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Molecular Characterization of the *CYTB* Gene of *Radix auricularia* Linnaeus, 1758 (Mollusca, Gastropoda, Lymnaeidae) in Al-Chibayish of Thi-Qar Province, Southern Iraq

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Abstract: *Radix auricularia* (*R. auricularia*) is widely distributed in Iraq, including Al-Chibayish marshes and found to be in many morphs. The study aimed to determine the regions of the mitochondrial genome of *R. auricularia* documented in NCBI and analyze the *CYTB* gene using bioinformatic tools. From November 2023 to May 2024, *R. auricularia snails* (480) were collected from six stations (A to F) in the Euphrates River in Al-Chibayish and had morphometric measurement variations. DNA was extracted from the station snails and the partial *CYTB* gene was amplified. For sequencing, four purified PCR products (approximately 400 bp) were randomly selected for each station. The bioinformatics results showed that the mitochondrial genome of *R. auricularia* contained 24 non-protein-coding genes and 13 protein-coding genes, including *CYTB*, *NAD5*, *NAD6* and *NAD4L*. The protein-coding genes were divided into two groups, overlapping (30.77%) and non-overlapping (69.23%). The *CYTB* and *NAD4L* genes partially overlapped and the *NAD6* and *NAD5* genes partially overlapped as well. The highest shell measurements were noted in the A station snails followed by E, C, F, B and D stations. Six unique haplotypes (H1 to H6) were identified in Al-Chibayish based on this portion of the *CYTB* gene. H1 was common and was distributed across five stations (A, C, D, E and F) whereas H2 and H3 were only found in the B station. However, H4, H5 and H6 were limited to the C, D and E stations, respectively. H1 and H4 were identical at amino acid levels. Similarly, H2 and H5 were also identical but H3 and H6 were partially identical. The partially overlapped *CYTB* gene is a suitable molecular marker in the identification of infraspecific *R. auricularia* snails in Al-Chibayish and could be broadly applied in the intraspecies recognition of molluscan taxa.

Keywords: Al-Chibayish, Cytochrome b, Haplotypes, *Radix auricularia,* Mitochondrial genome.

Introduction

Radix auricularia (Linnaeus, 1758), also known as *Lymnaea auricularia*, is a species of freshwater snail that belongs to Gastropoda. Like many other species of freshwater snails, *R. auricularia* can act as an intermediate host for a variety of parasites, including some trematode (fluke) species (Correa *et al*., 2011; Al-tooma *et al*., 2020; Al-Asadi, 2021; Abdullah *et al*., 2023). It is well known that the liver fluke, *Fasciola gigantica* Cobbold, utilizes *R. auricularia* as a key intermediate host. *R. auricularia* is widely distributed, appearing throughout portions of Africa, Asia, and Europe (Schniebs *et al*., 2022). In Iraq, Al-Mashhadani (1974) first mentioned the presence of *R. auricularia* based on

morphological characteristics. Previous studies showed that the novel forms of *L. auricularia* were identified based on the differences in the size of shells and snails such as *L. a.* var. *lapidaria* and *L. a.* var. *intercisa* (Plaziat and Younis, 2005; Naser *et.al*., 2008; Al-Asadi, 2021; Schniebs *et al*., 2022). These taxa differed from one another only slightly. Thus, drawing a border to identify among these infraspecies was sometimes difficult (Correa *et al*., 2011; Schniebs *et al*., 2022).

Molecular identification tools based on the ITS, 18S rRNA and COXI sequences have been widely applied in the taxonomical fields of living organisms, including Mollusca (Folmer *et al*., 1994; Correa *et al*., 2011; Schniebs *et al*., 2019; Mirfendereski *et al*., 2021; Schniebs *et al*., 2022). *R. auricularia* is extensively distributed in the southern regions of Iraq such Al-Chibayish marshes and Al-Sewaib River and is found to be in many morphs (Al-Salman *et al*., 2019; Al-Asadi, 2021). In the Al-Sewaib River, *R. auricularia* has been found in six different forms and these forms were almost studied based on morphological characteristics (Fig. 1) (Al-Asadi, 2021). Furthermore, bioinformatics analysis of the *COX1* gene demonstrated that the *Radix* spp. in Basrah province, Iraq only belonged to *R. auricularia* snails (Al-Asadi, 2021). These snails were nearer to those in Iran than to those in Russia and Europe. In Eurasia, Europe and Urals, the *CYTB* gene was used to identification *Radix* spp. collected from different locations (Schniebs *et al*., 2019; Schniebs *et al*., 2013; Schniebs *et al*., 2022). In Iraq, the majority of molecular marker genes, including the *CYTB* gene have not been employed in the molecular taxonomy of *Radix* spp. Hence, this study aimed to characterize the *CYTB* gene of *R. auricularia* and then aimed to employ it in the determination of *R. auricularia* haplotypes in the study region.

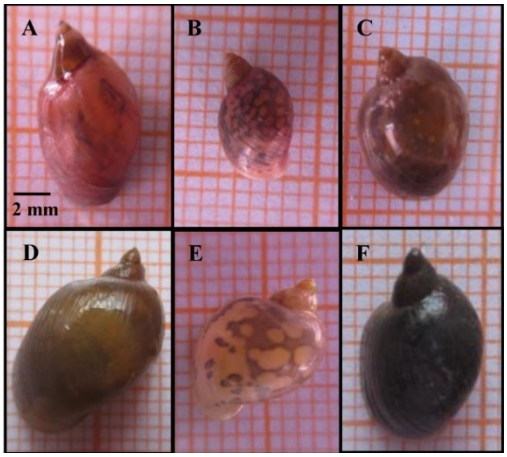

Fig. (1): *Radix auricularia* morphs in the southern regions of Iraq (Al-Asadi 2021).

