

Morphological and Molecular Characterization of the Terrestrial Snail *Xeropicta krynickii* (Krynicky, 1833) (Stylommatophora: Hygromiidae) from Northern Dhi-Qar Governorate, Iraq

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Abstract

This study documents the recording of the snail *Xeropicta krynickii* (Krynicky, 1833) from northern Dhi Qar Governorate, Iraq, during a twelve-month field survey (Sep. 2025 to Aug. 2026). Of 2,162 Hygromiidae snail specimens collected during the survey, only 46 (2.13%) were recorded. These specimens were found exclusively at the Al-Rifai station and only during the month of January (88.89% winter presence). The identification was based on distinctive morphological characteristics, most notably a fully open, wide umbilicus, along with morphometric measurements and their derived ratios. It was confirmed molecularly via the sequencing COI gene. The phylogenetic tree and haplotype network revealed seven genotypes (H1–H7), with the local specimens representing genotypes H4 and H5. Due to the extremely small sample size and its highly localized temporal and spatial distribution. Although restricted temporal occurrence precluded broader ecological inferences, these findings provide a critical baseline for terrestrial malacofaunal biodiversity and molecular documentation in Dhi Qar southern Iraq.

Keywords: *Xeropicta krynickii*, Hygromiidae, COI gene, Haplotype network, Dhi-Qar, Iraq.

التشخيص المظهري والجزيئي للقوقع الأرضي *Xeropicta krynickii* (Krynicky, 1833) (Stylommatophora: Hygromiidae) من شمال محافظة ذي قار، العراق

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الخلاصة

يوثق هذا البحث التسجيل للقوقع *X. krynickii* (Krynicky, 1833) من شمال محافظة ذي قار، العراق، ضمن مسح حقلي استمر اثني عشر شهرا خلال ايلول 2025 الى اب 2026. من أصل 2162 عينة من قواقع Hygromiidae جمعت خلال المسح، سجلت 46 عينة فقط لهذا النوع (2.13%)، اقتصر وجودها على محطة الرفاعي حصرا وعلى شهر كانون الثاني حصرا (نسبة حضور 88.89% في فصل الشتاء دون سواه). اعتمد التشخيص على الصفات المظهرية المميزة، أبرزها السرة الواسعة المفتوحة بالكامل، إلى جانب القياسات المورفومترية ونسبها المشتقة، وأكد جزيئيا عبر جين COI الميتوكوندري. كشفت شجرة العلاقات التطورية وشبكة الهابلوتايب عن سبعة أنماط وراثية (H1–H7)، مثلت العينات المحلية النمطين H4 و H5. نظرا لصغر حجم العينة الحاد وتركزها الزمني والمكاني الحاد، اقتصرَت الدراسة على التوثيق التصنيفي والجزيئي دون استدلالات بيئية إضافية.

1- Introduction

The genus (*X. Monterosato*, 1892) belongs to the family Hygromiidae and is distributed around the Black Sea and extending into the Mediterranean basin. This genus provided the first record of the species *X. krynickii* in Montenegro, representing a westward expansion of its native range. This suggests that the primary mechanism of dispersal for this genus is unintentional transport via human agricultural and commercial activities[1]. This pattern of human-induced dispersal confirms what Ostrovsky (2023) later documented[2]: a new record of a closely related species, *X. derbentina*, in Belarus, which he attributed to negative transmission via human activity (most likely railways), thus expanding the known geographical range of this diffusion mechanism northward. This conclusion is supported by population genetic studies of the closely related species *X. derbentina* conducted by Adamova et al. (2019), which revealed morphometric and genetic variation among introduced populations, reflecting a relatively recent settlement history compared to the native populations[3]. Although *X. krynickii* has been previously recorded in other parts of Iraq [4], this study represents its first recording specifically in Dhi-Qar Governorate. This province is already included in a broader checklist of Iraqi land snails compiled by Jihad (2024), which documented fifteen species from seven families in various locations across the country[5]. This study comes within the context of growing research activity on Iraqi land snails in recent years, including new records of introduced species and field surveys in different provinces [6]; [7]; [8], reflecting the expanding knowledge base regarding the biodiversity of this group in the country. In line with the methodological recommendation emphasized by Bashê & Al-Qassab (2024) regarding the necessity of integrating molecular and phenotypic diagnosis in recording Iraqi Hygromiidae snails due to the significant morphological similarity among their species[8], this research adopted an integrated approach to verify species identity. This morphological similarity is further confirmed by the presence of *X. krynickii* itself among six common land snail species described by Ali and Ramdane (2020) using a taxonomic key based on shell characteristics in Egyptian fields, reflecting a growing regional interest in documenting this particular species[9]. A similar integrative approach (joint morphological and molecular analysis) has also been used in recent studies of other introduced land snail genera outside Iraq, such as the characterization by Abobakr et al. (2022) of the invasive *Macrochlamys indica* in Saudi Arabia based on shell, body, and reproductive characteristics, along with the mitochondrial COI and I6S rRNA genes[10], and the study by Manganelli et al. (2025) which employed joint morphological and molecular analysis to resolve a taxonomic problem among closely related species of the genus *Monacha* (family Hygromiidae) on the Greek island of Corfu[11].

