Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 2391 – 2409 (2025)
www.ejabf.journals.ekb.eg

Morphometric and Molecular Identification *of Argulus japonicus* (Thiele 1900) on *Cyprinus carpio* from Three Different Areas in Erbil Province, Kurdistan Region-Iraq

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ARTICLE INFO
Article History:
Received: Aug. 15, 2025
Accepted: Sep. 27, 2025
Online: Oct. 12, 2025

Keywords:
Argulus japonicus, Aquaculture, Cytochrome oxidase, Ectoparasite

ABSTRACT

The current study aimed to characterize the morphological and molecular features of *A. japonicus* from *Cyprinus carpio* at three aquaculture sites in Erbil Province, Kurdistan Region, Iraq. A total of 1,382 fish were examined, in addition ectoparasite specimens were collected. The specimens were identified by scanning electron microscopy (SEM) and molecular analysis using PCR amplification and sequence of cytochrome c oxidase subunit I (COI) and 18S rRNA genes. At Ankawa Fish Hatchery, the highest percentage of *A. japonicus* infestation with a percentage of 41.26% marked a predilection site of attachment to the head and caudal fin of the host. SEM analysis showed clear morphological adaptations of the parasite to attach parasite and feed. Species identification was confirmed by molecular data and a strong phylogenetic distance was observed between *A. foliaceus* and closely related groups. In addition, the whole mitochondrial genome of *A. japonicus* was determined, which showed similar gene order and a high A+T bias. The combination of morphological and molecular analysis was essential to improve *in situ* identifying parasite species and document the distribution of the parasite. Thus, these findings would provide significant information for fish health management and control strategy in regional aquaculture systems.

INTRODUCTION

Commonly called fish lice, the ectoparasitic crustacean such as *Argulus* has contributed significantly to the sustainability of aquaculture through the reduction of fish populations and yields worldwide **(Al-Quraishy *et al.*, 2020)**. These parasitic crustaceans have long been recognized causing lethal effect on fish host, and thus, are responsible for major losses in the aquaculture industry **(Aalberg *et al.*, 2016; de la Cruz-Cervantes *et al.*, 2020)**.

Multiple Argulus species like *A. foliaceus* and *A. japonicas* examined from latitudinal gradients are determined to be tolerant to diverse ecological conditions **(Tam *et al.*, 2005; Wadeh**

***et al.*, 2008)**. In Iraq, and between the Erbil Province and the KRI in general, a favor in promoting the aquaculture system/culture practice has been carried on in line with the local needs of the fish and the rising demands on both sides for pushing the farming efficiency **(Al-Saeedi & Al-Nasiri, 2019)**. Nonetheless, the acceleration of aquaculture practice has increased fish stocking densities, which have increased the potential risk of parasitic infections, including those caused by *Argulus* species **(Hunt & Cable, 2020)**. Evidence of heavy infestation can manifest as acute wounds, increased production of mucus, loss of scales, and erosion of fins, which not only affect the health and welfare of fish but also create opportunities for secondary infections due to opportunistic bacteria and other pathogens **(Saurabh *et al.*, 2012; Saha & Bandyopadhyay, 2015)**.

The identification of *Argulus* species has traditionally been based on morphological features. However, species identification, especially at specific level, is generally difficult among some species within the genus *Argulus* due to the fact that they can show morphological variation due to the influence of environmental or stage of growth factor **(Shashank *et al.*, 2014)**. Thus, the classical morphological keys, although helpful, may not always provide complete clarity in discriminating between these parasites. Recent studies have combined molecular and conventional morphological methods to enhance species identifications. PCR amplification of genetic markers such as 18S rRNA and cytochrome c oxidase subunit I (COI) gene is considered as an essential tool for species identification of *Argulus* **(Pavan-Kumar *et al.*, 2020)**. At the molecular level, genetic diversity and phylogeny can be thoroughly studied, and this information is of great importance toward the reconstruction of the evolutionary history of some parasite species **(Tamura *et al.*, 2013)**. Further, the use of DNA barcoding has improved the accuracy of identifying species at different stages of life, and has given an alternative approach away from the traditional **(Aguilar *et al.*, 2017)**.

Parasitic infections have become prevalent in some districts of Kurdistan Region of Iraq, such as Erbil Governorate, due to the development of aquaculture in ponds and of caught fisheries in natural water bodies. This region is also relying on fish culture as a significant economic activity and *Cyprinus carpio* is one of the main cultured species. Heavy infestations of *Argulus* spp. in carp farms have also been found to lead to severe effects on carp health, including retarded growth and elevated mortality rates **(Northcott *et al.*, 1997; Taylor *et al.*, 2006)**. Hence, to elucidate the ultra-morphology and molecular characterization of *Argulus* infestations in *Cyprinus carpio* is crucial to contribute to safe and sustainable aquaculture in the region. In such a circumstance, the aim of this work was to study the ultra-morphological structure and molecular identification of *Argulus* infesting *C. carpio* from different fish farms in Erbil Province and the Kurds of Iraq Kurdistan Region.

MATERIALS AND METHODS

Sample collection

Live specimens of *Argulus* spp. were sampled from three sites within Erbil Province (Ankawa Fish Hatchery, Tarjan Fish Farm on Gwer Road, and Taqtaq Fish Farm on Koya Road) over a one-year period. Then, sampling was carried out monthly using a standard sampling procedure to standardize the results. Only one representative fish species of the common carp (*Cyprinus carpio*) was chosen to be investigated a part of the study. The fish hosts were collected with two nets, a wide mesh (10 cm × 10 cm) or narrow mesh (4 cm × 4 cm) net. Then, all fish taken were packed by hand into a cool box to minimize any mortality and desiccation during transport. Then, the samples were transferred to the laboratory of Salahaddin University for further preparation and processing.