Materials & Methods

The mitochondrial genome of *R. auricularia*

The entire mitochondrial genome of *R. auricularia* was obtained from GenBank (KP098540.1) through the NCBI website. The total length of this genome and the number of protein-coding genes, including the CYTB gene, and non-coding genes was determined using Unipro UGENE v44.0 .

Radix auricularia samples

R. auricularia snails were collected from six stations (A to F) at the Euphrates River in the Al-Chibayish, Thi-Qar province from November 2023 to May 2024. The GPS coordinates of stations were A (30.963943N, 47.016571E), B (30.962454N, 47.005177E), C (30.963127N, 46.998166E), D (30.962144N, 46.992934E), E (30.958172N, 46.988871E) and F (30.945536N, 46.981791E). The total number of collected snails was 480. These snails were brought to the laboratory in plastic containers with water and plants from the same sample collection place as previously described (Al-Asadi, 2021).

Morphological measurements

From each station (A to F), *R. auricularia* snails that were able to put egg masses were utilized in the measurement of body whorl, spire and shell dimensions (Al-Asadi, 2021). These measurements were determined by vernier calliper (mm) (AL-Asadi, 2011, 2021).

DNA extraction

DNA was obtained from head-foot tissues using the Geneaid extraction protocol from tissue according to the instruction of the manufacturer. DNA concentration was evaluated by applying NanoDrop^{TM} (Thermo Scientific™) and stored at -20°C.

Polymerase chain reaction

This assay was employed to amplify the *CYTB* gene of *R. auricularia*. This gene was targeted using the primer set 5`-AANAGGAARTAYCAYTCNGGYTG-`3 and 5`-TGTGGRGCNACYGTWATYACTAA-`3 (Merritt *et al*., 1998). An amplified reaction consisted of 10 pmole.µl^{-1} of each primer, 5 µl of genomic DNA and 12.5 µl of master mix (Promega) in a total volume of 25 µl. The thermal cycling conditions were 4 minutes at 94°C (pre-denaturation) and 40 cycles of 40 seconds at 94°C, 40 seconds at 48°C and 75 seconds at 72°C. The last step was 72°C for 6 minutes. The amplified products were electrophoresis on 1% agarose gel at an 85 voltage for 45 minutes and visualized using a UV-transilluminator (VWR). The visualized bands were imaged via a digital camera.

DNA sequencing and bioinformatics analysis

The *CYTB* amplified products were purified using a Promega clean-up kit to remove any leftover mixture components. After NanoDroping, 20 ng.µl^{-1} of purified products and 1.5 µl of 10 pmole.µl^{-1} of either forward or reverse primers were employed for sequencing in both directions in Macrogen company (South Korea). The DNA sequencing data of each sample was merged and edited, and the last data version was deposited in GenBank through the NCBI website. Each deposited sequence was aligned with other deposited sequences from current study samples and with GenBank samples. A haplotype network was generated for our study sequences against various R. auricularia isolates from GenBank using MEGA X and PopART 1.7.

Statistical analysis

This analysis was performed in SPSS version 22 and the graphing drawing using Graph Prism version 10. One-way ANOVA and Chi-square tests were applied in the current study. The data were considered significant when the *p*-value was < 0.05.

Results

The mitochondrial genome of *R. auricularia*

The bioinformatics analysis showed the total length of the mitochondrial genome was 13,745 bp. This genome length was divided into coding genes (10,666 bp) and non-coding genes (3,079 bp). The percentage of sequence length of coding genes (77.6%) was statistically significantly higher than the percentage of sequence length of non-coding genes (22.4%) (Fig. 2).

The analysis also revealed that the number of protein-encoding genes was 13 genes while the number of non-coding genes was 24 genes. The protein-encoding genes were *Cox1* to *Cox3*, *CYTB*, *ATP6*, *ATP8*, *NADI* to *NAD6* and *NAD4L*. However, the non-coding genes were one gene each for 12S *rRNA* and 16S *rRNA* and 22 genes for *tRNA*s (Fig. 3).

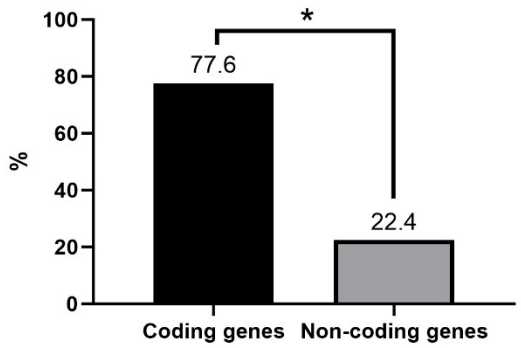

Fig. (2): The percentage of total coding and non-coding sequences in *R. auricularia* mitochondrial genome. X2 was 62.72 and *p* was 0.0001.