2. Materials and Methods of Work

1. 2 Study Area and Collection Method

The recording site is at Al-Rifai station, as shown in Figure 1. The survey was conducted monthly at four stations, with three sites per station and three sampling squares (1m²) per site. Their locations were chosen purposefully (Purposive Sampling), and the manual collection method was adopted according to the standard protocol followed in land snail surveys [12], with the coordinates of each site being recorded as detailed in (Table 1).

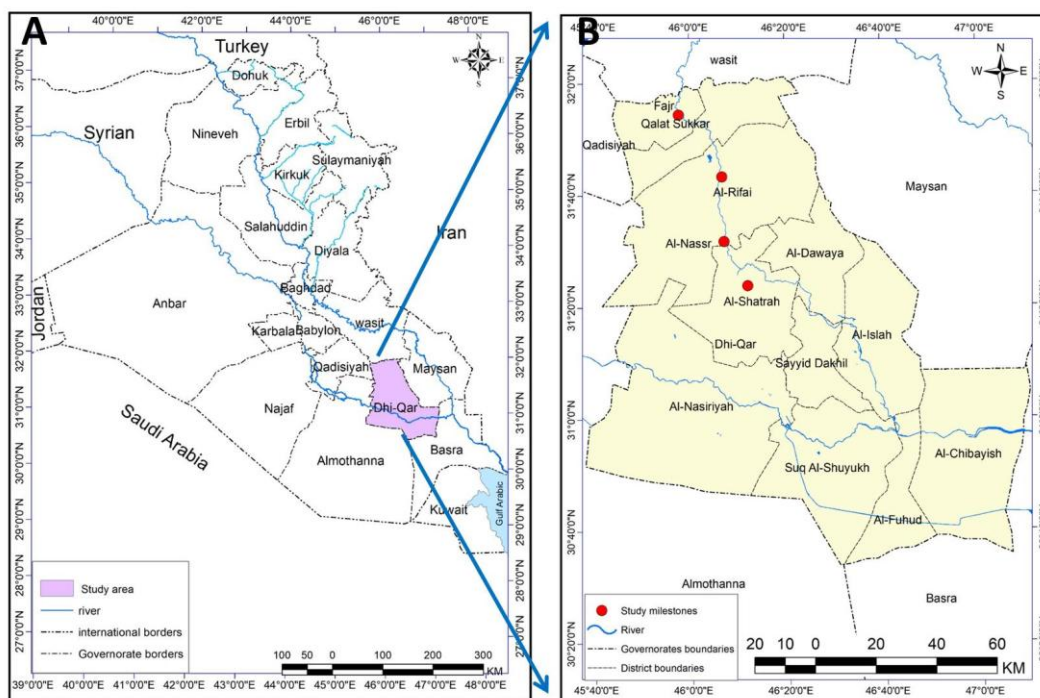


Figure -1 A. Map of Iraq showing the location of Dhi Qar Governorate in southern Iraq, B. Study Stations

