GMR
The Original

INTER AND INTRA SPECIES MORPHOLOGICAL AND MOLECULAR POLYMORPHY OF KHAWIA SPECIES

Aso Khalil A. Korde[1] , Samir Jawdat Bilal[1,2*]

[1]Fish Health and Production Department, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Erbil, Kurdistan Region-Iraq.
[2]Scientific Research Center, Salahaddin University-Erbil, Erbil, Kurdistan Region-Iraq.
*Corresponding author: Samir J. Bilal, samir.bilal@su.edu.krd

Abstract
The present study investigates interspecific and intraspecific variations (polymorphism) of Khawia spp. that infect cyprinid fishes in the Kurdistan Region of Iraq, using a combined taxonomic approach morphologically, the general features of the genetic constitution and ultrastructural morphological with SEM.
The study performed during September 2024 – August 2025, in both Greater Zab and Lesser Zab rivers the morphological and ultrastructure showed some variations scolex shapes and microtrichia. And some other features were studied, while the Molecular study on the 18S rRNA gene (approximately 600 bp) showed high homology (99.11 –100%) between Khawia armeniaca and K. sinenis (similar species). However, some minor nucleotide changes were found indicating intraspecific polymorphism (GC Content: 49.63%), (A: 170) Phylogenetic analysis confirmed that the samples sit in the monophyletic group Caryophyllaeidae.
These results highlight the limitations of using morphology alone due to surface variability (phenotypic plasticity) and demonstrate the importance of combining molecular and ultrastructural data in addition to the morphological characters for correct species identification. This study provides new insights into the diversity and genomic variation of Khawia in this region.

Keywords: Khawia, morphology, polymorphism, integrative taxonomy, SEM, 18S rRNA.

INTRODUCTION

In the past, the taxonomy of the genus Khawia was established using specific morphological characteristics; however, the development of molecular phylogeny has made the systematic integration of genetic material necessary (1; 2). Specifically, 18S rRNA gene tuals offer a potent framework to resolve species identification ambiguities that are frequently difficult to differentiate using light microscopy (3). This genetic marker has been crucial in the identification and characterization of Khawia species in the Iraqi Kurdistan Region because it contains both variable areas that enable precise species-level distinction and conserved areas that make it easy to match toilets. Scolex morphology within Khawia species are the mysterious differences. Furthermore, in order to accurately identify the boundaries of species within the genus, these issues necessitate a linked taxon approach that combines specific morleculi with complete morphology. This strategy is crucial since it increases the host species' capacity to alter Khawia morphology (morphological plasticity) (4).
A richer knowledge of genetic variation among Khawia populations in various geographic locales is made possible by this multidisciplinary approach, which is crucial for suggesting any formal taxonomic variation (5).The discovery of polymorphic variation that are phenotypically unknown is made possible by the advent of diagnostic molecular markers, especially in the 18S rRNA gene (6). Consequently, this is in the process of pre-binding the capture to a subject's capture (1; 6).
Some investigations conducted in the Kurdistan Region of Iraq have demonstrated the phenomena, of Morphological similarity with fish belonging to the family Cyprinidae discovered in water sources including the Greater & lesser Zab Rivers and others exhibiting khawia armeniaca.
Additionally, the necessity to switch to a taxonomy based on genetic phylogeny is demonstrated by the difference between different polymorphic presentations, which are frequently based on nucleotide variations (7).
Lastly, the probability of species misidentification due to host-influenced morphological alterations is decreased by total analysis of the 18S rRNA gene (1; 7). This study's the present work goals are review of genus Khawia species parasiting the freshwater species of Kurdistan region- Iraq and the Polymorphic variations of Inter and intra species basing on morphological and molecular characteristics.

Additionally, the difference between phylogenetic trees based on genomic data and those based on morphology suggests that homoplasy may be involved in several phenotypic variables used for categorization (1; 8). Thus, for a thorough new evaluation of Khawia species diversity, our study integrates extensive 18S rDNA genotyping Morphological characters (4; 7). This combination of techniques is crucial for treating systemic problems in Caryophyllidea cestode worms, especially when it's necessary to differentiate between cryptic lineages with extreme phenotypic diversity (7).
The present study aims to study the inter and intra specific variations of Khawia by combining of morphological ultra-structural and molecular characterizations.

MATERIALS AND METHODS

Erbil is home to a diverse set of water resources, including lakes, ponds, canals and several important river systems including two rivers (Greater Zab and Lesser Zab) that pass the province. For the Greater Zab It is situated between 36˚-37˚ north latitudes & for Lesser Zab It is situated between 34º-36º north latitudes and 43˚-44˚ & 43º-46º east longitude flow into the Tigris River (9; 10). (Fig 1)