Fish lice of the genus *Argulus* were harvested from affected fish and sampled from freshwater bodies of the region with forceps and stored in 2% formalin and 70% ethanol for analysis.

Sample preparation

Upon arrival to the Salahaddin University Laboratory, the samples were studied under a stereo-microscope in order to find *Argulus* spp. For molecular characterization, the preserved *Argulus* spp. microscope (SEM) investigation, fixed specimens were dehydrated in a series of ethanol gradient and successive change from 70, 80, 90, and 100% ethanol for 15min at each concentration. Following dehydration, the samples were dried using the critical point drying (CPD) method to prevent structural collapse. The prepared samples were then transferred to the Soran University Laboratory for morphological identification using SEM.

Molecular analysis

DNA extraction

Genomic DNA was extracted from *Argulus* parasite using the Beta Bayern tissue DNA preparation Kit (Beta Bayern GmbH, 90453 Bayern, Germany) following the protocol provided by the manufacturer. The parasite transferred to a 1.5ml microcentrifuge tube containing 400µl of Lysis Solution. The parasite was homogenized thoroughly to lyse the cells. Subsequently, 12µl of proteinase K solution (20 mg/ml) was added to the sample, and the mixture was vortexed to ensure proper mixing. Following this, the samples were incubated for 30 minutes at 80°C to promote complete lysis of cellular components. After incubation, the samples were allowed to cool to room temperature. For the binding of DNA to the column, 300µl of binding solution (BDB) was added

to the lysate, which was then vortexed vigorously for approximately 20-30 seconds and placed on ice for 5 minutes. The mixture was loaded on a spin column preplaced on a collection tube and centrifuged at 8,000 RPM for 2min. Flowthrough was discarded, and the spin column was reassembled with the collection tube. For washing, 300µl of Buffer BDW1 was added to the spin column which was then centrifuged at 10,000 RPM for 2 minutes. This was followed by another washing step with 300µl Buffer BDW2. DNA was recovered by eluting 50µl of pre-warmed Elution Buffer (BDE) onto the spin column, after incubating for 1 minute at room temperature, the column was centrifuged at 12,000 RPM for 1 minute. Purified genomic DNA was stored at −20°C until used for experiments.

Polymerase chain reaction (PCR) amplification

The PCR was used to amplify the Cytochrome Oxidase C subunit One (COI) gene. Each PCR reaction was conducted in a total volume of 50µl containing 25µl of 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S, Stenhuggervej 22), 10 pmol of each primer (COI-F: 5'-GGTCAACAAATCATTAAAGATATTGG-3' and COI-R: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), DNase-free water, and 5 µl of the template DNA extracted in the previous step. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 35 seconds, annealing at 59°C for 35 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 10 minutes, after which the thermal cycler was set to hold at 4°C indefinitely.

DNA sequencing

Sequencing of the PCR products was performed using the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at Macrogen Molecular Company of Korea. The sequence data were analyzed using the Finch TV program software for editing chromatograms and ensuring accurate base calling. The obtained gene sequences were subjected to analysis using the Basic Local Alignment Search Tool (BLAST) available at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi), which enabled comparison and alignment of our sequence data against existing biological sequences in the GenBank database to identify homology and phylogenetic relationships among the *Argulus* spp. samples.

Phylogenetic inferences

Phylogenetic analyses were performed to define evolutionary relationships among the species of *Argulus* spp. using the COI nucleotide sequences derived during sequencing. Sequences were aligned to the reference using the ClustalW method in MEGA 11 software **(Tamura *et al.*, 2021)** for accurate sequence alignment. The phylogenetic substitution model of best fit was estimated according to the Bayesian Information Criterion (BIC) in MEGA 11 and the Tamura-

Nei model was the most appropriate choice for our dataset as it considers the proportion of different nucleotides. The phylogenetic trees were generated by NJ and ML methods. Neighbor-joining (NJ) method was used to build the unrooted tree to show the genetic distances between the sequences, and maximum-likelihood (ML) method was used to generate a tree with branch length proportional to the extent of evolution between the species. The phylogenetic trees were tested for reliability by bootstrap analysis with 1,000 replicates and the support values for branches were given. The trees were displayed with MEGA 11, and exported for additional processing. The resulting phylogenetic trees were useful to show the clades, as to demonstrate the genetic differentiation among the species of *Argulus* spp., and helped elucidate the evolutionary history of this genus.

Scanning electron microscopy (SEM)

Following the method of **Everts and Avenant-Oldewage (2009)**, the trimmed samples were attached with double-sided conductive carbon tape to aluminum stubs to ensure a good connection and minimal movement through imaging. To minimize charging effects and because of the low conductivity of the samples, all the samples were coated with a thin layer of gold-palladium for sputtering using a Desk Super Coater DSR1 with argon gas at 0.1 mbar. The coating was carried out for 60 sec to deposit a uniform conductive layer. The samples were then analyzed under Quanta 450 SEM, Soran University Laboratory. Imaging was performed in high vacuum mode and at accelerating voltages between 10 and 20 kV to obtain the desired resolution and contrast. Fine surface structures and compositional contrast were visualized by secondary electron (SE) and backscattered electron (BSE) detectors, respectively. The working distance was set at 10- 15mm to attain desired image clarity and depth of field.

