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Morphological Variability and Molecular Studies of *Argulus japonicus* from *Cyprinus carpio* in Sulaymaniyah Province

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ABSTRACT

Background and Objective: *Argulus japonicus* is an important ectoparasitic crustacean that causes significant health and economic problems in freshwater aquaculture. This study aimed to determine the prevalence and provide morphological, ultrastructural, and molecular characterization of *A. japonicus* infecting common carp (*Cyprinus carpio*) in the Kurdistan Region of Iraq.

Material and methods: A total of 174 fish samples were collected between June 2021 and May 2022. The skin, fins, and gills were examined for *A. japonicus* infestation. Parasites were identified using light microscopy and scanning electron microscopy (SEM). Genomic DNA was extracted, and the nuclear 28S rDNA gene was amplified by polymerase chain reaction (PCR) and sequenced. The obtained sequences were compared with those available in GenBank, and phylogenetic analysis was performed.

Results: *A. japonicus* was detected with an overall prevalence of 8.62%. Morphological and ultrastructural analyses differentiated the adult male and female parasites and identified the metanauplius larval stage. The presence of claspers at the base of the swimming appendages and branched dorsal ridges represented key diagnostic characteristics for the identification of *A. japonicus*. Molecular analysis of the 28S rDNA gene showed 99–100% sequence identity with reference sequences available in GenBank, confirming species identity. The obtained sequences were deposited in GenBank, and phylogenetic analysis demonstrated close genetic relationships with previously reported *A. japonicus* isolates.

Conclusion: This study provides the first comprehensive morphological, ultrastructural, and molecular characterization of *A. japonicus* infecting *C. carpio* in Iraq. It also represents the first record of *A. japonicus* in common carp from the Kurdistan Region, expanding the known geographical distribution of this economically important parasite and providing valuable baseline data for future taxonomic and epidemiological studies.

Introduction

The freshwater common carp, *Cyprinus carpio* Linnaeus, 1758, is regarded as one of the most widely reared fish species in Iraq. It is well adapted to local aquatic environments due to its tolerance of low dissolved oxygen levels and low water temperatures in natural habitats [1]. This species was first introduced into Iraqi waters in 1955 at the Al-Zaafaraniya fish farm [2]. However, the culture sector in the Kurdistan Region has only recently begun to grow, having recently extended to numerous farms in the area [3]. *Argulus japonicus* Thiele, 1900, which belongs to the family Argulidae, is regarded as an exotic species in China, Japan, and East Asia, outside of its native area [4]. *A. japonicus* was discovered for the first time in Europe in 1921 in Spain. Since then, it has also been found in Belgium, Fennoscandinavia, Britain, France, Germany, Italy, Poland, Bosnia, Croatia, Serbia, and Slovakia [5].

Fish that are parasitized by crustaceans suffer metabolic disturbances and potentially fatal illnesses. Among the Branchiura, species of *Argulus* are the most common parasites infecting fish and are commonly referred to as fish lice or fish louse. More than 100 species of *Argulus* have been identified worldwide. They can infest freshwater and marine fish species in both natural and pond-raised settings [6]. Heavy infestations may be characterized by pronounced tissue damage, hypersecretion of mucus, desquamation of scales, and fin degeneration. Such pathological alterations impair the physiological condition and overall performance of fish while simultaneously increasing their susceptibility to secondary infections [7].

The ectoparasite *Argulus japonicus* has a direct life cycle; adult males have enormous testes, but adult females have small spermathecae; this difference may be seen in live specimens due to the parasite's transparency. The female will depart the host fish after mating and lay her eggs on nearby solid objects [8].

The overlapping physical features of the parasite and the fact that many species are only known from their original descriptions make parasite identification difficult [9]. There is no research on the ultra-morphological identification of *A. japonicus* in Iraq. However, there were just a few publications that studied the parasite body structures with SEM examination [10 and 11]. Based on the mitochondrial 16S rRNA, nuclear 18S rRNA, and nuclear 28S rRNA sequences that are now available in GenBank [12 and 13]. PCR amplification of genetic markers such as 18S rRNA and cytochrome c oxidase subunit I (COI) gene is considered an essential tool for species identification of *Argulus* [14]. Phylogenetic investigations of *Argulus* spp. have been conducted. Furthermore, according to [15], the morphological and genetic techniques are trustworthy diagnostic tools for identifying and characterizing *Argulus*. This work concentrates on the existing lack of molecular characterization of Iraqi *Argulus* species in order to determine genetic similarities within and among *Argulus* populations. The current study seeks to characterize the ultrastructure and molecular aspects of *A. japonicus* that infest freshwater *C. carpio* in Sulaymānīyah city.

Materials and methods

Description of Study Area

The sampling areas were located in ShakhaSwr of Sharbazher, specifically at ponds 1 (N 35.709994°, E 45.514334°) and 2 (N 35.708909°, E 45.514527°), as shown in Figure 1. Sulaymānīyah Province is located in the northeast of Iraq, situated between latitudes 35° 05’ and 36° 30’ and longitudes 44° 25’ and 46° 20’. It is situated near the Iraqi-Iranian border.

