ISSN: 2073-9524
eISSN: 2310-8746

Bioinformatics and Molecular Characterization Study of the *HSP60* Gene of *Pomacea canaliculata* (Lamarck, 1822)

Sarmad Awad Mozan AL-Asadi[1*], Noor J. A. Jouda[2], Sahar, M. A. AL-Mothafer[2], Muhammed J. Al-Emara[1], Ali AL-Ali[1]

[1]Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq.
[2]General Directorate of Education in Basrah, Iraq.

[*]Corresponding author: sarmad.mozan@uobasrah.edu.iq

Article history:

Received: 8 October 2024
Accepted: 17 March 2025
Published: 30 June 2025

Keywords: *A genotype, Heat shock protein 60, Intraspecies, Mitochondrial transit peptide.*

Abstract

A freshwater *Pomacea canaliculata* snail is widely spread across countries, including Iraq, and has the possibility for habitat alterations and lives under unusual conditions. This snail has not acquired any importance in Iraq at the molecular characterization level. Therefore, the study aimed to characterize the HSP60-coding gene in *P. canaliculata* at bioinformatics and molecular levels and determine whether HSP60 could be used as a taxonomical marker. *P. canaliculata* samples were collected from the Shatt Al-Arab River, Basrah province, and cDNA was synthesized from extracted RNA. *HSP60* primers were designed to amplify 1322 bases using PCR. Purified products were randomly selected for sequencing. The bioinformatics analysis showed that the *HSP60* gene (12 introns and 13 exons) belonged to the nuclear genome and its protein was located in mitochondria due to having the mitochondrial transit peptide. HSP60 protein could also form high molecular weight conformational changes during catalytic cycles. The results also displayed that the *HSP60* sequences from Basrah shared 100% identity. For the first time, the evolutionary network revealed four *P. canaliculate* genotypes and the A genotype (this study's samples) was unique compared with three different genotypes from China (B, C, and D). Likewise, the protein of the A genotype was also distinctive from Chinese proteins. These results suggest that there was only one genotype of *P. canaliculate* in Basrah based on the HSP60 gene/protein. HSP60 could be utilized as a taxonomical marker in studying the intraspecies in *P. canaliculate* and this could be applied to other gastropods.

https://dx.doi.org/10.52951/dasj.25170109

Introduction

Pomacea canaliculata (Lamarck, 1822) is also called an apple golden snail and belongs to Caenogastropoda, Ampullariidae. It is a freshwater snail and South America is its original habitat (Brito and Joshi, 2016). However, this snail is now widely spread across countries, including Iraq. *P. canaliculata* serves as a vector for certain human nematode parasites such as *Gnathostoma spinigerum* (Ali *et al*., 2020). The third-larva stage of *G. spinigerum* infects humans and causes human gnathostomiasis. In addition to this, *P. canaliculata* can also act as an

intermediate host to more than seven trematode parasites from digeneans, especially from the Echinostomatidae family (Martinez *et al*., 2024). In the last four decades, *P. canaliculata* has been widely used as a human food in East Asia (Xu *et al*., 2014). Nowadays, this situation has completely changed, and the golden apple snail has lost its economic importance and is listed at the top of dangerous species that damage the ecosystem (Martinez *et al*., 2024; Xu *et al*., 2014). *P. canaliculata* has the possibility for habitat alterations and lives under unusual conditions such as water pollution, food deficiency, high temperature and drought (Maldonado and Martin, 2019; Xu *et al*., 2014). Previous studies reported that the apple golden snail naturally stays alive at 37°C in South America and this might depend on certain proteins that deal with high temperatures such as stress proteins, heat shock proteins (HSPs) (Jeyachandran *et al*., 2023; Seuffert *et al*., 2010; Xu *et al*., 2014).

