdar, M., and Junejo, Y. (2020). Molecular identification of *Argulus japonicus* in red cap oranda goldfish (*Carassius auratus*) in Multan, Pakistan. Zeugma Biological Science. 1(2): 42-46.

Kismiyati, Andika, A.R., Kusnoto. (2023). The percentage of male and female *Argulus* infesting cyprinidae fish in Magelang regency, Central Java, Indonesia. Indian Journal of Animal Research. 57(9): 1236-1239. doi: 10.18805/IJAR.BF-1510.

Kumari, P., Kumar, S., Ramesh, M., Shameena, S., Deo, A.D., Rajendran, K.V. and Raman, R.P. (2019). Antiparasitic effect of aqueous and organic solvent extracts of *Azadirachta indica* leaf against *Argulus japonicus* in *Carassius auratus*. Aquaculture. 511: 634175. https://doi.org/10.1016/j.aquaculture.2019.05.060.

Laltlanmawia, C., Saha, H., Ghosh, L., Saha, R.K. and Malla, S. (2023). Unveiling the mechanisms of Aeromonas hydrophila COF_AHE51 induced mortality in *Labeo rohita*: Hematobiochemical and immune-pathological perspectives. Indian Journal of Animal Research. 57(10): 1305-1313. doi: 10.18805/IJAR.B-5153.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. and Schad, G. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology. 68(1): 131-133. https://doi.org/10.2307/3281335 .

Møller, O.S. (2011). *Argulus foliaceus*. In: Fish Parasites: Pathobiology and Protection. [Woo, P.K.T. and Buchmann, K. (eds)], CAB International, Wallingford, UK. https://doi.org/10.1079/9781845938062.0327. pp. 327-336.

Mousavi, H.E., Behtash, F., Rostami-Bashman, M., Mirzargar, S.S., Shayan, P. and Rahmati-holasoo, H. (2011). Study of *Argulus* spp. infestation rate in Goldfish, *Carassius auratus* (Linnaeus, 1758) in Iran. Human and Veterinary Medicine. 3(3): 198-204. http://www.hvm.bioflux.com.ro/.

Nagasawa, K. (2021). Argulus japonicus (Branchiura: Argulidae) parasitic on largemouth bass *Micropterus salmoides* in Japan, with the morphology of the adult female of the argulid. Crustacean Research. 50: 119-129.

Nagasawa, K. and Yuasa, A. (2019). 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.https://doi.org/10.18353/crustacea.49.0_1.

Noaman, V., Chelongar, Y. and Shahmoradi, A.H. (2010). The first record of *Argulus foliacesus* (Crustacea: Branchiura) infestation on lionhead goldfish (*Carassius auratus*) in Iran. Iranian Journal of Parasitology. 5(2): 71-76.

Patra, A., Mondal, A., Banerjee, S., Adikesavalu, H., Joardar, S.N. and Abraham, T.J. (2016). Molecular characterization of *Argulus bengalensis* and *Argulus siamensis* (Crustacea: Argulidae) infecting the cultured carps in West Bengal, India using 18S rRNA gene sequences. Molecular Biology Research Communications. 5(3): 156.

Poly, W.J. (2008). Global diversity of fish lice (Crustacea: Branchiura: Argulidae) in freshwater. Hydrobiologia. 595: 209-212. https://doi.org/10.1007/s10750-007-9015-3

Rahman, M.A., Alom, M.Z., Yeasmin, M.S. and Khan, S. (2019). Status, occurrence, intensity and impact of *Argulosis* in different brood stock ponds. MOJ Ecology and Environmental Sciences. 4: 225-229. https://doi.org/10.15406/mojes.2019.04.00158

Saha, M. and Bandyopadhyay, P.K. (2015). First report of three species of Argulus (Crustacea: Branchiura) infesting on red-can Oranda gold fish (*Carassius auratus auratus*) in India. Biolife. 3(4): 813-819.