RESULTS AND DISCUSSION

Prevalence of infection of *Argulus japonicus*

The investigation into *Argulus japonicus* infections across various fish farms demonstrated notable differences in prevalence and distribution patterns. Table (1) illustrates the distribution of *Argulus japonicus* of infected fish from three different locations Ankawa Fish Hatchery, Gwer Road (Tarjan Fish Farm), and Taqtaq Koya Road. The data show that at Ankawa Fish Hatchery, 41.26% of the infected fish were found, whereas Taqtaq Koya Road accounted for 39.35%. Conversely, Gwer Road exhibited the lowest prevalence, with only 19.39% of the infected fish sampled from that location. In the present study, the Ankawa Fish Hatchery exhibited the highest overall infection prevalence. which may be attributed to warmer summer temperatures (24–28 °C) that support rapid parasite development and increased egg hatching, as previously demonstrated by **Hakalahti and Valtonen (2003)** and **Subburaj *et al.* (2019)**.

Table 1. Prevalence and site infection of *Argulus japonicus* species on *Cyprinus carpio*

Location	No. host	Host (%)	Gender	Infected	Infected (%)	Head	Skin	Caudal fins	Dorsal fins	Anal fins
Ankawa fish hatchery	570	41.2	male	280	49.1	40	118	53	22	47
			female	290	50.9	41	136	61	29	23
Gwer road (Tarjan fish farm)	268	19.39	male	47	17.5	8	21	9	5	4
			female	63	23.5	6	25	18	10	4
			Non- specific*	158	59.0	36	63	40	10	9
Taqtaq Koya road	544	39.35	Male	195	35.8	21	93	47	23	11
			female	165	30.3	8	114	22	8	13
			Non- specific*	184	33.8	5	130	33	10	6

* *Non-specific* indicates that the sex of the specimen (male or female) was not identified or not reported in the original source.

The notable infection in "non-specific" areas observed at the Gwer Road site may reflect the parasite's migratory behavior within host tissues, a pattern also discussed by **Everts and Avenant-Oldewage (2009)** in their analysis of developmental variation and tissue tropism. Additionally, the relatively balanced infection prevalence between male and female fish, as shown in Fig. (1), indicating that at Ankawa Fish Hatchery, females constitute 51% of the infected population, while males account for 49%. This finding corresponds with the observations of **Tandel *et al.* (2021)**, who found no significant sex-based difference in *A. japonicus* infestation in cold water species. Thus, the spatial distribution patterns in Fig. (1) are consistent with the parasite's known ecological plasticity, temperature-driven reproductive strategies, and anatomical adaptations, highlighting the need for targeted monitoring of high-risk body regions during seasonal outbreaks.

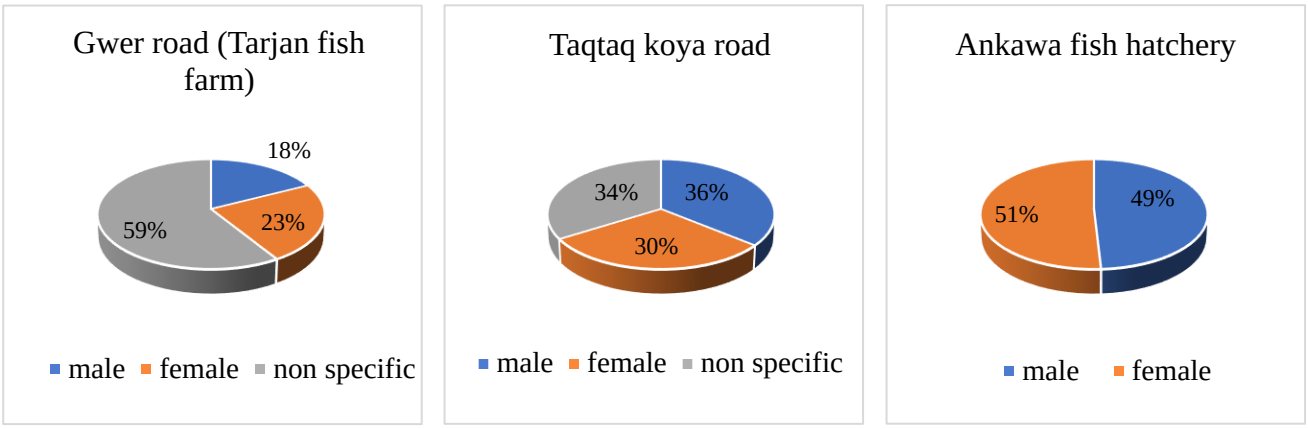

Fig. 1. Percentage of infected fish in three different locations: Ankawa Fish Hatchery, Taqtaq Koya Road and Tarjan fish farm Gwer Road