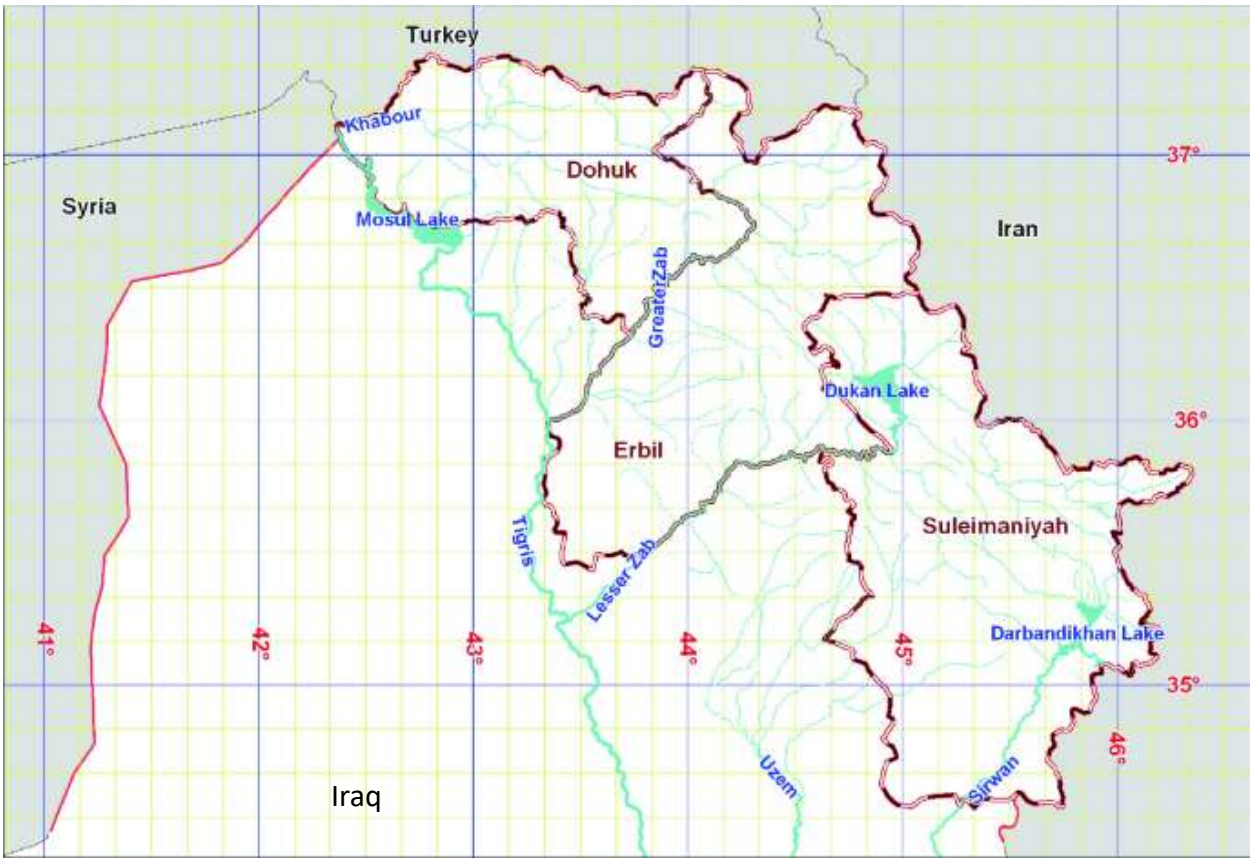


Fig. (1) Map of Iraq showing Kurdistan region showing Greater Zab and Lesser Zab Rivers according to (10).

A total 62 of specimens of Capoeta trutta were randomly collected monthly by using gill nets from local commercial fishermen for a period starting from September of 2024 to August 2025. Fish specimens `were placed in plastic tanks supplied with local river water and brought alive to the Parasitology Laboratory of The Research Center at Salahaddin University-Erbil identified twice according to fishbase (11). The fishes were checked for parasites internally. collected fishes were opened ventrally and Intestines were separated, kept wet in a Petri dish with normal saline 25-28C, and examined under a dissecting microscope according to, parasite fixation and preservation were done accordingly (1) and (12). The identified parasites were classified based on the measurements of their scolex and shape structures, as detailed. The prevalence, mean intensity, and abundance of the parasites were all studied according to (13).

MORPHOLOGICAL IDENTIFICATION

The detached Khawia specimens were washed in a normal saline solution, for morphological study fixing in hot formalin (4%) and presented in 70% ethanol, stained with Acetocarmine and amounted in Canada balsam (14)
Regarding the preparation of Scolex for scanning electron microscope (SEM) examination, protocols often use specific chemicals to maintain ultrastructural integrity. After fixation, the samples are thoroughly washed and dried through a graded alcohol (ethanol) series (e.g. from 30% to 100%) to replace the water with an organic solvent (15). To ensure that the sample remains intact during transfer to the high vacuum of the scanning electron microscope, or critical point drying (15) were done. Finally, the scoleces are mounted on aluminum bases (aluminum stubs) and covered (sputter-coated) with a conducting metal such as gold to prevent the accumulation of electrical charges during observation (15). Image were done with Quanta 450 SEM in Laboratory of The Research Center at Soran University.

MOLECULAR STUDY

For molecular study, 18s rRNA were amplified and sequenced with forward (Table 1). DNA was extracted using agen kit following the leaflet protocol, A region of 18S rDNA was amplified by polyme rase chain reaction (PCR). The used primers were universal, forward primer (5'-GGTTCTCCTAGCGTTGCCTT-3'), and reverse primer (5'-GCACCACCAACCACCAAATC-3')) (Table 1). PCR reaction and condition were performed using SimpliAmp. PCR amplification was done in a total volume of 50 µl of reaction mixture containing; 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 Picomol (pmol) primers table (1), DNase free water and template DNA by Bioresearch PTC-200 Gradient thermocycler as follows: 95°C for 5 min (initial denatura-tion), 35 cycles of denaturation at 95°C for 38 sec, annealing temperatures at 58°C for 38 s and extension at 72°C for 60 s, and 72°C for 10 min (final extension) as shown in Table (2). Agarose gel electrophoresis was employed to check the efficiency of PCR reactions. The samples were prepared and run in the 1.5% gel of agarose, and then stained with ethidium bromide that makes the DNA visible under UV light, with the ex - pected size of the PCR product at 600 bps in the present study The NCBI (National Center for Biotechnology Information) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) website to comparing and alignment laboratory was used to find the nucleotide order of 18S rDNA in the sample. The PCR fragments of the sample were from the agarose gel and used as a source of DNA template for the sequence specific PCR amplification, polymorphy Were studied according to result sequences.