HSPs exist in all living organisms and have a wide range of important physiological functions (Singh *et al*., 2024; Wei *et al*., 2024). These proteins have been involved in protein folding, reproduction, thermoprotection, development, immune defense and toxic stress response (Jeyachandran *et al*., 2023). They also function as chaperones in different cellular processes. Heat shock proteins have been generally subdivided into many groups based on their molecular weights (Wei *et al*., 2024). These groups are HSP40, HSP60, HSP70, HSP90 and HSP100. In addition to this, there is also a group of small heat shock proteins that exist in all living organisms and their molecular weights are less than 44 kDa (Gu *et al*., 2023). Generally, HSP60, HSP70 and HSP90 have been extensively studied in mammals but a few studies have targeted these proteins in snails, in particular *P. canaliculata* (Xu *et al*., 2014; Zheng *et al*., 2012). *P. canaliculata* has been reported in Iraq since 1990 and has invaded many regions, including the Shatt Al-Arab (Ali *et al*., 2020). However, there are no studies that have characterized any of these *HSPs* in Iraqi *P. canaliculata*. Thus, the present study aimed to employ bioinformatics in studying the *P. canaliculata* HSP60 gene/protein and characterize it in snails collected from Shatt Al-Arab. Additionally, it aimed to determine whether or not the HSP60 can be used as a taxonomical marker.

Materials and Methods

Bioinformatics analysis

A search on the *Pomacea canaliculatia HSP60* gene was done via the GenBank database, and it showed only one complete gene sequence originally from China (accession no. KM504522.1). This gene consists of 10117 bases and was utilized in bioinformatics analyses. These analyses included the prediction of subcellular location, peptide cleavage site, and the structural model of HSP60 (AL-Asadi and Awad, 2024; AL-Asadi *et al*., 2019).

Sample collection and RNA extraction

Fifty *Pomacea canaliculatia* samples were collected from the Shatt AL-Arab River in the AL-Salhi region of Basrah province in May 2018. These snails were transferred in plastic containers to the laboratory and were washed three times utilizing normal saline. RNA was

extracted from *P. canaliculate* snails using the GENEzol™ TriRNA Pure kit (Geneaid) according to the manufacturer's instructions (Xu *et al*., 2014; Zheng *et al*., 2012). The concentration of RNA was estimated using a Nanophotometer. RNA was then stored at -80°C.

cDNA Synthesis

CDNA was synthesized from 1 µg RNA using the AccuPower® RocketScriptTM RT PreMix kit (Bioneer) based on the manufacturer's instructions. The oligo dt_{20} and random hexamer primers were also included in each reaction.

Polymerase chain reaction

cDNA prepared as mentioned above was used as a template to amplify the coding sequences of the HSP60 gene (AL-Asadi *et al*., 2019). Primer sets were designed in this study based on the mRNA sequences of *P. canaliculata* heat shock protein 60 (Accession No. KM405323.1). The forward primer was 5'- CTTATGCTTCAGGGGGTTGA-3' and the reverse primer was 5'-ATAGTCAGTGCTGGCACACG-3'. The total reaction volume was 50 µl, consisting of 25 µl (Promega master mix), 5 µl of cDNA, 1 µl of each primer and 18 µl nuclease-free water. The amplification was performed in the PCR thermal cycler (Applied Biosystems™) under the following conditions: one cycle of 95°C (5 minutes), 40 cycles (95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute), one cycle of 72°C (10 minutes). The products were analyzed on a 1% agarose gel and visualized via the UV-transilluminator. The electrophoresis conditions were 85 voltages and 120 mA for 45 minutes. The bands were captured using the Sony Cyber-shot digital camera.

DNA sequencing analysis

The PCR products were purified using a Promega clean-up kit. Twelve purified PCR products were randomly selected for sequencing in both ways using gene-specific primers. The sequencing was done in the Macrogen company (South Korea). The sequencing data were trimmed and edited using Sequencher 5.4.6. software and the sequences were deposited in GenBank. They aligned up and compared with each other and with those from GenBank using MEGA-X.