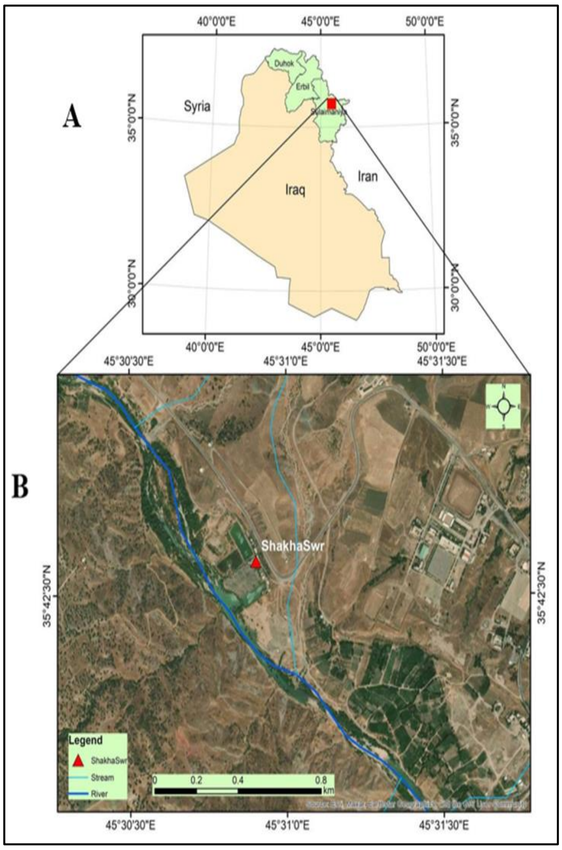

Figure 1: (A) Map of Iraq, showing the Sulaymaniyah Province. (B) Map showing the study area (Google Maps).

Sample collection

A total of 174 live common carp (*C. carpio*) were collected from ShakhaSwr by using hand nets, and fish specimens were gathered throughout fieldwork from June 2021 to May 2022. The fish were immediately transported to the advanced zoology lab in the Biology Department of the College of Science at the University of Sulaimani within a short period.

Morphological identification

Light microscopy

The occurrence of *Argulus* spp. was observed visually over the whole body, on the ventral and lateral body surface, gills, and caudal fins of the fish. Each recovered *Argulus* specimen was carefully collected with forceps and preserved in 70% ethanol for subsequent morphological examination and PCR analysis. Morphological characteristics of the parasite were observed under a light microscope. The photos were taken with an iPhone Digital camera model Xs Max, a 12 megapixel.

The morphological description of *Argulus* was based on adult males, adult females, and larval stages, following the references [16 and 17].

Scanning electron microscopy

For SEM, an adult male of *A. japonicus* was washed five times with 0.2 M cacodylate buffer (pH 7.3), fixed in 2.5% glutaraldehyde, and post-fixed in 1% osmium tetroxide at 4°C. The samples were dehydrated through a graded ethanol series [18], dried using the CO_2 critical point method, sputter-coated with gold, and subsequently examined using a scanning electron microscope (SEM; Quanta 450, USA).

Molecular data amplification and sequencing

Total genomic DNA was extracted from the parasite body using the Prep Genomic DNA Extraction Kit (Addbio, Korea) following the manufacturer's standard laboratory protocol. DNA was stored at − 20 °C for subsequent molecular analysis. DNA fragments of about 700 base pairs (bp) of the 28S gene nuclear rDNA were amplified by Polymerase chain reaction (PCR) using the Thermal Cycler Veriti (Applied Biosystems, USA) using forward primer FA (5'-GAC CCG TCT TGA AAC ACG GA-3') and reverse primer RA (50 -CTA GTT GCT TCG GCA GGT GAG-30) [19]. In accordance with the protocol, the amplifications were carried out as follows: 94°C for 2 minutes of initial denaturation, 35 cycles of 94°C for 30 seconds of denaturation, 50°C for 30 seconds of annealing, and 72°C for 40 seconds of extension, followed by a final extension at 72°C for 10 minutes. The PCR products were separated using 1.5% agarose gel electrophoresis (Cleaver, Scientific Ltd., U.K.) in 1x TAE buffer, 80V for 60 min, and followed by staining with 0.5 µg/mL ethidium bromide (TransGen, China), then visualized under ultraviolet illumination using Gel Doc XR+ (Bio-Rad, USA), and fragment sizes were verified using bands of a 100 bp DNA ladder (Promega, U.S.A). The amplicon was then purified using the QIAquick PCR Purification kit (QIAGEN, Germany). The amplified five specimens were sequenced on a Genetic Analyzer (Applied Biosystems/3500, Korea), then manually checked and edited for accuracy using Bio Edit software. Sequences were compared with similar sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) to confirm their identity and were deposited in GenBank under the accession numbers.

Phylogenetic analysis

For the phylogenetic study, the sequence data of the 28S rDNA of the *Argulus* fragment obtained from collected freshwater cyprinid fish *C. carpio* were installed into the MEGA X version 11 software program [20]. The sequences were aligned using the CLUSTALW alignment program to construct the trees of evolutionary development. The trees of all isolated species were constructed based on the Maximum Likelihood (ML) [21].

Results

Prevalence and Host Record

Out of 174 examined specimens of *Cyprinus carpio*, 15 individuals were infested with *Argulus japonicus*, resulting in an overall prevalence of 8.62%. A total of 25 parasites were recovered, including 20 males and 5 females. Infested fish exhibited parasites firmly attached to the body surface, particularly on the fins and lateral skin regions (Figures 2A, 2B). These findings confirm *C. carpio* as an additional host for *A. japonicus* in Iraq and represent the first documented record of this parasite in the Kurdistan Region.