Table (1): Pair of primers used in 18S rRNA amplification with their profile temperatures

Gene	Primer Sequence (5′→3′)	Amplicon Size (bp)	PCR Thermal Profile
18S rRNA	F: 5′-GGTTCTCCTAGCGTTGCCTT-3′	600	95°-5 min; 95°-38 sec, 58°-38 sec, 72°-1 min; 72°-10 min; 4° ∞
	R: 5′-GCACCACCAACCACCAAATC-3′		

Table (2) 18S rRNA PCR Amplification Reagents

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	3
3	Reverse Primer	20 Pmol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
Total			50

RESULT AND DISCUSSION

MORPHOLOGICAL IDENTIFICATION

The present study included revealing of 49 specimens of Capoeta trutta and showed presence of two cestode species belonging to the genus Khawia namely K. armeniaca and K. sinensis with prevalence of (6.12%) and (2.04%) respectively, also intensity (0.16) and (0.06) respectively for the description of these parasites a combination of morphologically, histologically, surface ultra-structure by scanning electron microscope (SEM) and molecular data for insuring of the species taxa of them. According to earlier descriptions for cestodes, the scolex was slightly enlarged and lacked bothria or sucker (16; 17).
Morphological analysis of collected tapworms revealed distinctive features of the scolex, one of the most important diagnostic tools in the Caryophyllidea. The Body of K. armeniaca 15–47 mm long; maximum width 1.12–3.10 mm. Scolex bulbate, slightly wider (width 1.14–2.10 mm) than neck (width 0.44–1.60 mm), with oval anterior end. Testes

numerous, estimated number 130–440 (n = 7), oval to almost spherical, 102–315 times 66–242, begin posterior to vitelline follicles, reaching posteriorly up to anterior margin of cirrus-sac (1; 16). Cirrus-sac widely oval to spherical, thick-walled, 322–1,146 times 436–937. Ovary bilobed, butterfly-shaped, i.e., with short and wide lateral arms, follicular, near posterior extremity. Total width of ovary 0.85–2.11 mm; lateral arms 0.61–1.82mm long and 0.17–0.71 mm wide. Vagina tubular, nearly straight, proximally joining with uterus to form uterovaginal canal. Seminal receptacle 144–508 times 77–271. Vitelline follicles cortical, oval, variable in size, 47–280 times (32–221), begin 0.88–2.35 mm from anterior extremity, reach posteriorly to anterior half of cirrus-sac; follicles may be present alongside preovarian uterine loops (25; 18). Postovarian follicles relatively few, 26–98 (n = 8) in number, occupying small area near posterior extremity, few solitary vitelline follicles located around posterior arms of ovary or do not even reach to ovary. The uterus is tubular, reaching from the anterior to the posterior margin of the cirrus sac. Eggs large, oval, operculate, unembryonated, 44–66 times 25–46 (n = 34) intrauterine eggs (1; 16).

The Body of K. sinensis, 41–112 mm long; maximum width 1.2–2.2 mm. The scolex is festoon-like, with deep (up to 0.4 mm long) folds, mostly 6–10 in number, on the anterior margin (front edge), wider than the neck, and 1.0–2.2 mm wide (19; 20; 21). Neck: 0.5–0.9 mm wide. Testes numerous, estimated number 350–650 (n = 4), oval, 139–149 × 108–143, medullary, beginning 2.0–7.5 mm from the anterior extremity, 0.1–2.4 mm posterior to the first vitelline follicles, reaching up to the anterior margin of the cirrus sac. The cirrus sac is oval to almost spherical, thick-walled, 0.4–0.7 × 0.2–0.6 mm. Ovary bilobed, H-shaped, total width 0.9–1.1 mm; lateral arms 1.2–3.1 mm long and 0.18–0.45 mm wide; anterior arms usually longer and wider than posterior ones, which may be bent inwards to touch each other (18). The vagina is tubular and nearly straight, proximally joining with uterus to form uterovaginal canal that opens into the genital atrium. Seminal receptacle dorsal to ovarian isthmus, 210–373 × 120–260 mm. Vitelline follicles, oval, 106–123 × 84–98, start anterior to the first testes, 0.9–4.3 mm from the anterior extremity, and reach posteriorly alongside the cirrus sac up to the anteriormost uterine coils. A few (1–13) follicles alongside preovarian uterine loops. Preovarian follicles are cortical; postovarian follicles extensive, usually cortical, with some follicles in the medulla (Oros et al., 2010). The uterus is sinuous. Eggs are oval, operculate, and unembryonated, 48–48 × 24–29 (n = 30, intrauterine eggs) and 30–40 × 18–20 (freshly laid eggs) (18).

The morphological characteristics of K. armeniaca were examined using a scanning electron microscope (SEM), with a focus on the tegument's epidermis and anterior body area as shown in figure (1). Numerous microtriches were dispersed throughout the entire body surface, covering the tegument. These tiny projections are thought to be crucial for the intake of nutrients and work together to establish parasites in the host environment. Microthrix were found all over the body, but they were more common in the anterior than the posterior regions. Previous research on Caryophyllidea cystodes found in fish belonging to the family Cyprinidae has also documented such tegumentary structure (12). Additionally, a thorough ultra-morphological examination of khawia was made possible by the high-resolution pictures produced by SEM as shown in (2-4). Specimens of K. armeniaca were gathered from Cyprinidae host fishes in the Kurdistan Region's various aquatic habitats. Important morphological characteristics of the scolex and body surface, which are crucial for differentiating this species from other closely related species, are clearly visible in these micrographs. A strong morphological foundation that may be utilized in conjunction with genetic phylogeny investigations is also provided by comprehensive data regarding their distribution, scolex surface topography, and related glandular structures. A trustworthy foundation for comprehending species divergence and taxonomic relationships within the genus Khawia is provided by this combination of morphological and molecular data (4; 19).