Notably, Gwer Road displayed a high incidence (59%) in "non-specific" hosts, indicating a broad host range for *A. japonicus*, which aligns with previous observations that this parasite infects diverse species such as the goldfish, rohu, catla, mrigal, and koi carp **(Sahoo *et al.*, 2012; Kumari *et al.*, 2019)**. However, *A. japonicus* is emerging as a major ectoparasite in Iraqi aquaculture, earlier reports from Iraq primarily documented *Argulus foliaceus*, which has been found on both marine and freshwater fish species, such as *Boleophthalmus dussumieri* and *Planiliza subviridis*, particularly in the Shatt al-Arab River and Basrah Province **(Mhaisen, 1986; Al–Janabi, 2010; Khamees *et al.*, 2015)**. Furthermore, according to **Mhaisen (2024)**, studies on fish parasitology in Iraq, at least three main *Argulus* species: *A. foliaceus*, *A. japonicus*, and *A. coregoni*, were identified along with a few unidentified forms. *A. foliaceus* is the most widespread, reported from up to 18 fish species, particularly those in the Cyprinidae family such as *C. carpio* (common carp) and other native and farmed fish species. Beside it, *A. foliaceus* in Kurdistan was reported from the skin of *C. carpio* from Ainkawa Fish Hatchery and from skin and fins of the same fish from Agriculture College fish farm, University of Salahaddin, Erbil **(Mustafa, 2016)** as well as from skin and fins of *M. mastacembelus* from Greater Zab River.

The parasite's low host specificity and adaptability to various host types have also been emphasized by **Saha and Bandyopadhyay (2015)**, who recorded its occurrence in both cyprinid and non-cyprinid species. In addition, the spatial differences observed may be affected by environmental conditions as previous research has shown that *A. japonicus* prefers warmer temperature water in which hatching rates and survival are increased **(Hakalahti and Valtonen, 2003; Subburaj *et al.*, 2019)**. These conditions were present during the sampling period, suggesting that climatic and ecological factors, alongside host biology, contribute to the observed distribution patterns. Collectively, the data in Fig. (1), supported by prior research, underscore the importance of host diversity, environmental temperature, and site-specific conditions.

Fig. (2) presents the distribution percentage of the anatomical distribution of *A. japonicus* on *C. carpio*, revealing a strong preference for attachment to the head skin (51%) and caudal fins (21%), followed by the pectoral and dorsal fins (8%) and anal fins (8%). The head skin exhibits the highest concentration of parasites, indicating a preference for this area, possibly due to both accessibility and suitable microenvironments for attachment and feeding. These findings are in line with earlier observations by **Sahoo *et al.* (2013a, b)**, who reported that *A. japonicus* predominantly attaches to soft, vascularized body surfaces such as the dorsal, caudal, and pectoral fins; These are among the most commonly infested regions in *Labeo rohita* and snow trout (*Schizothorax richardsonii*), supporting the consistent host-site preferences of *A. japonicus* across multiple cyprinid hosts. The high infestation in the head and skin regions was similarly documented in the current study's broader host analysis (Table 1), where 48.2% of parasites at the Ankawa site were concentrated on the head and skin, reaffirming these as high-risk zones for parasite anchorage. Morphologically, *A. japonicus* exhibits adaptations such as a broad carapace and well-developed maxillae with blunt teeth, which enhance its grip on smooth, curved surfaces like the cranial and caudal regions **(Fryer, 1968; Sahoo *et al.*, 2012)**. Moreover, the observed infestation pattern correlates with localized tissue damage and pathological responses including epidermal hemorrhages and fin erosion, as described in prior studies and reaffirmed in this investigation, underscoring the clinical relevance of parasite localization for disease management. Collectively, Fig. (2) emphasizes the need for targeted treatment and surveillance focusing on high-load anatomical sites to effectively control *A. japonicus* outbreaks in cultured carp populations.

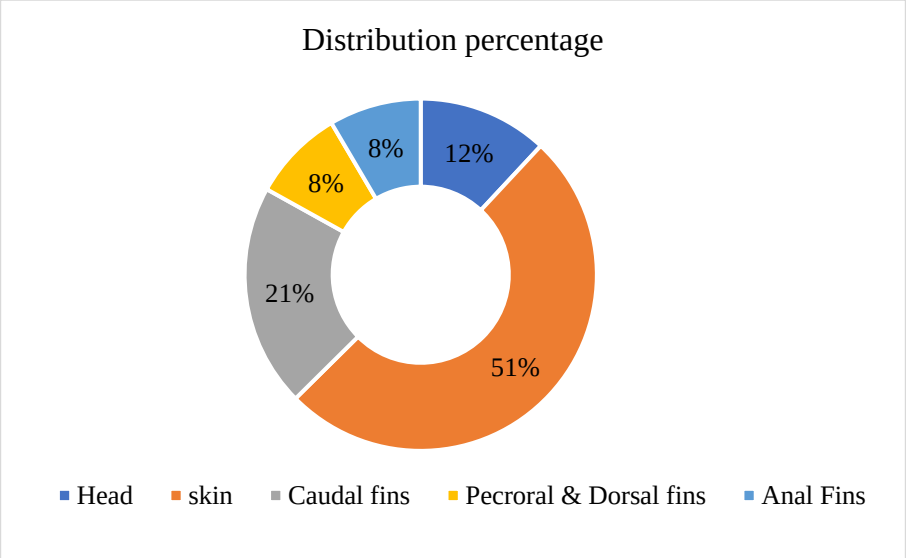

Fig. 2. The distribution percentage of *Argulus japonicus* on different body parts of *Cyprinus carpio*

Molecular identification

In this study, the genes of mitochondrial cytochrome c oxidase subunit I (COI) were amplified and sequenced to perform molecular diagnosis of parasitic samples. The amplified fragment resulted in a 720 bp product, which is in accordance with studies of crustacean parasites where the COI gene has become a common genetic marker for species identification **(Altschul *et al.*, 1997)**. Fig. (3a) depicts PCR amplification of partial COI gene of specimens of *Argulus japonicus*, in landslide two containing 100bp ladder with a molecular ladder from 100 to 3000 bp and wells 1-6 with gene bands of about 720 bp. The clear, strong bands seen in these lanes suggest that the COI gene fragment desired was successfully amplified, and serve to confirm the molecular level identity of the parasite. No amplification is observed in the negative control (C), as would be expected, which demonstrates the accuracy of the reaction and the lack of contamination. The common band patterns among the analyzed *A. japonicus* samples show genetic relatedness among the specimens of *A. japonicus* studied.

