

Morphological and Molecular Characterization of *Dicrocoelium dendriticum* isolated from livestock in Erbil, Iraq

Maryam Thamir Abduljabbar^{1✉}, Qaraman Mamakhidr Koyee²

Department of Biology, College of Science, Salahaddin University-Erbil, Iraq.

ORCID: ¹ <https://orcid.org/0009-0000-2560-9662>, ² <https://orcid.org/0000-0003-1265-4282>

Corresponding Email: maryam.abduljabbar@su.edu.krd

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Abstract:

Background: One of the most significant ruminant helminthic parasites that are present across the world are liver flukes, which cause economically significant diseases in domestic animals. Among the liver flukes are *Dicrocoelium* spp., which inhabit the gallbladder and hepatic bile ducts of both domestic and wild ruminants. Numerous species of the *Dicrocoelium*, are responsible for the zoonotic disease known as dicrocoeliosis.

Aims: The primary goal of this research is given comprehensive characterization of *D. dendriticum* isolated from the liver of sheep and cattle in Erbil, Kurdistan region-Iraq, through morphological examination and molecular analysis, including phylogenetic analysis, haplotype network, and secondary structure prediction

Results: Morphological and molecular data confirmed the identity of the Iraqi isolates of *D. dendriticum*, and phylogenetic tree showed that Iraqi *D. dendriticum* clusters closely related with *D. dendriticum*, forming a distinct subclade supported by bootstrap values ranging from 65% to 100%. Haplotype analysis and RNA secondary structure indicate genetic variation.

Conclusions: In animal slaughterhouses, clinical diagnosis of *D. dendriticum* is difficult, particularly due to its small size and the presence of mild infections. Therefore, more information about this zoonotic parasite is necessary.

Keyword: *Dicrocoelium dendriticum*, phylogenetic tree, haplotype analysis, RNA secondary structure, Iraq



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Introduction:

Digenean trematode parasites were as liver flukes cause economically significant diseases in domestic animals (Howell & Williams, 2020; Karim et al., 2023). Numerous species of the genus *Dicrocoelium* including: *D. chinensis*, *D. hospes*, and *D. dendriticum*, Inhabitants of the gallbladder and hepatic bile ducts of domestic and wild ruminants, are responsible for the zoonotic disease known as dicrocoeliosis (Kreshchenko et al., 2022; Scala et al., 2019). *Dicrocoelium* spp. have indirect life cycle that can take up to six months to complete. Adults in the bile ducts are monoecious, sexually reproducing, and self-fertile. Eggs containing fully formed miracidia (Khan et al., 2021). Farm animals are important for human nutrition as well as the growth and development of society. About 15% of energy and 30% of protein globally come from animal products like milk and meat, which are also an important source of nutrients and energy. With rising populations and incomes, the usage of animal protein per person in emerging nations is expected to rise by about 73% by 2050, along with the world's meat consumption (Arbabi et al., 2018; Azeez & Yassin, 2024). However, in reality, dicrocoeliosis is seldom linked to reductions in animal production performance, as the illness is still underrecognized in field settings due to its subclinical development. Veterinarians and farmers may undervalue the significance of dicrocoeliosis in ruminants because its pathological effects can occasionally be overshadowed by concurrent liver infections (such as Cystic echinococcosis, *Taenia hydatigena*, cysticercosis, and fasciolosis). Even infections with 4000 parasites can produce moderate symptoms, while infected animals with a parasitic load of less than 1000 *D. dendriticum* individuals often do not exhibit any clinical signs (Scala et al., 2019). *Dicrocoelium* is more commonly associated with asymptomatic or spurious infections than other liver flukes like *Clonorchis* and *Fasciola*, and it is regularly found in the feces of individuals who have consumed infected cattle liver (Paranjpe et al., 2020).

For a long time, this parasite was mistaken for an immature form of *Fasciola hepatica* due to the common coexistence of both trematodes in the liver of ruminants (Manga-González & Ferreras, 2019). The primary methods for diagnosing ruminant dicrocoeliosis include post-mortem examination of the animal's liver and gallbladder, collection of parasites identified in these organs, identification of the eggs in the host's feces, and immunological techniques (Manga-González & Ferreras, 2014). As an alternative, nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) sequences are used in molecular data to indicate genetic variations of parasites (Zhao et al., 2013). Despite all that, it has been shown that mitochondrial genomes are valuable source of genetic markers for taxonomic identification, investigations of intra- and interspecific variation, systematics, and phylogenetic study of trematodes at various taxonomic levels, including those belonging to the dicrocoeliidae (Suleman et al., 2020). However, 28S rDNA evolves more slowly than the mitochondrial genes used to delineate species; it can be used to infer phylogenies above the species level (Čkrkić et al., 2020).

The primary goal of this study is performing comprehensive morphological examination and to understand the genetic diversity and phylogenetic relationship of Iraqi *D. dendriticum* and compare

them to those reported from other countries using 28S rDNA through haplotype network, and secondary RNA structure prediction.

Materials and Methods:

Study area and sampling

Infected Liver samples with dicrocoeliosis were collected from sheep and cattle, that were slaughtered at the Erbil Governorate's slaughterhouses between July and September of 2024. The samples were collected directly from the liver and carefully transferred to 99% ethanol as a preservative solution.

Morphological identification

Collected liver flukes were examined microscopically using dissecting microscope and light microscope using diagnostic keys described by Khan et al. (2021).

DNA Extraction and PCR amplification

Genomic DNA extraction was carried out from whole worm bodies, which were crushed between two pieces of clean foil made of aluminum. The DNA was then isolated using the DNaesy Blood and Tissue kit (Qiagen, Germany), according to the manufacturer's protocol.

Partial large subunit (LSU) rDNA (variable regions D1–D3) was employed to determine the species of parasite. The primer pairs used included LSU-5 (5-TAGGTCGACCCGCTGAAYTTAAGCA-3) and 1500R (5'-GCTATCCTGAGGGAACTTCG-3') (Littlewood et al., 2000; Olson et al., 2003). About 25 µl of PCR mix was made to amplify the partial sequences of 28S rDNA which included: 12.5 µl of master mix (Ampliqon PCR Enzymes & Reagents, Denmark), 0.2 µM of each primer, 3 µl of DNA, and 6.5 µl of ddH₂O.

PCR (PCRmax Alpha thermal cycler, UK) reaction performed using modified method of Littlewood et al. (2000) under the following cycle conditions: denaturation for 5 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1min at 58°C, 2 min at 72°C; and a 10 min extension at 72°C. To validate the PCR results, a 1.3% agarose gel was employed and Gel purification kit (QIAquick Gel Extraction, Qiagen, Germany) was employed in accordance with the manufacturer's instructions to purify PCR products.

Sequencing and Accession number

Out of three PCR products, the one with high quality was selected for DNA sequencing. Which was carried out using an ABI 3730XLs nucleotide sequence analyzer through MacroGen Inc. (Korea). The obtained sequencing results were improved by eliminating any low-quality portions by using Geneious Prime software, and consensus sequences are produced using the same program. The final sequences obtained after trimming low quality region 1061 bp reverse and 712 bp forward. After that, the

sequence was put to the BLAST and aligned with other stored sequences of *Dicrocoelium* spp. from GenBank. Finally, improved versions of the sequences through sequential steps were submitted to the GenBank database to receive an accession number.