ULTRASTRUCTURAL IDENTIFICATION WITH SEM

The Scanning electron microscope (SEM) suggest that there are variations in scolex shape and surface topography. The scolex is convex and spreading, with deep long ridges and a layered surface. Inter-and intra-species as shown in figure (2-4) Variation Although the overall body plan is identical within the genus, differences in the pattern of stratification and the extent of apical expansion were observed among specimens, indicative of Morphological polymorphism (7).

In recent ultrastructural examinations using scanning electron microscopy (SEM), significant morphological variations have been documented between and within Khawia species. In K. armeniaca, the scolex is typically described as semi-bulbate and a fossette with a smooth anterior margin (Kibet et al., 2021) as shown in figure (2). However, newer studies highlight its remarkable intraspecific polymorphism, particularly in the anterior body region; specimens may exhibit a simple, blunt scolex or one with more pronounced features such as deeply inflected cavities or surface incisions depending on the host and geographic origin (15). In contrast, K. sinensis often presents a wider scolex relative to the body as shown in figure (4), frequently characterized by well-developed sutures and anterior projections at the proximal end (Oros et al., 2010). These sutures are critical for attachment and vary in growth rates and prominence between different specimen types. Recent molecular and morphological revisions suggest that what was previously classified as a single widespread species, such as K. armeniaca, may actually represent a species

complex, further explaining the high degree of variation in scolex depth, urethral-like incisions, and gland development (15; 21; 22; 23).

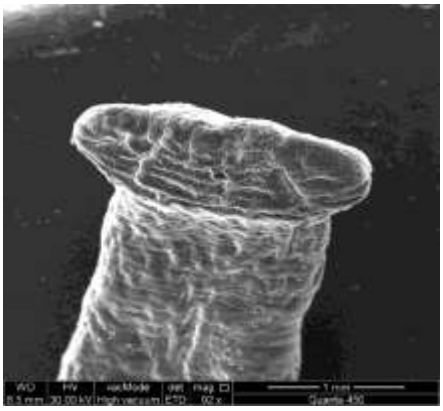


Figure (2) SEM image of Khawia armeniaca showing the morphology of the scolex and anterior part of the body.

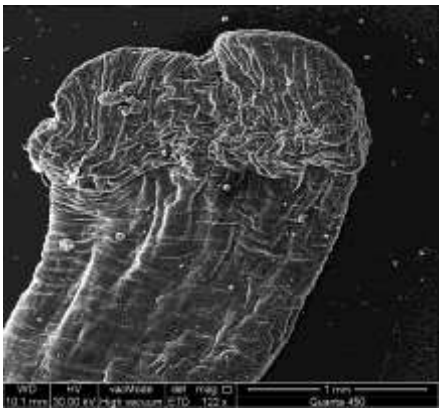


Figure (3) SEM image of Khawia armeniaca showing the morphology of the scolex and anterior part of the body.

Figure (4) SEM image of Khawia sinensis showing the morphology of the scolex.

MOLECULAR IDENTIFICATION

A universal primer of partial 18S rRNA gene were used for species identification as well as variations of nucleoids. The primers could yield a band of 600 bp. The PCR product was electrophoresed and visualized by 1.5% Agarose gel and the results showed 600 bp amplicons fig (5). DNA sequencing, using only forward primer of both genes, was performed separately by ABI 3130X genetic analyzer (Applied Biosystem). The PCR products of the three samples were used as a source of DNA template for sequence-specific PCR amplification.. The findings of the sequence comparison revealed a significant degree of similarity (99.11–100%) with previously published K. armeniaca (100) and K. sinensis (99.11) as shown in (Table 4).

The present sequences were aligned query and compared with GenBank sequence to find out more similarity and nucleotide variation with other targets. BankIt, a WWW-based submission tool with wizards to guide the submission process was used. The GenBank database was intended for new sequence of data that was determined and annotated by the submitter. All sequences were uploaded to GenBank as shown in (Table 3). (20; 21; 24).

These findings support the specimens' taxonomic identification and show that molecular markers are a trustworthy technique for differentiating closely related cestode species (20). Partial 18s rRNA sequence alignment revealed some nucleotide differences among the acquired sequences, indicating genetic inter species polymorphism and variation, the present study specimens were collected from same host and region, hence, suggestion of polymorph is so suggested. These differences, which may be connected to host and regional differentiation, have also been noted in recent molecular investigations of Caryophyllidea cestodes (25). The chromatograms were converted to FASTA format using Finch TV chromatogram viewer software. The DNA sequences in the ABI file were manually edited using BioEdit v.7.0.5. Results of sequence editing were analyzed using BLAST (Basic local alignment search tool) NCBI to indicate homology from the closest species as shown in (Table 4). The phylogenetic tree was constructed using the maximum likelihood method, with (MEGA 11) software program (26; 24).

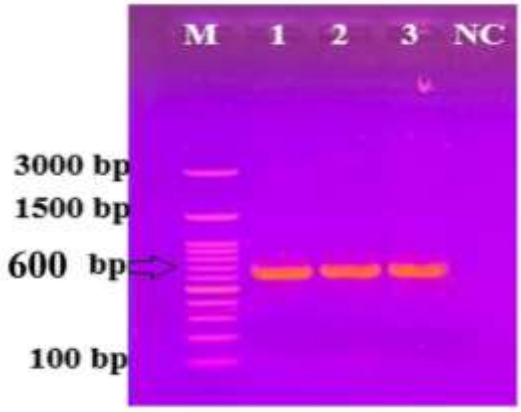


Figure (5) PCR amplification 18S rRNA with amplicon size 600 bp gene from parasites. M; indicate ladder (3000 bp-100 bp) and NC is a negative control**.**

Table (3) GenBank accession no. of 18S rRNA partial gene of Khawia sp.