Results and Discussion

Bioinformatics analysis

The analysis revealed that the *P. canaliculata HSP60* gene (accession no. KM504522.1, China) had thirteen exons and twelve introns (Figure 1). These exons encoded for 574 amino acids (accession no. AIZ03408.1, China). It was documented that the entire mitochondrial genome of *P. canaliculata* did not have any *HSP* genes (Zhou *et al*., 2016). This could indicate that all *HSP* genes, including *HSP60*, belong to the nuclear genome. The HSP60 protein was predicted to have the mitochondrial transit peptide (MTP). This peptide consisted of 24 amino acids (MYRLPNILRSAVARKFAPSLARCY) and was cleaved at the tyrosine^{24} (Y^{24}) amino acid (Figure 2). The MTP is always present at the N-terminal end of the protein. While MTP transfers

the protein to its subcellular location, it cleaves at a certain amino acid, releasing the mature protein in its proper location (Sidorczuk *et al*., 2023). It is well known that the signal peptide directs a protein to the endoplasmic reticulum whereas MTP directs a protein to Mitochondria and chloroplasts (Sidorczuk *et al*., 2023). Like other gastropods, *P. canaliculata* did not have Chloroplasts. Thus, the subcellular location of the HSP60 protein was mitochondrion and its molecular weight was 58.22 kDa.

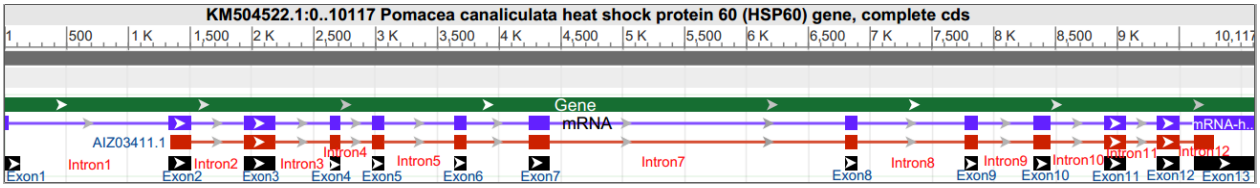

Figure 1. The diagram shows the complete HSP60 gene in *P. canaliculata*

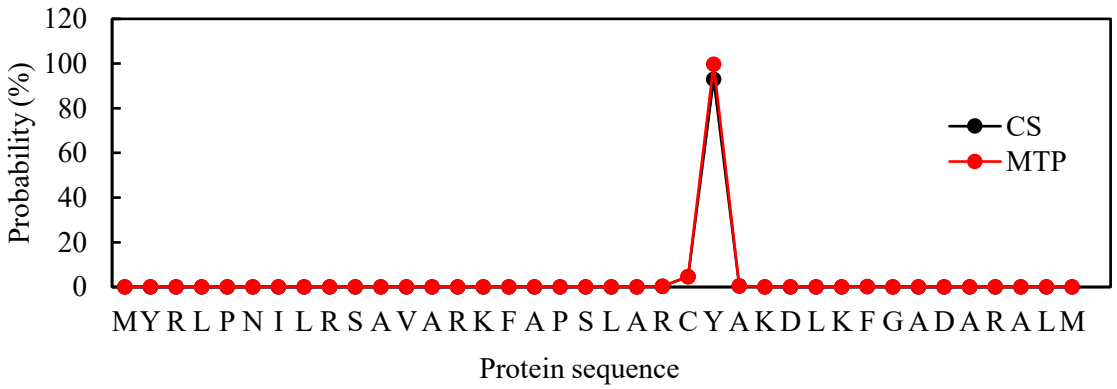

Figure 2. The mitochondrial transit peptide (MTP) and the cleavage site (CS) of HSP60 protein

