IJMPES International Journal of
Medical Parasitology &
Epidemiology Sciences

http://ijmpes.com
doi 10.34172/ijmpes.6208
2025;6(4):111-120
eISSN 2766-6492

Original Article

Morphological and Molecular Identification of Fish Lice (*Argulus coregoni, A. follicaeus, A. japonicus*) in Ornamental Freshwater Fish

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Abstract

Introduction: Fish lice of the genus *Argulus* are among the most important ectoparasites affecting freshwater ornamental fish, causing economic losses and threatening fish health. The present study aimed to identify and characterize three species of fish lice (*Argulus coregoni, A. foliaceus,* and *A. japonicus*) collected from ornamental freshwater fish in local markets.

Methods: A total of 442 ornamental fish, including goldfish (*Carassius auratus*), koi carp (*Cyprinus carpio*), Black Moor, and other varieties, were examined for parasitic infestation. Morphological observations were carried out using a light microscope equipped with a Lusida digital camera at 40 × magnification. Diagnostic features such as carapace shape, appendage arrangement, and oral structures allowed the identification of *A. japonicus* and *A. foliaceus*, whereas *A. coregoni* could not be reliably distinguished based on morphology alone. Molecular analysis was performed using PCR amplification of ribosomal DNA markers. The 18S rRNA gene (850 bp) was successfully amplified in *A. coregoni* and *A. foliaceus*, while the 28S rDNA gene (400 bp) was used for *A. japonicus*. Sequencing confirmed the species identity, and all obtained sequences were deposited in the NCBI GenBank with accession numbers.

Results: BLAST analysis demonstrated high similarity between the local isolates and reference sequences from Iran, Indonesia, and India. Phylogenetic trees revealed that *A. coregoni* and *A. foliaceus* clustered closely with international isolates, while *A. japonicus* formed a separate branch, reflecting nucleotide variations in its 28S region.

Conclusion: The integration of morphological and molecular approaches provided accurate identification of the studied *Argulus* species. The molecular data, supported by phylogenetic analysis and sequence registration in GenBank, not only confirmed species identity but also highlighted genetic relationships with isolates from other geographical regions. These findings contribute to the understanding of fish lice diversity in ornamental freshwater fish and provide a valuable reference for future parasitological and molecular studies.

Keywords: Fish lice, Ornamental fish, Morphological diagnosis, Molecular identification, 18S rRNA, 28S rDNA

Received: July 16, 2025, **Revised:** September 18, 2025, **Accepted:** November 12, 2025, **ePublished:** November 23, 2025

Introduction

Fish lice belonging to the genus *Argulus* (family Argulidae) are cosmopolitan ectoparasites that parasitize a wide range of freshwater and marine fish, including economically valuable aquaculture species and ornamental fish (1,2). These parasites are responsible for serious health problems in infested hosts, including tissue damage, anemia, reduced growth, immunosuppression, and facilitation of secondary infections by bacteria, fungi, and viruses (3–5). In aquaculture, infestations of *Argulus* often lead to reduced productivity and increased mortality, resulting in significant economic losses (6). The spread of *Argulus* is further aggravated by the ornamental fish trade, which serves as an essential pathway for introducing new parasites into different regions of the world (7).

Three species are of particular concern in freshwater ornamental fish: *Argulus coregoni*, *A. foliaceus*, and *A. japonicus*. Each of these species has been widely reported in Europe, Asia, and other regions. However, their occurrence in ornamental fish is particularly problematic given the commercial importance of these hosts (8, 9). *A. foliaceus* is one of the most widely distributed species in Europe and Asia, commonly infecting cyprinids, including goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*) (10). *A. japonicus*, originally described in East Asia, has been reported from multiple continents due to fish translocation and the ornamental fish industry, making it an invasive species of global concern (11, 12). *A. coregoni*, on the other hand, is frequently associated with salmonids and cyprinids and is considered a severe parasite in temperate regions (13).

Traditionally, *Argulus* identification has relied on morphological methods. Morphological diagnosis is typically based on observable characters such as the shape and size of the carapace, the segmentation and ornamentation of appendages, the structure of

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mouthparts, and the position of the compound eyes (14, 15). In particular, the post-antennal spine, antennules, and arrangement of thoracic legs are considered essential for species-level identification (16). However, several authors have highlighted that morphological characters alone are often unreliable for distinguishing closely related species, especially when specimens are small, damaged, or at juvenile stages (17, 18). Phenotypic plasticity and overlapping morphological traits among *Argulus* species have been documented as significant limitations, leading to misidentification and underestimation of parasite diversity (19). For example, Kesik et al emphasized that some species, including *A. japonicus*, can be misclassified solely on morphological grounds, and that molecular confirmation is essential to ensure accuracy (20).