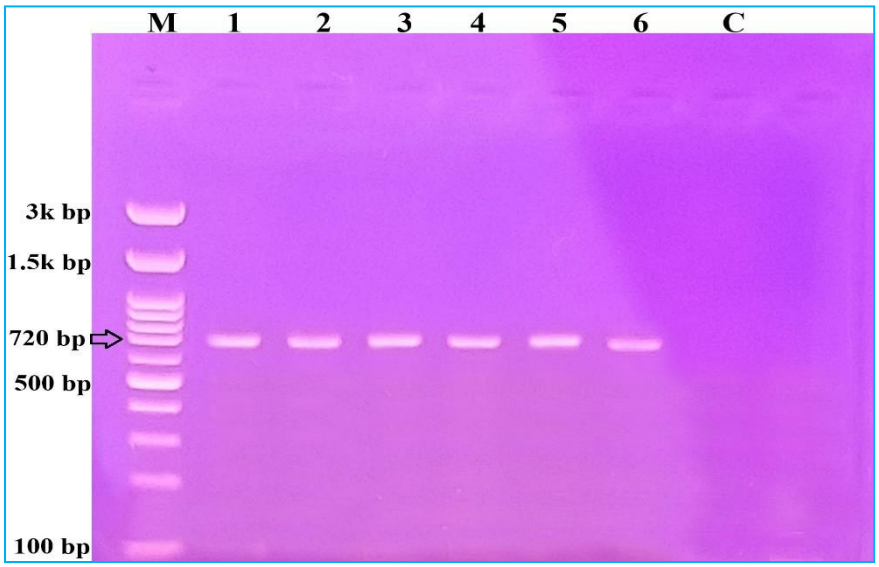

Fig. 3. PCR amplification of partial COI gene from parasite, wells include M; Ladder (3000-100 bp), lane1-6; gene bands with the size of 720 bp amplified and C indicate negative control without the band

The whole mitochondrial genome of *A. japonicus* was efficiently sequenced and characterized and found to be 15,045 bp in length, which is in accordance with the length of the typical crustacean mitogenomes (about 15,000–16,000 bp) **(Lavrov *et al.*, 2004; Shen *et al.*, 2015; Luchetti *et al.*, 2019)**. The genome contains 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs), which is in accord with the typical mitochondrial genomic architecture observed in related crustaceans **(Lavrov *et al.*, 2004; Luchetti *et al.*, 2019)**. The gene arrangement and gene position on the J- and N-strands assist the position

reported for other *Arguloida* species, reflecting the conserved genomic order of this group **(Luchetti *et al.*, 2019; Sterling-Montealegre & Prada, 2024)**.

Overlaps among some PCGs (e.g., atp8-atp6 and nad4L-nad4) observed in this species are common in the compact mitogenome, which economizes genetic space with overlapping genes, which was previously described in other crustaceans such as *A. americanus* **(Lavrov *et al.*, 2004; Luchetti *et al.*, 2019)**. Furthermore, the detection of two non-coding regions, with the largest presumed as the control region (647 bp), aligns with typical mitochondrial structure, serving as the replication and transcription regulation site, similar to other crustaceans **(Luchetti *et al.*, 2019; Baeza, 2022)**. The nucleotide composition of *A. japonicus* exhibits an A+T bias (71.64%), with a notable G-skew (GC skew = −0.307), which is characteristic of crustacean mitochondrial genomes and influences codon usage patterns **(Lavrov *et al.*, 2004; Luchetti *et al.*, 2019; Sterling-Montealegre & Prada, 2024)**. RSCU analysis revealed a preference for codons ending with U, such as UUA and UCA, and amino acids like Leucine, Lysine, Phe, and Asparagine, consistent with the nucleotide bias and codon usage trends seen in other crustaceans **(Luchetti *et al.*, 2019)**. In addition, the start codons were mainly ATG, but alternative starts were ATC, ATA, and ATT as well as incomplete stop codons (T), which are probably completed via post-transcriptional polyadenylation, a mechanism well-described in the cox genes of crustaceans **(Passmore & Coller, 2022; Koludarova & Battersby, 2024)**.

Analysis of sequence divergence of PCGs showed that cox1 is the most conserved gene in crustaceans, it could be used as a molecular marker in species and phylogenetic analysis **(Camousseight *et al.*, 2012; Pan *et al.*, 2014)**. In contrast, nad6 had the lowest sequence similarity, indicative of a relatively rapid evolutionary rate and a possibility that it might vary at the species or population level. The high conservation of cox1 indicates its value as a marker for DNA barcode in *A. japonicus*, promoting molecular diagnosis and population genetics, such as those seen in other crustacean groups **(Kim *et al.*, 2013; Luchetti *et al.*, 2019)**.