Phylogenetic analysis

The phylogenetic tree was constructed using MEGA 12 software. It was generated using the Maximum Likelihood method and the Kimura (1980) 2-parameter model (Kimura, 1980) of nucleotide substitutions, and the tree with the highest log likelihood (-4,766.16) is presented. The percentage of replicate (1,000 replicates) trees in which the associated taxa clustered together is indicated above the branches (Felsenstein, 1985). The initial tree for the heuristic search was obtained by selecting the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) and a Maximum Parsimony (MP) tree. The NJ tree was constructed using a matrix of pairwise distances computed using the p-distance (Nei & Kumar, 2000). The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The analytical procedure encompassed 17 nucleotide sequences with 1,979 positions in the final dataset. NCBI BLAST was used to obtain the sequences of the neighboring species *D. dendriticum*. Sequence of *Fasciola hepatica* used as outgroup (accession number JQ999969).

Haplotype network

To visualize the genetic relationship among rDNA sequences of *Dicrocoelium*, a haplotype network was constructed. The analysis requires that sequences be free of ambiguous nucleotides because their presence would lower the quality of the haplotype network, and the reference sequences utilized are displayed in Table 1. MEGA 12 is used in the first phase to align the sequences, followed by DnaSP6 (Rozas et al., 2017) for DNA polymorphism and Popart (Leigh & Bryant, 2015) for haplotype network construction.

Table (1): The reference sequences used in haplotype network of *D. dendriticum*

Host	Accession number	Location
Sheep	OL455743, JQ966973, JN835427, JN835425, JN835430, JN835432, JN835436, JN835438, JN835440	Iran
	MH048705	India
	MG004688	Italy
	HM026461	Slovakia
Goat	JN835426, JN835428, JN835429, JN835431, JN835435, JN835437, JN835439	Iran
	KC774514, KC774523, KC774524	China
	MH683568	Turkey
Cow	MH683567	Turkey
	LC159514	China
Serow	AB369980, AB369981, AB367789	Japan
Yak	LC159516	China
Deer	MH683570	Turkey
Unknown host	MN831473, MN831475, MN831474	Iran
Cattle	PV862035, PV862036	Iraq

RNA secondary structure

The Vienna RNA Package's online RNA fold Web Server (Lorenz et al., 2011) was used to estimate the secondary structure of *Dicrocoelium* 28S rDNA. It is necessary to change the thymine (T) nucleotide with uracil (U) in order to transform rDNA into RNA prior to analysis, and the default parameter was used in the analysis.

Ethics approval: The current study was approved by the Research Ethics Committee Office (RECO) of the Department of Biology, College of Science, Salahaddin University-Erbil. The ethical approval was issued on 7 October 2024 under the reference number 4S/357. All parasite samples were collected from livestock that had been legally slaughtered for food consumption, and none were injured or harmed specifically for the purpose of this study.

Results:

Morphological Analysis

Slaughtered animals' liver and bile duct sections with *Dicrocoelium* sp. have been shown in Figure 1. The adult worm had a semi-transparent appearance and lanceolate body shape, measuring approximately 5-9.9 mm in length and 1-2 mm in width. The oral sucker is smaller than the ventral sucker. The two testes are situated immediately below the acetabulum, and their shapes range from

circular to weakly or heavily lobed, with round ovary situated below the testes. The vitellaria extended laterally along the liver fluke body, and the uterus filled the trematode's hind body and occupied the central portion, including ascending and descending branches, and was filled with numerous eggs (Figure 2a). The eggs of *Dicrocoelium* sp. are operculated with thick shells, and the average size of the eggs ranged from 35 to 45 μ m in length and 22 to 30 μ m in width (Figure 2b).

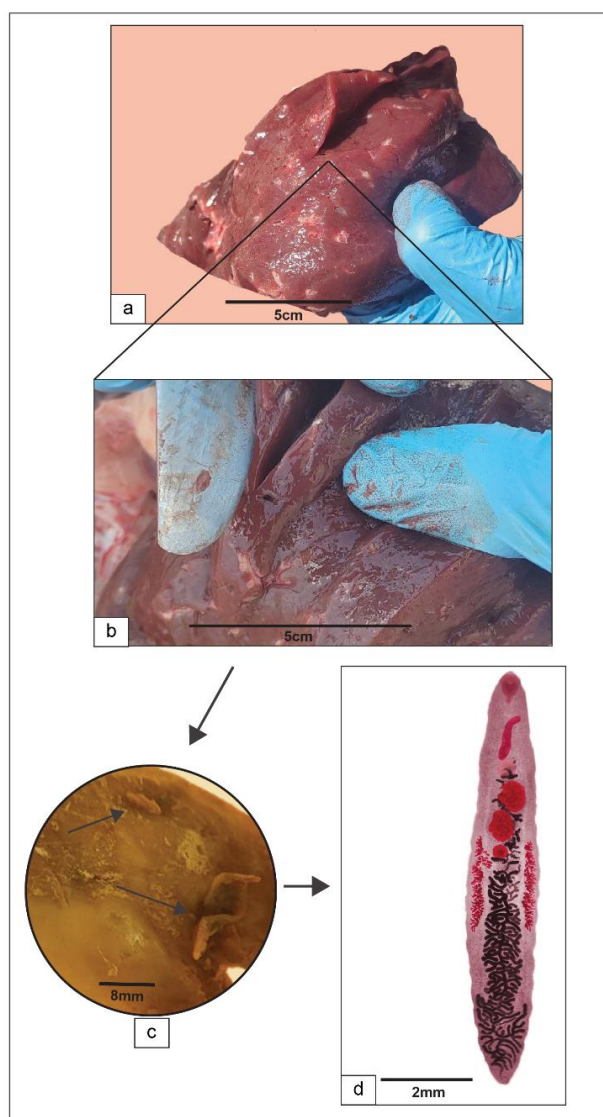


Figure (1): A piece of liver tissue displaying the parasite *Dicrocoelium*'s extraction site
a, b: Examined liver infected with *D. dendriticum*
c: The arrows indicate *D. dendriticum* on pieces of liver tissue under dissection microscope
d: *D. dendriticum* under dissection microscope; the image was digitally colored for better visualization by Adobe Photoshop.

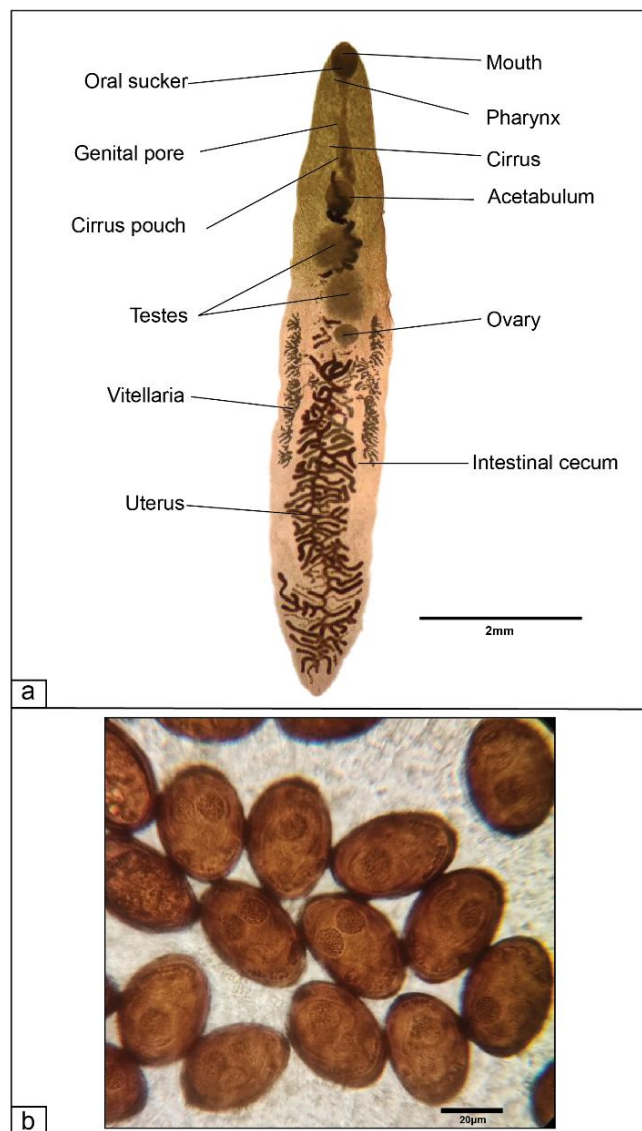


Figure (2): *Dicrocoelium* sp. under dissecting microscope highlighting the key microscopic anatomical features and operculated eggs under light microscope.

a: *D. dendriticum* under dissecting microscope (2.5X).

b: Eggs of *D. dendriticum* under light microscope (100X).