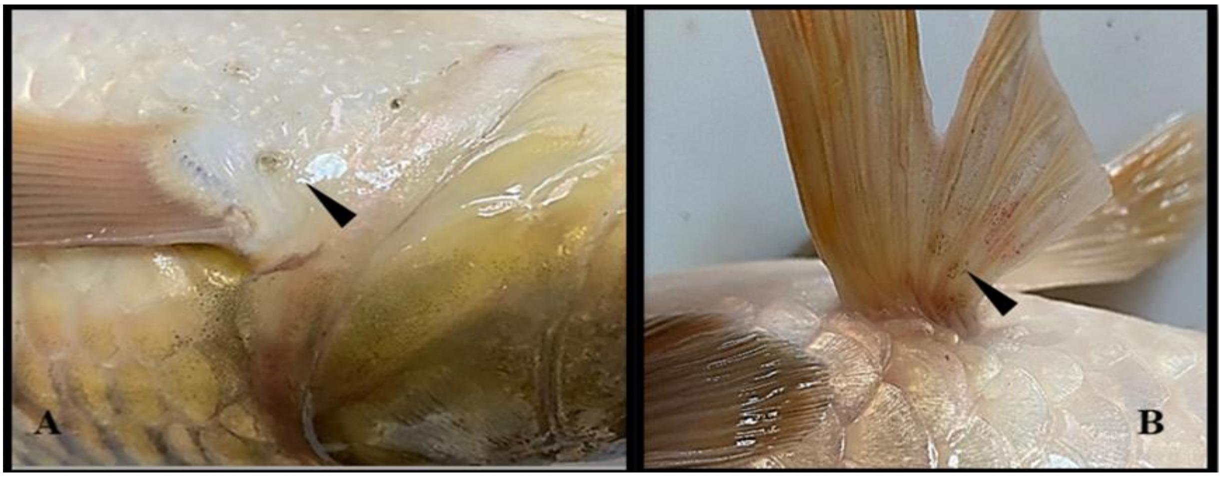

Figure 2: *C. carpio* infested with *Argulus japonicus*, (A) Skin, (B) Pelvic fin.

Morphological Characterization

The general morphology of the ventral surface in both adult male and female specimens matched the characteristics that identify A. japonicus. The body was flattened dorsoventrally, and a large carapace covered it anteriorly. Also, the same structures and locations of antenna, compound eyes, maxilla, proboscis, melanophore patches, respiratory areas, and swimming leg structures were identified (Figures 3A and 3B).

The male reproductive organs, including testes and seminal vesicles, were clearly visible through the transparent body wall (Figure 3A), while in females, spermathecae were observed (Figure 3B), confirming sex differentiation.

The result showed branched dorsal ridges on the dorsal surface (Figure 3C).

In males, paired claspers were present at the base of the swimming appendages (Figure 3D).

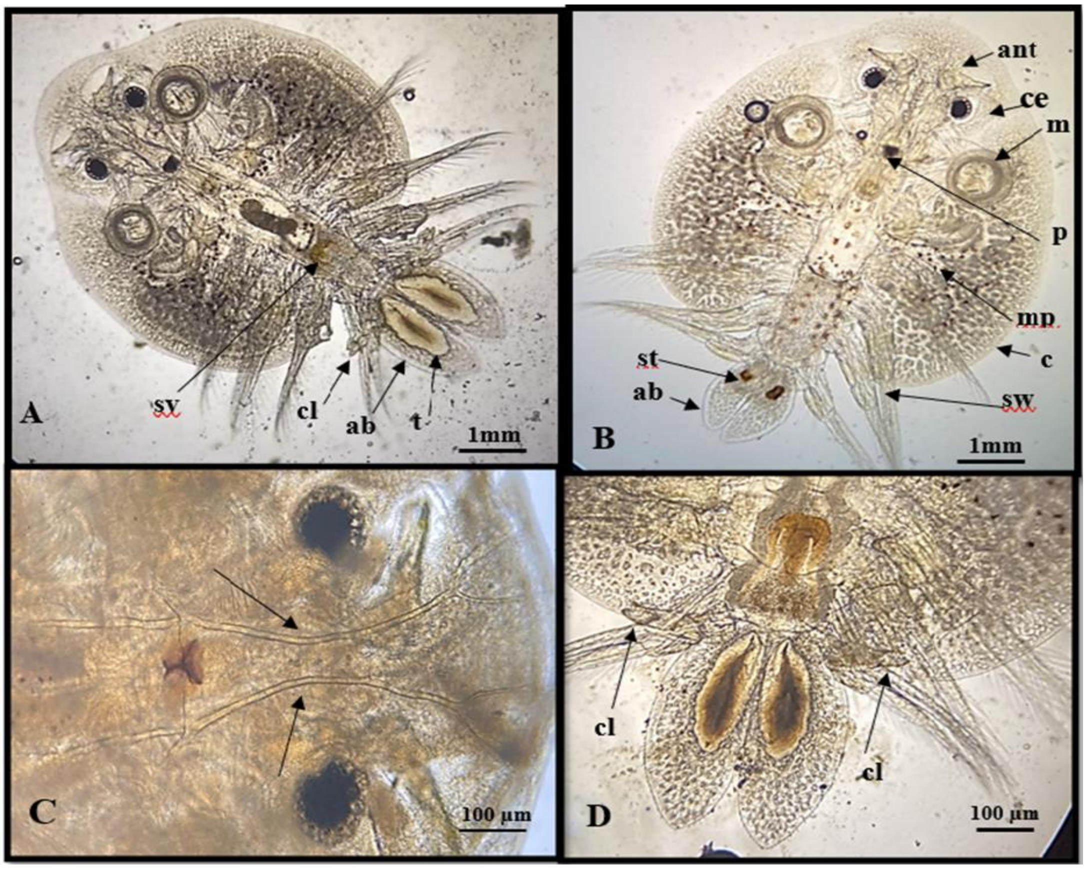

Figure 3: Ventral surface of *Argulus japonicus*, (A) Adult male and female (10X), (B) Branched dorsal ridge (400X), (C) Clasper of male (400X).

ant: antenna, ce: compound eyes, m: maxilla, mp: melanophore pigments, c: carapace, ab: abdomen, sw: swimming leg, p: proboscis, sv: seminal vesicle, t: testis, cl: male clasper, st: spermatheca.