Table 1-Sample collection areas

No.	Station's name	Location within the governorate	Area type	St. coordinates
1	Al-Fajr District	North of Dhi Qar Governorate	Agricultural	N 31° 50' 4.202" E 45° 55' 8.636"
2	Al-Rifai District	North of Dhi Qar Governorate	Agricultural	N31°45' 21.237" E46° 6' 21.624"
3	Al-Nasr District	North of Dhi Qar Governorate	Rural	N31°32' 23.958" E46° 07' 37.884"
4	Al-Shatra District	North of Dhi Qar Governorate	Agricultural	N31°24' 36.646" E 46°8' 21.336"

2.2 Sample Preservation

Samples for molecular analysis were preserved in 75% ethanol after cleaning according to the Al-Khafaji (2016) protocol [13]. Empty shells were kept dry.

2.3 Morphological Diagnosis and Morphometric Measurements

Shell shape, color, number of whorls, suture depth, aperture shape, and umbilical condition were examined under a Zoom Stereo Microscope (A23.1501 Series). The results were compared with established taxonomic keys [14]; [15]. Basic morphometric dimensions were measured, and derived ratios (Shell Height/Shell Width (SH/SW), Aperture height/ Aperture width (AH/AW), Aperture Height/ Shell Height (AH/SH), Aperture Width/ Shell Width (AW/SW)) were calculated for use as complementary diagnostic criteria.

2.4 DNA Extraction and PCR Reaction

Genomic DNA was extracted from land snail tissue according to the solid tissue protocol using the gSYNC™ DNA Extraction Kit. A portion of the COI gene was amplified using the forward primer LCO1490 and the reverse primer HC02198 in a 25 µL reaction mixture containing 12.5 µL of GoTaq Green Master Mix (Promega, USA), 1 µL of each primer, 4.5 µL of nuclease-free water, and 6 µL of genomic DNA. The PCR reaction was performed under the following thermal conditions: pretreatment of the protein at 95°C for 2 minutes, followed by 35 cycles (heat treatment at 95°C for 60 seconds, annealing at 40°C for 60 seconds, and extension at 72°C for 90 seconds), and a final extension at 72°C for 10 minutes. The PCR products were separated on a 1.5% agarose gel. Similar gene amplification of the COI gene has been used in recent gene coding studies on other land snails, including Feng et al. (2026) on the invasive Achatinidae snails of Hainan Island, China[16], and Galan et al. (2018) on the gene coding of three snail species using the same gene[17], which supports the reliability of this methodological selection.

2.5 Evolutionary and Haplotype Analysis

For 2 specimens, the sequences were analyzed in MEGA 12 software, and an evolutionary relationship tree was constructed using the maximum sparing method with Bootstrap support for 1000 iterations. A haplotype network was also constructed with DNA Nucleotide alignment.

3. Results

3.1 Morphological Diagnosis

Kingdom: Animalia

Phylum: Mollusca

Class: Gastropoda

Order: Stylommatophora

Family: Hygromiidae

Genus: Xeropicta

Species: Xeropicta krynickii Krynicki, 1833)

3.2 Morphological Description and Morphometric Measurements

46 specimens were recorded (23 live, 23 dead), their presence limited to the Al-Rifai station and to the month of January only out of the twelve months of the study period, with a 100% seasonal presence in winter only. The shell is right-handed, low-set, spherical, firm, milky white to pale cream with varyingly distinct brown spiral bands, number of whorls 5-6. The umbilicus is wide and fully open, showing the internal whorls, which is the most important diagnostic feature distinguishing the species. The soft body has a head that is light gray to yellowish in color, bearing two pairs of tentacles. The muscular foot is broad and extended and relatively light in color, as shown in (Figures 2 and 3). The morphometric measurements ranged within the limits shown in (Tables 2 and 3): SH 3.71–6.22 mm, SW 5.88–10.57 mm, AH 2.47–4.04 mm, AW 2.98–5.25 mm, SH/SW 0.58–0.64, AH/AW 0.76–0.86, AH/SH 0.55–0.66, AW/SW 0.41–0.50.