Molecular analysis

The result sequences recorded in the NCBI with accession numbers PV862035 and PV862036. To validate the accuracy of DNA sequencing, chromatograms were used (Figure 3 and Figure 4). Each colored peak representing one of the four nucleotides, and when the peak appears sharp and evenly,

they indicate high quality sequencing output (Koyee & Abdullah, 2023). The analysis showed that 99.5% of the harvested sequence was *D. dendriticum* as demonstrated in Figure 5.

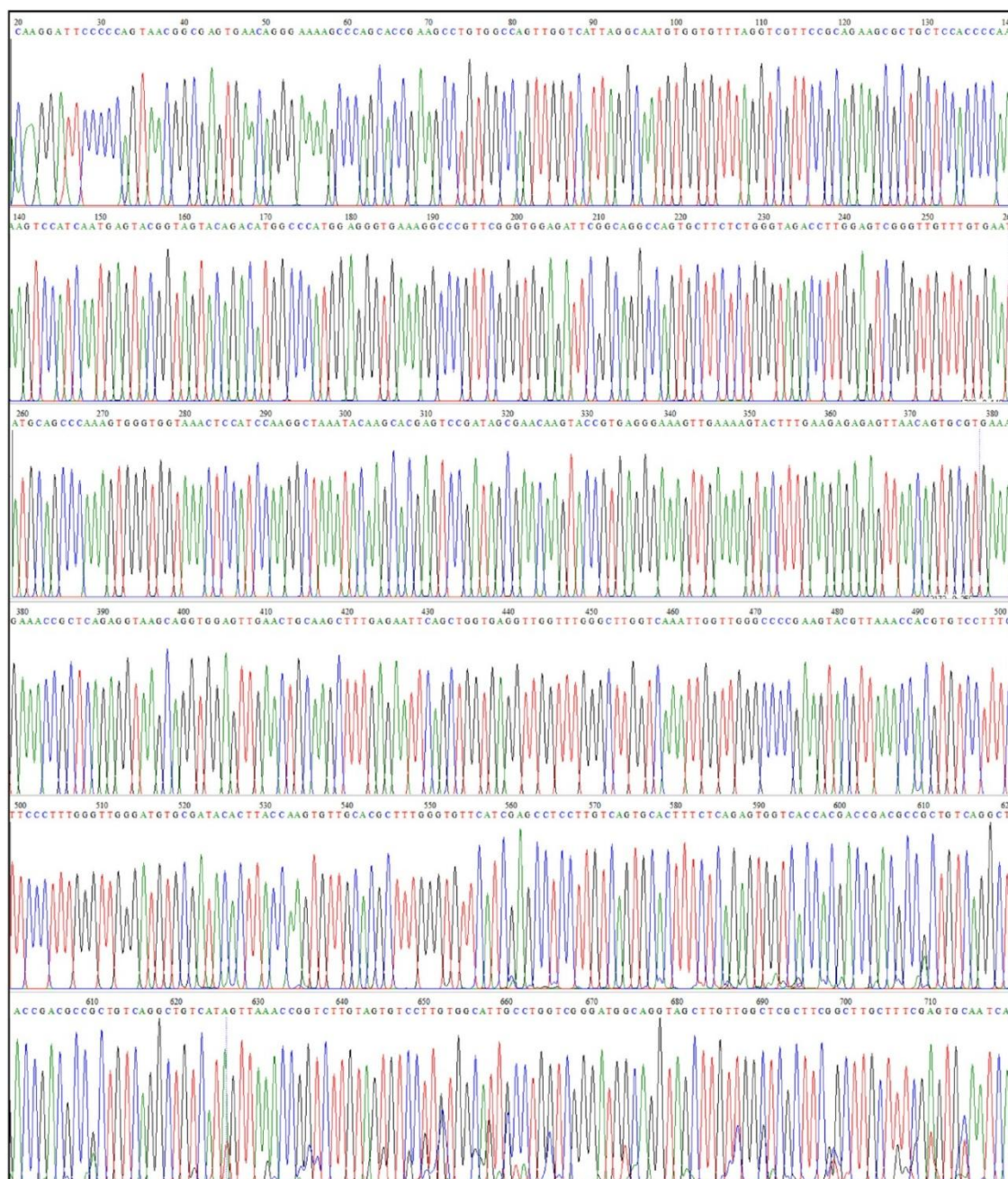


Figure (3): The chromatogram of PCR products, forward sequence (Accession number: PV862035).