Phylogeny

The phylogenetic analysis of the 18S rRNA gene sequences revealed a clear clustering pattern among the examined *Argulus* species. The query sequence PV707243, identified as *A. japonicus*, clustered tightly with other *A. japonicus* sequences such as KF747847.1, KF747850.1, KF747848.1, PV707242, PV569545, and KF747849.1. These sequences shared high bootstrap support values (up to 98%), indicating strong genetic similarity and a monophyletic grouping within the *A. japonicus* clade. In contrast, sequences corresponding to *Argulus foliaceus*, including KF747853.1, EU370442.1, and MW423690–692.1, formed a separate clade, supported by moderate to high bootstrap values (89–94%), suggesting clear genetic divergence from *A. japonicus*. Notably, the query sequence PV707243 did not group with any *A. foliaceus* sequences, confirming its identification as *A. japonicus*. Furthermore, the outgroup used in the analysis, OM835790.1 (*Lernaea cyprinacea*), was distinctly separated from both *Argulus* clades, validating

the tree's rooting and supporting the genetic distinctiveness of *Lernaea*. The presence of lower bootstrap values (33–66%) at certain internal nodes between *A. japonicus* and *A. foliaceus* clades may reflect evolutionary divergence or limited conserved regions across these species. Overall, the tree provides molecular evidence supporting the taxonomic distinction between *A. japonicus* and *A. foliaceus*, and confirms the identity of the query sequence as *A. japonicus* through its close clustering with established reference sequences.

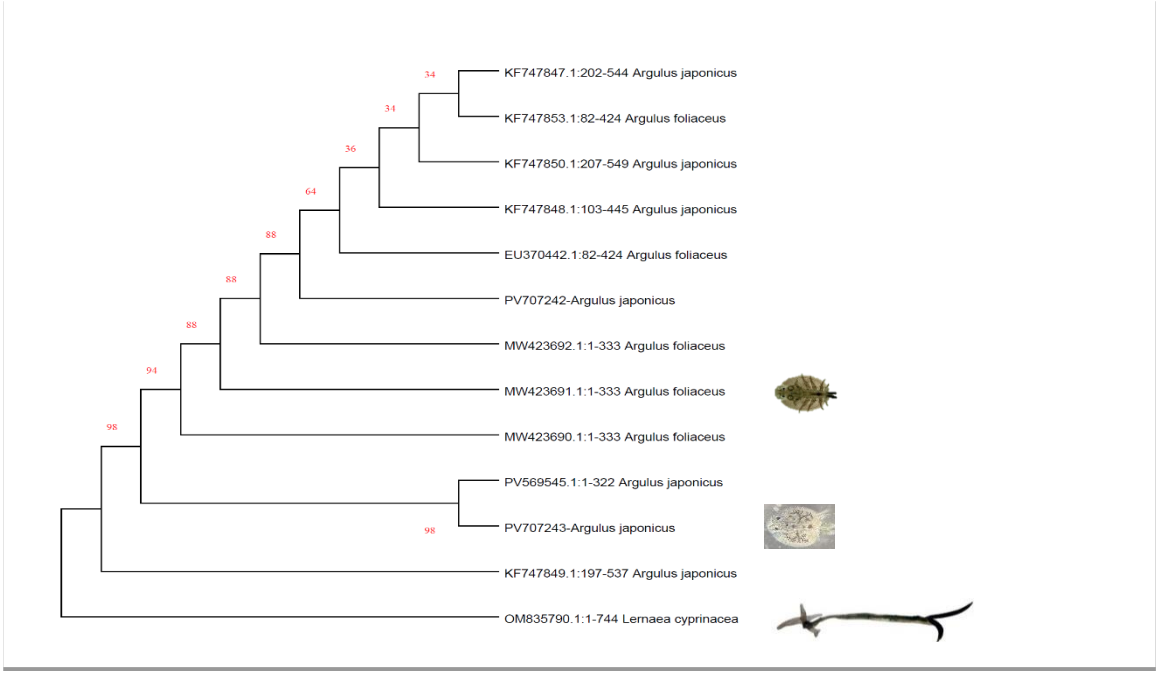

Fig. 4. Phylogenetic tree showing evolution relationship of PV707243 and PV707242 with other related isolates based on 18sRNAsequences. Sequences were compared with the *Lernaea* sp. as outgroup of phylogenetic analysis. Numbers next to the branches indicate bootstrap values, and scale bar represents

Morphological description

The morphology of *Argulus japonicus* was evaluated through both light microscopy and scanning electron microscopy (SEM). This combined approach provided detailed resolution of external structures critical for taxonomic differentiation and functional interpretation. In this study, SEM was utilized to observe and document fine morphological structures, including surface spination, antennal segmentation, the arrangement of sensory setae, and the structural complexity of the mouthparts and maxillae. The SEM observations supported and further extended previously reported morphological observations. The body of the illustrate specimen was dorsoventrally plethangid, and the carpace was almost rounded, covering the first to third pairs of thoracopods. SEM examination showed that both the ventral side of the frontal region and the lateral margins

of the carapace were covered by densely set and acute-pointed spines, details difficult to see under light microscope due to high density (Fig. 5).