The structural model of the *P. canaliculata* HSP60 protein

The predicted structural model of the *P. canaliculata* HSP60 protein was built based on the crystal structure of the human mitochondrial HSP60 protein (PDB: 7AZP). The monomer subunit of the structure model was predicted to have seventeen alpha-helices and twelve beta-sheets (Figure 3A). It is well known that these structures of alpha-helices and beta-sheets help a protein to gain more constant /stable and they also aid a protein to gain the functional structural form (Rudnev *et al*., 2021). This protein could also form a homo-dimer (two subunits) and high molecular weight conformational changes during catalytic cycles such as forming a single ring of seven subunits called a homo-heptamer (Figure 3B, C). The formation of high molecular weight structures leads to the HSP60 protein acting as a chaperone (Wei *et al*., 2024). The chaperone activity induces the correct folding of natural proteins. Additionally, this activity maintains the function of proteins under stress conditions and protects them from incorrect folding (Al-Asadi *et al*., 2019; Jeyachandran *et al*., 2023; Singh *et al*., 2024).

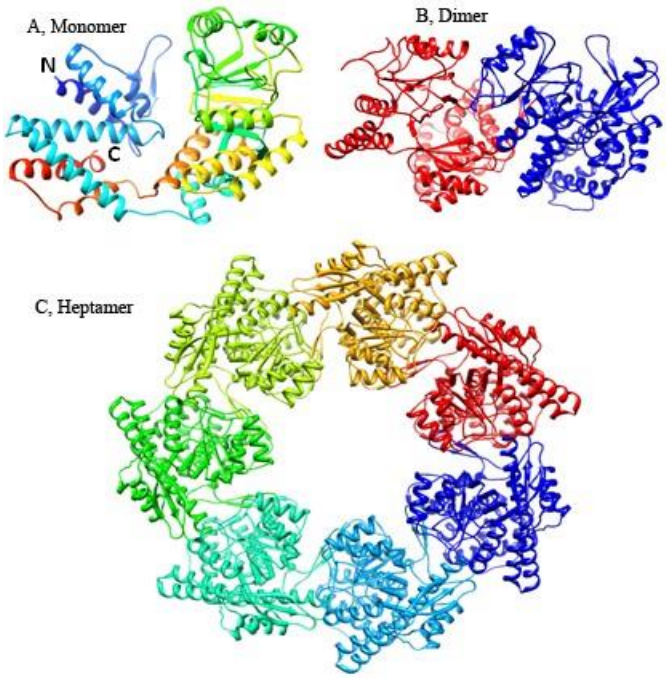

Figure 3. A structural model of the *P. canaliculata* HSP60 protein. A represents the monomer subunit; B indicates a homo-dimer and C indicates a high molecular weight homo-heptamer

Detection of the *HSP60* mRNA and sequencing

The HSP60 mRNA was partially amplified from cDNA using PCR and the agarose gel showed sharp bands at the size of 1322 bp (Figure 4, boxed bands). These bands were within the correct size for the designed primers. The band within 1322 bp was purified from gels and 12 of them were randomly selected for sequencing and deposited in GenBank under the accession numbers (Table 1). The deposited samples had 100% identity at the mRNA and protein levels. Consequently, a consensus sequence was used in the next analyses.