Name	**Accession No.**	**Gene name**
Khawia armeniaca	PX754524	18S rRNA
Khawia armeniaca	PX754525	18S rRNA
Khawia sinensis	PX754526	18S rRNA

Table (4) Percentage distribution of samples of Khawia sp. into the same parasite according to blast of Genbank NCBI of partial 18S rRNA gene.

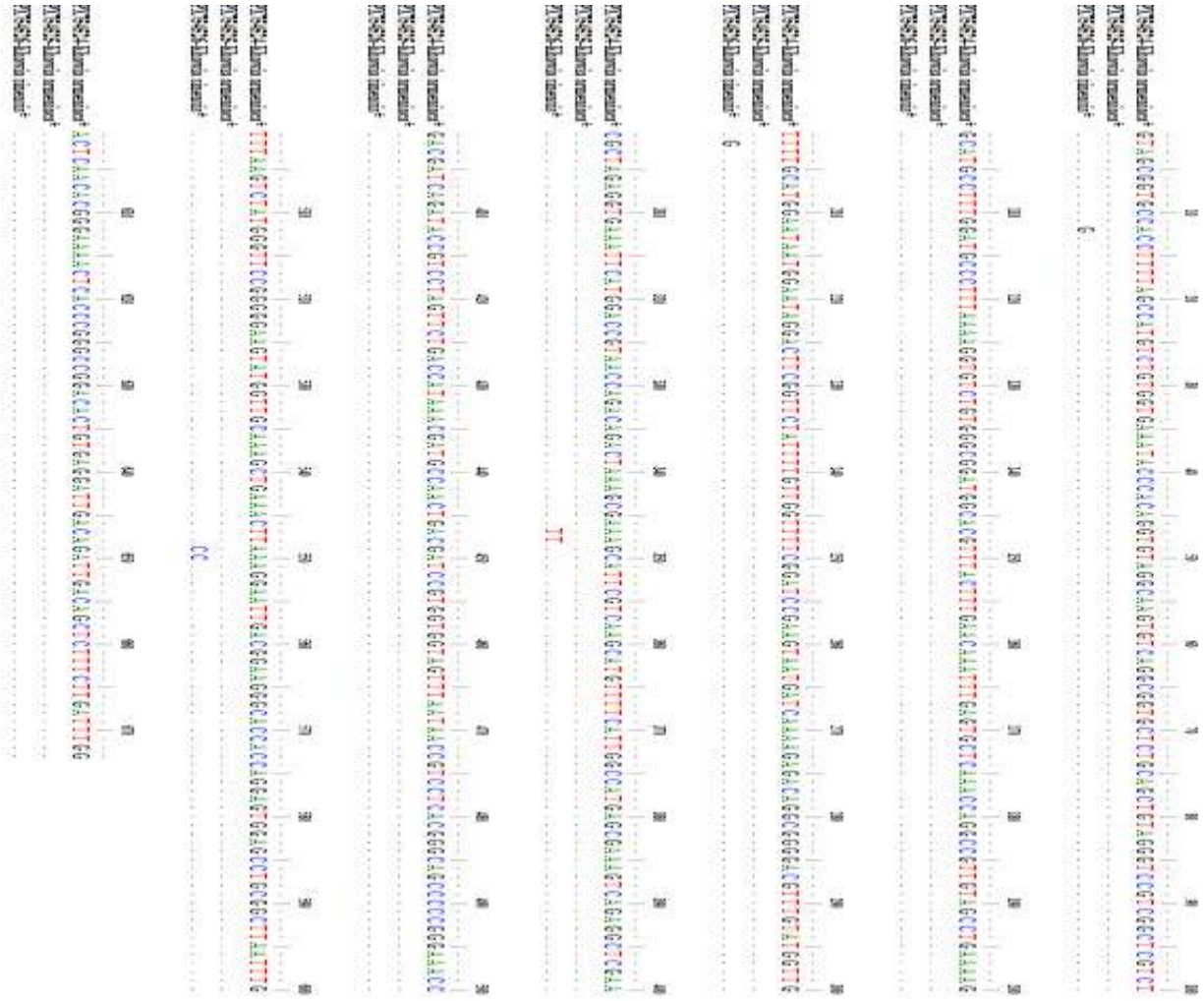
Parasite samples Accession Number	Query Cover %	Identic Number %	Genbank Accession Number	Genbank Identification
PX754524 PX754525	100	100	PV558933	Khawia armeniaca
	100	100	JN004246	
	100	100	PV558932	
	100	100	PV558934	
	100	100	MW027428	
	100	99.70	PV558935	
PX754526	100	99.11	MW027430	Khawia sinensis

The phylogenetic tree constructed by Neighbor-Joining (NJ) in the MEGA 11 program classified the samples in Iraq Erbil into two different groups Figure (6). The samples showed perfect similarity (100%) to GenBank sequences for K. armeniaca strain (PV558933) and had high similarity (99.11%) to K. sinensis (PX754526) strain in (Table 3). The Samples PX754524 and PX754525 were co-classified manually to 100% of the known (homologous) GenBank sequences for Khawia armeniaca species (PV58933). The Sample PX754526 showed curvature with high type (99.11%) Xavia sinensis (MW027430), confirming the distinctive characteristics of the trend diversity (27).

