Utilizing Cytochrome C subunit 1 in the identification of Radix auricularia in the AL-Chibayish marshes of Thi-Qar province, Iraq

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Abstract

This study came to show the presence of Radix auricularia snails in the Al-Chibayish Marshes, east of Thi Qar province, southern Iraq. After many previous studies denied the existence of this species of snail in southern Iraq, where 2140 snails were collected during the period (from November 2021 to October 2022) from the Chibayish Marshes. east of Thi Qar province, southern Iraq, then sorted them according to form and color into four distinct dark red (A), black ((B), dark orange (C), brown (D). After that, the DNA was extracted, and the molecular study of the COX1 gene was performed, where the results of the sequence of this gene and through the evolutionary tree showed that the study samples belong to the form R. auricularia. There is also a percentage of agreement of these samples with the reference sample (93.23-100) % and with each other (98.54-100) %. It is a high match rate. It was confirmed that all samples of the current study belong to the form R. auricularia. The samples were then registered in the International Gen Bank. In this study, the presence of R. auricularia snails was confirmed in the Al-Chibayish Marshes in Thi-Qar province, which represents one of the provinces of southern Iraq.

Keywords: *Cytochrome C subunit 1, Radix auricularia, Lymnaeidae.*

INTRODUCTION

Diversified snails belonging to the family Lymnaeidae, including Radix auricularia (12).Which is one of the most important intermediate hosts for many parasites, the most important of which is Faciola gigantica (15).It is of health, veterinary and economic importance and is the cause of fascioliasis, which affects animals and humans(13).It has spread recently(18).Yakchali et al. (2014) confirmed the role of Lymnae auricularia as a strong vector of faciola to both animals and humans, he confirmed the use of conventional and molecular methods To determine the epidemiological status of the disease and the hosts of this species of parasite. In many studies, species belonging to the family Lymnaeidae, especially species belonging to the genus Radix spp. Depending on the external appearance of the shell and the internal anatomy of the genitals(16).However, these studies have become unreliable and have been questioned(8).Many studies have confirmed morphological changes in the form and size of the shell in relation to snails R.auricularia(16).Recent studies have proven that they have large heterogeneous phenotypic values subject to environmental conditions from chemical factors such as pH, water salinity, and physical factors such as

temperature, humidity, parasitism, predation and feeding conditions(2).Which gives a weak taxonomic value in naming and identification, especially in the absence of molecular technology and bioinformatics (19). This caused confusion to many researchers in this field AL-waaly et al. (2014) and Naser et al. (2008) have pointed out that R.auricularia is not present In the southern regions of Iraq, despite the presence of parasitic infections with giant liverworms in the central and southern provinces of Iraq(3).Therefore, molecular studies and bioinformatics, which are reliable taxonomic tools, have been used, especially in the classification of species belonging to the Lymnaeidae family(11).In this field, the cytochrome C gene is often used in the mitochondria because this type of gene is characterized by maintaining the coding of proteins. Proteins, insensitivity to ambient environmental conditions and the ability to separate overlap between Radix species(7).The stability and objectivity of the results and their significant role in determining the evolution and development in the Lymnaeidae family(5).

MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

2140 snails were collected from the Chibayish Marshes during the period (from November 2021 to October 2022). Then the samples were sorted in plastic tubs (25 mm x 25 mm x 60 mm). It is divided into four clear shapes, different in color: dark red (A), black (B), moderately dark orange (C), and brown (D).

2.2 DNA EXTRACTION AND POLYMERASE CHAIN REACTION

The DNA was extracted at the rate of three replicates per color from the head area - presented to the collected snails using the extraction kit and according to the manufacturer's recommendations, the nuclear material was digested within 24 hours for each sample and then the COX1 area was amplified with PCR using primers LCO (5-GGTCAACAAATCATAAAGATATTGG-3) and HCO (5 -TAAACTTCAGGGTGACCAAAAAATCA-3) and protocols of Folmer et al. (1994). PCR reactions were performed with primer, With a total volume of 50 µL containing 25 µL Master mix (Promega), 2 µL Forward Primer, 2 µL Reverse Primer, 15 µL Nuclease free water and 6 µL Template DNA Temperature cycling for the COX1 was as follows: 95°C for 2 min, 95°C for 1min, 40°C for 1min, 72°C for 1.5min, repeated for 30 cycles, and final extension at 72°C for 7 min .