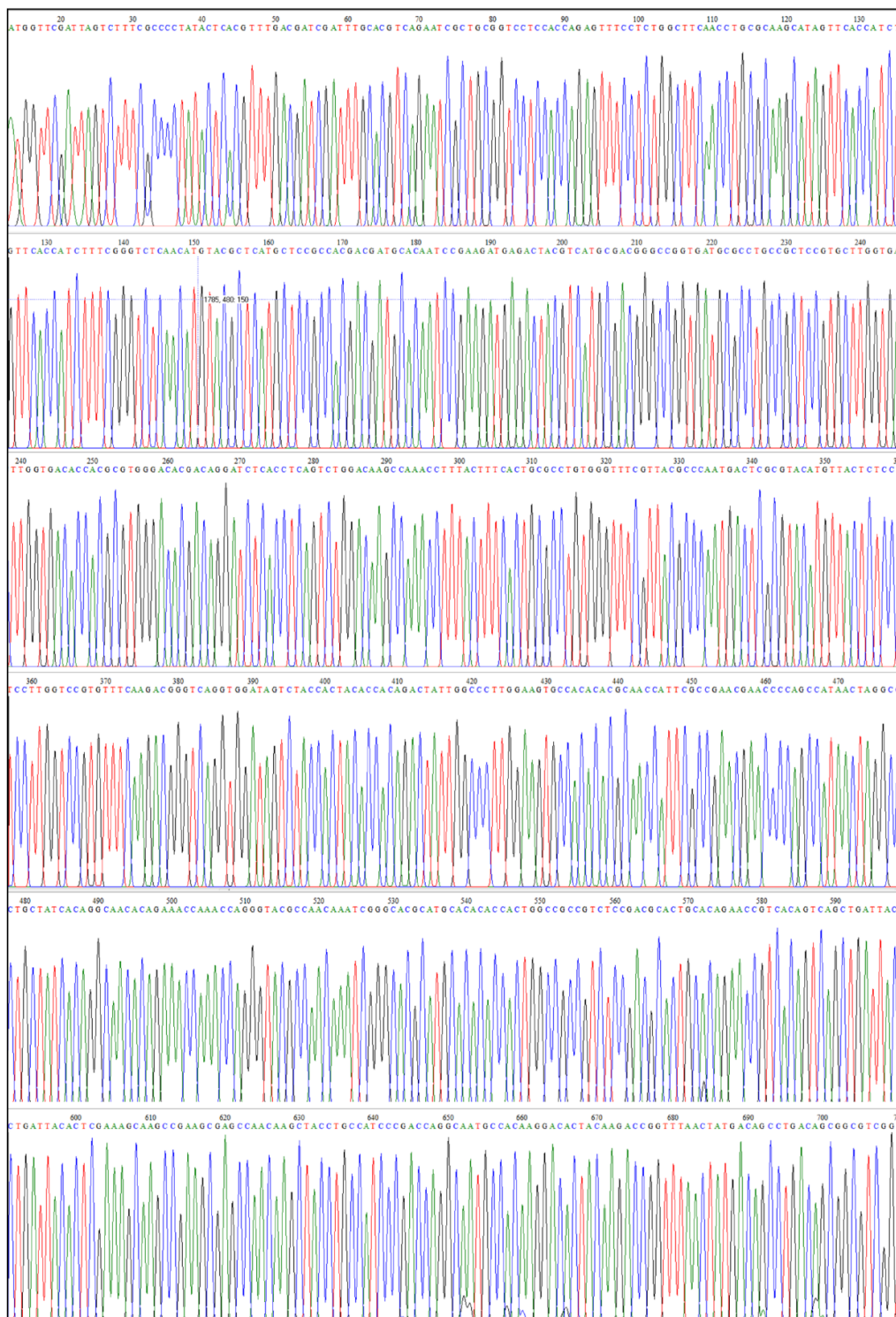


Figure (4): The chromatogram of PCR products, reverse sequence (Accession number: PV862036).

Score	Expect	Identities	Gaps	Strand
1267 bits(686)	0.0	691/694(99%)	0/694(0%)	Plus/Plus
Query 19	CGGCGAGTGAACAGGGAAAAGCCCAGCACCGAAGCCTGTGGCCAGTTGGTCATTAGGCAA	78		
Sbjct 1	CGGCGAGTGAACAGGGAAAAGCCCAGCACCGAAGCCTGTGGCCAGTTGGTCATTAGGCAA	60		
Query 79	TGTGGTGTGTTAGGTCGTTCCGCAGAAGCGTGCTCCACCCCAAGTCCATCAATGAGTACG	138		
Sbjct 61	TGTGGTGTGTTAGGTCGTTCCGCAGAAGCGTGCTCCACCCCAAGTCCATCAATGAGTACG	120		
Query 139	GTAGTACAGACATGGCCCATGGAGGGTGAAAGGCCCGTTGCGGTGGAGATTCGGCAGGCC	198		
Sbjct 121	GTAGTACAGACATGGCCCATGGAGGGTGAAAGGCCCGTTGCGGTGGAGATTCGGCAGGCC	180		
Query 199	AGTGCTTCTCTGGGTAGACCTTGAGTTCGGGTGTTTGTGAATGCAGCCCAAAGTGGGTG	258		
Sbjct 181	AGTGCTTCTCTGGGTAGACCTTGAGTTCGGGTGTTTGTGAATGCAGCCCAAAGTGGGTG	240		
Query 259	GTAAACTCCATCCAAGGCTAAATACAAGCACGAGTCCGATAGCGAACAAGTACCGTGAGG	318		
Sbjct 241	GTAAACTCCATCCAAGGCTAAATACAAGCACGAGTCCGATAGCGAACAAGTACCGTGAGG	300		
Query 319	GAAAGTTGAAAAGTACTTTGAAGAGAGAGTTAACAGTGCCTGAAACCGCTCAGAGGTAAG	378		
Sbjct 301	GAAAGTTGAAAAGTACTTTGAAGAGAGAGTTAACAGTGCCTGAAACCGCTCAGAGGTAAG	360		
Query 379	CAGGTGGAGTTGAACTGCAAGCTTTGAGAATTCAGCTGGTGAGGTTGGTTTGGGCTTGGT	438		
Sbjct 361	CAGGTGGAGTTGAACTGCAAGCTTTGAGAATTCAGCTGGTGAGGTTGGTTTGGGCTTGGT	420		
Query 439	CAAATTGGTTGGGCCCCGAAGTACGTTAAACCACGTGTCCTTTCCCTTTGGGTTGGGATG	498		
Sbjct 421	CAAATTGGTTGGGCCCCGAAGTACGTTAAACCACGTGTCCTTTCCCTTTGGGTTGGGATG	480		
Query 499	TGCGATACACTTACCAAGTGTGACGCTTTGGGTGTTTCATCGAGCCTCCTTGTCAGTGC	558		
Sbjct 481	TGCGATACACTTACCAAGTGTGACGCTTTGGGTGTTTCATCGAGCCTCCTTGTCAGTGC	540		
Query 559	ACTTTCTCAGAGTGGTCACCACGACCGACGCCGCTGTGAGGCTGTCATAGTTAAACCGGT	618		
Sbjct 541	ACTTTCTCAGAGTGGTCACCACGACCGACGCCGCTGTGAGGCTGTCATAGTTAAACCGGT	600		
Query 619	CTTGTAAGTGTCTTGTGGCATTGCCTGGTCGGGATGGCAGGTAGCTTGTGGCTCGCTTC	678		
Sbjct 601	CTTGTAAGTGTCTTGTGGCATTGCCTGGTCGGGATGGCAGGTAGCTTGTGGCTCGCTTC	660		
Query 679	GGCTTGCTTTTCGAGTGCAATCAACTGACTGTGAC	712		
Sbjct 661	GGCTTGCTTTTCGAGTGCAATCAGCTGACTGTGAC	694		

Figure (5): Pairwise alignment of *D. dendriticum* 28S rDNA sequence. Query represents the sample sequence while the subject (Sbjct) represents the sequence that was taken from GenBank. The identity percentage was shown as 99% with the sbjct sequence (AF151939). Without any deletions noticed from total 712 amplified bp.

In the present study, the consensus sequence of Iraqi *D. dendriticum* was used and clustered closely with *D. dendriticum*, particularly isolates from Iran (OL455743, MN83147X) and Turkey (MH683567), forming a distinct subclade with a bootstrap value ranging from 65% to 100%. It is clearly the Iraqi specimen separated from other *D. dendriticum* sequences (MT539115, JQ081959, and AF151939), demonstrating bootstrap values of 83% and 100%, and separated from a cluster of *D. hospes* (MT8587XX), indicating possible genetic divergence within the species. *Fasciola hepatica* is used as an outgroup, which guides the direction of evolutionary divergence. Consequently, the phylogenetic tree supports that the consensus sequence of the *D. dendriticum* Iraqi specimen belongs to *D. dendriticum* (Figure 6).