Molecular techniques have therefore emerged as powerful tools to overcome the limitations of morphology. Ribosomal DNA markers such as the 18S rRNA and 28S rDNA genes have been widely used for species-level identification of parasitic crustaceans, including *Argulus* (21, 22). These markers provide high-resolution data for differentiating closely related taxa, detecting cryptic species, and facilitating phylogenetic studies. In addition, mitochondrial DNA sequences, such as cytochrome oxidase I (cox1), and complete mitogenomes have been increasingly used to resolve species boundaries and explore evolutionary relationships (23). For instance, Wang et al. reported the complete mitogenome of A. japonicus, highlighting its potential for molecular systematics and diagnostics (14,24). Other studies have combined morphological identification with ribosomal marker sequencing to confirm the presence of *A. japonicus* and *A. foliaceus* in ornamental fish populations (25).

Beyond taxonomy, molecular data provide insights into the genetic diversity and geographic structure of *Argulus* species. Comparative studies have shown that isolates from different regions often exhibit high sequence similarity, indicating limited genetic divergence, while others form distinct phylogenetic branches, reflecting local adaptation or cryptic diversity (22, 23, 26). For example, phylogenetic analyses of *A. foliaceus* and *A. coregoni* demonstrated close clustering with Iranian and Indonesian isolates, whereas *A. japonicus* often separates into independent branches due to unique nucleotide variations (20, 24). These findings highlight the importance of molecular approaches not only for accurate identification but also for understanding evolutionary dynamics.

The economic and ecological implications of *Argulus* infestations have also driven interest in epidemiological studies. Several investigations have examined seasonal variations, prevalence rates, sex ratios, and host preferences of *Argulus* in different environments (6, 7, 11). Such data are crucial for developing control strategies, as infestations often peak during warmer seasons, coinciding with increased fish trade and aquaculture activity. Moreover, the high reproductive capacity of *Argulus* females and their ability to attach egg strings to substrates make eradication challenging (12, 27). In response, recent studies have explored novel management methods, including the application of herbal extracts such as turmeric (*Curcuma longa*) oil, which has shown promising antiparasitic activity against *Argulus* under laboratory conditions (27).

Collectively, the literature indicates that integrative approaches combining morphological and molecular techniques provide the most reliable framework for identifying *Argulus* species. While morphological traits remain useful for rapid diagnosis, molecular markers confirm species identity, uncover cryptic diversity, and clarify phylogenetic relationships. Expanding molecular databases, registering local isolates in GenBank, and conducting comparative phylogenetic analyses are therefore essential steps in strengthening global knowledge of fish lice biodiversity and epidemiology. Such integrated strategies not only enhance taxonomic accuracy but also support sustainable aquaculture by informing control and prevention programs for ornamental freshwater fish (21, 23, 26).

Materials and Methods

Sample Collection

A total of 442 ornamental freshwater fish, including goldfish (*Carassius auratus*), koi carp (*Cyprinus carpio*), Black Moor, and other ornamental species, were examined for ectoparasitic infestations. Infected fish were transported alive to the laboratory in aerated containers. Parasites were gently removed from the external surface and fins using fine forceps under a stereomicroscope, rinsed thoroughly with distilled water, and preserved in 70% ethanol until further analyses.

Morphological Examination

Morphological identification of *Argulus* specimens was performed using a Lusida compound light microscope equipped with a digital camera at 40× magnification. Specimens were temporarily mounted on glass slides in distilled water for observation. Key diagnostic features, such as the shape of the carapace, the presence and arrangement of post-antennal spines, the structure of the antennules and antennae, the mouthparts, and the thoracic appendages, were examined in detail.

Diagnostic keys and taxonomic descriptions published in the literature were followed to identify the parasites at the species level whenever possible (9–12, 18). High-resolution photographs were taken to document morphological traits and to support species confirmation. When diagnostic features were insufficient for reliable classification, molecular methods were used for confirmation.

Molecular Analysis

DNA Extraction

Genomic DNA was extracted from individual lice using a commercial Tissue DNA Mini Kit (Geneaid, Taiwan) following the manufacturer's protocol. Briefly, each specimen was homogenized in lysis buffer with Proteinase K, incubated at 60°C, and processed through silica spin columns. DNA was eluted in 50 µl of preheated buffer (60°C) and stored at –20°C until further use.

PCR Amplification

Two ribosomal markers were targeted: 18S rRNA (850 bp) and 28S rDNA (400 bp). Specific primers previously described for branchiuran parasites were used (Table 1). Each reaction was performed in 25 µl volumes containing 13 µl of 2 × Master Mix, 1 µl of each primer, 4 µl of DNA template (30–100 ng), and 6 µl of nuclease-free water. The PCR program was optimized for each primer set based on annealing temperatures (Table 2).

Agarose Gel Electrophoresis

PCR products were analyzed using electrophoresis on 1.5% agarose gels in 1 × TBE buffer. Gels were stained with SafeView dye, electrophoresed at 70 V and 65 mA for 45 minutes, and visualized under a UV gel documentation system (Cleaver, UK). A 100 bp DNA ladder was used as a size marker to confirm the expected amplicon length.