The findings also showed that the protein-coding genes were divided further into two groups (Table 1). These groups were overlapping genes (OLGs) and non-overlapping genes (NOLGs). NOLGs were about 69.23% (9 genes) whereas OLGs were about 30.77% (4 genes). All genes, except *CYTB* and *NAD4L*, *NAD5* and *NAD6*, belonged to the NOLGs group. Whereas the exceptions *CYTB* and *NAD4L*, *NAD5* and *NAD6* belonged to the OLGs group. The total length of the *NAD4L* gene was 438 bp, 131 of which at the 3`-end were shared with the *CYTB* gene. The *CYTB* and *NAD4L* genes partially overlapped (Fig. 4). Similarly, the *NAD6* gene had 459 bp, 14 bp of which partially overlapped with the *NAD5* gene at the 3`-end (Table 1).

Morphometric characteristics

The morphometric measurements are shown in Fig. 5. The highest shell length rate (14.8 mm) was noted in the snails collected from the A station and it was statistically significant when compared with the rate of the shell length in other stations (B to F). The lowest shell length rate (10.4 mm) was documented in the snails obtained from the D station and it had no statistical differences with the snails obtained from the B (11.09 mm) station compared with other snail stations (Fig. 5A).

Fig. (3): The mitochondrial genome of *R. auricularia* (GenBank accession number KP098540).

Table (1): The overlapping and non-overlapping genes in the mitochondrial genome.

Gene	Length (bp)	Overlapping gene	
		Shared with	Length (bp)
CoxI	1527	-	
Cox2	633	-	
Cox3	780	-	
CYTB	1080	*NAD4L*	131 bp at the 5`-end
ATP6	642	-	
ATP8	186	-	
NADI	861	-	
NAD2	903	-	
NAD3	363	-	
NAD4	1275	-	
NAD5	1650	*NAD6*	14 bp at the 5`-end
NAD6	459	*NAD5*	14 bp at the 3`-end
NAD4L	438	*CYTB*	131 bp at the 3`-end

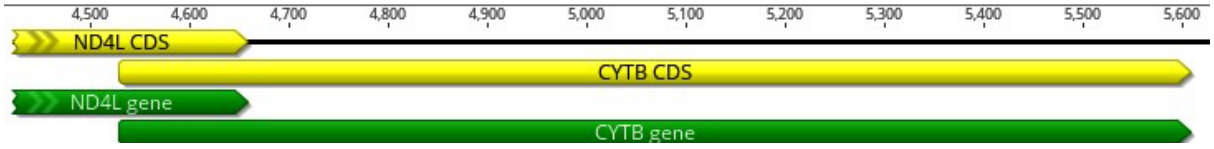

Fig. (4): Overlapping genes (*CYTB* and *NAD4L*) in the mitochondrial genome of *R. auricularia*. *NAD4L* and *CYTB* genes are green in colours and their proteins are yellow.

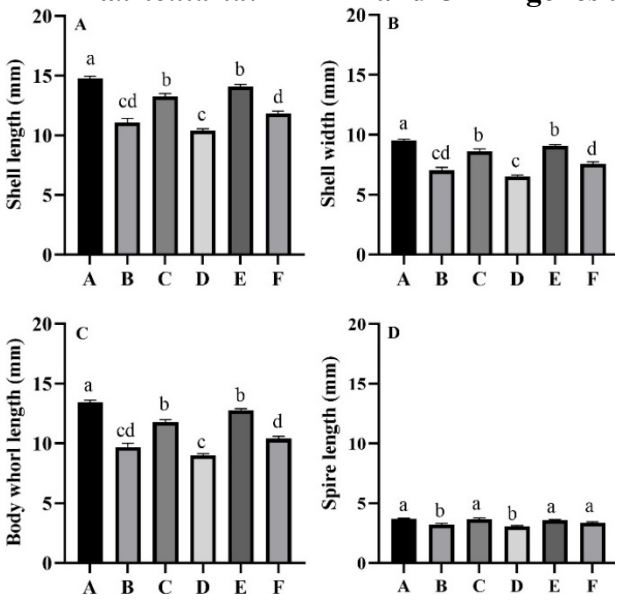

Fig. (5): The morphometric characteristics of the *R. auricularia* shell at stations A to F. Data represent mean ± SE of the mean (n = 60). A refers to shell length and B refers to shell width. C and D refer to body whorl length and spire length, respectively.

Similarly, the highest shell width rate (9.53 mm) was observed in the snails collected from the A station and it was statistically significant when compared with the rate of the shell width in the B to F stations. The lowest shell width rate (6.5 mm) was noted in the snails obtained from the D station and it had no statistical differences with the snails obtained from the B station (7.02 mm) when compared with other snail stations (Fig. 5B). Like previous measurements, the body whorl length was completely revered the same patterns noted in shell lengths and widths. Unlike other measurements, the spire length was almost similar in the A, C, E and F stations and they were statistically higher than the rate in the B and D stations, showing similar patterns.

Detection of the CYTB gene

The DNA obtained from *R. auricularia* was presented to amplify via PCR, utilizing sense and anti-sense primers targeting the *CYTB*

gene. The products were examined on an agarose gel, appearing as a single band for each product. Each band had a size of about 400 bp (Fig. 6).