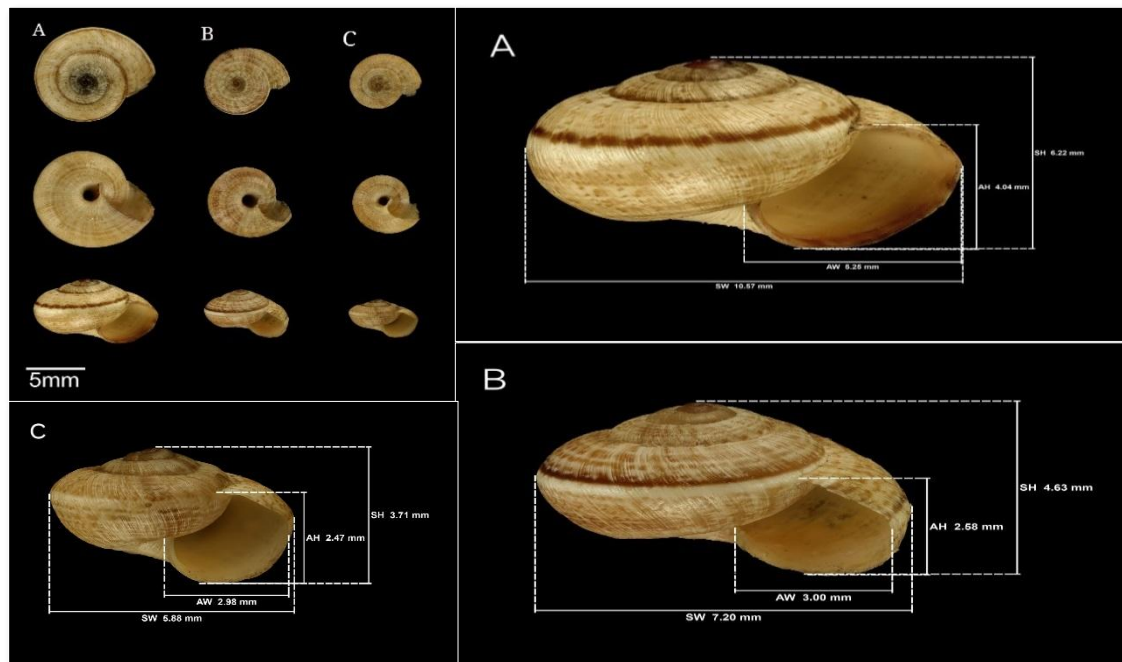


Figure - 2 Samples of the species *X. krynickii*



Figure -3 The soft body details and shell decoration of the species *X. krynickii*

Table 2- Morphometric Measurements of the species *X. krynickii*

specimens	Shell height SH(mm)	Shell width SW(mm)	Aperture Height AH (mm)	Aperture Width AW(mm)	Number of whorls W
A	6.22	10.57	4.04	5.25	6
B	4.63	7.20	2.58	3.00	5
C	3.71	5.88	2.47	2.98	5

Table 3- Morphometric ratios between different measurements of species *X. krynickii*

Specimens	SH\SW	AH\AW	AH\SH	AW\SW
A	0.58	0.76	0.64	0.49
B	0.64	0.86	0.55	0.41
C	0.63	0.82	0.66	0.50

3.3Molecular Results

COI gene amplification showed clear single bands of the expected size for all analyzed samples, as shown in Figure 4. The maximal parsimony tree (Bootstrap = 1000), shown in Figure 5 placed the local samples within a statistically supported genetic cluster alongside *Xeropicta* reference sequences from GenBank, accession numbers:

H1: 3 [PP528206.1, PP528208.1 and PP528207.1]

H2: 1 [PP528205.1]

H3: 1 [PP528204.1]

H4: 1 [PZ069756.1]

H5: 1 [PZ069755.1]

H6: 1 [KU234582.1]

H7: 1 [KU234583.1]

Analysis of the haplotype network, shown in (Figure 6), revealed seven patterns (H1–H7), with the local samples representing patterns H4 and H5. Nucleotide alignment showed a perfect match between H4 and H5 and all reference patterns except for pattern H2, which differed from them in four positions (33, 34, 65, 154).