2.3 DNA SEQUENCING AND BIOINFORMATICS ANALYSES

The DNA sequence analysis was carried out in Macrogen (South Korea) genetics of the study target and in both directions using the same prefixes that were previously used in PCR technology. The PCR products were purified using Promeca's purification kit, the concentration of deoxy ribonucleic acid was determined using a NanoDrop device, and the analysis of the DNA sequence was completed. Biometric analysis was carried out utilizing a variety of tools, including the Molecular Evolutionary Genetics analysis, after the raw sequences produced by Macrogen had been read and processed.

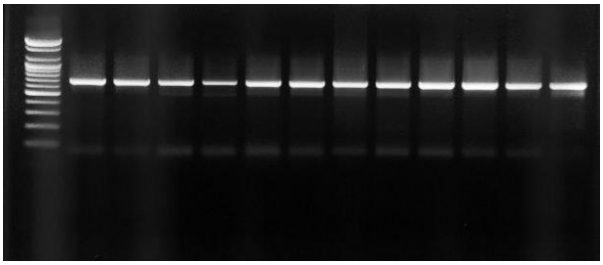
RESULTS

3.1 COX1 GENE AMPLIFICATION RESULTS

The results of the electrophoresis of the COX1 gene for the DNA of the study samples. The results showed that this region was

enlarged in all forms of R.auricularia snails By having a distinct clear band for each sample at ~700 bp in size.

Figure 1. Shows Electrostatic migration of the COX1 gene for Radix spp snail forms, while the numbers shown in the figure represent the sequence of samples (1-3) replicates of Figure A, (4-6) repeaters of Figure B, (7-9) repeaters of Figure C, (10-12) repeaters of Figure D.



3.2 Sample recording

Study samples collected from the Chabayish Marshes were recorded in the NCBI gene biodata bank for the COX1 gene sequences of R. auricularia snails and according to the sample sequence.

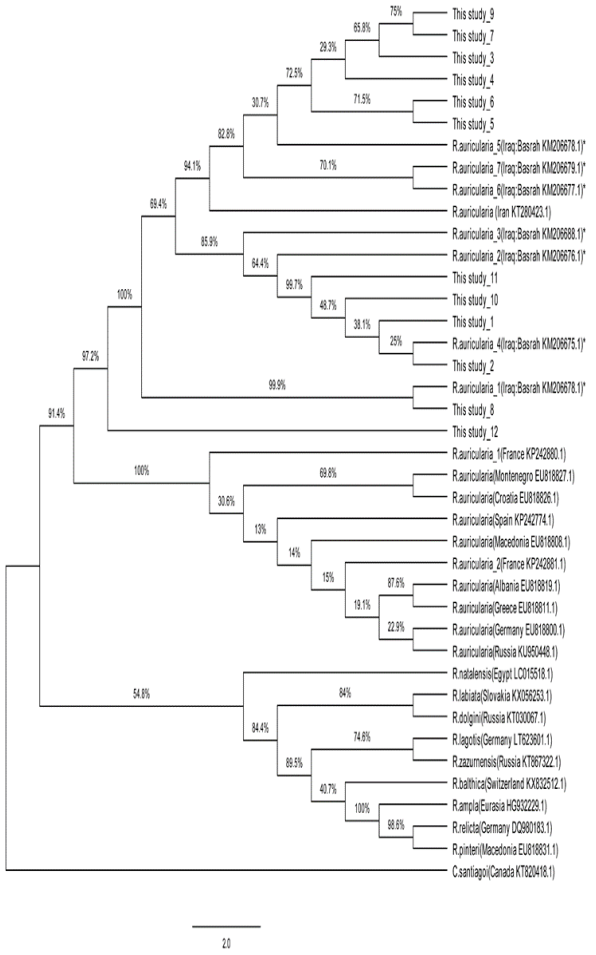
Table 1. Registration numbers of study samples in the NCBI genebank

Sample sequence	Sample form	NCBI sample registration number
1	A1	OP563712
2	A2	OP563713
3	A3	OP563714
4	B1	OP563715
5	B2	OP563716
6	B3	OP563717
7	C1	OP563718
8	C2	OP563719
9	C3	OP563720
10	D1	OP563721
11	D2	OP563722
12	D3	OP563723

3.3 rRNA- ITS2 BIOINFORMATIC

The results of the current study of the molecular aspect, based on the analysis of the phylogenetic tree of the species of Radix spp. COX1 gene, showed that it contains two main branches, the first main branch contains the species R.natalensis, R.labiata, R.dolgini, R.lagotis, R.zazurnensis, R.balthica, R.ampla, R.relicta, R.pinteri, while the second branch contains the type R.auricularia where the study samples met with the type R.auricularia belonging to the province of Basra and the Republic of Iran.