DNA Sequencing

Positive PCR products were purified and sequenced bidirectionally by Macrogen (Korea) using the same primers as in PCR. Sequences were aligned and compared with reference data in the NCBI GenBank database using BLAST for species confirmation. All validated sequences were assigned accession numbers upon submission to GenBank.

Results

Morphological Diagnosis

A total of 442 ornamental fish were examined for ectoparasitic infestations. The infected hosts included 353 goldfish (*Carassius auratus*), 150 Black Moor goldfish, and 148 koi carp (*Cyprinus carpio*). Additional infected species were recorded in small numbers, including red cap goldfish, telescope goldfish, subinkin goldfish, pearlscale goldfish, and striped catfish (*Pangasius hypophthalmus*). These ornamental species served as the primary source for the recovery of *Argulus* parasites.

Microscopic examination of the lice was conducted using a light microscope equipped with a Lusida camera at 40 × magnification. This setup enabled visualization of body structures, appendages, and oral parts; however, not all species could be reliably identified solely by morphology.

Fish Louse: Argulus japonicus

Specimens of *A. japonicus* were characterized by a dorsoventrally flattened oval body with a broad carapace. The first and second antennae were visible, as well as the post-antennal spine. The oral region included the suctorial tube, the accessory mandible, and its basal plate. Thoracic appendages, including the second, third, and fourth legs, were distinguishable, and the ventral view revealed the abdominal outline with paired suckers. These morphological details are illustrated in Figures 1 and 2.

Fish Louse: Argulus foliaceus

Female specimens of *A. foliaceus* displayed a wide carapace covering much of the thorax. The dorsal and ventral habitus were visible, with prominent compound eyes and clear respiratory areas on the ventral side. The oral structures included the mouth tube, supporting rods, and mandibles. The maxilla showed terminal segments with two scale-like denticles, while the four pairs of thoracic legs were distinguishable under the microscope. The caudal ramus was also observed dorsally. These traits are represented in Figures 3 and 4.

Fish Louse: Argulus coregoni

This species could not be identified morphologically under light microscopy at 40 × magnification. Its identification was achieved solely by molecular methods, as diagnostic morphological traits were insufficiently resolved.

Molecular Diagnosis

Fish Louse: Argulus japonicus

PCR amplification of the 28S rDNA gene (400 bp) was successfully achieved for samples 22, AA, and 3H, since amplification of the 18S rRNA gene did not provide

Table 2. Thermal cycling conditions for PCR.

Step	Temperature (°C)	Time	Cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	51–53	30 sec	35
Extension	72	40 sec	35
Final extension	72	10 min	1

Table 1. Primers used for PCR amplification of 18S rRNA and 28S rDNA genes.

Target gene	Product size (bp)	Annealing temp (°C)	Primer sequence (5′–3′)	Reference
18S rRNA	850	51	F: TCACGGGTAACGGGGAATCA R: CTGGTGAGGTTTCCCGTGTTG	Tandel et al 2021 (5)
28S rDNA	400	53	F: ACCCGCTGAATTTAAGCAT R: CTCTTCAGAGTACTTTTCAAC	Khaty, 2022 (27)

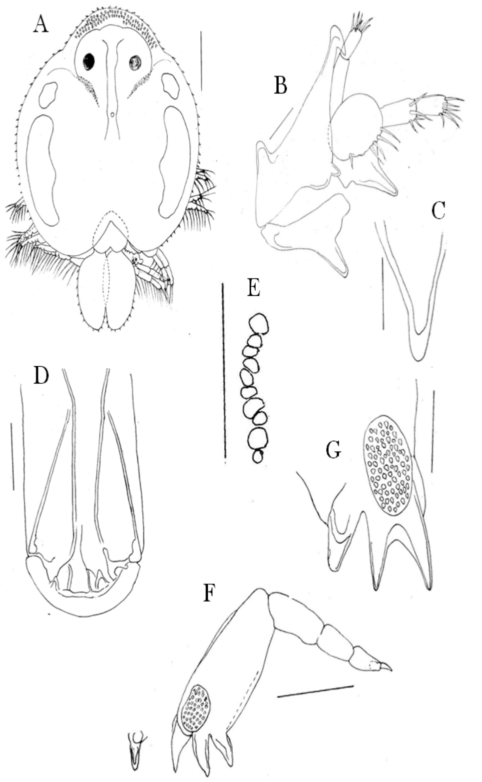

Figure 1. Male of *Argulus japonicus* A: complete body dorsal view; B: antennules and antennae; C: post-antennal spine; D: mouth; E: suction tube; F: accessory mandible; G: basal plate of accessory mandible (scale bars: A, E, F = 500 µm; B, D = 125 µm; C, G = 25 µm)