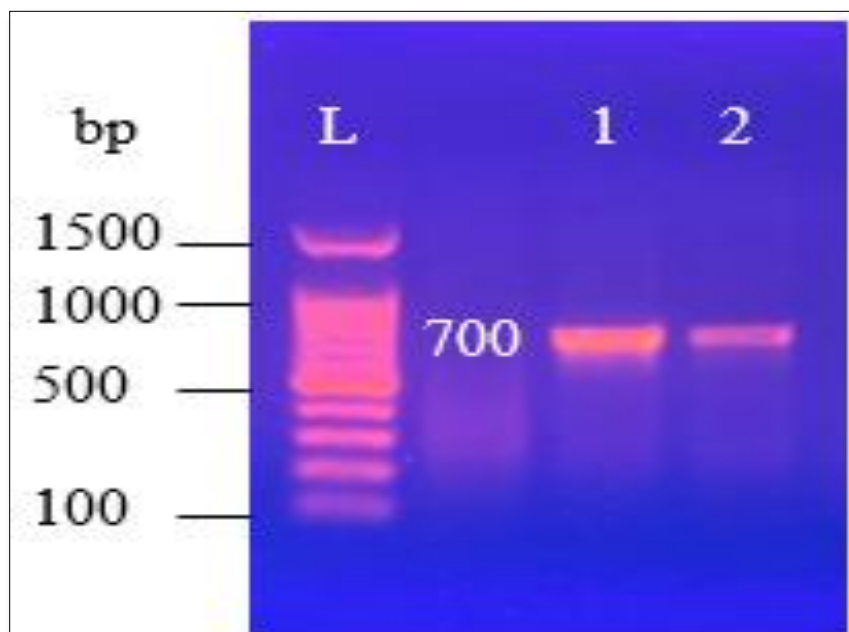


Figure -4 Electrophoresis of the COI gene amplification product from the current study snail, *X. krynickii*. Numbers 1 and 2 indicate *X. krynickii* replicates. The letter L represents *DNA ladder* reference range of 100 to 1500 base pairs.