Figure 2. Shows Evolution tree for study samples and reference samples of R. aricularia snails in Basra province and the Republic of Iran.



3.4 Identity matrix the study samples with the reference sample R.auricularia

The results of the COX1 gene sequences of the study samples showed a match of (93.24-100)% with the samples of R.auricularia for Basra province and the Republic of Iran. And the existence of a match of (98.54-100) % between the study samples with each other.

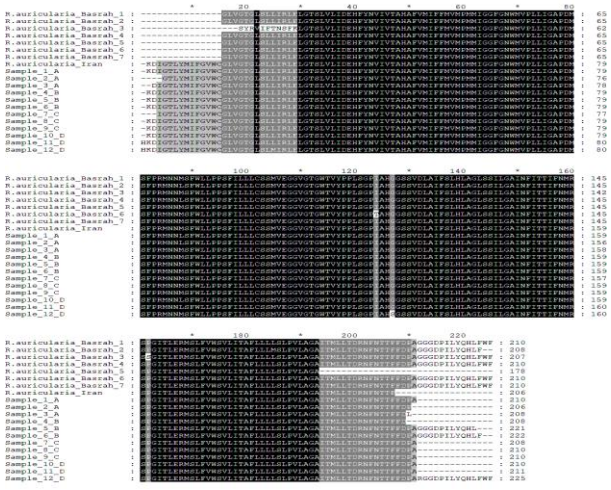
Table 3. Percent identity matrix showing a relation between the study samples with the type of R.auricularia for Basra province and with each other

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1: R.auricularia_Basrah_1	100	99.04	94.2	99.52	100	99.52	100	100	99.49	99.49	99.49	100	100	100	100	100	100	99.49	99.49	98.57
2: R.auricularia_Basrah_2	99.04	100	93.17	99.52	98.88	98.56	99.04	98.96	99.49	99.49	98.46	98.97	99.03	99.04	98.98	98.98	98.98	99.49	99.49	97.6
3: R.auricularia_Basrah_3	94.2	93.17	100	93.72	93.14	93.72	94.2	93.65	93.26	93.23	93.23	93.72	94.12	94.15	93.78	93.78	93.78	93.26	93.26	93.24
4: R.auricularia_Basrah_4	99.52	99.52	93.72	100	99.44	99.05	99.52	99.48	100	100	98.97	99.48	99.52	99.52	99.49	99.49	99.49	100	100	98.1
5: R.auricularia_Basrah_5	100	98.88	93.14	99.44	100	99.44	100	100	99.44	99.44	100	100	100	100	100	100	100	99.44	99.44	98.88
6: R.auricularia_Basrah_6	99.52	98.56	93.72	99.05	99.44	100	99.52	99.48	98.98	98.97	98.97	99.48	99.52	99.52	99.49	99.49	99.49	98.98	98.98	98.1
7: R.auricularia_Basrah_7	100	99.04	94.2	99.52	100	99.52	100	100	99.49	99.49	99.49	100	100	100	100	100	100	99.49	99.49	98.57
8: R.auricularia_Iran	100	98.96	93.65	99.48	100	99.48	100	100	99.51	99.51	100	100	100	100	100	100	100	99.51	99.51	99.03
9: Sample_1(A)	99.49	99.49	93.26	100	99.44	98.98	99.49	99.51	100	100	99.04	99.52	99.52	99.52	99.52	99.52	99.52	100	100	98.57
10: Sample_2(A)	99.49	99.49	93.23	100	99.44	98.97	99.49	99.51	100	100	99.03	99.51	99.51	99.51	99.51	99.51	99.51	100	100	98.54
11: Sample_3(A)	99.49	98.46	93.23	98.97	100	98.97	99.49	100	99.04	99.03	100	100	99.52	99.52	99.52	99.52	99.52	99.04	99.04	98.56
12: Sample_4(B)	100	98.97	93.72	99.48	100	99.48	100	100	99.52	99.51	100	100	100	100	100	100	100	99.52	99.52	99.04
13: Sample_5(B)	100	99.03	94.12	99.52	100	99.52	100	100	99.52	99.51	99.52	100	100	100	100	100	100	99.52	99.52	98.64
14: Sample_6(B)	100	99.04	94.15	99.52	100	99.52	100	100	99.52	99.51	99.52	100	100	100	100	100	100	99.52	99.52	98.65
15: Sample_7(C)	100	98.98	93.78	99.49	100	99.49	100	100	99.52	99.51	99.52	100	100	100	100	100	100	99.52	99.52	99.04
16: Sample_8(C)	100	98.98	93.78	99.49	100	99.49	100	100	99.52	99.51	99.52	100	100	100	100	100	100	99.52	99.52	99.05
17: Sample_9(C)	100	98.98	93.78	99.49	100	99.49	100	100	99.52	99.51	99.52	100	100	100	100	100	100	99.52	99.52	99.05
18: Sample_10(D)	99.49	99.49	93.26	100	99.44	98.98	99.49	99.51	100	100	99.04	99.52	99.52	99.52	99.52	99.52	99.52	100	100	98.57
19: Sample_11(D)	99.49	99.49	93.26	100	99.44	98.98	99.49	99.51	100	100	99.04	99.52	99.52	99.52	99.52	99.52	99.52	100	100	98.58
20: Sample_12(D)	98.57	97.6	93.24	98.1	98.88	98.1	98.57	99.03	98.57	98.54	98.56	99.04	98.64	98.65	99.04	99.05	99.05	98.57	98.58	100