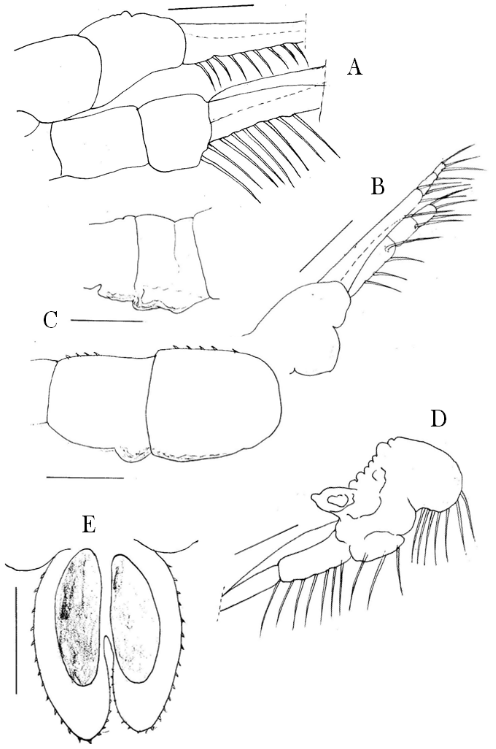

Figure 2. Male branchiuran *Argulus japonicus* showing detailed morphological structures. Panels A and B illustrate the second leg, whereas panel C presents the anterior portion of the third leg. Panel D shows the fourth leg, and panel E depicts the ventral view of the abdomen. The scale bars correspond to 500 µm for A, B, and D, and 125 µm for C and E

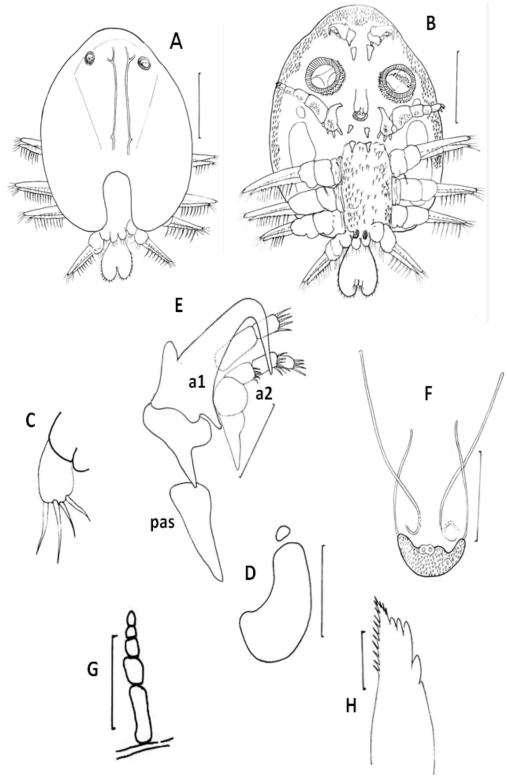

Figure 3. Female of *Argulus foliaceus* A: habitus, dorsal view; B: habitus, ventral view; C: caudal ramus, dorsal view; D: respiratory areas, ventral view; E: antennule, antenna, and post-antennal spine, ventral view; F: mouth tube; G: supporting rods; H: mandible (scale bars: A, B, D, F = 500 µm; C, G, H = 25 µm; E = 150 µm)

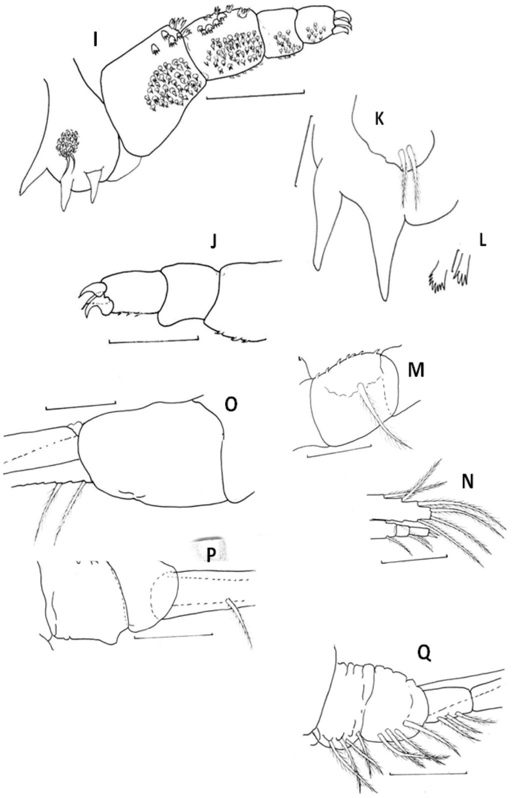

Figure 4. *Argulus foliaceus*, Female. I maxilla, ventral view; J tip of terminal segments of maxilla, ventral view; K base of maxilla, dorsal view; L two scale- like denticles on maxilla, ventral view; M first leg; O second leg; P third leg; Q fourth leg (Scale bars 150 in I, 50 in J, K and L; 150 µ in M-Q)

sufficient concentration for sequencing (Figure 5). This confirmed the molecular diagnosis of *A. japonicus*.