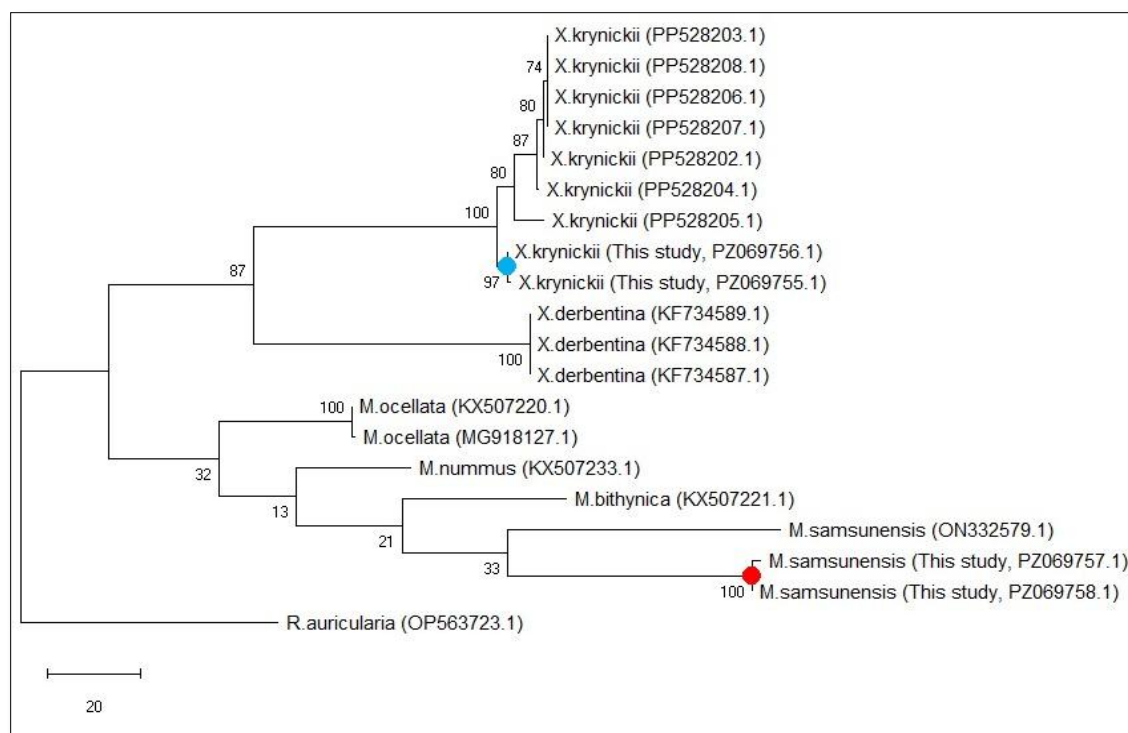


Figure -5 The evolutionary relationship tree of the studied samples based on the COI gene using the Maximum Parsimony method.

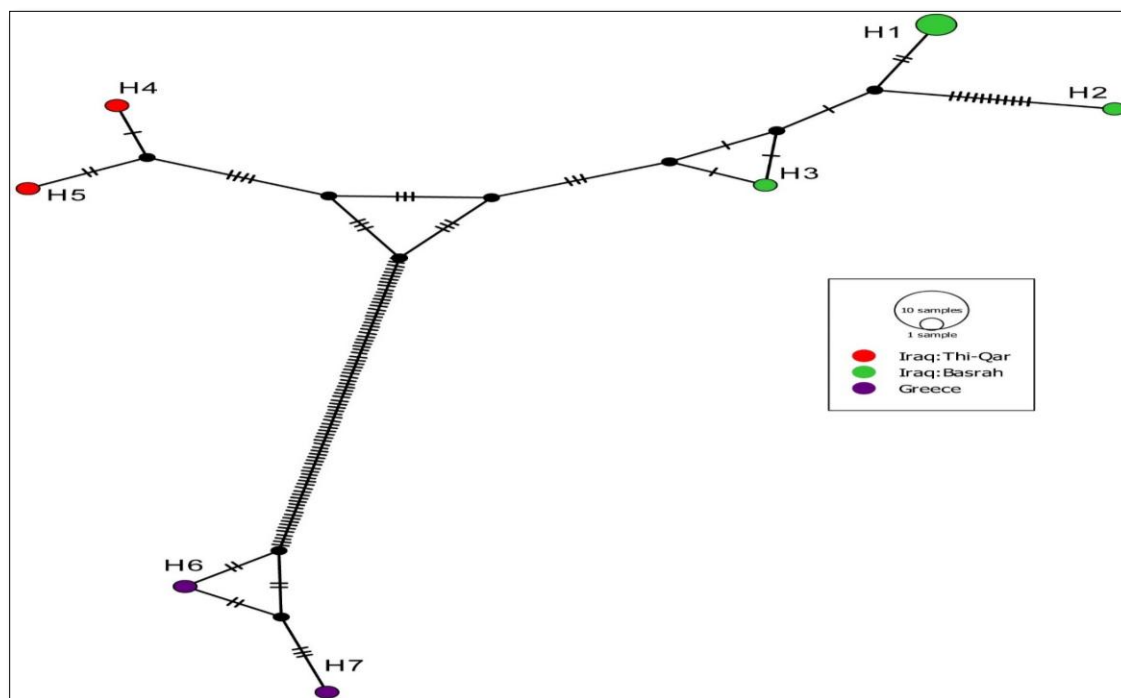


Figure -6 The evolutionary network of haplotype patterns of *X. krynickii* (H)

4. Discussion

The first recording of *X. krynickii* in northern Dhi-Qar Governorate is consistent with the westward geographical expansion pattern documented by De Mattia and Pešić (2014) for this species[1], which they primarily linked to human-induced dispersal mechanisms via agricultural and commercial transport, this contradicts with what Ostrovsky (2023) observed regarding a similar northward spread of a closely related species (*X. derbentina*) as far north as Belarus via passive human transport[2], suggesting that this mechanism is not limited to a single geographical direction but is a general pattern in the dispersal behavior of the species as a whole. The fact that the sighting was limited to a single month (88.89 % presence in winter, with no presence in other seasons) raises a legitimate question about the nature of this presence. It either reflects a small, recently established population that has not yet expanded geographically and temporally in the region, or it reflects a partial limitation in field detection capabilities due to the small size of the specimen and the limited surface activity pattern of this species compared to other associated species in the same location.