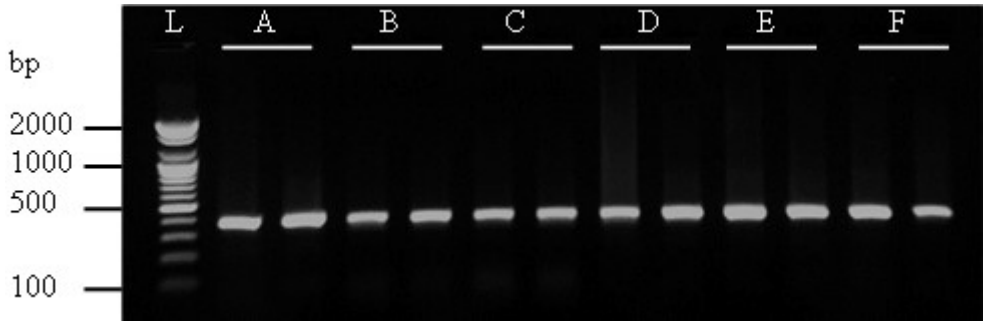

Fig. (6): Gel electrophoresis shows the amplification of the *CYTB* gene in *R. auricularia* collected from 6 stations (A to F). L represents the molecular ladder (2000 bp, Bioneer).

Sequence and bioinformatics analyses

Four representative samples for each station were used to enrich this. These samples were randomly selected and sequenced in both directions. The sequence of each sample was deposited in GenBank under the unique DNA accession number, shown in Table 2. Additionally, the deduced amino acids for each sample were mentioned in the unique protein accession number (Table 2).

The haplotype network analysis of the current study sequences is shown in Fig. (7). Our findings identified six haplotypes in Al-Chibayish. These haplotypes were distributed among the six stations. Haplotype 1 (H1) was found in five stations: A (PP646453.1, PP646454.1, PP646455.1 and PP646456.1), C (PP646461.1 and PP646462.1), D (PP646465.1and PP646466.1), E (PP646469.1 and PP646470.1) and F (PP646473.1, PP646474.1, PP646475.1 and PP646476.1).). H1 was absent from the B station and its percentage was 58.3% of the total percentage (Table 3). While H2 (PP646457.1 and PP646458.1) and H3 (PP646459.1 and PP646460.1) were only found in the B station and they had about 8.3% for each haplotype compared to the total percentage. Similarly, H4 (PP646463.1 and PP646464.1), H5

Table (2): The Accession numbers of the study samples were placed in GenBank.

Station	Accession No. (DNA)	Accession No. (Protein)
A	PP646453.1	WZP31480.1
	PP646454.1	WZP31481.1
	PP646455.1	WZP31482.1
	PP646456.1	WZP31483.1
B	PP646457.1	WZP31484.1
	PP646458.1	WZP31485.1
	PP646459.1	WZP31486.1
	PP646460.1	WZP31487.1
C	PP646461.1	WZP31488.1
	PP646462.1	WZP31489.1
	PP646463.1	WZP31490.1
	PP646464.1	WZP31491.1
D	PP646465.1	WZP31492.1
	PP646466.1	WZP31493.1
	PP646467.1	WZP31494.1
	PP646468.1	WZP31495.1
E	PP646469.1	WZP31496.1
	PP646470.1	WZP31497.1
	PP646471.1	WZP31498.1
	PP646472.1	WZP31499.1
F	PP646473.1	WZP31500.1
	PP646474.1	WZP31501.1
	PP646475.1	WZP31502.1
	PP646476.1	WZP31503.1

(PP646467.1 and PP646468.1) and H6 (PP646471.1 and PP646472.1) were only present in C, D, and E stations, respectively(Table 3). These three haplotypes had 8.3% each compared to the total percentage.

The haplotype network analysis of the current study sequences is shown in Fig. (7). Our findings identified six haplotypes in Al-Chibayish. These haplotypes were distributed among the six stations. Haplotype 1 (H1) was found in five stations: A (PP646453.1, PP646454.1, PP646455.1 and PP646456.1), C (PP646461.1 and PP646462.1), D (PP646465.1and PP646466.1), E (PP646469.1 and PP646470.1) and F (PP646473.1, PP646474.1, PP646475.1 and PP646476.1).). H1 was absent from the B station and its percentage was 58.3% of the total percentage (Table 3). While H2 (PP646457.1 and PP646458.1) and H3 (PP646459.1 and PP646460.1) were only found in the B station and they had about 8.3% for each haplotype compared to the total percentage. Similarly, H4 (PP646463.1 and PP646464.1), H5 (PP646467.1 and PP646468.1) and H6 (PP646471.1 and PP646472.1) were only present in C, D, and E stations, respectively(Table 3). These three haplotypes had 8.3% each compared to the total percentage.

Furthermore, the current study sequences (24) were compared with the available sequences from GenBank which are from Russia (45 sequences), Germany (1 sequence) and Eurasia (4 sequences). The results showed 36 haplotypes for *R. auricularia* which were one for Russia and Eurasia, one for Russia and Germany, three for Eurasia, six for Iraq and twenty-five for Russia (Fig. 8). The Iraqi six haplotypes were clustered away from other haplotypes and they had 39 mutated positions compared with the closer haplotypes from Russia (H20 and H28).

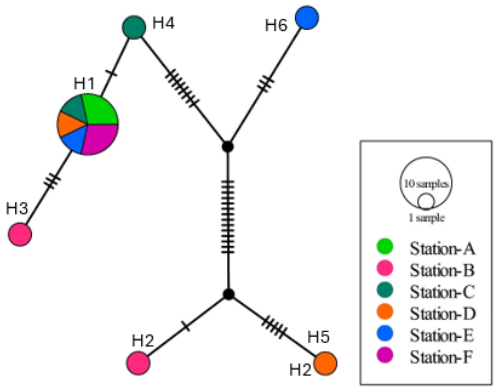

Fig. (7): The haplotype network of *R. auricularia* in the study stations was built based on the *CYTB* gene. The median vector is indicated by dark circles and mutated positions are indicated by dashes.