Fish Louse: Argulus foliaceus

Amplification of the 18S rRNA gene (850 bp) was successful for samples 14 and 20, producing distinct bands that confirmed the molecular identity of *A. foliaceus* (Figure 6).

Fish Louse: Argulus coregoni

In *A. coregoni*, the 18S rRNA gene (850 bp) was successfully amplified in multiple samples (15, 19, 21, 23, 1H, H3, B, B1, C, NB), confirming its molecular diagnosis (Figure 7).

All successfully sequenced PCR products of the studied parasites were deposited in the National Center for Biotechnology Information (NCBI) database. Each isolate was assigned a unique accession number, serving as a permanent identifier for future molecular and taxonomic studies. This registration ensures that the obtained sequences are publicly accessible and comparable with international data (Table 3).

Phylogenetic Analysis

Fish Louse: Argulus japonicus

Phylogenetic analysis of the sequenced 28S rDNA gene showed that local isolates (PV569546, PV569547, PV569545) formed an independent sub-branch, distinct from the closest Indian isolate MK775552, due to nucleotide variations (Figure 8).

Fish Louse: Argulus foliaceus

For *A. foliaceus*, sequencing the 18S rRNA gene confirmed close relationships with reference isolates from Iran, Indonesia, and China. One local isolate (PV569625) clustered with these references, while isolate PV569628 formed a separate sub-branch due to minor nucleotide differences (Figure 9).

Fish Louse: Argulus coregoni

All sequenced isolates of *A. coregoni* showed 100% similarity with Iranian and Indonesian references (JQ740820, MW375605) based on BLAST analysis. Phylogenetic analysis demonstrated that local isolates clustered tightly with the Iranian isolate, forming a distinct branch, whereas other Argulus species formed separate branches (Figure 10).

Table 4 summarizes the molecular identification of Argulus foliaceus, A. coregoni, and A. japonicus isolates obtained in the present study. The accession numbers assigned to our sequences are compared with reference sequences from GenBank, showing high identity values ranging from 96.92% to 100%. The results confirm the accuracy of molecular diagnosis and reveal close genetic relationships with isolates from Iran, India, and Indonesia.

Discussion

The present study provides comprehensive insight into the identification of *Argulus coregoni*, *A. foliaceus*, and *A. japonicus* in ornamental freshwater fish using a combination of morphological and molecular approaches. The findings emphasize the diagnostic value of microscopy and sequencing, highlight the prevalence of infestations in Iraq, and reveal genetic relationships with isolates from other regions.

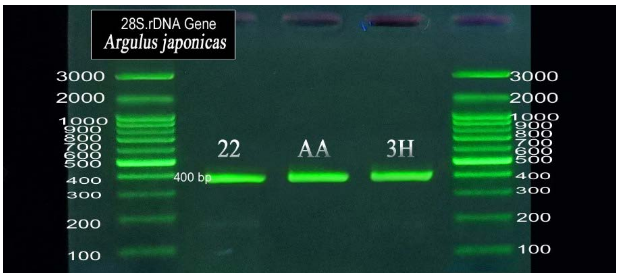

Figure 5. Electrophoresis on a 1.5% agarose gel for the PCR product of the 28S rDNA gene (400 bp) for *Argulus japonicus*

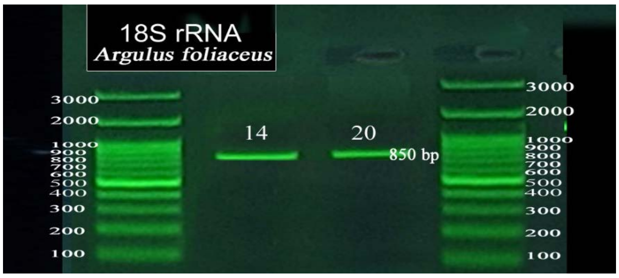

Figure 6. Electrophoresis on a 1.5% agarose gel for the PCR product of the 18S rRNA gene (850 bp) for *Argulus foliaceus*

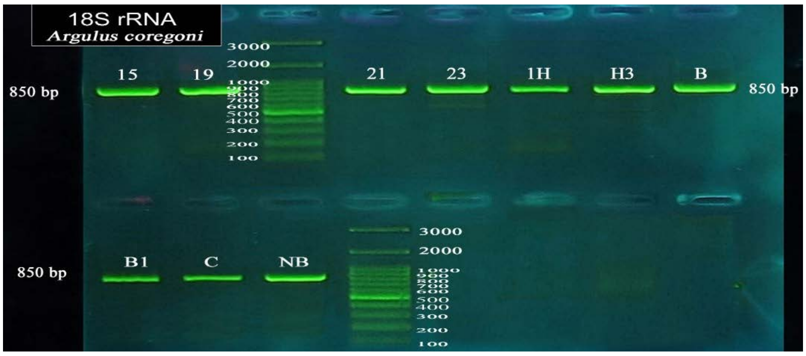

Figure 7. Electrophoresis on a 1.5% agarose gel for the PCR product of the 18S rRNA gene (850 bp) for *Argulus coregoni*.