The strong genetic similarity between the local genotypes (H4, H5) and most of the reference genotypes (except H2), shown in Figure 6, supports a scenario of relatively recent geographic introduction, consistent with the population genotypes documented by Adamova et al. (2019) for the closely related species *X. derbentina*[3]. This limited genetic divergence also aligns with what molecular phylogenetic studies have documented within the Hygromiidae family itself. Neiber and Hausdorf (2017), in their phylogenetic analysis of the genus *Monacha* (the most diverse genus in the family),

showed that the center of genetic diversity for the family is located in Anatolia, with limited geographic branching patterns in peripheral populations far from this center[18]. This pattern is similar to what has been observed here between the few local subclades (H4, H5) and the rest of the global subclades. Furthermore, the taxonomic basis established by Hausdorf (2000) for the closely related species of the genus *Monacha* in the Western Caucasus provides a similar framework for understanding patterns of limited geographic distribution within the family[19]. Given the small sample size available (only 46 individuals) and its sharp temporal and spatial (only one station) concentration, this study deliberately limits itself to the taxonomic and molecular documentation of the species, leaving the broader ecological question for future, more extensive studies. This aligns with the general methodological recommendation emphasized by Bashê and Al-Qassab (2024) that the sample size should be broadened before generalizing any ecological conclusion to newly recorded species[20]. This record adds to the growing taxonomic record of Iraq's land snails, reinforcing the need for a comprehensive and up-to-date national checklist similar to that started by [5].

5. Conclusion

This research documents *Xeropicta krynickii* (Krynicky, 1833) as a new record of northern Dhi Qar Governorate, Iraq, with integrated morphological and molecular evidence, with the detection of two local haplotype patterns (H4, H5) that are genetically close to most known global patterns.

References

- [1] W. De Mattia and V. Pešić, "Xeropicta (Gastropoda, Hygromiidae) goes west: the first record of *X. krynickii* (Krynicky, 1833) for Montenegro, with a description of its shell and genital morphology, and an additional record of *X. derbentina* (Krynicky, 1836) for Italy," *Ecologica Montenegrina*, vol. 1, pp. 193-200, 2014.
- [2] A. M. Ostrovsky, "Xeropicta derbentina (Krynicky, 1836) (Gastropoda: Eupulmonata: Geomitridae) in Belarus—new data," *Folia Malacologica*, vol. 31, no. 1, pp. 43–47, 2023, doi: 10.12657/folmal.031.006.
- [3] V. V. Adamova, E. A. Snegin, and P. Ukrainskiy, "Morphometric and genetic variability of the alien land snail *Xeropicta derbentina* (Gastropoda, Pulmonata, Hygromiidae) populations," *Ruthenica, Russian Malacological Journal*, vol. 29, no. 3, pp. 193-200, 2019. DOI: 10.35885/ruthenica.2019.29(3).3
- [4] K. Al-Khafaji, "The First record of *Xeropicta mesopotamica* (Mousson, 1874) from Hareer region, southern marshes of Iraq," *Mesopotamian Journal of Marine Science*, vol. 24, pp. 122-125, 2009.
- [5] H. M. Jihad, "ADDITIONAL INFORMATION WITH A CHECKLIST OF LAND SNAILS IN IRAQ," *Bulletin of the Iraq Natural History Museum (P-ISSN: 1017-8678, E-ISSN: 2311-9799)*, vol. 18, pp. 465-476., 2024.
- [6] H. M. Jihad and H. B. Ali, "New Record Of The Land Snail *Polygyra Cereolus* (Megerle Von Mühlfeld, 181 8) (Gastropoda, Stylommatophora, Polygyridae) For Malacofauna Of Iraq," *Bulletin of the Iraq Natural History Museum (P-ISSN: 1017-8678, E-ISSN: 2311-9799)*, vol. 16, pp. 421-428, 2021.