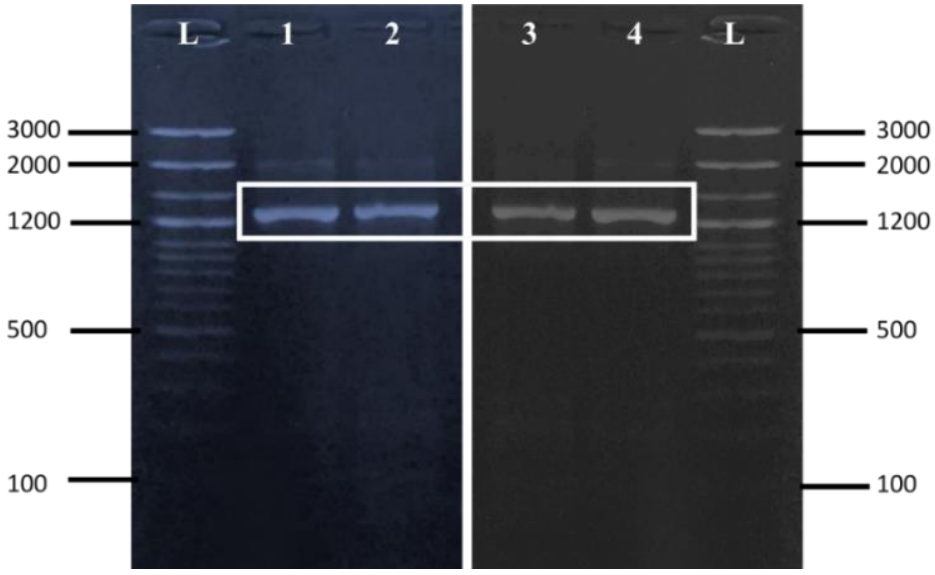

Figure 4. Agarose gel shows the *HSP60* gene of *P. canaliculate*. Lanes 1-4 represent samples, and boxed bands represent 1322 bp fragments that cut off the agarose gel

Table 1. Accession numbers of samples deposited in GenBank

Sample	mRNA		Protein	
	Accession no.	Size (bp)	Accession no.	Size (aa)
1	OR515623.1	1322	WNY14698.1	440
2	OR515624.1	1322	WNY14699.1	440
3	OR515625.1	1322	WNY14700.1	440
4	OR515626.1	1322	WNY14701.1	440
5	OR515627.1	1322	WNY14702.1	440
6	OR515628.1	1322	WNY14703.1	440
7	OR515629.1	1322	WNY14704.1	440
8	OR515630.1	1322	WNY14705.1	440
9	OR515631.1	1322	WNY14706.1	440
10	OR515632.1	1322	WNY14707.1	440
11	OR515633.1	1322	WNY14708.1	440
12	OR515634.1	1322	WNY14709.1	440

Comparison of the *HSP60* mRNA

The comparison of the *P. canaliculate* HSP60 mRNA was generated based on the 12 deposited samples from this study and all GenBank samples (KM405323.1, KM504522.1 and XM_025240009.1) that were originally from China (Figure 5). The results showed that all present study sequences were separated from other GenBank sequences in a unique genotype (A) whereas the three GenBank sequences were also separate from each other in three different genotypes (B, C and D). It was reported that gastropod members such as *Radix auricularia* had variations at a genotypic level of the *COX1* gene (Al-Asadi, 2021). Like *R. auricularia*, the *P. canaliculate* snail also had variations at a genotypic level of the *HSP60* mRNA. This implies that the *HSP60* mRNA/DNA could be employed in intraspecies diagnosis of apple golden snail members and could be extended to other gastropod species. Additionally, the sequences of the A genotype shared 100% identity with each other, and they shared 99.24, 99.17 and 98.87% identities with sequences of the C (KM504522.1), B (XM_025240009.1) and D (KM405323.1) genotypes, respectively. The current study samples (genotype A) had less than 1.5% differences compared with other genotypes (C, B and D) and these slight differences could belong to the polymorphism phenomenon which is very common in gastropods (Ajuria Ibarra and Reader, 2013; Al-Asadi, 2021; Gefaell *et al*., 2023).

The polymorphism phenomenon was investigated further by looking at nucleotide replacements in the *HSP60* mRNA (Figure 5). The four genotypes had some mutations (dashed lines), and their analyses showed that the A genotype sequences had 10, 11 and 15 single nucleotide polymorphisms (SNPs) compared with the C, B and D genotypes, respectively (Table 2). SNPs are very common in invertebrates, especially in gastropods (Ajuria Ibarra and Reader, 2013; Al-Asadi, 2021; Gefaell *et al*., 2023). The A genotype possessed ten polymorphic sites and one insertion polymorphism at site 1320 compared with the B genotype. The ten polymorphic sites were divided into two mutational groups, transversion and transition. The transversion