Table 3. Nucleotide sequence accession numbers for 28S rDNA and 18S rRNA genes of the studied *Argulus* parasites submitted to NCBI.

Scientific Name	Accession Number for our study	ID	No.
Argulus foliaceus	PV569625	14	1
Argulus coregoni	PV569626	15	2
Argulus coregoni	PV569627	19	3
Argulus foliaceus	PV569628	20	4
Argulus coregoni	PV569629	21	5
Argulus japonicus	PV569546	22	6
Argulus coregoni	PV569630	23	7
Argulus japonicus	PV569547	AA	8
Argulus coregoni	PV569624	1H	9
Argulus japonicus	PV569545	3H	10
Argulus coregoni	PV569634	H3	11
Argulus coregoni	PV569631	B	12
Argulus coregoni	PV569632	B1	13
Argulus coregoni	PV569633	C	14
Argulus coregoni	PV569634	NB	15

Morphological Considerations

Morphological identification remains a cornerstone of parasitological diagnostics, providing rapid, cost-effective information on parasite identity. In this study, *A. foliaceus* was recognized by its rounded abdominal lobes and relatively short posterior extensions of the carapace, consistent with previous descriptions from European waters (9,12). *A. japonicus* was distinguished by elongated posterior lobes extending beyond the abdomen, in line with its global invasive profile (7,8). These features were visible under the Lusida compound light microscope with digital camera, allowing high-resolution imaging for comparative taxonomy.

However, *A. coregoni* could not be reliably distinguished morphologically due to overlapping traits with *A. foliaceus*. Several authors have reported similar difficulties, emphasizing that post-antennal spine morphology and carapace outline can be highly variable and insufficient for species-level separation (21,25). Such challenges confirm that reliance on morphology alone can lead to misidentification, particularly when specimens are juvenile or ethanol-preserved (11,29).

Molecular Insights

Molecular diagnostics using ribosomal markers provided explicit species confirmation. Amplification of the 18S rRNA (850 bp) gene successfully identified *A. coregoni* and *A. foliaceus*, while 28S rDNA (400 bp) confirmed *A. japonicus*. The reliability of these loci for branchiuran parasites has been demonstrated in previous studies (13, 14, 26).

Electrophoresis confirmed product size but did not provide comparative insights, which were obtained only after sequencing and BLAST analysis (30-32). Phylogenetic trees revealed that *A. coregoni* clustered tightly with Iranian and Indonesian isolates, reflecting high sequence similarity and limited divergence (16, 33). *A. foliaceus* grouped with isolates from Iran and China, though minor nucleotide variations created separate sub-branches (19, 34). *A. japonicus*, on the other hand, displayed unique sub-branching relative to Indian isolates, consistent with reports of genetic variability across its invasive range (15, 31, 32).

The deposition of all sequences in GenBank strengthens the global reference library, providing essential resources for future comparative taxonomy (24, 26). This step ensures that local data contribute to broader evolutionary and epidemiological understanding.

The detection of all three *Argulus* species in ornamental fish in Iraq is noteworthy. Surveys across different provinces have reported *A. foliaceus* as the most common species, followed by *A. japonicus*, while *A. coregoni* is less frequently encountered (6, 27, 28). The present findings agree with previous Iraqi reports, including studies from Mosul where *A. japonicus* reached a prevalence of over 60% in goldfish populations (26, 33).

Globally, prevalence rates vary widely depending on host species, environmental conditions, and geographical region. In South Asia, infestations of cultured carp species range from 10–55% (35), while European studies often report prevalence below 15% (9). The worldwide distribution of *A. japonicus*, facilitated by the ornamental fish trade, has raised concerns due to its invasive capacity and adaptability (8, 31). The occurrence of multiple species in Iraq highlights both local endemism and potential introduction via fish imports.

Infested fish in this study exhibited skin irritation, hemorrhagic spots, and abnormal swimming behavior, consistent with argulosis pathology reported elsewhere (1, 3, 7). These lesions predispose fish to opportunistic bacterial and fungal infections, thereby compounding disease burden (4, 23). Feeding by *Argulus* through its proboscis not only causes direct tissue damage but also contributes to anemia and stress in heavily infested hosts (5, 22).