Table (3): The percentage of haplotypes in study stations.

Haplotype	Stations						Total	%
	A	B	C	D	E	F		
H1	4		2	2	2	4	14	58.3
H2		2					2	8.3
H3		2					2	8.3
H4			2				2	8.3
H5				2			2	8.3
H6					2		2	8.3
Total	4	4	4	4	4	4	24	100
Chi-square value= 152.8 , *p* value = 0.0001								

Identity Matrix

The partial *CYTB* nucleotides of six haplotypes were translated to amino acids and compared with those amino acids for the CYTB protein from the genome (CYTBG) in Fig. (4). The percent identity matrix showed that H1 and H4 shared 100% identity and shared 93.3% identity with CYTBG. Similarly, H2 and H5 shared 100% identity and shared 90.2% identity with CYTBG (Table 4). H3 and H6 shared 97.7 % identity with each other and shared 92.4 and 93.9% identities with CYTBG, respectively. Furthermore, the percentage of identity ranged from 92.4 to 100% within the current study haplotypes and it ranged from 90.2 to 93.9% between the present haplotypes and CYTBG.

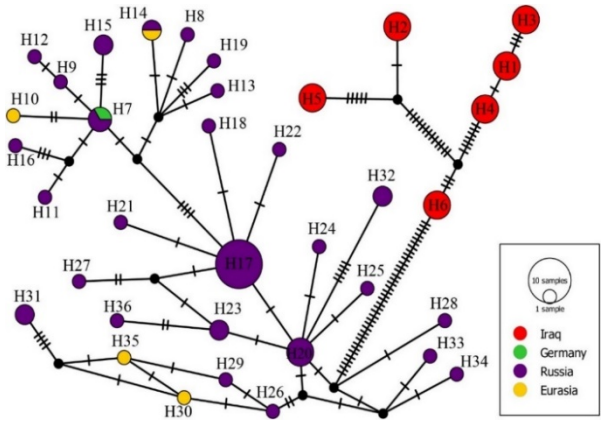

Fig. (8): A comparison network of the current study *R. auricularia* haplotypes against the available sequences from GenBank. This network was built based on the *CYTB* gene. The median vector is indicated by dark circles and mutated positions are indicated by dashes.

Table (4): The percent identity matrix of the partial CYTB proteins of haplotypes and GYTBG.

	CYTBG	H1	H2	H3	H4	H5	H6
CYTBG	100	93.2	90.2	92.4	93.2	90.2	93.9
H1		100	93.2	99.2	100	93.2	98.5
H2			100	92.4	93.2	100	94.7
H3				100	99.2	92.4	97.7
H4					100	93.2	98.5
H5						100	94.7
H6							100

Comparison of amino acids

The partial CYTB proteins of current haplotypes were lined up with the CYTBG protein (Fig. 9). The findings showed that H1 to H6 amino acid sequences had some variations from CYTBG amino acids in 9 to 13 sites. H1 to H6 had six identical differences. In these haplotypes, tryptophan^{125} (W^{125}), threonine^{128} (T^{128}), isoleucine^{148}(I^{148}), phenylalanine^{218} (F^{218}), leucine^{220} (L^{220}), serine^{241} (S^{241}) amino acids found in CYTBG were replaced with valine^{125} (V^{125}), alanine^{128} (A^{128}), V^{148}, L^{218}, F^{220} and T^{241} amino acids, respectively. Additionally, in H1, $S1^{43}$, Asparagine^{197} (N^{197}) and S^{200} amino acids found in CYTBG were substituted with A^{143}, T^{197} and T^{200} amino acids, respectively. In H2 and H5, A^{175}, S^{195}, N^{197}, V^{219}, glycine^{224} (G^{224}), A^{227} and L^{228} amino acids were changed in order with G^{175}, G^{195}, A^{197}, L^{219}, S^{224}, V^{227} and V^{228} amino acids. H3 and H4 also had four identical differences. The S^{143}, N^{197}, S^{200} and L^{228} amino acids found in CYTBG were replaced by A^{143}, T^{197}, T^{200} and A^{228} in both H3 and H4. Furthermore, H3 also had an S^{174} residue instead of a G^{174} residue. Like H1, H3 and H4, H6 possessed an S^{143} residue and like H2, it had an A^{197} residue. Like H3 and H4, H6 contained an A^{228} residue.

Fig. (9): An alignment of partial CYTB amino acids of haplotypes with CYTBG amino acids.