mutations were three (A193>C, A1193>C and T1201>G) while the transition mutations were seven (A194>G, A196>G, G211>A, C212>T, G1036>A, T1207>C and T1217>C). similarly, the A genotype had ten polymorphic sites compared with the C genotype, nine of which were also found in the B genotype (A193>C, A1193>C, T1201>G, A194>G, A196>G, G211>A, C212>T, T1207>C and T1217>C) and the tenth one was a transition mutation (A712>G). The A genotype had thirteen polymorphic sites compared with the D genotype, one insertion polymorphism (G) at site 799 and one deletion polymorphism at site 791. Nine of thirteen polymorphic sites were found in B and C genotypes and the four extra polymorphic sites were transversion mutations (A1172>C, A174>T, A1177>T and T1180>A). A previous study noted that the polymorphism occurred in the apple golden snail at the allelic level using 16 microsatellite markers; however, it did not specify these changes at the level of genes and proteins (Chen *et al*., 2011). Here, the current study findings specified for the first time the existence of polymorphisms in the *HSP60* mRNA/gene in the apple golden snail, collected from the Shatt AL-Arab river. Like other barcoding genes such as *COX1*, *NAD1* and *CYTB*, the *HSP60* mRNA/DNA could be used as a barcoding gene in intraspecies diagnosis of apple golden snail members in other regions of Iraq.

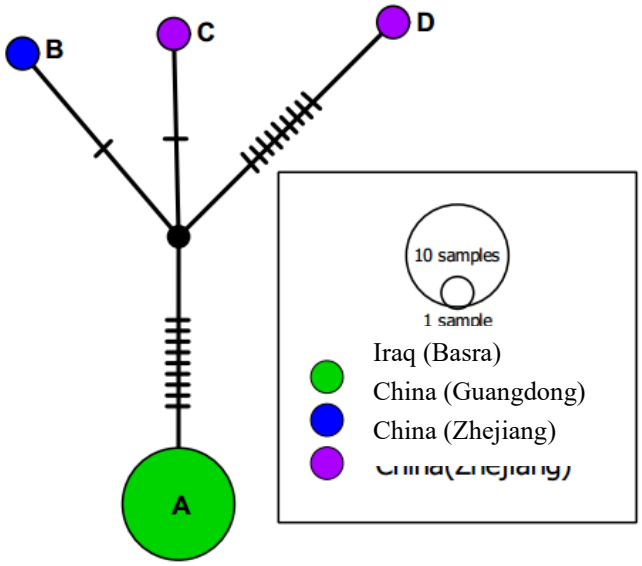

Figure 5. The network shows the evolutionary relation between the *P. canaliculate* HSP60 mRNA of 12 present study samples (A), GenBank samples: XM_025240009.1(B), KM504522.1(C) and KM405323.1 (D). A dish represents mutations between genotypes

Table 2. Nucleotide polymorphisms among the four groups

Site	This study	GenBank		
	A (12 sequences)	B (XM_025240009.1)	C (KM504522.1)	D (KM405323.1)
193	C	A	A	A
194	G	A	A	A
196	G	A	A	A

211	A	G	G	G
212	T	C	C	C
712	G	G	A	G
791	-	-	-	G
799	G	G	G	-
1036	A	G	A	A
1172	C	C	C	A
1174	T	T	T	A
1177	T	T	T	A
1180	A	A	A	T
1193	C	A	A	A
1201	G	T	T	T
1207	C	T	T	T
1217	C	T	T	T
1320	C	-	C	C

Comparison of the HSP60 protein

To gain more understanding of whether the substitutions in the HSP60 mRNA sequences of the present study samples extend their effects on the HSP60 amino acid sequences or not. The 12 HSP60 mRNA sequences of the A genotype were translated to amino acids (accession numbers, Table 1) and utilized in comparison with the amino acid sequences of the GenBank samples, B (XP_025095794.1), C (AIZ03411.1) and D (AIZ03408.1) genotypes. The four genotypes had greater identities at the amino acid level. The A genotype (present study sequences) shared 98.86% identity with the B and C genotypes and 98.41% with the D genotype. The B and C genotypes had 99.55% identity with each other and 99.09% with the D genotype. As can be seen, the protein of A genotype had less than 1.6% differences with other genotypes.