The Maximum Likelihood approach in the MEGA program was used to conduct phylogenetic analysis based on 18S rRNA sequences. The discovered sequences are distributed within the Khawia group and are similar to K. armeniaca sequences found in Cyprinidae fishes in the Eurasian region, according to the phylogenetic tree. The examined samples validate their taxonomic identity within the Caryophyllaeidae family by forming a highly supported monophyletic group with previously described Khawia sequences. According to recent molecular research on parasite systematics, the phylogenetic tree's form also demonstrated a distinct divergence between both recorded in the present study Khawia species or Caryophyllidea (28; 16).

The present study improved that both K. armeniaca and K. sinensis are mono morphic and developed from the sane ancestors and clade Fig (7). These findings demonstrate that a trustworthy framework for accurately identifying and classifying Khawia species that attack freshwater fishes is provided by the combination of morphological, ultrastructural, and g
enetic data.

Figure (6) Multiple nucleotide sequence alignment analysis of 18S rRNA gene among three submitted sequences Khawia sp.



>PX754525-Khawia armeniaca*Length: 673 bp,Type: DNA, Molecular Weight: 167329.48 Da, GC Content: 49.63% Base Composition: A: 170 (25.26%), T: 169 (25.11%), G: 193 (28.68%), C: 141 (20.95%). >PX754526-Khawia sinensis Length: 673 bp, Type: DNA, Molecular Weight: 167304.48 Da, GC Content: 50.22%, Base Composition: A: 165 (24.52%), T: 170 (25.26%), G: 195 (28.97%), C: 143 (21.25%) as show in Table (5) (30).

Table (5) nucleotide frequencies 18S rRNA

Nucleotide	AT%	GC%	T	C	A	G	Variation	Total bp	Gene name
Khawia armeniaca	50.37%	49.63%	169	141	170	193	0.74%	673	18S rRNA
Khawia sinensis	49.78%	50.22%	170	143	165	195	0.44%	673	

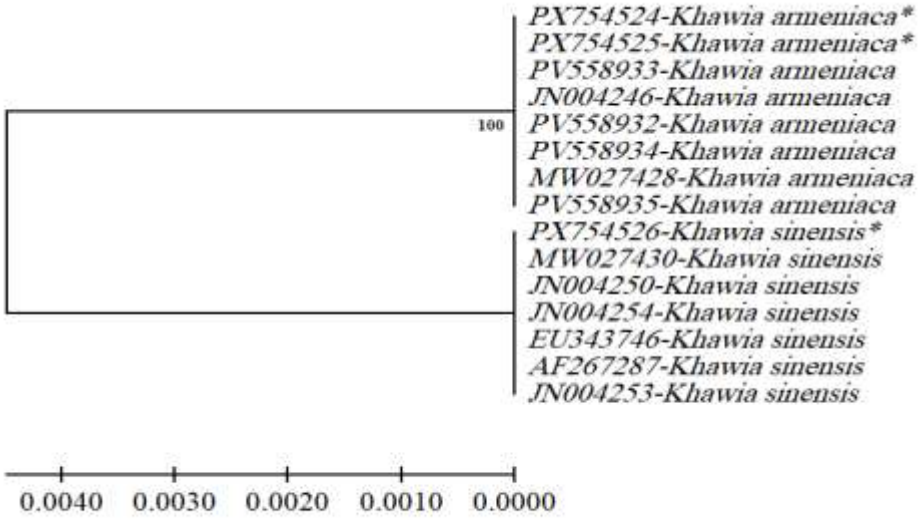


Figure (7) Employing Neighbor joining of Mega 11 program, shows phylogenetic positioning of Iraq: Erbil Khawia sp. with similar GenBank sequences of 18S rRNA that are available in GenBank.

This study indicates that the morphological polymorphism found in the scolex of Khawia species, is often a functional adaptation to self-adhesion within the host gut. SEM imaging recent Studies confirms that these surface changes are not necessarily related to genetic divergence. (2). This dissociation between morphology and genes is crucial for the identification of the Khawia assemblage, where external appearance may mislead common taxonomic efforts. but the Present study showed inter Species variation between K. armenica and K. sinensis also intra-species variation between different K. armenica specimens even their morphological is same

Morphologically, the animals exhibited characteristics typical of the khawia species, including an elongated, sessile body and a scolex devoid of suckers. The descriptions originate from (16; 28). Relying solely on morphology is erroneous; morphological changes (morphological plasticity) induced by the host and environment are associated with recognition inaccuracies.

In the SEM study, many microtriches were identified on the body tegument, especially in the anterior region as shown in figures (2-4). These frauds are significant for sustenance and accommodation in the host, analogous to prior research (26). The ultrastructural variations indicate that SEM is crucial for differentiating closely related species.

Significant nucleotide variations and particular polymorphisms were found in K. armeniaca samples taken from fish belonging to the Cyprinidae family from aquatic sources like the Great Zabi River at the Khabat region. By comparing these nucleotide towels, scientists can identify specific mutations that explain the evolutionary relationships between various Khawia species and their fish readers (Cyprinidae). This has resulted in the discovery of cryptic diversity and similar, albeit morphologically similar species (14; 29).

The discovery of multiple shapes polymorphisms within different and between different species in the 18S rRNA gene is highly possessive as shown in fig (6) (Table 5). A separate unit has a full length of 673 bp (t pair) for the sequence fragment, GC variation of 4.63% in khawia These polymorphisms, which are seen in the order of genes as shown in figure (6), serve as molecular signatures that allow us to separate these species when they appear physically. (30; 27).