The larval developmental stages were also detected. The metanauplius (first larval stage) is shown in Figure 4A, characterized by a relatively simple body structure and early appendage development. The juvenile stage (second larval stage) is presented in Figure 4B, showing further differentiation of appendages and body segmentation.

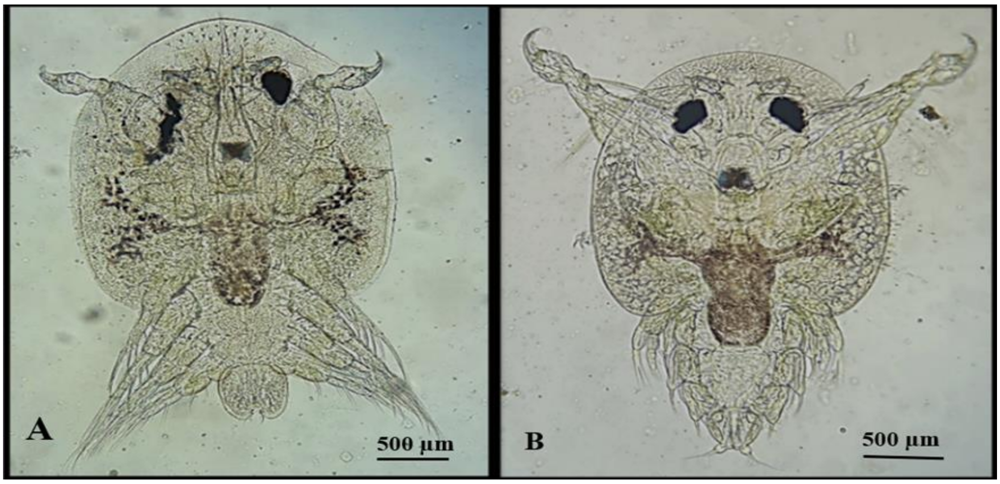

Figure 4: The ventral surface of larval stages of *Argulus japonicus*, (A) Stage I larva, metanauplius (100X). d- Stage II larva, juvenile stage (100X).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy provided detailed ultrastructural features of adult male specimens. The dorsal view (Figure 5A) shows the smooth carapace covering the anterior region of the body. Two prominent compound eyes were clearly visible in the frontal region (Figure 5B). The ventral surface exhibited well-developed attachment organs, including paired suction discs (maxillary suckers) and modified appendages. Thoracic appendages and abdominal lobes were also distinctly observed (Figure 5C).

Figure 5: Scanning electron micrograph of adult male A. japonicus from C. carpio, (A) dorsal view, (B) Location of compound eyes indicated by an arrow, (C) General morphology, ventral view.

The findings of the SEM results show the structural components of the first and second antennae (Figure 6A). Sharp and pectinate scales are uniformly distributed on the ventral surface of the frontal area (Figure 6B). The first antenna consists of two segments; the first is somewhat curved, and the second has spines that resemble hooks. The second antenna had five segments, with two proximal segments wider than the three terminal segments.

However, the ultrastructural features represented five segments of the second maxilla. The terminal section comprises three spiniform projections and a large basal segment (basal podomere) that is equipped with a sort of scale. The ventral surface of the second maxilla is decorated with various pectinate scale types that include forked structures (Figure 6C). Two sharp claws with hook-like points protruded from the terminal section of the maxilla, which resembled a blunt, fused extension (Figure 6D).

Despite the representation of the ultramorphological structure of the first maxilla, which was altered into sucking discs supported by multiple rods, each sucker consists of a cylindrical stalk with a flattened rim. Five tiny, overlapping sclerites were visible on each rod (Figure 6E).