Discussion

The mitochondrial genome of living organisms contains important taxonomical genes, in particular the *COXI*, *CYTB* and *NADI* genes, applied in many studies (Krishna Krishnamurthy and Francis, 2012; Al-Asadi *et al*., 2021; Mirfendereski *et al*., 2021; Kasalo *et al*., 2023; AL-Asadi & Awad, 2024). The *CYTB* gene was employed in studying the diversity of *Radix* spp. in the ecosystem (Schniebs *et al*., 2013; Schniebs *et al*., 2019; Schniebs *et al*., 2022). Here in this study, the mitochondrial genome of *R. auricularia* was analyzed and showed 13 protein-coding genes. These genes were divided into two groups, overlapping and non-overlapping genes. *CYTB*, *NAD4L*, *NAD5* and *NAD6* genes overlapped partially and counted about 30.77% of protein-coding genes whereas the rest of the genes were non-overlapped and they reached up to 69.23%. Previous studies noted the presence of overlapping genes in some eukaryotes, viruses and prokaryotes and these overlaps could be totally or partially (Wright *et al*., 2022). In the current study, overlaps were only partial. This suggests that the mitochondrial genome of *R. auricularia* had fewer coding sequences compared to the number of sequences in each protein. Thus, *R. auricularia* got over the short issue in coding sequencing by using four overlapping genes, including the *CYTB* gene. Previous studies focused on the importance of this gene in the intraspecies recognition of molluscan taxa (Merritt *et al*., 1998; Schniebs *et al*., 2019).

R. auricularia snails had differences in morphometric measurements and the highest shell measurements were noted in the A station snails followed by E, C, F, B and D stations, indicating there were morphometric variations in *R. auricularia* snails. This was consistent with our previous observation of *R. auricularia* in the Al-Sewaib River (Al-Asadi,

2021). The cytochrome b findings revealed six haplotypes in the current study stations. They were distributed on these stations. H1 existed in five stations and the haplotypes (4, 5 and 6) also existed in the C, D and E stations, respectively. However, the rest of the haplotypes were only found in the B station. Previous studies noted that the haplotypes could be found in different sites (Schniebs *et al*., 2022). This is consistent with the current study results.

When a comparison of the current six haplotypes with other haplotypes from Russia, Eurasia and Germany was made, it appeared that the Iraqi haplotypes from Al-Chibayish were unique and had 39 mutated sites with the closest haplotypes (H25 and H28). These differences could be due to geographical entities. It was observed based on the *COXI* gene that the Iraqi *R. auricularia* snails obtained from Basrah shared more than 96% identity with the Iranian *R. auricularia* snails compared with 86 – 87% identity with those snails from Europe and Russia (Al-Asadi, 2021). Our findings with the *CYTB* gene suggest that the Middle East region could contain very similar *R. auricularia* haplotypes, which vary from their counterparts in Europe, Eurasia and Russia.

The six haplotypes were also compared with each other based on the deduced amino acids. The results showed that H1 and H4 were identical as well as H2 and H5 were also identical, which showed no variations at the amino acid levels. On the contrary, H3 and H6 shared 97.7% identity and they differed in three amino acids (2.3%). These differences were the G^{174} residue found in H6 and other haplotypes replaced by an S^{174} residue in H3 and the A^{197} and S^{200} residues found in H2, H5 and H6 substituted with T^{197} and T^{200}residues, respectively. The glycine (G) amino acid is from the hydrophobic aliphatic R group while the serine (S) and threonine (T) amino acids are from the hydrophilic uncharged R group (Al-Asadi, 2021). These changes in the amino acids at positions 174 and 197 led to nonsynonymous mutations which could change the CYTB protein activity. However, the changes in position 200 led to synonymous mutations due to both the serine and threonine amino acids are from the same group (Al-Asadi, 2021; Al-Asadi *et al*., 2021; Al-Asadi & Awad, 2024).

Furthermore, when the amino acids of these haplotypes were paralleled with the amino acids of the CYTBG protein, the findings revealed that the values of identity ranged from 90.2 to 93.9%. H1 and H4 had nine changes in amino acids, four of which (V^{128}, T^{197}, T^{200} and T^{241}) were synonymous mutations compared with the CYTBG protein whereas the rest of the five changes were nonsynonymous mutations. These nonsynonymous mutations were either from nonpolar aliphatic R groups (V^{125}, A^{128}, A^{143} and L^{218}) or from aromatic R groups (L^{218}) (Al-Asadi *et al*., 2021; Al-Asadi & Awad, 2024). These five replacements could affect protein function and activity. H2 and H5 had 13 amino acid replacements, four of which (G^{175}, L^{219}, V^{227} and T^{241}) were synonymous mutations whereas the rest of the nine changes were nonsynonymous mutations. Like in H1 and H4, these nonsynonymous mutations were either from nonpolar aliphatic R groups (V^{125}, A^{128}, A^{143}, G^{195}, A^{197} and L^{218}), from aromatic R groups (F^{220} and F^{228}) or polar, uncharged R groups (S^{224}) (Al-Asadi *et al*., 2019; Al-Asadi *et al*., 2021, Al-Asadi & Awad, 2024). These changes could also affect the CYTB activity of H2 and H5. H3 had 10 amino acid replacements and H6 had 8 amino acid changes. Like H1 and H4, H3 shared the same amino acid changes, except for an extra change at position S^{174}. Similar to H1 and H4, H6 shared the same amino acid changes, except at

positions (A^{197}). These changes might be related to various morphs of *R. auricularia* observed in a previous study (Al-Asadi, 2021). This needs to be investigated.