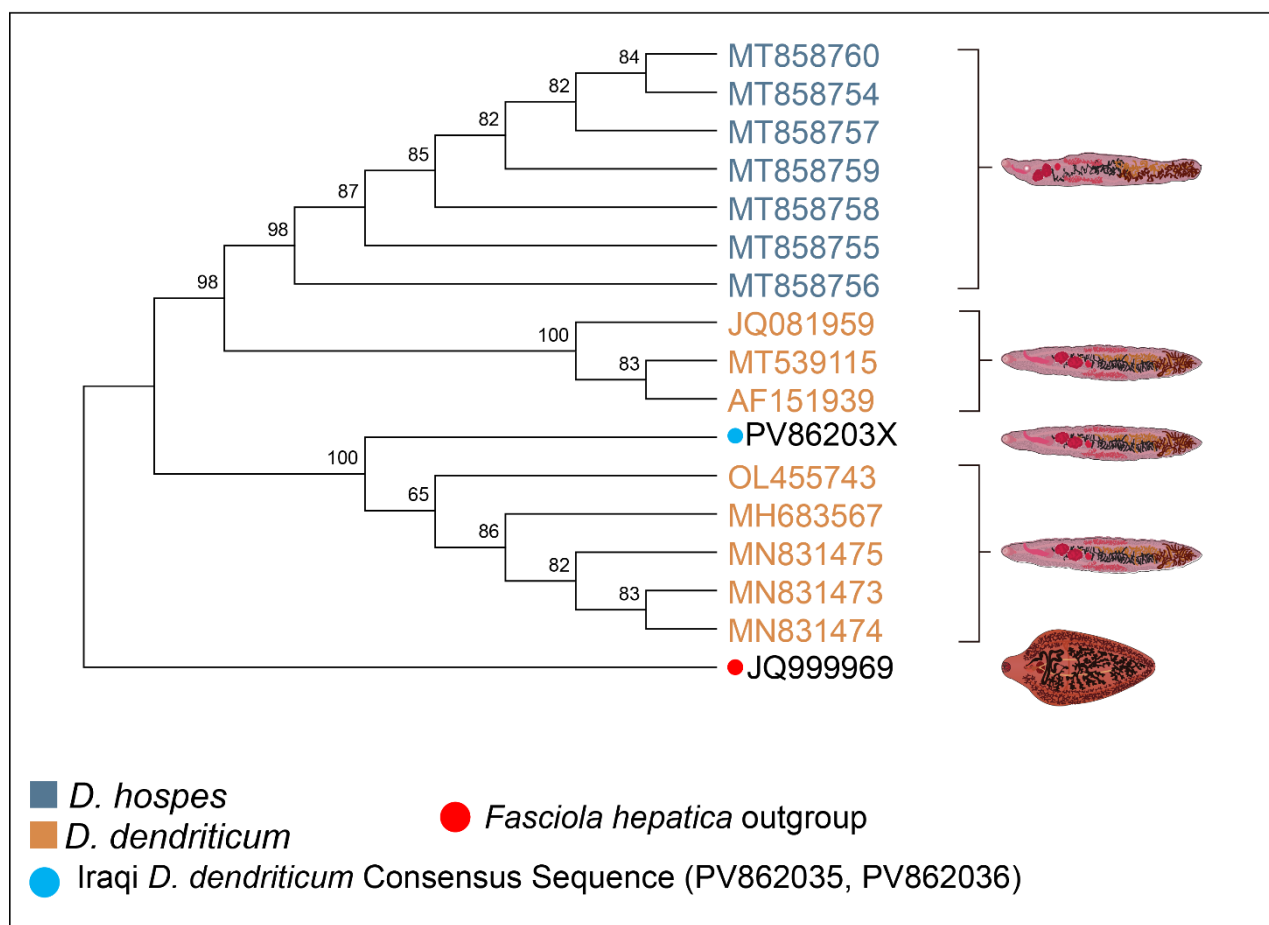


Figure (6): Evolutionary analysis using the maximum likelihood approach for 17 taxa, where 16 nucleotide sequences were taken from the BLAST alignment, the sequence labeled "PV86203X" standing for an Iraqi isolate of *D. dendriticum* denoted as a light blue dot, and the last one, which was *F. hepatica*, used as an outgroup represented as a red dot, while *D. hospes* represented by gray-blue and *D. dendriticum* represented by orange. Evolutionary analyses were carried out in MEGA12 (Kumar et al., 2024). using up to 12 parallel computing threads.

Haplotype network

The Iraqi *D. dendriticum* haplotype network analysis reveals distinct genetic differences among *D. dendriticum* reference sequences from various host species and countries. The number of mutational stages that separate the Iraqi *D. dendriticum* haplotype from others (shown by hash marks) supports the idea that the Iraqi sample is a unique haplotype that has not been shared with any other location. A unique haplotype is represented by each circle, and the size of each circle reflects the number of sequences that comprise that haplotype. Additionally, as illustrated in Figure 7, The current study represents Iraqi *D. dendriticum*. Circle colors, represent different host species. The Iraqi *D. dendriticum* sample forms a distinct haplotype that is far from the other cluster that is shared by sheep, goats, and cattle. Furthermore, as illustrated in Figure 8, The current study represents Iraqi *D. dendriticum*, and Colors of circles indicate various geographic distributions. The Iraqi sample forms a distinct haplotype that is far from the other cluster that is composed of Iranian, Turkish, Chinese, and Japanese samples with a high number of mutational steps (as indicated by hash marks), which indicate a high level of sequence divergence.