Economically, argulosis is recognized as one of the major parasitic threats in freshwater aquaculture. Losses include reduced growth rates, increased mortality, and the need for costly treatments. Estimates from Asian aquaculture suggest annual losses amounting to several hundred dollars per hectare due to *Argulus* infestations (2, 29). In the ornamental fish trade, infestations also reduce the marketability of fish, posing challenges for both breeders and exporters (8).

The central challenge in *Argulus* taxonomy is the morphological similarity among species, particularly between *A. foliaceus* and *A. coregoni*. Overlapping characters and intraspecific variability make morphological identification alone unreliable (10,11,25). The present

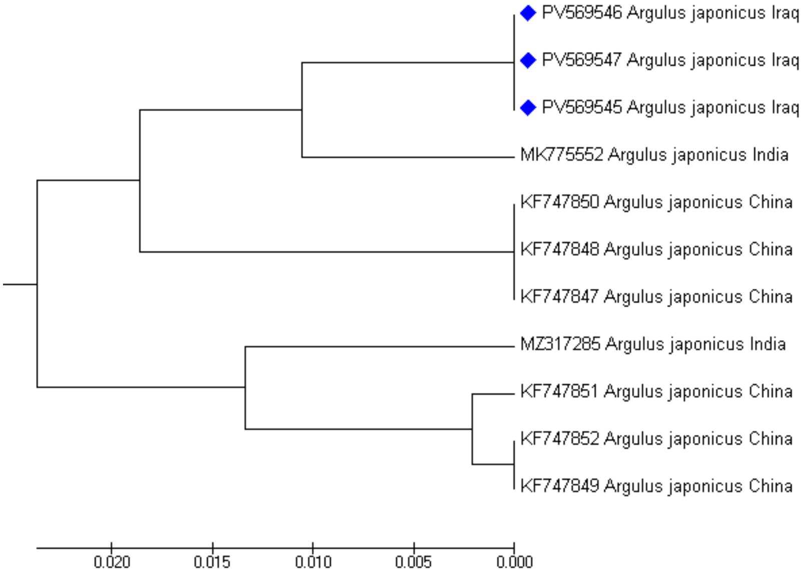

Figure 8. Phylogenetic tree of *Argulus japonicus* isolates compared with reference sequences from GenBank

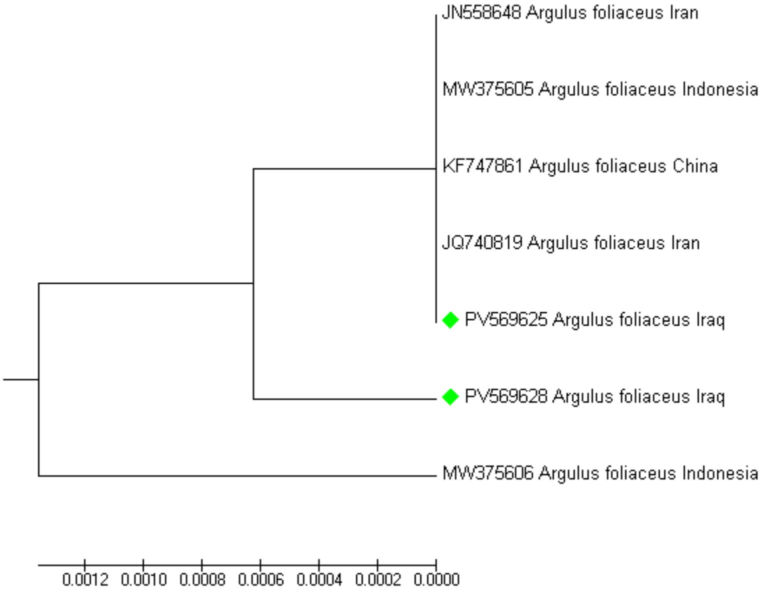

Figure 9. Phylogenetic tree of *Argulus foliaceus* isolates compared with reference sequences from GenBank

study illustrates that without molecular confirmation, *A. coregoni* would have remained misclassified.

Molecular tools, especially ribosomal DNA sequencing, provide precise species identity and reveal phylogenetic relationships (13,16,26). The integration of both approaches—morphological for initial screening and molecular for confirmation—represents the most robust diagnostic strategy. This dual framework aligns with recommendations from Duman et al (26) and Sivasankar et al (25), who advocate integrative taxonomy for ectoparasitic crustaceans.

The combination of morphological and molecular tools enhances diagnostic precision and provides insights relevant to aquaculture management. Future efforts should focus on expanding genetic databases by routinely depositing local isolates into GenBank, thereby strengthening global reference datasets (24, 35, 36).

Conclusion

The present study successfully identified three species of fish lice infesting ornamental freshwater fish: *Argulus japonicus, A. foliaceus,* and *A. coregoni*. Morphological examination using light microscopy at 40 × magnification enabled the recognition of diagnostic traits in *A. japonicus* and *A. foliaceus*, while *A. coregoni* could not be distinguished morphologically. Molecular diagnosis through PCR amplification and sequencing of ribosomal DNA markers (18S rRNA and 28S rDNA) provided definitive identification for all three species.