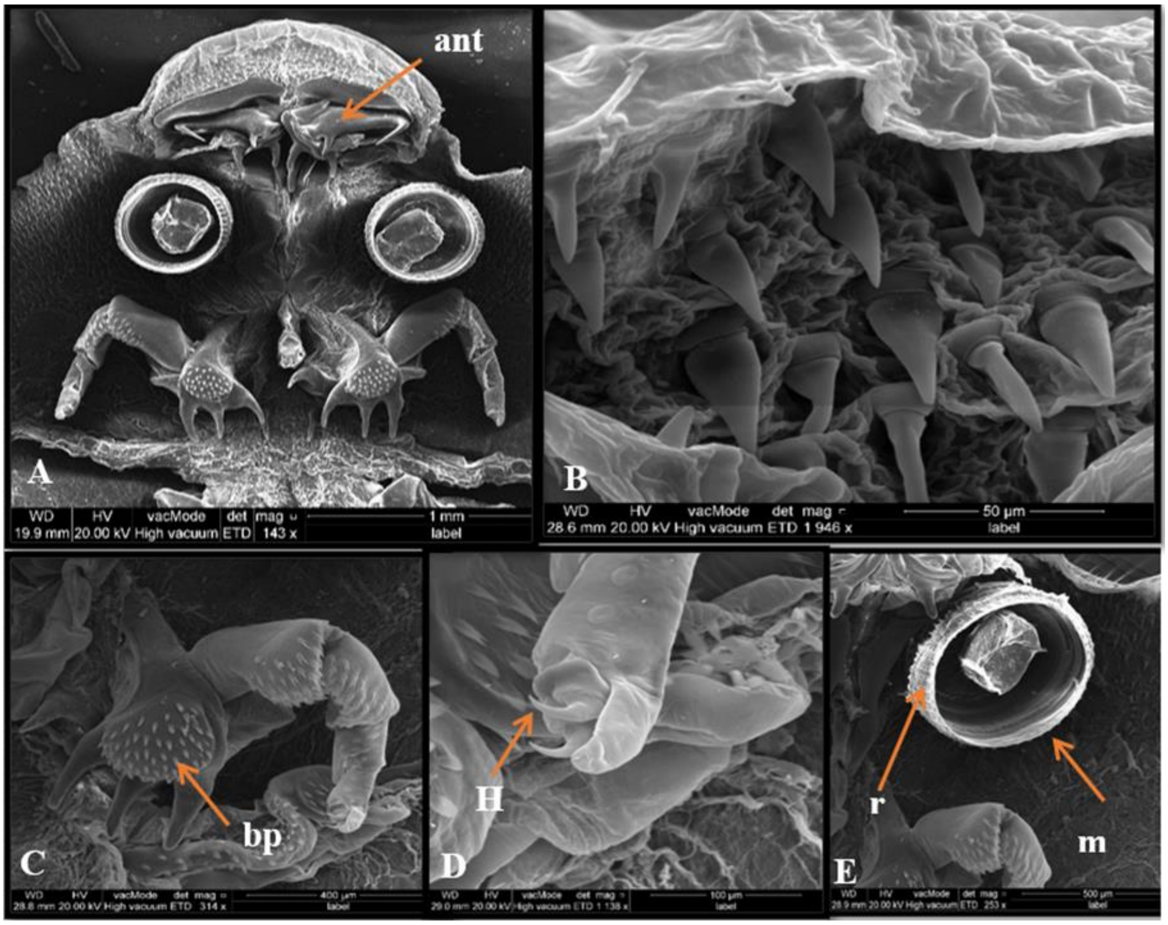

Figure 6: Scanning electron micrograph of (A) first and second antennae structure, (B) viewing the frontal region covered by pectin scales, (C) showing the structure of the second maxilla, with different types of pectinate denticles, (D) Terminal segments of the second maxilla, terminal hooks indicated by an arrow, (E) The first maxilla contains a sucking disk of the rod structure of overlapping sclerites.
ant: antenna, r: rod structure, bp: basal podomere, m: Maxilla, h: hook.

Molecular Identification and Phylogenetic Analysis

The *Argulus* spp. were amplified with a sequence length of 700 bp of 28S rDNA (Figure 7) from five isolates. Sequences that were obtained for the isolates were compared to those available in the NCBI database that are closely similar. The result showed 99%–100% identity to the 28S rRNA of *A. japonicus*, which was previously submitted under the accession numbers KF747847, KF747848, KF747849, and

KF747852. The sample nucleotide sequences in this study were submitted to the GenBank database with the accession numbers OQ608213, OQ608214, OQ608215, OQ608216, and OQ608217. Based on the morphological characteristics and genetic analysis, the present sample is identified as *Argulus japonicus*. The molecular identification of the samples studied represents the first work on *A. japonicus* in Iraq.

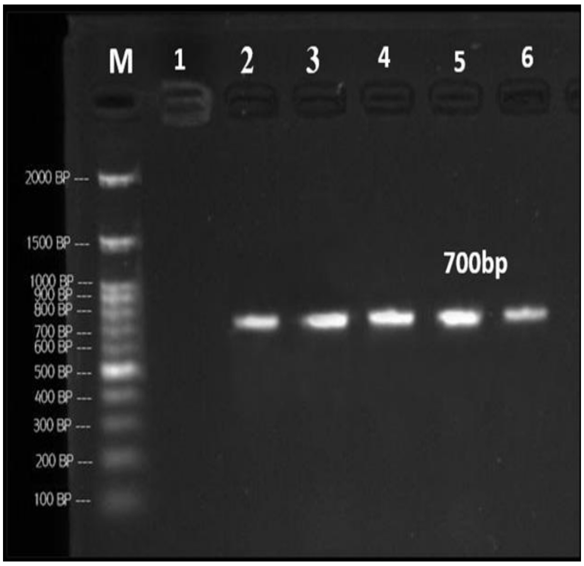

Figure 7: Gel electrophoresis of PCR product. Lane M: 100bp DNA ladder; lane 1 represents the negative control, lanes 2-6 show amplicons of 700 bp of 28S rDNA from *A. japonicus*, respectively.

According to the phylogenetic analysis, the obtained 28S rDNA sequence was compared with reference sequences available in GenBank using BLAST analysis (Table 1). The results showed very high similarity with *Argulus japonicus*, with percentage identities ranging from 99.54% to 100% (accession numbers KF747848, KF747849, KF747850, and KF747852), which were deposited and previously investigated in China, confirming that the examined specimen belongs to *A. japonicus* (Figure 8).

In contrast, lower similarity values were observed with other species of the genus *Argulus*, including *Argulus flavescens* (95.63–95.97%), *Argulus foliaceus* (97.27–99.54%), *Argulus americanus* (96.53%), *Argulus* bicolor (91.24%), and *Argulus* sp. (95.85%). The relatively lower similarity percentages with these species indicate genetic divergence and support the correct identification of the present isolate as *A. japonicus*.

For phylogenetic tree construction, *Lamproglena chinensis* (DQ107545.1) was used as an outgroup to root the tree and confirm evolutionary relationships.

Table 1: Accession numbers of 28S rDNA of *A. japonicus* from the present study that were used to build phylogenetic trees with different *Argulus* spp. Downloaded from the GenBank database.