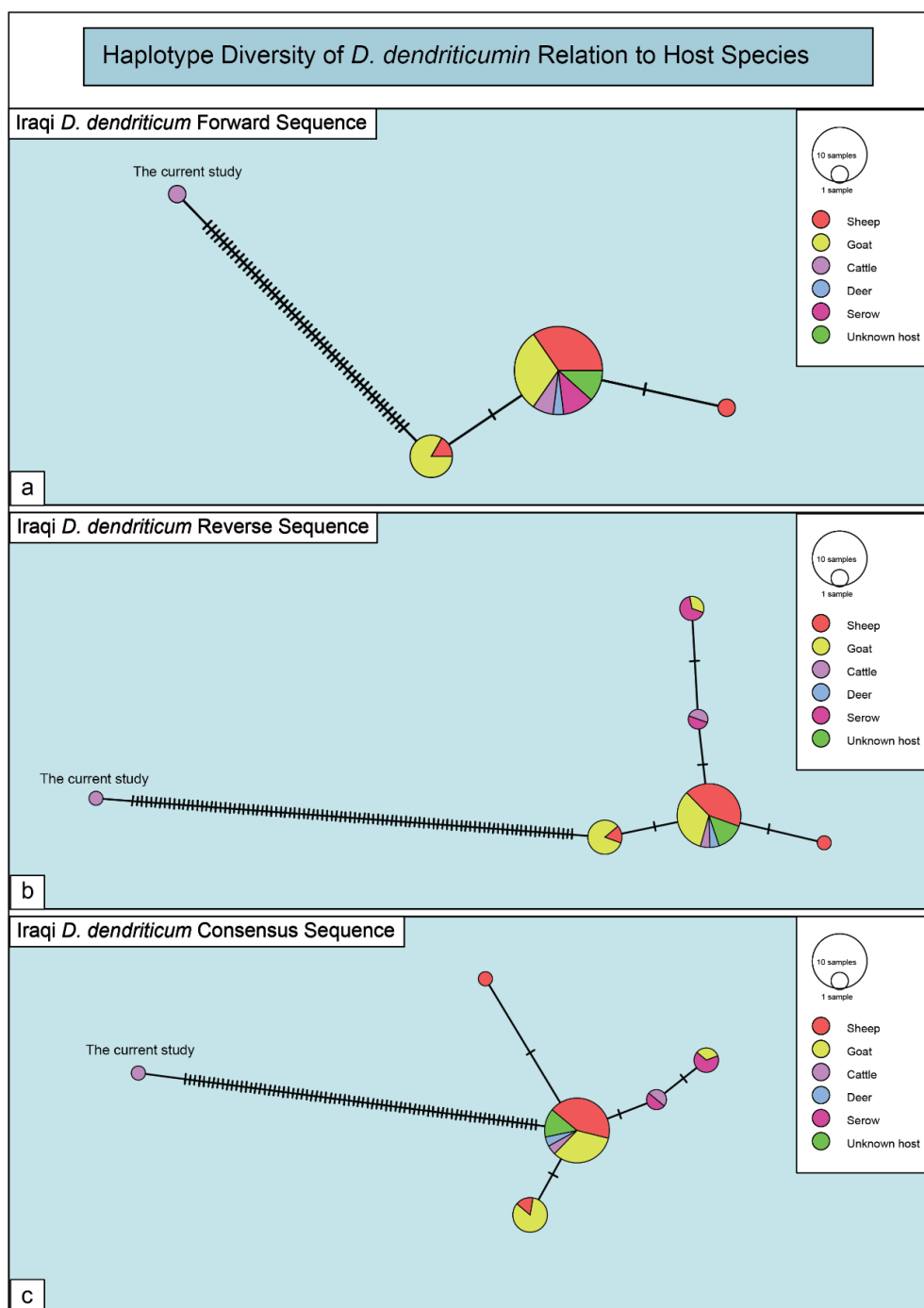


Figure (7): Median joining haplotype network of Iraqi *D. dendriticum* based on 28S rDNA demonstrating relationship among haplotype in relation to host species.

a: Forward sequence, b: Reverse sequence, c: Consensus sequence.

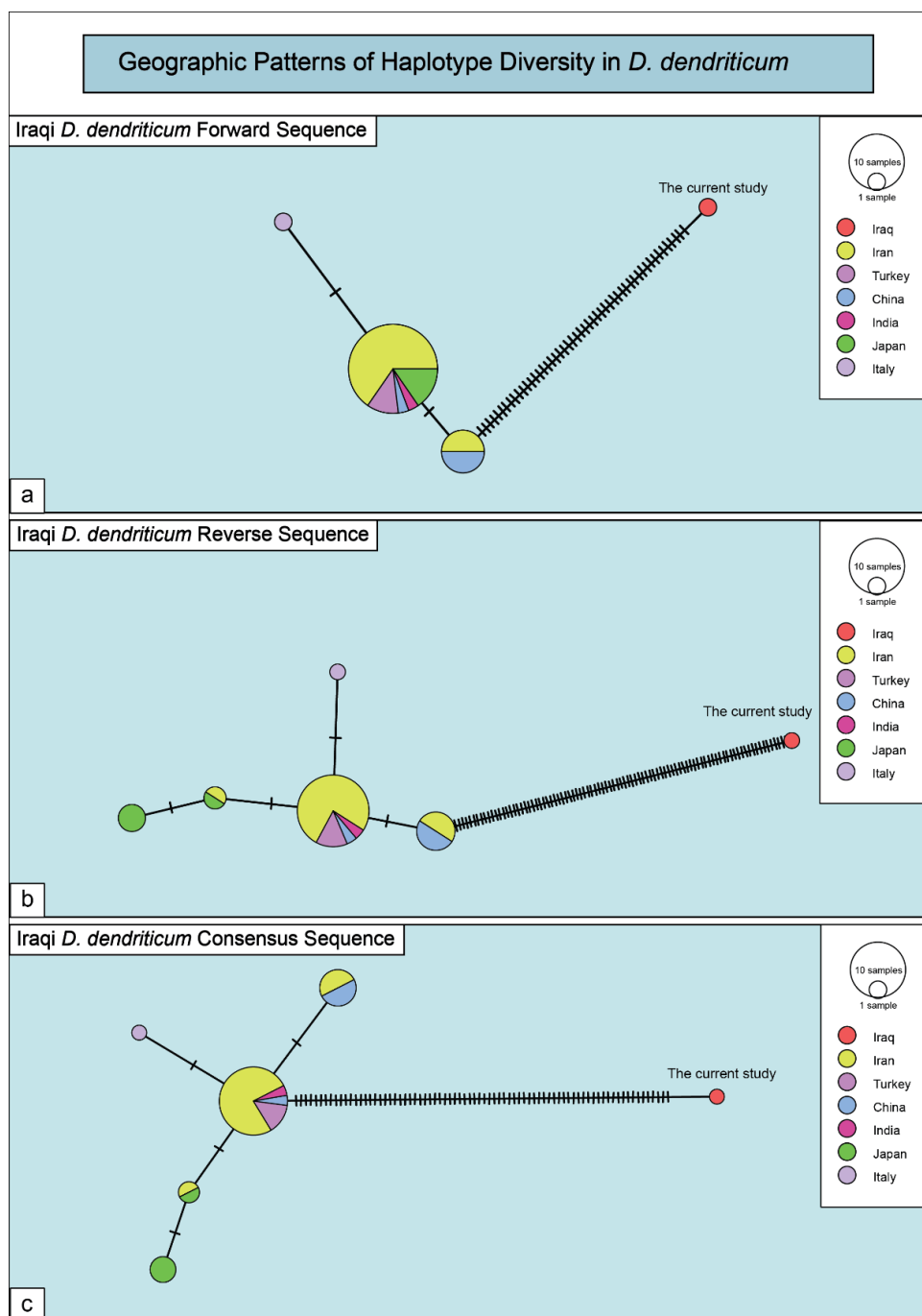


Figure (8): Median joining haplotype network of Iraqi *D. dendriticum* based on 28S rDNA demonstrating relationship among haplotype in relation to geographic.

a: Forward sequence, b: Reverse sequence, C: consensus sequence.

RNA Secondary Structure Analysis

The RNA secondary structure of consensus sequences of *D. dendriticum* was predicted using RNA fold. Each dot, as shown in Figure 9, represents a nucleotide, and the color gradient reflects the probability of base pairing. Red color indicates high pairing probability, which is close to 1, and blue color indicates low pairing probability, which is close to 0. Numerous stem-loop sections, also known as hairpin loops, are present in the structure, suggesting that the rRNA contains stable secondary motifs. Proper rRNA folding and function depend on these structures.

The secondary structural profile along the sequence is displayed in a mountain plot as shown in Figure 10. The red color represents minimum free energy (MFE) structure, the green color represents partition function (pf), and the blue color represents centroid structure. Each nucleotide position's structural variability is depicted in the entropy plot. Higher entropy values indicate greater structural flexibility, and conversely, lower values indicate base pairing that is more stable and consistent (Mathews et al., 2004).

The centroid secondary structure of the Iraqi *D. dendriticum* sequence was compared with other *D. dendriticum* sequences (as shown in Figure 11) that were published in GenBank. The current study represents Iraqi *D. dendriticum*. Visually, there is structural diversity among the sequences. Sequence divergence or evolutionary adaptation may be reflected in minor and substantial variations in secondary structure morphology.