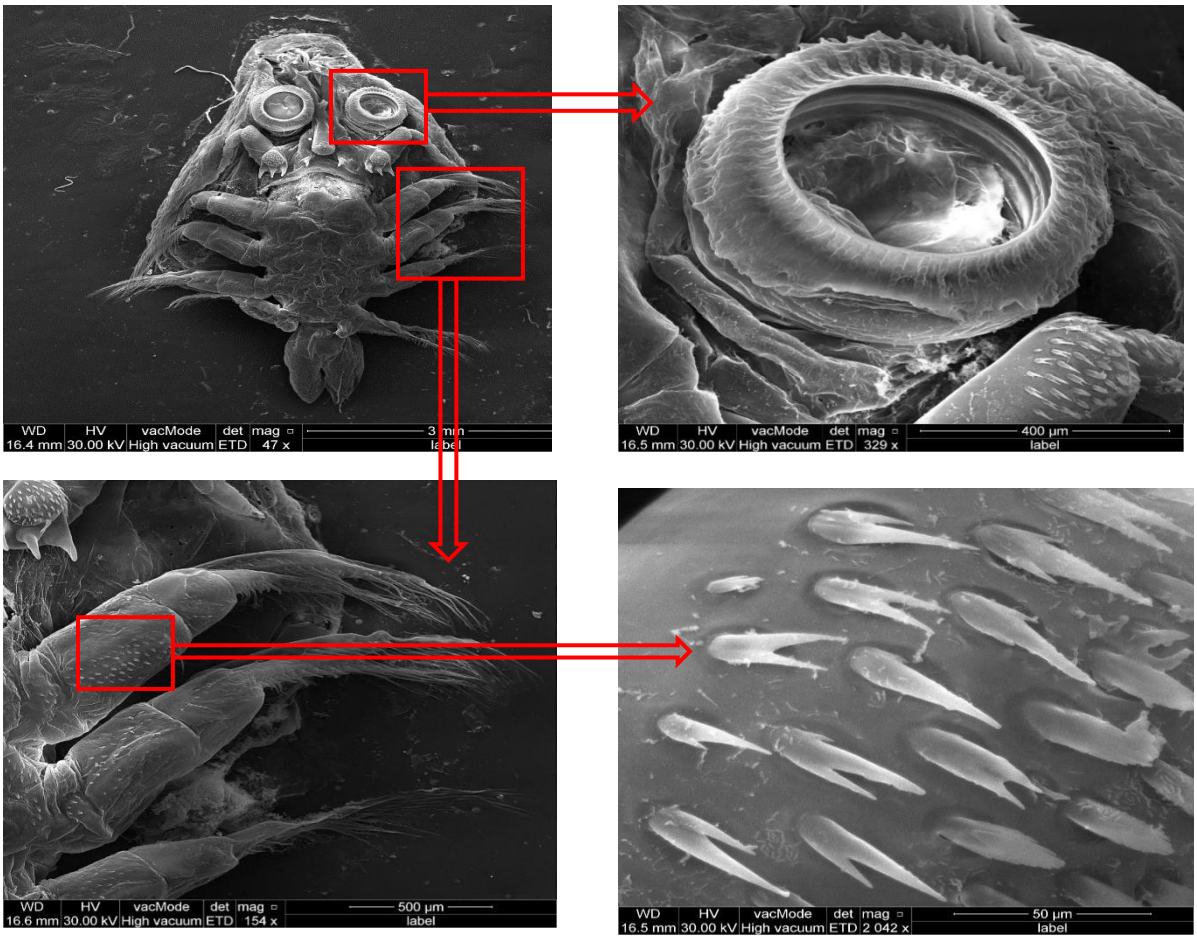

Fig. 5 The scanning electron microscopy (SEM) for *Argulus japonicus*

Morphological data of *Argulus japonicus*, a common ectoparasite of freshwater fish, have been described from several places around the world such as Japan, India, Hungary and very recently Iraq. Body flattened in the dorsoventral direction in all examined populations, with a circular carapace usually covering the first to third pairs of legs, and bilobed abdomen and rounded at the end **(Nagasawa *et al.*, 2024)**. The following diagnostic characters are generally accepted: dorsal compound and naupliar eyes; a preoral stylet positioned posteriorly to the second antennae; and unpaired areas associated with well-defined ventrolateral, anterior, and posterior zones **(Keve *et al.*, 2025)**. The thorax comprises four segments, each bearing biramous legs, with the fourth pair forming prominent natatory lobes. The first maxillae develop into characteristic cup-like suckers, and the second maxillae possess robust, segmented structures with claw-like terminal appendages

used for attachment **(Dekari *et al.*, 2024)**. Variation in supporting rod numbers within the first maxillae was observed, similarly, **Nagasawa *et al.* (2024)** reported a count of 45–54 rods in Japanese specimens, aligning closely with the Hungarian study (52–53 rods) and Indian isolates (typically within 45–53). This consistency strengthens the use of supporting rod count as a reliable morphological marker for species-level identification. Sexual dimorphism is also notable. Males possess clasping structures on the second, third, and fourth legs distinct from the females and terminal hooks on the second maxillae that facilitate mating **(Keve *et al.*, 2025)**. Females tend to be slightly larger, and in gravid stages exhibit a visibly distended abdomen (Fig. 6). Body length among females ranged between 3.1 and 6.27mm across studies, while males were generally smaller (3.81–5.49mm).