The stability of the 18S rDNA region is crucial for distinguishing K. armeniaca from the worldwide distributed K. sinensis. While SEM imaging helps visualize the polymorphism of the surface adapter (scolex), it is the sequence fragment (673 bp) and its unique spit structure that allows the researchers to confirm species identity with 100% bootstrap support. This molecular approach is of growing importance in the Kurdistan Region, where anthropogenic factors and fish migration patterns lead to mixed outbreaks of native and invasive helminths (31; 32).

The investigation of the 18S rRNA gene revealed a remarkable similarity (99–100%) with K. armeniaca and its related species. This verifies that this gene is among the most effective markers for cestode identification (28). Nevertheless, several nucleotide variations were noted, potentially signifying intraspecific polymorphism or the onset of genetic differentiation within a specific region.

Phylogenetic analysis reinforces the "expected lines" of caryophyllid evolution, in which species belonging to the same genus are clustered closely together. The 100% bootstrap support for the K. armeniaca collection indicates a high level of genetic stability for this species in the Erbil region (Al-Zubaidi & Al-Niaeemi, 2024) as shown in (Table 3). The presence of K. sinensis, a species often linked to global movements of cyprinid fishes alongside the native regional species K. armeniaca indicates a diversity in parasitic organisms that may be influenced by both local environmental and anthropogenic factors (2).

This study demonstrated that Khawia species in Erbil from the Kurdistan Region exhibit identical physical traits, although possess certain genetic variations. The integration of SEM and 18S rRNA gene analysis constitutes an effective methodology for the identification and sequencing of these parasites. In the future, more molecular markers, such as COI, should be utilized, and samples from diverse places and hosts should be incorporated to enhance the comprehension of polymorphism and evolutionary links.

REFERENCES

(1) Scholz, T., Brabec, J., Oros, M., Bazsalovicsoň, E., Ermolenko, A. V., Hanzelová, V., and KováHromadová, I. (2011). Khawia spp. revision. (Cestoda: Caryophyllidea), cyprinid fish parasites, together with a molecular phylogeny and identification key. Folia Parasitologica, 58(3), 197. https://doi.org/10.14411/fp.2011.020

(2) Uhrovič, D., Oros, M., Kudlai, O., & Scholz, T. (2025). The diversity of Khawia tapeworms (Cestoda: Caryophyllidea) of barbels (Cyprinidae: Barbinae) in the western Palearctic: the existence of vicariant lineages and the description of a new species. Parasitology International, 94, 102712. https://doi.org/10.1016/j.parint.2024.102712

(3) Jang, Y., Lee, C. S., Nam, S. W., Kim, J., Cho, H. S., and Lee, J. (2025). Novel species and intraspecific taxa in Desmodesmus (Scenedesmaceae) are described morphologically and molecularly. ALGAE, 40(3), 163. https://doi.org/10.4490/algae.2025.40.8.7

(4) Bilal, S. J., Al-Saeed, A. T., Al-Taee, A.-R. A., Al-Niaeem, K. S., Ali, L. A., Abdullah, S. M. A., Abdul Rahman, Z. F. A., Rahemo, Z. I. F. (2013). An ultra- and molecular analysis of some nematodes and cestodes that parasitize certain freshwater fish in the Kurdistan region of Iraq.

(5) Brabec, J., Scholz, T. & Oros, M. (2023). Recent advances in the systematics and molecular phylogeny of Caryophyllidea (Platyhelminthes: Cestoda). Parasitology International, 92, 102707.

(6) Koutná K, Benovics M, Scholz T. (2025).The diversity of Khawia tapeworms (Cestoda: Caryophyllidea) of barbels (Cyprinidae: Barbinae) in the western Palearctic: the existence of vicariant lineages and the description of a new species from the Iberian Peninsula. Parasitology. 2025;152(6):618-631. doi:10.1017/S0031182025100206

(7) Uhrovič, D., Oros, M., Kudlai, O., Choudhury, A., & Scholz, T. (2021). Molecular evidence of three closely related species of Biacetabulum Hunter, 1927 (Cestoda: Caryophyllidea): a case of recent speciation in different fish hosts (Catostomidae)? Parasitology, 148(9), 1040–1056. https://doi.org/10.1017/s0031182021000743.

(8) Caña-Bozada, V.H., Mera-Loor, G.B., Hernández-Mena, D.I., Justine, J.-L. & Álvarez-Presas, M. (2025). Evaluating topological variability in Neodermata phylogenies using mitochondrial and ribosomal gene markers. PLOS ONE, 20(10): e0333282.

(9) Abdullah, A., & Mohammed, A. (2022). Water Scarcity in Erbil City: Problems and Innovative Solution. 4th International Conference on Communication Engineering and Computer Science (CIC-COCOS'2022), 105–113. https://doi.org/10.24086/cocos2022/paper.760 Cited by: 1

(10) Mamand, B. S., & Mawlood, D. K. (2024). Identifying sources of groundwater and recharge zone using stable environmental isotopes in the Erbil basin-northern Iraq. Kuwait Journal of Science, 51, 100128. https://doi.org/10.1016/j.kjs.2023.09.002

(11) Froese, R., & Pauly, D. (Eds.). (2026). FishBase. World Wide Web electronic publication. www.fishbase.org, version (03/2026).

(12) Kuchta, R., and Brabec, J. & Scholz, T. (2022). Ribosomal DNA sequences are used to infer the molecular phylogeny of the Caryophyllidea (Platyhelminthes: Cestoda). Systematic Parasitology, 98, pp. 1–15.