Parasite	Gene Bank accession no. (28S rDNA)	Host	Source	Identity (%)
A. japonicus	KF747848	Not given	Direct submission	100%
A. japonicus	KF747849	Not given	Direct submission	100%
A. japonicus	KF747850	Not given	Direct submission	99.84%
A. japonicus	KF747852	Not given	Direct submission	99.54%
A. flavescens	MN688125	*Acipenser oxyrinchus desotoi*	(Andres *et al.*, 2019)	95.82%
A. flavescens	MN688126	*Acipenser oxyrinchus desotoi*	(Andres *et al.*, 2019)	95.97%
A. flavescens	MN688127	*Acipenser oxyrinchus desotoi*	(Andres *et al.*, 2019)	95.63%
***Argulus* sp.**	AY210804	Not given	(Mallatt *et al.*, 2004)	95.85%
A. bicolor	MN688129	*Hypanus sabinus*	(Andres *et al.*, 2019)	91.24%
A. foliaceus	OM721927	*Squalius cephalus*	(Öztürk, 2022)	97.27%
A. foliaceus	KF747853	Not given	Direct submission	99.54%
A. foliaceus	EU370442	Not given	(von Reumont *et al.*, 2009)	97.27%
A. americanus	MN668128	*Amia calva*	(Andres *et al.*, 2019)	96.53%
***Lamproglena chinensis* (out group)**	DQ107545.1	*Ophiocephalus argus*	(Song *et al.*, 2008)	-

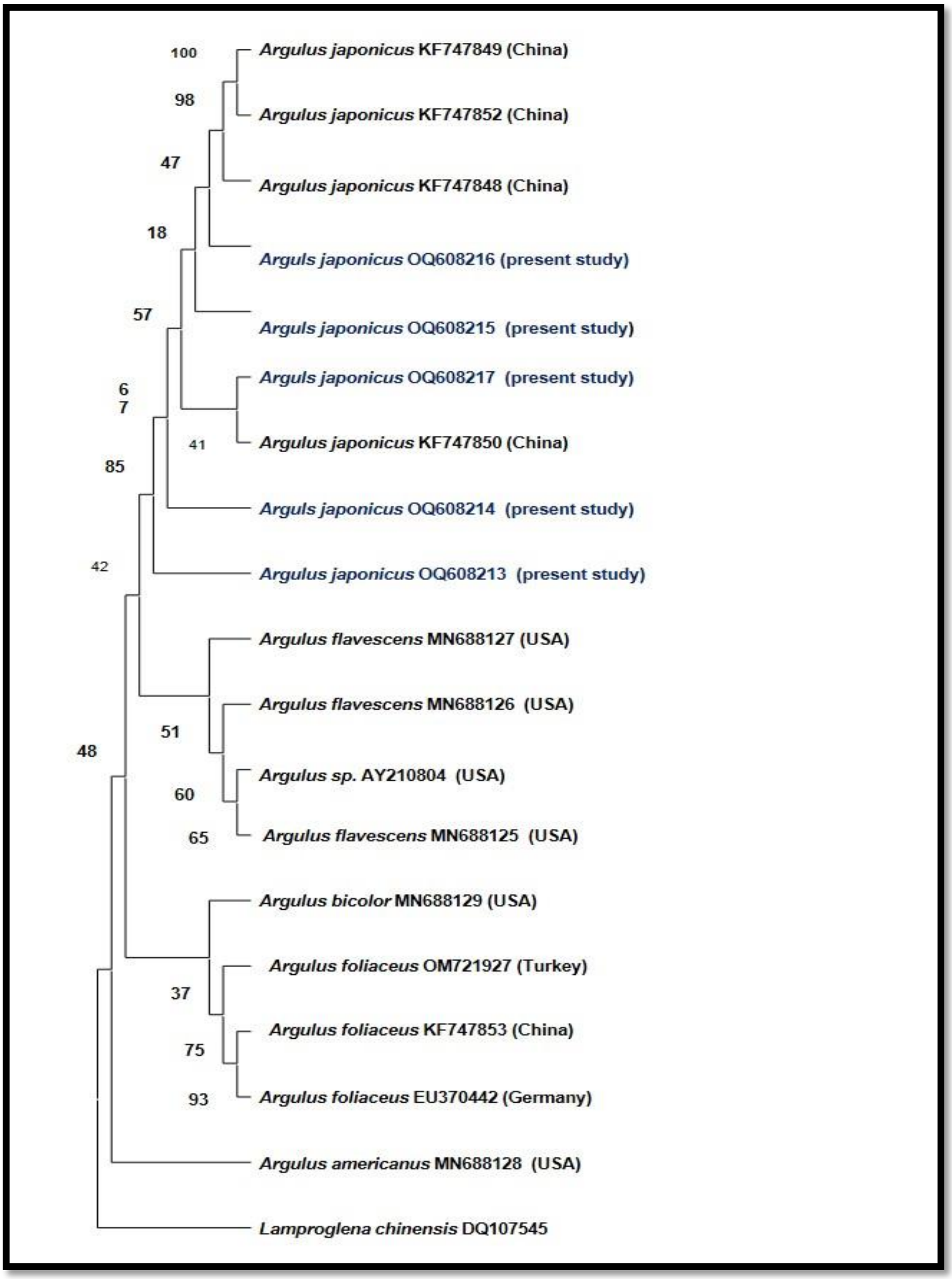

Figure 8: Phylogenetic tree showing the evolutionary relationship between *A. japonicus* from the study with other related isolates based on 28S rDNA sequences. The tree was constructed with the maximum likelihood method using MEGA X 11.

Discussion

The prevalence rate (8.62%) recorded in this study is comparable to values reported from certain regions but considerably lower than those documented in others. For example, a recent investigation in aquaculture facilities in the Kurdistan Region of Iraq documented a notably higher prevalence of *A. japonicus* in *C. carpio*, reaching up to 41.26% at specific sites, with parasites likewise attaching predominantly to fins and skin (head and caudal regions), supporting our observation of preferred attachment sites on the fish body surface [22]. A separate study in Nepal found that *A. japonicus* was present in common carp, with an 80% prevalence rate [23]. However, the sex ratio in *Argulus* infestations may vary considerably and can be affected by factors such as host species, developmental stage of the parasite, and seasonal variations [24]. Although infection rates vary widely among regions and management systems. Such variation may be attributed to differences in environmental conditions, water temperature, seasonal fluctuations, fish stocking density, management practices, and host immune response [25].