Conclusions

In conclusion, *R. auricularia* is widely distributed in Iraq, including Al-Chibayish marshes of Thi-Qar province and is found to be in various morphs. The results showed that the *CYTB* gene of *R. auricularia* overlaps with the *NAD4L* gene at the 5`-end and it reveals six haplotypes (H1 to H6) of *R. auricularia* snails collected from six different stations at the Euphrates River in Al-Chibayish. H1 was found in five stations whereas the rest of the haplotypes were limited to the station. H1 and H4 were identical at amino acid levels. Similarly, H2 and H5 were also identical but H3 and H6 were partially identical. The *CYTB* gene of *R. auricularia* is a useful taxonomical marker in the recognition of infraspecific *R. auricularia* snails in the Euphrates River in Al-Chibayish and can be widely applied in the intraspecies recognition of molluscan taxa.

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Contributions of Authors

The author designed the experiment, analyzed the data and wrote the manuscript.

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Conflicts of interest

There is no conflict of interest.

Ethical approval

This project did not require ethical approval.

References

Abdullah, Y. S., Bilal, S. J., Soor, T. A. H., & Mohammad, Y. O. (2023). Surface ultrastructure and molecular studies of *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 (Trematoda: Clinostomidae) metacercariae in some freshwater fishes from Sulaimani province, Iraq. *Basrah Journal of Agricultural Sciences*, *36*(2), 81-98. https://doi.org/10.37077/25200860.2023.36.2.07

Al-Asadi, S. A. M. (2011). Effect of calcium chloride on hatching, growth and survival of snails *Lymnaea auricularia*-Intermediat host of *Fasciola gigantica*. *Marsh Bulletin*, *6*(2), 125-133. https://iasj.net/iasj/article/73979

Al-Asadi, S. A. M. (2021). Morphological and bioinformatics study for *Radix auricularia* snails in freshwater in basrah province, Iraq. *Iraqi Journal of Agricultural Sciences*, *52*(1), 146-154. https://doi.org/10.36103/ijas.v52i1.1246

Al-Asadi, S. A. M., Malik, A., Bakiu, R., Santovito, G., Menz, I., & Schuller, K. (2019). Characterization of the peroxiredoxin 1 subfamily from *Tetrahymena thermophila*. *Cellular and molecular life Sciences*, *76*, 4745-4768. https://doi.org/10.1007/s00018-019-03131-3

Al-Asadi, S. A. M., & Awad, A.-H. H. (2024). Complete characterization of NADH dehydrogenase subunit 1 gene in human hydatid cysts. *Baghdad Science Journal*, *21*(5), 1457-1457. https://doi.org/10.21123/bsj.2023.8094

Al-Asadi, S. A. M., Hansh, W. J., & Awad, A.-H. H. (2021). Employing NADH dehydrogenase subunit 1 in the determination of *Echinococcus granulosus* strain in sheep, cattle and human in Thi-Qar province, Iraq. *Baghdad Science Journal*, *18*(2), 0238-0238. https://doi.org/10.21123/bsj.2021.18.2.0238

Al-Mashhadani, H. M. (1974). Proceedings: morphology and ecology of Lymnaeid snails of Iraq with special reference to fascioliasis. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *68*(1), 10-11. https://doi.org/10.1016/0035-9203(74)90231-4

Al-Salman, A. N., Farid, W. A., & Ali, W. A. A. (2019). Effect of endosulfan pesticide on the oxygen consumption rate of three species of snails collected from middle part of Shatt Al-Arab River. *Basrah Journal of Agricultural Sciences*, 32, 323-331. https://doi.org/10.37077/25200860.2019.181

Al-Tooma, M. A., Al-Binder, S. T., & Al-habeeb, M. A. L. (2020). Larval stages of trematodes isolated from the *Lymnaea auricularia* (freshwater snail) in Basra. *Journal of Education for Pure Science-University of Thi-Qar*, *10*(1), 218-229. https://jceps.utq.edu.iq/index.php/main/article/view/48

Correa, A. C., Escobar, J. S., Noya, O., Velásquez, L. E., González-Ramírez, C., Hurtrez-Boussès, S., & Pointier, J. P. (2011). Morphological and molecular characterization of Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. *Infection, Genetics and Evolution*, *11*(8), 1978-1988. https://doi.org/10.1016/j.meegid.2011.09.003

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, *3*(5), 294-299. https://pubmed.ncbi.nlm.nih.gov/7881515/

Kasalo, N., Skejo, J., & Husemann, M. (2023). DNA barcoding of pygmy hoppers—The first comprehensive overview of the BOLD Systems' data shows promise for species Identification. *Diversity*, *15*(6), 696. https://doi.org/10.3390/d15060696

Krishna Krishnamurthy, P., & Francis, R. A. (2012). A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodiversity and conservation*, *21*(8), 1901-1919. https://doi.org/10.1007/s10531-012-0306-2

Merritt, T., Shi, L., Chase, M., Rex, M., Etter, R., & Quattro, J. (1998). Universal cytochrome b primers facilitate intraspecific studies in molluscan taxa. *Molecular marine biology and biotechnology*, *7*, 7-11. https://pubmed.ncbi.nlm.nih.gov/9597773/

Mirfendereski, R., Hashemi, S., Shirali, S., Shemshadi, B., & Lawton, S. P. (2021). DNA barcoding of Iranian radicine freshwater snails begins to untangle the taxonomy and phylogeography of intermediate hosts of schistosomiasis and fasciolosis from the Middle East and across Central Asia. *Infection, Genetics and Evolution*, *89*, 104728. https://doi.org/10.1016/j.meegid.2021.104728

Naser, M. D., Yasser, A. G., Al-Khafaji, K. K., Aziz, N. M., & Gmais, S. A. (2008). The genus *Lymnaea* (Lamarck, 1799) from southern Mesopotamia: Are the morphological and anatomical studies enough to solve its complexity. *Marina Mesopotamica*, *23*(2), 349-362.