To focus on these changes, an alignment of the A protein with the B, C and D proteins was performed (Figure 6). The results showed there were four substitutions in the A protein sequences compared with other sequences. These substitutions were arginine^{65} (R^{65}), aspartic acid^{66} (D^{66}), isoleucine^{71} (I^{71}) and leucine^{398} (L^{398}) residues instead of asparagine^{65} (N^{65}), N^{66}, alanine^{71} (A^{71}) and I^{398} residues, respectively. in contrast, the B protein only differed from others in an I^{440} residue, and the C protein differed in a lysine^{238} (K^{238}) residue. Whereas the D protein differed from others in three amino acids, glycine^{264} (G^{264}), serine (S^{265}) and R^{266}. The two replacements found in the A protein (I^{71} and L^{398}) belonged to the hydrophobic and aliphatic R groups, which are the same groups for A^{71} and I^{398} found in the B, C and D proteins (Al-Asadi, 2021; AL-Asadi and Awad, 2024). This suggests that the A protein activity could not be affected at these two amino acid positions due to having similar structures and functions. Contrarily, the other two replacements in the A protein (R^{65} and D^{66}) belonged to two different groups. The R^{65} residue belonged to the positively charged R groups. While the D^{66} residue belonged to the negatively charged R groups (Al-Asadi, 2021; AL-Asadi and Awad, 2024). Both R^{65} and D^{66} residues were away from the hydrophilic and uncharged R groups of N residue found in the B, C and proteins.

This indicates that the A protein activity could be affected at these two amino acid positions because they have different structures and functions.

Figure 6. Amino acid sequence alignment of HSP60 from *P. canaliculate* of the present study samples (A) and the GenBank samples (B, C, and D)

Conclusions

In conclusion, the bioinformatics analysis shows that the *P. canaliculate HSP60* gene, containing 13 exons and 12 introns, belongs to the nuclear genome. The HSP60 protein possesses mitochondrial transit peptide (24 amino acids) and its subcellular location in mitochondria. The current study findings show that the *P. canaliculate HSP60* samples from Basrah province had 100% identities with each other and they had only one genotype. The world genotypic network shows for the first time that the Basrah genotype (A) was unique compared with three different genotypes from China (B, C, and D). Similarly, at the level of amino acids, the Basrah A protein was also unique compared with its counterparts from China. All of these results suggest that in Basrah, there was only one genotype of *P. canaliculate* based on the HSP60 gene/protein and this gene could be employed as a taxonomical marker in studying the intraspecies in *P. canaliculate* and this could be applied to other gastropod species.

Conflict of Interest

There was no conflict of interest between the authors.

Acknowledgements

We would like to thank Basim Kareem Shatti (General Directorate of Education in Basrah, Iraq) for his help with the sample collection.

References

Ajuria Ibarra, H., and Reader, T. (2013). Reasons to be different: do conspicuous polymorphisms in invertebrates persist because rare forms are fitter? *Journal of Zoology*, *290*(2), 81-95. https://doi.org/10.1111/jzo.12034

Al-Asadi, S. (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., Malik, A., Bakiu, R., Santovito, G., Menz, I., and 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., and 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

Ali, M., Mohammed, H., and Ahmed, H. (2020). Temperature Impacts on the Cardiac Activity of the Snail *Pomacea canaliculata* from Shatt Al-Arab Region, Basrah, Iraq, *Baghdad Science Journal, 17*(2), 437-443. https://doi.org/10.21123/bsj.2020.17.2.0437

Brito, F. C., and Joshi, R. C. (2016). The golden apple snail *Pomacea canaliculata*: a review on invasion, dispersion and control. *Outlooks on Pest Management*, *27*(4), 157-163. https://doi.org/10.1564/v27_aug_03

Chen, L., Xu, H., Li, H., Wu, J., Ding, H., and Liu, Y. (2011). Isolation and characterization of sixteen polymorphic microsatellite loci in the golden apple snail *Pomacea canaliculata*. *International Journal of Molecular Sciences*, *12*(9), 5993-5998. https://doi.org/10.3390/ijms12095993