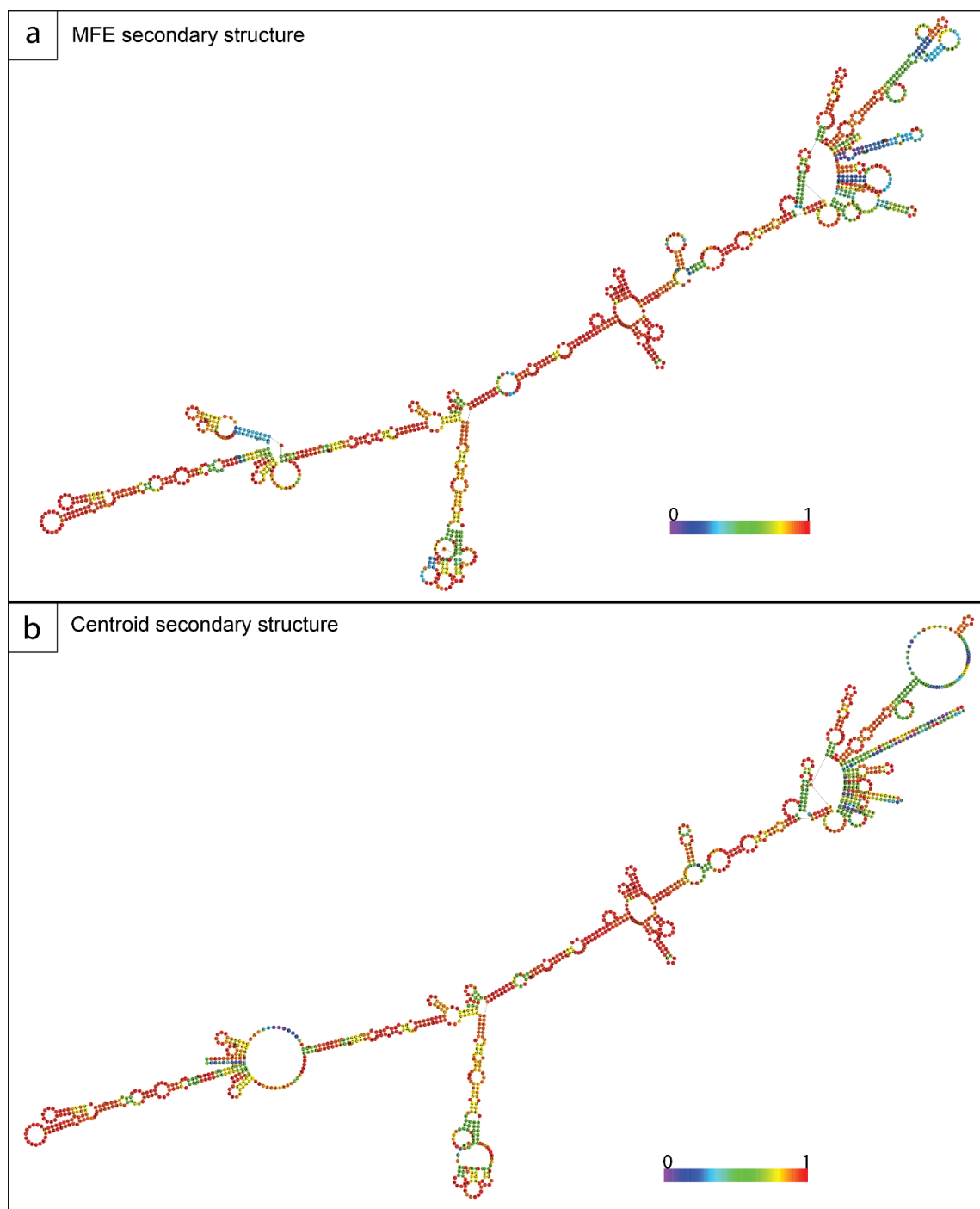


Figure (9): Predicted RNA Secondary structure of 28S rRNA consensus sequence from *D. dendriticum*.

a: MFE secondary structure, b: Centroid secondary structure

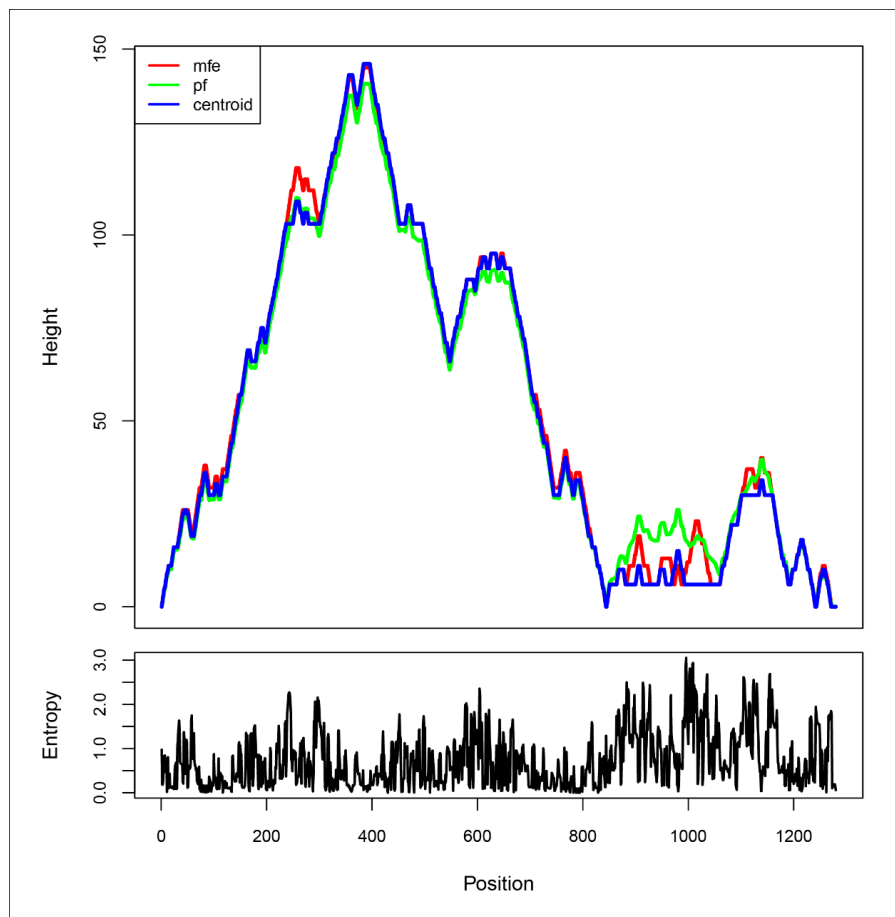


Figure (10): Mountain plot and entropy of *D. dendriticum* RNA consensus secondary structure.

Minimum free energy (red), partition function (green), and centroid (blue) predictions are compared in the plot. It reveals RNA structural stabilit.