Argulus japonicus was first recorded in Iraq from *Silurus glanis* collected from the Tigris River in Mosul [26]. Subsequently, it was reported to infect *Aspius vorax* and *Carassius auratus*. Two other species of the genus *Argulus* were reported in the fishes of Iraq: *A. foliaceus* on the skin, dorsal fin, and gills of *Carasbarbus luteus* and *C. carpio*, and *A. coregoni* on *B. xanthopterus* [27]. Therefore, *Cyprinus carpio* in the current investigation represents a new host record for this parasite in Iraq. Moreover, this study constitutes the first documented occurrence of *A. japonicus* in the Kurdistan Region.

The examined specimens demonstrated distinctive morphological characteristics, including a dorsoventrally flattened body, a circular carapace generally extending over the first to third pairs of legs, and a bilobed abdomen with a rounded posterior margin. These features are in agreement with the findings reported by [28]. The differentiation between male and female *Argulus* species is primarily based on morphological criteria, including the structure and position of copulatory appendages, the distribution of melanophore patches, the configuration of respiratory areas, and the morphology of the swimming legs [29].

Although *A. japonicus* and *A. foliaceus* are occasionally misidentified due to their morphological similarities. Another study indicated that males of *A. japonicus* can be reliably distinguished by the presence of a small coxal process (clasper) located at the base of the swimming appendages [30]. This diagnostic feature was clearly observed in the present specimens. The abdominal lobes are distinctly sharper and more pointed compared to those of *A. foliaceus* [31].

One of the principal diagnostic features distinguishing *A. japonicus* from closely related species, such as *A. siamensis*, is the presence of branching dorsal ridges, as previously described in [32]. The same morphological characteristic was determined. Minor morphological variations may reflect geographical influences and environmental adaptation.

The description of the first larval stage of the metanauplius as sub-oval in shape, transparent, with two highly visible black compound eyes, and the juvenile stage was reviewed by [33].

SEM analysis further supported species identification by revealing detailed ultrastructural features. The dorsal surface of the adult male displayed compound eyes located in the frontal region, consistent with the study reported by [10]. The carapace covered nearly one pair of swimming legs, reflecting the relatively small size of both the carapace and the specimen, as noted by [17]. The carapace generally covers approximately half of the body; in *A. dartevellei*, it may extend to the anterior portion of the abdomen, whereas in *A. elongatus*, it is restricted to the base of the first pair of legs [34].

The ventral surface revealed the complete body of the male parasite, highlighting the antennae, maxillae, suction disc, and a carapace encompassing approximately four pairs of swimming legs, in agreement with previous descriptions [35].

The ultrastructural features of the first and second antennae revealed sharp, pectinate scales evenly distributed over the ventral surface of the frontal region. The first antenna comprises two segments: the proximal segment is slightly curved, and the distal segment bears hook-like spines. The second antenna consists of five segments, with the two proximal segments broader than the three distal segments. Furthermore, the SEM findings revealed that the second maxilla consists of five segments, with the terminal segment bearing three spiniform projections and a large basal podomere bearing scale-like structures. The ventral surface of the second maxilla is adorned with various pectinate scales, including forked forms. Two sharp, hook-like claws extend from the terminal segment, resembling a blunt, fused projection. These morphological features correspond to those described by [34].

The first maxilla is modified into suction discs reinforced by multiple rods, each consisting of a cylindrical stalk with a flattened rim, as supported by previous findings [31]. Five tiny, overlapping sclerites were visible on each rod. Variation in the number of suction cup rods was found, including 50-52 [17], 44-50 [36], and 48-52 [11]. In this study, the number of these rods was not quantified. The number of setae (spines) on the first leg endopod and the number of setae on the caudal rami may be important traits for *Argulus* spp. taxonomy or systematics, the presence of three setae on the endopod of the first leg of *A. japonicus* was found by [37].

The molecular identification of *Argulus japonicus* based on 28S rDNA sequencing in this research confirmed its species identity and allowed comparison with other *Argulus* taxa. The molecular identification of the samples studied represents the first work on *A. japonicus* in Iraq. Previous work has shown that 28S rDNA sequences can reveal distinct phylogenetic branches among *Argulus* species, highlighting nucleotide variation that differentiates local isolates from international reference sequences [38].

The direct sequencing of the rRNA gene is generally recognized as a reliable and precise marker for identification [39]. All *Argulus* species and their immature stages were identified using DNA barcode technology [40]. Representative sequence data for only a few argulid species are available [9]. Morphological and molecular approaches are reliable diagnostic tools for the identification and characterization of *Argulus* species. In addition, the NCBI GenBank database provides eight 18S rRNA gene nucleotide sequences for *Argulus japonicus* [15]. Recently, [29] submitted two GenBank accession numbers (MW857090 and MW857091) of the 18S rRNA gene of *A. japonicus*, obtained from *Schizothorax richardsonii* in India, to the NCBI database.

According to the phylogenetic analysis, *A. japonicus* is similar to accession numbers that were deposited and previously investigated in China. The results determined a distinct cluster with other species of *Argulus* from different countries, including the USA, Turkey, Germany, and Japan. The phylogenetic tree represents the evolutionary history of organisms at the genus or species level [41]. Their reconstruction is based on morphological, molecular, and observable similarities [42]. Populations with a higher degree of similarity are more homogeneous [43].

Regarding the initial submission of a partial sequence of *Argulus* sp. 28S rRNA gene to the GenBank, two studies published the first molecular phylogeny of Branchiura, Pentastomida, and other Maxillopoda based on mitochondrial 16S rRNA gene, nuclear 18S gene, and 28S rRNA gene [12 and 13]. The genetic diversity of the fish louse *A. japonicus* from Africa, the Middle East, and Asia was identified in its mitochondrial DNA (mtDNA) regions [44].