- [7] H. M. Jihad and N. HusseinYousif, "A survey of gastropods in the greenhouses of Iraq," *Bulletin of the Iraq Natural History Museum (P-ISSN: 1017-8678, E-ISSN: 2311-9799)*, vol. 18, pp. 565-576., 2025.
- [8] S. E. Al-Qassab, "First record of the pointed snail *Cochlicella acuta* (O. F. Müller, 1774) from Iraq," *Journal of Wildlife and Biodiversity*, vol. 10, pp. 192-200, 2026.
- [9] R. F. Ali and R. Ramdane, "Taxonomic key as a simple tool for identifying and determining the abundant terrestrial snails in Egyptian fields (Gastropoda, Pulmonata: Succineidae, Geomitridae, Helicidae, Hygromiidae)," *Egyptian Academic Journal of Biological Sciences, B. Zoology*, vol. 12, pp. 173-203, 2020.
- [10] Y. Abobakr, A. S. Al-Sarar, A. A. Alzabib, and A. A. Saleh, "Morphological and molecular characterization of the invasive pestiferous land snail *Macrochlamys indica* Godwin-Austen, 1883 (Gastropoda: Ariophantidae) from Saudi Arabia," *Agriculture*, vol. 12, p. 1756, 2022.
- [11] G. Manganelli, J. R. Pieńkowska, D. Barbato, A. Benocci, K. Sosnowska, R. Anderson, *et al.*, " Exploring *Monacha* species from the island of Corfu (NW Greece) by an integrative approach: new insights on *M. claustralis* (Rossmässler, 1834), *M. parumcincta* (Rossmässler, 1834) and allied species (Gastropoda, Eupulmonata, Hygromiidae)," *ZooKeys*, vol. 1250, p. 201, 2025.
- [12] F. Götmark, T. Von Proschwitz, and N. Franc, "Are small sedentary species affected by habitat fragmentation? Local vs. landscape factors predicting species richness and composition of land molluscs in Swedish conservation forests," *Journal of Biogeography*, vol. 35, pp. 1062-1076, 2008.
- [13] K. K. Al-Khafaji, "First record of terrestrial snail *Eobania vermiculata* (OF Muller, 1774)(Gastropoda: Helicidae) from Basrah areas, Iraq," *Arthropods*, vol. 5, p. 125, 2016.
- [14] F. W. Welter-Schultes, *European Non-Marine Molluscs: A Guide for Species Identification—Bestimmungsbuch für europäische Land- und Süßwassermollusken*, 1st ed. Göttingen, Germany: Planet Poster Editions, 2012.
- [15] L. Chiberry, J. Sánchez, V. Núñez, S. Rodriguez, M. Pellegrino, and S. Poljak, "Contributions to the knowledge of DNA barcodes and diversity of intertidal and shallow-water mollusks in the Beagle Channel," *Polar Biology*, vol. 49, p. 5, 2026.
- [16] X. Feng, Y. Wang, Y. Xu, and Y. Lu, "DNA barcoding and genetic characterisation of giant African land snails (Achatinidae) on Hainan Island, tropical China, by using COI," *Molluscan Research*, vol. 46, pp. 76-83, 2026.
- [17] G. L. Galan, N. P. Mendez, and R. Y. Dela Cruz, "DNA barcoding of three selected gastropod species using cytochrome oxidase (COI) gene," *Annals of West University of Timișoara, Series of Biology*, vol. 21, no. 1, pp. 93–102, 2018.
- [18] M. T. Neiber and B. Hausdorf, "Molecular phylogeny and biogeography of the land snail genus *Monacha* (Gastropoda, Hygromiidae)," *Zoologica Scripta*, vol. 46, pp. 308-321, 2017.
- [19] B. Hausdorf, "The genus *Monacha* in the Western Caucasus (Gastropoda: Hygromiidae)," *Journal of natural History*, vol. 34, pp. 1575-1594, 2000.
- [20] S. K. Bashê and S. E. Al-Qassab, "Morphological and molecular identification of some terrestrial snails: first report," *Zanco Journal of Pure and Applied Sciences*, vol. 36, pp. 94-104, 2024.