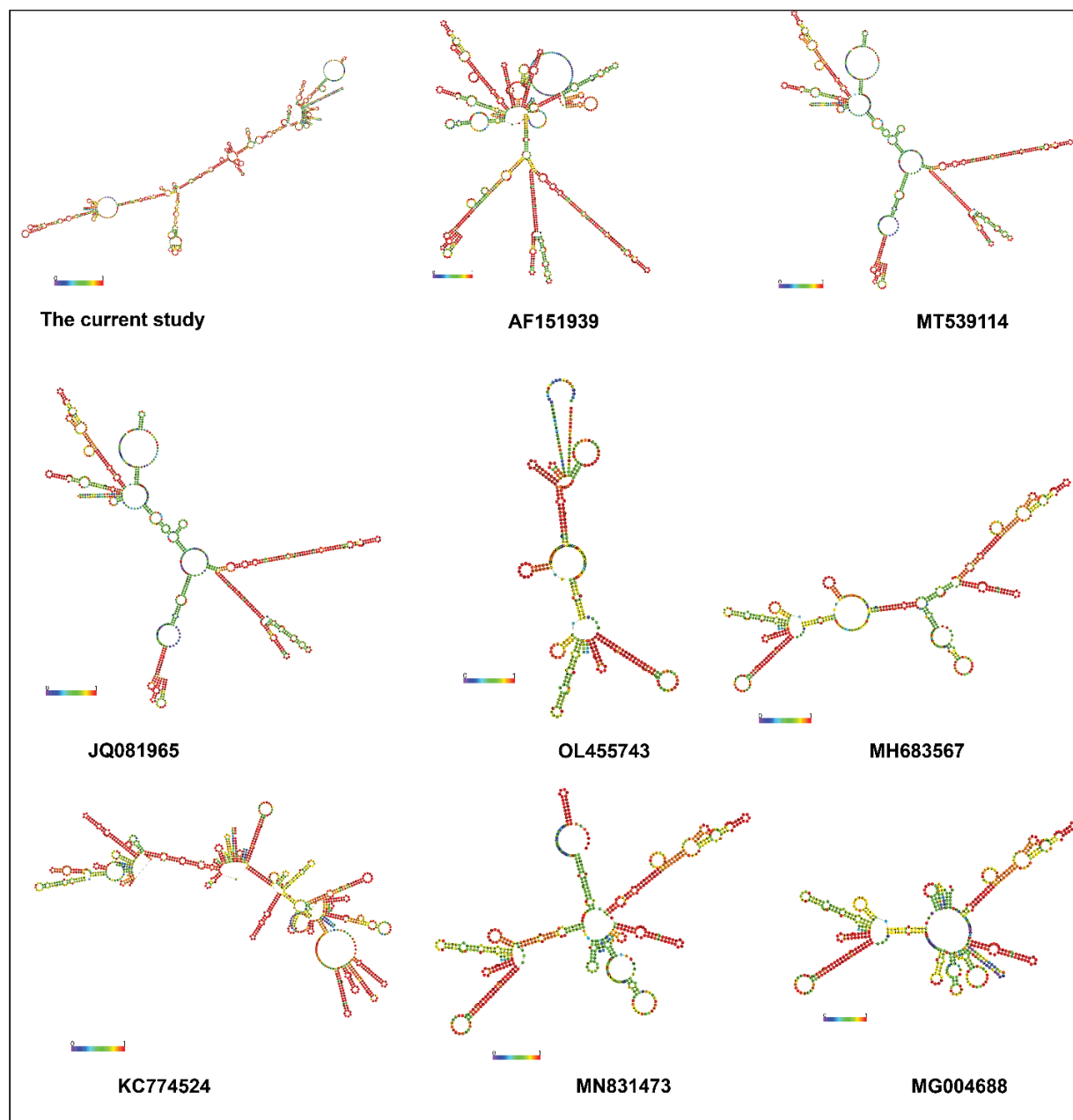


Figure (11): Visual comparison among Iraqi *D. dendriticum* and previously published sequences.

Discussion:

The heavy infection of the zoonotic disease dicrocoeliosis can cause severe liver damage. The fluke's small size and smooth, spineless surface result in far less mechanical and toxic damage than other disorders like fascioliasis and opisthorchiasis (Cranwell et al., 2010; Mowlavi et al., 2015). For morphological study, the morphological characterization of Iraqi isolates clearly confirmed that the

species is *D. dendriticum*. The size, shape, and anatomical features of the internal organs, such as testes, ovary, suckers, uterus, and vitellaria, which are characteristics of *Dicrocoelium* spp., matched those described by Moure et al. (2017) and Kruchynenko et al. (2020). However, previous research has demonstrated that traditional morphological methods, which primarily concentrate on identifying eggs during coprological examination or recovering adults in the liver during necropsy, are difficult to use to infer inter- or intra-species differences of *Dicrocoelium* species (Zhao et al., 2013). Molecular tools, typically DNA sequencing, could offer an alternative technique for identifying species of the parasites that are closely related. Out of all the target sequences used for phylogenetic and polymorphism analyses, rDNA is a potent tool to show significant intra- and interspecific variability of different parasite species because it contains regions with different rates of evolution, ranging from highly conserved (18S, 5.8S, and 28S) to highly variable (transcribed and nontranscribed or intergenic spacer regions), which are by far the most often studied regions for the exploration of species boundaries in digeneans (Maurelli et al., 2007). The region included in the first three variable domains of 28S rDNA (D1–D3) has been widely used to estimate the relationships among and within Platyhelminthes (Lee et al., 2007). In the current study, the molecular analysis based on 28S rDNA confirmed that the Iraqi isolate belongs to *D. dendriticum*. This finding was further supported by phylogenetic analysis; the consensus sequence of Iraqi *D. dendriticum* formed a distinct subclade with a bootstrap value ranging from 65% to 100% with *D. dendriticum*, particularly isolates from Iran and Turkey.

On the other hand, haplotype networks can be used to illustrate molecular data for phylogeographic or intraspecific studies. By easily visualizing the relationships between individuals, populations, and species, these networks provide information regarding speciation, migration, and population structure. Haplotype networks derived from Iraqi *D. dendriticum* based on 28S rDNA sequences clearly demonstrated genetic heterogeneity among isolates from various host species and geographical locales. The observed clustering patterns can be a reflection of localized cycles of transmission or host-specific adaptation. These results give support to the value of haplotype network analysis in figuring out the genetic diversity and structure of parasite populations. The current investigation relies on qualitative interpretation. Quantitative comparisons are challenging due to the combined genetic and topological components of haplotype networks, even though measuring genetic diversity from these networks is standard (Garcia et al., 2021).

Additionally, recent data suggests that an unexpectedly high percentage of DNA in higher eukaryotes, possibly 90%, is converted to RNA. An organism's developmental complexity has been found to positively correlate with a higher percentage of noncoding than coding RNA. According to some theories, this pattern indicates that noncoding RNA (ncRNA) is "new genetics" and could be the source of eukaryotic complexity (Mathews et al., 2010). Despite all that, according to recent research, functional ncRNAs are involved in many biological processes, from cell differentiation to development, and their dysfunction is linked to a number of diseases. These functions include transcriptional regulation and guiding modification. Since it is generally known that the activities of

ncRNAs are closely linked to their structures rather than their primary sequences, learning about their structures can help us understand their functions. However, due to the high experimental costs and resolution limitations on RNA measurements, there are significant challenges in establishing RNA tertiary structures using experimental assays like nuclear magnetic resonance and X-ray crystal structure analysis. These restrictions have not yet been fully addressed, despite recent significant advancements in cryo-electron microscopy studies on RNA tertiary structure identification. As a result, computer prediction of RNA secondary structures, which are sets of base pairs with hydrogen bonds between the nucleotides, is commonly used in place of such experimental trials (Sato et al., 2021). Consensus sequences of *D. dendriticum* were used to predict and compare RNA secondary structures, which shed light on possible structural divergence or conservation. These structural variations could be the result of functional specialization within populations or evolutionary adaptations.

Conclusions:

The findings of this study provide morphological and molecular characteristics of *D. dendriticum*. The morphological observation was consistent with the standard taxonomic description of *D. dendriticum*, and molecular analysis confirmed its identity as the Iraqi isolate *D. dendriticum*. Phylogenetic analysis, haplotype network, and RNA secondary structure collectively demonstrated clear genetic differentiation of the Iraqi isolates from those of neighboring countries. Unlike other flukes, the absence of clear clinical signs in mild infections often hinder its detection. Improved knowledge of the parasite may facilitate the design of sensitive and reliable diagnostic tools for veterinary practice.

Recommendations: Perform complete genome sequencing or analysis of additional genes such as COI and ITS to enhance understanding about the genetic diversity of *Dicrocoelium dendriticum* in Iraq, and compare them with global isolates.

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Authors Contributions: Both authors contributed to the study conception and design. Qaraman Mamakhidr Koyee designed the study and review and editing of original article. sample collection, methodology, design of figures and the first draft of the manuscript was written by Maryam Thamir Abduljabbar. Both authors read and approved the final manuscript.

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