Sahoo, P.K., Hemaprasanth, Kar B., Garnayak, S.K. and Mohanty, J. (2012). Mixed infection of *Argulus japonicus* and *Argulus siamensis* (Branchiura, Argulidae) in carps (Pisces, Cyprinidae): loss estimation and a comparative invasive pattern study. Crustaceana. 1449-1462. https://doi.org/10.1163/156.854012X651501.

Sahoo, P.K., Mohanty, J., Garnayak, S.K., Mohanty, B.R., Banya, K., Hema, P. and Jena, J.K. (2013). Estimation of loss due to argulosis in carp culture ponds in India. Indian Journal of Fisheries. 60(2): 99-102.

Sahoo, P.K., Paul, A., Sahoo, M.K., Pattanayak, S., Rajesh Kumar, P. and Das, B.K. (2020). Incidences of infectious diseases in freshwater aquaculture farms of Eastern India: A passive surveillance based study from 2014-2018. Journal of Aquaculture Research Development. 11(1): 579. https://doi.org/10.35248/2155-9546.20.11.579.

Saravanan, K., Kumar, S., Praveenraj, J., Meena, B.L., Kasinath, B.L., Kiruba-Sankar, R. and Roy, S.D. (2021). Molecular characterization of muscle infecting *Myxobolus* sp. causing outbreak in Labeo rohita, Rohu: First report from Andaman Islands. Indian Journal of Animal Research. 55(2): 211-216. doi: 10.18805/ijar.B-3931.

Shimura, S. (1981). The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura). Journal of Natural History. 15(2): 331-348. https://doi.org/10.1080 002229381007 70251.

Soes, D.M., Walker, P.D. and Kruijt, D.B. (2010). The Japanese fish louse *Argulus japonicus* new for The Netherlands. Lauterbornia. 70: 11-17.

Steckler, N. and Yanong, R.P. (2013). Argulus (Fish Louse) Infections in Fish: FA184, 11/2012. Edis, 2013(2).

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 24(8): 1596-1599. https://doi.org/10.1093/molbev/msm092.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 30(12): 2725-2729. https://doi.org/10.1093/molbev/mst197.

Tandel, R.S., Chanu, K.V., Hussain Bhat, R.A., Dash, P., Shah, T.K. and Thakuria, D. (2021). Morphometric and molecular identification of *Argulus japonicus* (Thiele 1900) in vulnerable Himalayan snow trout, *Schizothorax richardsonii* (Gray 1832). Research. 52(12): 6770-6778. https://doi.org/10.1111/are.15486.

Thakur, K., Sharma, A., Sharma, D., Brar, B., Choudhary, K., Sharma, A.K., Mahajan, D., Kumar, R., Kumar, S. and Kumar, R. (2023). An insight into the interaction between *Argulus siamensis* and *Labeo rohita* offers future therapeutic strategy to combat argulosis. Aquaculture International. 31(3):1607-1621. https://doi.org/10.1007/s10499-022-01043-x.

Tokşen, E. (2006). *Argulus foliacesus* (Crustacea: Branchiura) infestation on oscar, *Astronotus ocellatus* (Cuvier, 1829) and its treatment. Ege Journal of Fisheries Aquatic Sciences. 23(1):177-179. http://jfas.ege.edu.tr/.

Valarmathi, K. (2017). Crusta Cea: BranChiura: Argulidae (FishliCe). Freshwater Faunal Diversity in India. 247.

Wadeh, H., Yang, J.W. and Li, G.Q. (2008). Ultrastructure of *Argulus japonicus* Thiele, 1900 (Crustacea: Branchiura) collected from Guangdong, China. Parasitology Research. 102: 765-770. https://doi.org/10.1007/s00436-007-0837-8.

Wadeh, H., Alsarakibi, M. and Li, G. (2010). Analysis of genetic variability within *Argulus japonicus* from representatives of Africa, Middle East and Asia revealed by sequences of three mitochondrial DNA genes. Parasitology Research. 107: 547-553. https://doi.org/10.1007/s00436-010-1891-1.

Wafer, L.N., Whitney, J.C. and Jensen, V.B. (2015). Fish lice (*Argulus japonicus*) in goldfish (*Carassius auratus*). Comparative Medicine. 65(2): 93-95.