(13) Margolis, L. G.; Esch, W. J.; Holmes, C.; Kuris, A. M. and Schad, G. A. (1982). The Use of Ecological Terms in Parasitology (Report of an Ad Hoc Committee of the American Society of Parasitologists). The Journal of Parasitology, 68(1)::131-133. https://doi.org/10.2307/3281335
(14) Bilal, S. J., Abdullah, S. M. A., & Rahemo, Z. I. F. (2017). Further histological and ultrastructural observations on the cestode, Senga mastacembeli sp. n. with the revision of genus specieses. Eurasian Journal of Science and Engineering, 3(1), 27–37. https://doi.org/10.23918/eajse.v3i1sip27
(15) Shigoley, M. I., Kmentová, N., Ndegwa, D. M., Topić, M., Thys, K. J. M., & Vanhove, M. P. M. (2025). The diversity of Khawia tapeworms (Cestoda: Caryophyllidea) in the western Palearctic: morphological variability and genetic evidence for a species complex. Parasitology International, 100, 102845. https://doi.org/10.1016/j.parint.2024.102845
(16) Scholz, T. & Oros, M. (2021). Recent developments in taxonomy and phylogeny of freshwater fish caryophyllidean tapeworms. International Journal of Parasitology, 51, 897–908.
(17) Scholz, T., Waeschenbach, A., Oros, M., Brabec, J., & Littlewood, D. T. J. (2025). The diversity of Khawia tapeworms (Cestoda: Caryophyllidea) of barbels (Cyprinidae: Barbinae) in the Western Palearctic: The existence of vicariant lineages and the description of a new species from the Iberian Peninsula. Parasitology, 152(6), 1–14. https://doi.org/10.1017/S003118202500045X
(18) Scholz, T., Oros, M., & Kuchta, R. (2026). Khawia japonensis (Cestoda), the Asian parasite of common carp, continues to spread in Central European countries: Distribution, infection indices, and histopathology. Journal of Helminthology, 100(1), e15. https://doi.org/10.1017/S0022149X2500012X
(19) Koutná, K. et al. (2024). Genetic and morphological variability of the tapeworm Khawia armeniaca (Cestoda: Caryophyllidea) reveals distinct lineages across its distribution range. conference abstract. https://www.muni.cz/en/research/publications/2484207?utm_source=chatgpt.com
(20) Oros, M., Scholz, T., Hanzelová, V., & Mackiewicz, J. S. (2010). Scolex morphology of monozoic cestodes (Caryophyllidea) from the Palaearctic Region: A useful tool for species identification. Folia Parasitologica, 57(1), 37–46. https://doi.org/10.14411/fp.2010.005
(21) Sayers, E.W., Cavanaugh, M., Clark, K., Pruitt, K.D., Schoch, C.L., Sherry, S.T. and Karsch-Mizrachi, I. (2022) GenBank. Nucleic Acids Research, 50(D1), D161-D164. https://doi.org/10.1093/nar/gkab1135
(22) Oros, M., Uhrovič, D., Choudhury, A., Mackiewicz, J. S., & Scholz, T. (2020). Scolex morphology of monozoic tapeworms (Caryophyllidea) from the Nearctic Region: taxonomic and evolutionary implications. Folia Parasitologica, 67, Article 003. https://doi.org/10.14411/fp.2020.003
(23) Kibet, C. J., Zhao, W. T., Sarwar, H., & Nie, P. (2021). Redescription of the monozoic cestode Khawia armeniaca (Cholodkovsky, 1915) Shulman, 1958 (Cestoda: Caryophyllidea) from cyprinid fish in Lake Tana, Ethiopia. Journal of Lake Sciences, 33(3), 913–922. https://doi.org/10.7541/2021.2020.095
(24) Camacho, G. I., Sanabria, M. A., & Rodríguez, C. E. (2023). Morphology and molecular phylogeny of trypanorhynchid larvae (Cestoda) infesting marine fish species. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 75(1), 71–82. https://doi.org/10.1590/1678-4162-12711
(25) Scholz, T. & R. Kuchta, 2022. Caryophyllidean cestode diversity and host relationships in freshwater fish from Europe. International Parasitology, 89, 102583.
(26) Stecher, G., and Tamura, K. together with Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis, version 11 (2021). Evolution and Molecular Biology, 38(7), 3022-3027. Doi: https://doi.org/10.1093/molbev/msab120
(27) Mustafa, J. S., & Mawlood, D. K. (2024). Developing three-dimensional groundwater flow modeling for the Erbil Basin using Groundwater Modeling System (GMS). Journal of Groundwater Science and Engineering, 12(2), 178–189. https://doi.org/10.26599/jgse.2024.9280014
(28) Brabec, J., and Kuchta, R. & Scholz, T. (2022). The surface appearance and ultrastructure of cyprinid fish parasitized by caryophyllidean cestodes. Research on Parasitology, 121, 1043–1054.
(29) Dumendiak, S., et al. (2025) Hidden diversity of cestodes in wild African carnivores. International Journal for Parasitology: Parasites and Wildlife.
(30) Al-Zubaidi, A. M. H., & Al-Niaeemi, B. H. (2024). Molecular phylogeny and genetic diversity of caryophyllid cestodes in northern Iraq: A MEGA 11-based analysis. Baghdad Science Journal, 21(3), 890–905.
(31) Shareef, C. M., & Aziz, F. H. (2023). New records of non-diatom algae in aquatic habitat along Greater Zab River path from Bekhma to Gwer District in Erbil Province, Iraq. Baghdad Science Journal, 20(5). https://doi.org/10.21123/bsj.2023.7657
(32) Kang, D., Choi, J.H., Kim, M., Yun, S., Oh, S., Yi, M.-H., Yong, T.-S., Lee, Y.A., Shin, M.H. & Kim, J.Y. (2024) 18S rRNA metabarcoding optimization for concurrent intestinal parasite identification. Scientific Reports, 14(1), 25049. https://doi.org/10.1038/s41598-024-76304-1