Plaziat, J. C., & Younis, W. R. (2005). The modern environments of Molluscs in southern Mesopotamia, Iraq: A guide to paleogeographical reconstructions of quaternary fluvial, palustrine and marine deposits. Carnets de Géologie/Notebooks on Geology, CG2005 (A01), 1-18. https://hal.science/hal-00142754/

Schniebs, K., Peter, G., Vinarski, M. V., & Hundsdoerfer, A. K. (2013). Intraspecific morphological and genetic variability in the European freshwater snail *Radix labiata* (Rossmaessler, 1835)(Gastropoda: Basommatophora: Lymnaeidae). *Contributions to Zoology*, *82*(1), 55-68. https://brill.com/view/journals/ctoz/82/1/article-p55_4.xml?language=en

Schniebs, K., Glöer, P., Vinarski, M. V., Beran, L., & Hundsdoerfer, A. K. (2019). Intraspecific morphological and genetic variability in the palaearctic freshwater snail *Radix ampla* (Hartmann, 1821)(Gastropoda: Basommatophora: Lymnaeidae). *Journal of Conchology*, *43*(3), 245-267. https://pureportal.spbu.ru/en/publications/intraspecific-morphological-and-genetic-variability-in-the-palaearctic-freshwater-snail-radix-ampla-hartmann-1821-gastropoda-basommatophora-lymnaeidae(11db8228-ccc6-4fbd-b7ad-d48b775a6ab6).html

Schniebs, K., Sitnikova, T. Y., Vinarski, M. V., Müller, A., Khanaev, I. V., & Hundsdoerfer, A. K. (2022). Morphological and genetic variability in *Radix auricularia* (Mollusca: Gastropoda: Lymnaeidae) of Lake Baikal, Siberia: The story of an unfinished invasion into the ancient deepest lake. *Diversity*, *14*(7), 527. https://doi.org/10.3390/d14070527

Wright, B. W., Molloy, M. P., & Jaschke, P. R. (2022). Overlapping genes in natural and engineered genomes. *Nature Reviews Genetics*, *23*(3), 154-168. https://doi.org/10.1038/s41576-021-00417-w

التوصيف الجزيئي لجين *CYTB* في (Mollusca, Gastropoda, Lymnaeidae) *Radix auricularia* Linnaeus, 1785 في الجبايش، محافظة ذي قار، جنوب العراق

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المستخلص: تنتشر قواقع *Radix auricularia* (*R. auricularia*) بشكل واسع في العراق وبضمنها اهوار الجبايش وتتواجد بعدة اشكال. لذا هدفت الدراسة الحالية الى تحديد مناطق جينوم المايتوكندريا لهذه القواقع الموثق في NCBI واستخدام المعلومات الحياتية لتحليل الجين *CYTB*. جمع خلال الفترة من كانون الأول 2023 الى أيار 2024، 480 قوقعا من *R. auricularia* من ست محطات (Aالى F) في نهر الفرات بمدينة الجبايش، وأظهرت القواقع تباينا في القياسات المظهرية. استخلص DNA من قواقع المحطات الست وضخم جزء من الجين *CYTB* ثم اختير عشوائيا من كل محطة أربع نواتج منقاه من PCR (400 زوج قاعدي) وانجز تتابع القواعد النيتروجينية لها. أظهرت نتائج المعلومات الحياتية أن جينوم الميتوكوندريا يحتوي على 24 جيناً غير مشفر للبروتين و13 جيناً مشفراً للبروتين، بضمنها*CYTB* و*NAD5* و*NAD6* و*NAD4L*. قسمت الجينات المشفرة للبروتين الى مجموعتين، جينات متداخلة (30.77%) وغير متداخلة (69.23%). اذ لوحظ ان الجينين *CYTB* و*NAD4L* يتداخلان جزئيا مع بعضهما وكذلك الحال بالنسبة الى *NAD6* و*NAD5*. اذ لوحظ اعلى قياسات في قواقع المحطة A تليها قواقع المحطات E وC وF وB وD. اعتمادا على جين *CYTB* تم تشخيص ست أنماط فريدة (H1 إلى H6) من هذه القواقع في الجبايش. النمط الفرداني H1 كان الأكثر شيوعا وانتشر عبر المحطات الخمس (A وC وD وE وF) في حين النمطين الفردانيين H2 وH3 انتشرا فقط في المحطة B. الأنماط الفردانية H4 وH5 وH6 اقتصر انتشارها على المحطات C وD وE، على التوالي. النمطان الفردانيان H1 وH4 كانا متطابقان على مستويات الأحماض الأمينية وكذلك الحال بالنسبة الى النمطين الفردانيين H2 وH5. ولكن النمطين الفردانيين H3 وH6 اظهرا تطابقا جزئيًا على مستوى الاحماض الامينية. يعد جين *CYTB* المتداخل جزئيًا واسما جزيئياً مناسبة في التعرف على القواقع تحت النوع *R. auricularia* في الجبايش وبالإمكان تطبيقه على نطاق واسع في التعرف على الأنواع الداخلية لأصناف الرخويات.

الكلمات المفتاحية: الجبايش، سايتوكروم b، الأنماط الفردانية، *Radix auricularia*، جينوم المايتوكندريا.