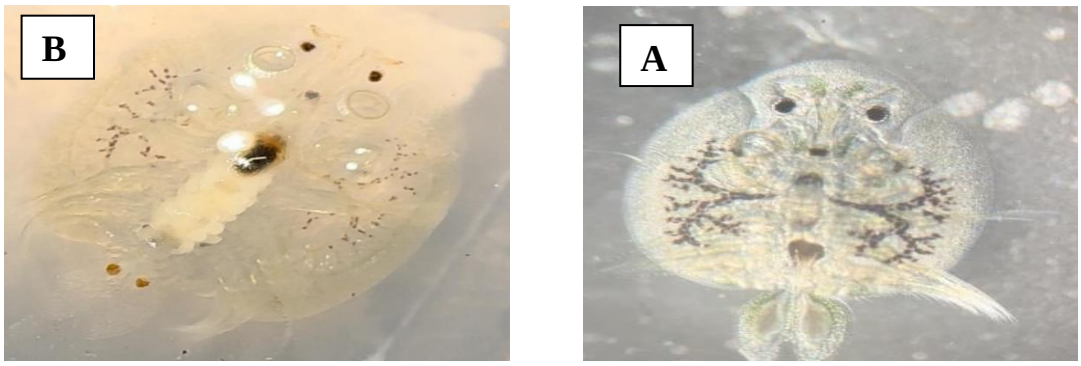

Fig. 6 The light microscopy for: **A**. Male; **B.** Female of *Argulus japonicus*

Respiratory areas in *A. japonicus* were consistently described as reniform and posteriorly located, a distinguishing feature compared to related species like *A. coregoni* and *A. foliaceus* which display different configurations of the carapace and respiratory zones **(Dekari *et al.*, 2024)**. The posterior lobes of the carapace in *A. japonicus* extend beyond the onset of the abdomen, and its abdominal lobes are characteristically more pointed than in *A. foliaceus* **(Noaman *et al.*, 2010; Dekari *et al.*, 2024)**.

The morphology of *A. japonicus* supports its ecological adaptability and wide host range. In India, it parasitizes a variety of cultured and ornamental fish including the goldfish, rohu, catla, and koi carp **(Dekari *et al.*, 2024)**. Morphological variation among regional isolates, such as the widened bases of post-antennal spines observed in Hungarian specimens **(Keve *et al.*, 2025)**, may indicate emerging subspecies distinctions, leading to the proposal of *A. japonicus* subsp. *europaeus* in Europe, based on consistent molecular and morphological divergence that may play a role in shaping the epidemiology of *A. japonicus* within aquaculture systems.

CONCLUSION

This study verified the presence of *Argulus japonicus* in *Cyprinus carpio* from fish farms in Erbil Province, using detailed morphological and molecular examination. Ankawa Fish Hatchery presented the highest rate of infection, probably because the environmental circumstances favored the occurrence. SEM and genetic sequencing (COI and 18S rRNA) confirmed species identification and demonstrated distinct phylogenetic separation from closely related ones. The fact that the head and caudal fin are more frequented by parasites indicates important infection burden zones. The study, as a whole, underscores the role of morphological and molecular techniques for precise diagnosis, and thereby advocates the need for selective parasite control strategies in aquaculture.

Funding

No funding was received for conducting this study.

Conflict of interest

The authors declare that they have no conflicts of interest related to this study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

All the data regarding the current study are provided within the manuscript.

Materials availability

Not applicable.

Code availability

Not applicable.

Author contribution

Both authors (RK and SHA) are contributed equally to this work regarding the experimental design, samples preparation and their analysis, in addition to writing the manuscript and reading thouroughly.

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دراسة المورفولجية والجينة لـ(Thiele 1900) *Argulus japonicus* لاسماك *Cyprinus carpio* في ثلاث مناطق مختلفة لمحافظة اربيل , اقليم كوردستان-العراق

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ملخص

هدفت الدراسة الحالية إلى وصف الخصائص المورفولوجية والجينية لطفيلي *A. japonicus* من أسماك الكارب *Cyprinus carpio* لثلاثة مواقع في محافظة أربيل، إقليم كردستان، العراق. تم فُحص ما يقارب 1,382 سمكة، وجُمعت عينات من

الطفيليات الخارجية. حُددت الطفيليات لعينات الاسماك باستخدام المجهر الإلكتروني الماسح والتحليل االجيني باستخدام تضخيم تفاعل البوليميراز المتسلسل وتسلسل جينات الوحدة الفرعية الأولى من إنزيم أوكسيديز السيتوكروم سي (COI) وجينات الرنا الريبوسومي 18 S. في مفرخ أسماك عنكاوا، سُجلت أعلى نسبة إصابة بطفيلي *A. japonicus* تقدر ب 41.26%، مع ميل واضح لمكان الالتصاق برأس وزعانف ذيل العائل. أظهر تحليل المجهر الإلكتروني تكيفات مورفولوجية واضحة للطفيلي للالتصاق بالطفيلي والتغذية. تم تأكيد تحديد الأنواع من خلال البيانات الجينية، ولوحظت مسافة تطورية كبيرة بين *A. foliaceus* والمجموعات ذات الصلة الوثيقة. بالإضافة إلى ذلك، تم تحديد الجينوم الميتوكوندريا الكامل لـ*A. japonicus* ، والذي أظهر ترتيبًا جينيًا متشابهًا وتحيزًا عاليًا لـ A+T. كان الجمع بين التحليل المورفولوجي والجيني ضروريًا لتحسين تحديد أنواع الطفيليات في الموقع وتوثيق توزيعها. وبالتالي، وفرت هذه النتائج معلومات مهمة لصحة و ادارة الأسماك واستراتيجية مكافحتها في أنظمة الاستزراع المائي في اقليم كوردستان العراق.

الكلمات المفتاحية:، *Argulus japonicus* الاسماك ، COI ،الطفيليات الخارجية.