The registration of obtained sequences in the NCBI

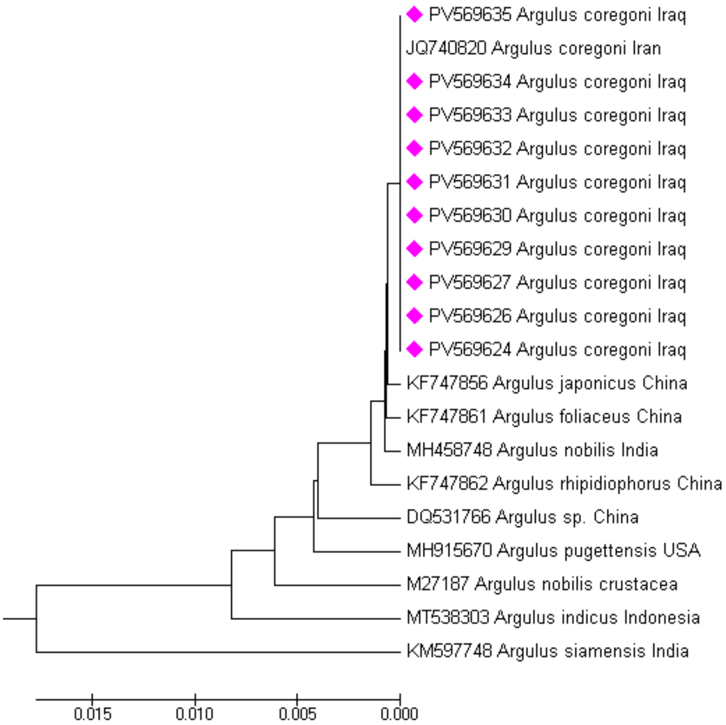

Figure 10. Phylogenetic tree of *Argulus coregoni* isolates compared with reference sequences from GenBank

Table 4. Sequence similarity percentages of 18S rRNA and 28S rDNA genes of the studied Argulus isolates compared with reference sequences available in the NCBI GenBank database

BLAST NCBI				**Our study**		
Country	**Reference copy**	**Scientific Name**	**Percentage of Identity %**	**Accession Number for our study**	**ID**	**No.**
Indonesia	MW375605.1	*Argulus foliaceus*	100%	PV569625	14	1
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569626	15	2
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569627	19	3
Indonesia	JQ740819.1	*Argulus foliaceus*	99.88%	PV569628	20	4
Indonesia	MW375605.1	*Argulus coregoni*	100%	PV569629	21	5
India	MK775552.1	*Argulus japonicus*	96.92%	PV569546	22	6
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569630	23	7
India	MK775552.1	*Argulus japonicus*	96.92%	PV569547	AA	8
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569624	1H	9
India	MK775552.1	*Argulus japonicus*	96.92%	PV569545	3H	10
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569634	H3	11
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569631	B	12
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569632	B1	13
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569633	C	14
Iran	JQ740820.1	*Argulus coregoni*	100%	PV569634	NB	15+

GenBank and their phylogenetic analysis confirmed the close genetic relationships between local isolates and those from other regions (Iran, Indonesia, and India). The separation of *A. japonicus* into an independent branch highlights nucleotide variations within this species.

Overall, the combined use of morphological and molecular approaches proved essential for reliable identification of *Argulus* species. This integrative strategy enhances taxonomic accuracy, supports epidemiological monitoring of parasitic infestations in ornamental fish, and provides a valuable genetic reference for future parasitological and aquaculture research.

Recommendations

- Adopt integrated diagnosis: Combine morphological and molecular methods for accurate identification of

Argulus species.

- Enhance monitoring and quarantine: Regular screening of ornamental fish and strict quarantine of imports to prevent parasite spread.
- Expand genetic data: Continue depositing sequences in GenBank to strengthen reference databases for global comparison.

Authors' Contribution

Conceptualization: Ghadeer Noman Jasim.
Data curation: Ghadeer Noman Jasim.
Formal analysis: Ghadeer Noman Jasim.
Investigation: Ghadeer Noman Jasim.
Methodology: Ghadeer Noman Jasim.
Project administration: Khaled Thamer Mattar Alshaebani.
Resources: Ghadeer Noman Jasim.
Software: Ghadeer Noman Jasim.
Supervision: Khaled Thamer Mattar Alshaebani.
Validation: Ghadeer Noman Jasim.
Visualization: Ghadeer Noman Jasim.
Writing–original draft: Khaled Thamer Mattar Alshaebani.
Writing–review & editing: Khaled Thamer Mattar Alshaebani.

Competing Interests

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

Funding

It is funded by the authors of this article.

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