3.5 Alignment of rRNA-ITS2 region sequences of current study snails with R.auricularia

The results of the line-up of the sequences of the current study samples with the reference samples of R.auricularia conch in the province of Basra and the Iranian Republic showed that all the study samples belong to the type R.auricularia with one difference between the sequences of sample 12 examined with the reference samples at position 127 contain the amino acid Glycine (G) and have been replaced by the amino acid Serine (S) in sample 12.

Figure 3. Shows COX1 gene alignments of study samples with R.auricularia reference samples in Basra province and the Republic of Iran



DISCUSSION

Fascioliasis, or what is known as liver sepsis, has spread recently in various parts of the world in general(10).It has spread in the regions of southern Iraq in particular(3).Which leads to health, veterinary and economic losses(17).And the direct cause of it is the parasite F.gigantica, which takes from the snail of R.auricularia an intermediate host to complete its life cycle (1).In order to control the epidemiology of this disease, the intermediate host has been studied in several previous studies, both morphologically and anatomically, However, these studies have been questioned because of the different values that the shell of this species of snail possesses and its impact on the environmental conditions surrounding it. Among these studies astudy by Nasser (2008) and Al-Waaly (2014), where they indicated that this type of cochineal is not found in the central and southern regions of Iraq. Therefore, this study came to investigate the presence of R.auricularia snails in the Al-Chibayish Marshes, east of Dhi Qar province, southern Iraq.This was done through the molecular study of study samples collected from the Chibayish Marshes and the analysis of bioinformatics of the COX1 gene. The results of the bioinformatics analyzes of the COX1 gene, which is characterized as one of the most conservative protein-coding genes in animals, showed, which has the possibility of separating the overlap between species(11).Most of the snails of the same form carry the same sequence of amino acids and for all the different colors, except for one sample that belongs to the brown form (D). This confirms that all snails with different forms belong to the species R.auricularia, and these results are consistent with what was mentioned by AL-Asadi (2021), As the samples of the current study are identical to the species R.auricularia found in the province of Basra and Iran AL-Asadi (2021) It has the same amino acids as the COX1 protein, and polymorphism occurs, but with a low rate of 8.3% at the level of amino acids in COX1 proteins, these results do not agree with what was mentioned by AL-Asadi (2021). The reason may be due to the different environmental pressures on the snails. Therefore, the snails collected from the Chibayish Marshes belong to the species R.auricularia. Therefore, the results of the current study agree completely with the results of the study of AL-Asadi (2021) and completely contradict with the results of the study of AL-Waaly et al. (2014) and Naser et al. (2008),the absence of R.auricularia snails in the southern governorates of Iraq.

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