Gefaell, J., Galindo, J., and Rolán-Alvarez, E. (2023). Shell color polymorphism in marine gastropods. *Evolutionary Applications*, *16*(2), 202-222. https://doi.org/10.1111/eva.13416

Gu, C., Fan, X., and Yu, W. (2023). Functional diversity of mammalian small heat shock proteins: a review. *Cells*, *12*(15), 1-24. https://doi.org/10.3390/cells12151947

Jeyachandran, S., Chellapandian, H., Park, K., and Kwak, I. S. (2023). A review on the involvement of heat shock proteins (extrinsic chaperones) in response to stress conditions in aquatic organisms. *Antioxidants*, *12*(7), 1-22. https://doi.org/10.3390/antiox12071444

Maldonado, M. A., and Martin, P. R. (2019). Dealing with a hyper-successful neighbor: effects of the invasive apple snail *Pomacea canaliculata* on exotic and native snails in South America. *Curr Zool*, *65*(3), 225-235. https://doi.org/10.1093/cz/zoy060

Martinez, L. E., Gilardoni, C., Medina, C. D., Cremonte, F., and Etchegoin, J. A. (2024). Histopathological Lesions Caused by a Digenean Trematode in a Pest Apple Snail, *Pomacea canaliculata*, in Its Native Geographic Distribution Area. *Animals (Basel)*, *14*(8), 1-16. https://doi.org/10.3390/ani14081191

Rudnev, V. R., Kulikova, L. I., Nikolsky, K. S., Malsagova, K. A., Kopylov, A. T., and Kaysheva, A. L. (2021). Current approaches in supersecondary structures investigation. *International Journal of Molecular Sciences*, *22*(21), 1-23. https://doi.org/10.3390/ijms222111879

Seuffert, M. E., Burela, S., and Martín, P. R. (2010). Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *Journal of Thermal Biology*, *35*(2), 77-84. https://doi.org/10.1016/j.jtherbio.2009.11.003

Sidorczuk, K., Mackiewicz, P., Pietluch, F., and Gagat, P. (2023). Characterization of signal and transit peptides based on motif composition and taxon-specific patterns. *Scientific Reports*, *13*(1), 1-10. https://doi.org/10.1038/s41598-023-42987-1

Singh, M. K., Shin, Y., Ju, S., Han, S., Choe, W., Yoon, K.-S., Kim, S. S., and Kang, I. (2024). Heat shock response and heat shock proteins: Current understanding and future opportunities in human diseases. *International Journal of Molecular Sciences*, *25*(8), 1-31. https://doi.org/10.3390/ijms25084209

Wei, H., Zhang, Y., Jia, Y., Chen, X., Niu, T., Chatterjee, A., He, P., and Hou, G. (2024). Heat shock protein 90: biological functions, diseases, and therapeutic targets. *MedComm*, *5*(2), 1-30. https://doi.org/10.1002/mco2.470

Xu, Y., Zheng, G., Dong, S., Liu, G., and Yu, X. (2014). Molecular cloning, characterization and expression analysis of HSP60, HSP70 and HSP90 in the golden apple snail, *Pomacea canaliculata*. *Fish and shellfish immunology*, *41*(2), 643-653. https://doi.org/10.1016/j.fsi.2014.10.013

Zheng, G., Dong, S., Hou, Y., Yang, K., and Yu, X. (2012). Molecular characteristics of HSC70 gene and its expression in the golden apple snails, *Pomacea canaliculata* (Mollusca: Gastropoda). *Aquaculture*, *358*, 41-49. https://doi.org/10.1016/j.aquaculture.2012.06.002

Zhou, X., Chen, Y., Zhu, S., Xu, H., Liu, Y., and Chen, L. (2016). The complete mitochondrial genome of *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Mitochondrial DNA Part A*, *27*(2), 884-885. https://doi.org/10.3109/19401736.2014.919488