Additionally, complete mitogenome analyses have established the genetic relationships of *A. japonicus* within the family Argulidae, reinforcing the utility of molecular markers in elucidating evolutionary relationships among *Argulus* spp. [45]. Consistent with regional molecular studies, the phylogenetic results demonstrate a clear genetic separation between *A. japonicus* and other congeners such as *A. foliaceus*, supporting their distinction at the species level and contributing to a broader understanding of the evolutionary development of *Argulus* species in Iraq and neighboring regions [22].

However, the limited availability of 28S rDNA sequences in public databases restricts broader phylogeographic interpretation. Future studies should incorporate additional molecular markers. Genetic data and the phylogeographical structure of a species give the fundamental knowledge needed to comprehend the evolution of a species and its biogeographical history. Yet, this is the first record of *A. japonicus* molecular characterization based on 28S rDNA sequences from Kurdistan and their phylogenetic relationship with other *Argulus* spp. in the GenBank database. The molecular technique appears to be a reliable diagnostic tool for *Argulus* characterization and identification of *Argulus* species [46].

The integration of morphological, ultrastructural, and molecular approaches in this research enhances the accuracy of species-level identification and contributes valuable data to the regional parasite biodiversity record.

Conclusion

This investigation confirms *Cyprinus carpio* as an additional host of *Argulus japonicus* in Iraq and documents the first record of this parasite in the Kurdistan Region. These findings expand the known host range and geographical distribution of the species within the country. The combined use of morphological examination and molecular analysis enabled the precise identification of the studied *Argulus* specimens. Molecular characterization, supported by phylogenetic reconstruction and sequence submission to GenBank, not only verified species identity but also revealed genetic affiliations with isolates from other geographical regions. Collectively, these results enhance current knowledge of fish lice diversity in freshwater fish and provide a valuable reference framework for future parasitological and molecular studies. Furthermore, the implementation of effective biosecurity measures and parasite control strategies in carp aquaculture systems is essential to limit parasite transmission and prevent outbreaks.

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Declaration of interests

The authors declare that they have no conflicts of interest.

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Data and material availability

All data generated and analyzed during this study are included in this published article.

Author contribution

R.M. Mohammed**:** Sample collection, laboratory techniques, manuscript writing, and revising. Sirwan M. Muhammed Ameen and Shamall M. A. Abdullah **:** Suggested the title of the research, manuscript writing, and revising, with morphological identification of the parasite.

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التغايرات المورفولوجية والدراسات الجزيئية لـ *Argulus japonicus* المعزول من أسماك الشبوط *Cyprinus carpio* في محافظة السليمانية

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الملخص

الخلفية والهدف: يُعد *Argulus japonicus* أحد أهم القشريات الطفيلية الخارجية التي تُصيب أسماك المياه العذبة، ويسبب خسائر صحية واقتصادية في قطاع الاستزراع السمكي. هدفت هذه الدراسة إلى تحديد نسبة انتشار هذا الطفيلي وإجراء توصيف مورفولوجي، وفوق مورفولوجي، وجزيئي له في أسماك الشبوط (*Cyprinus carpio*) في إقليم كردستان–العراق.

المواد وطرائق العمل: تم جمع 174 سمكة شبوط (*C. carpio*) خلال الفترة الممتدة من حزيران 2021 إلى أيار 2022. وفُحص الجلد والزعانف والخياشيم للكشف عن الإصابة بالطفيلي. أُجري التوصيف المورفولوجي باستخدام المجهر الضوئي والمجهر الإلكتروني الماسح (SEM). كما استُخلص الحمض النووي الجينومي، وأُجري تضخيم منطقة 28S rDNA النووية باستخدام تقنية تفاعل البلمرة المتسلسل (PCR) ، ثم حُدد التسلسل النيوكليوتيدي، وأُجريت المقارنة مع التسلسلات المسجلة في بنك الجينات (GenBank) ، إضافةً إلى إجراء التحليل الوراثي التطوري.

النتائج: بلغت نسبة الانتشار الكلية للإصابة بـ *A. japonicus* 8.62% وأظهر الفحص المورفولوجي والفوق مورفولوجي الفروقات الرئيسة بين الذكور والإناث، كما تم التعرف على طور اليرقة الأول (Metanauplius). وشملت الصفات التشخيصية المميزة وجود الملقطيات عند قاعدة الزوائد السباحية والتفرعات في الحواف الظهرية، مما ساعد على تمييز الطفيلي عن الأنواع الأخرى التابعة لجنس Argulus. وأكد تحليل تسلسل 28S rDNA هوية الطفيلي، إذ أظهرت النتائج نسبة تطابق تراوحت بين 99–100% مع التسلسلات المسجلة في المركز الوطني لمعلومات التقانة الحيوية (NCBI). كما أودعت التسلسلات الناتجة في بنك الجينات (GenBank) ، وأظهر التحليل الوراثي التطوري تقارباً وراثياً مع العزلات المسجلة سابقاً.

الاستنتاج: توفر هذه الدراسة أول توصيف شامل يجمع بين الخصائص المورفولوجية، والفوق مورفولوجية، والجزيئية للطفيلي ***A. japonicus*** في العراق. كما تمثل أول تسجيل لإصابة أسماك الشبوط (***C. carpio***) بهذا الطفيلي في العراق، وأول تسجيل له في إقليم كردستان، مما يوفر بيانات مرجعية مهمة للدراسات التصنيفية والوبائية المستقبلية.

الكلمات المفتاحية: *Cyprinus carpio*، *Argulus japonicus*، المجهر الإلكتروني الماسح (SEM)، 28SrDNA ، تسجيل جديد.