ORIGINAL STUDY

The Assessment of Morphological, Anatomical, and Phylogenetic Relationships of Three Helicid Snails in Egypt

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Abstract

Land snails provide valuable models for eco-genetic research because of their morphology and banding variability. Snails have shown unusually high intraspecific variation in anatomical traits leading to confusion for taxonomical studies, although their taxonomic resolution is low specifically in Egypt. This study aims to investigate the morphological, anatomical, and phylogenetic relationships for the first time among the snails *Eobania vermiculata*, *Eremina desertorum*, and *Helix aspersa* within the family Helicidae. The morphological indices revealed similarities among *E. vermiculata*, *E. desertorum*, and *H. aspersa*, However, scanning electron microscope examinations indicated that the marginal teeth of the radulae in *E. vermiculata* and *H. aspersa* were more closely related than those of *E. desertorum*. Scanning electron microscope analysis of the darts demonstrated a close resemblance in shape and length between *E. desertorum* and *E. vermiculata*, in contrast to *H. aspersa*. Molecular techniques, including 18 s rRNA and random amplified polymorphic DNA analysis, indicated that *E. vermiculata* and *E. desertorum* were closely related to *H. aspersa*, dealing with the dart-shaped results. This study highlighted the importance of integrating morphological or anatomical research with DNA techniques to explore relationships among closely related species. It also gives information for future studies on the evolutionary relationship of land snails in Egypt.

Keywords: Dart, Gastropoda, Molecular techniques, Morphological characters, Radula

1. Introduction

Comprehending the mechanisms leading to morphological and genetic variety is a fundamental objective in evolutionary biology. The evaluation of the roles played by selection and history in the genesis of diversification frequently employed land snails as model animals (Fathey *et al.*, 2016). Class Gastropoda is the only mollusc that has successfully invaded the land with more than 80 000 described species and can be considered the second most species-rich animal group after insects (Krings *et al.*, 2019). One of the most diverse groups of land snails is the superfamily Helicoidea which has many commercial species distributed worldwide except from most of sub-Saharan Africa, and some islands of the South Pacific (Ahyong *et al.*, 2023). Within the family Helicidae, land snails have been widely used for phylogeographical, ecological genetics, and phylogenetic investigations (Schweiger *et al.*, 2004).

The shape of gastropods' shells is incredibly variable, making them one of the most diversified animal groups. Their shells show ornamental variations and vary in size from ~1 to over 1 m. The

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morphology of the shell can be extensively associated with the life mode of gastropods (Frýda, 2013). The shells of *Eobania vermiculata* (Müller 1774) (*E. vermiculata*), *Eremina desertorum* (Forsskål, 1775) (*E. desetorum*), and *Helix aspersa* (Müller 1774) (*H. aspersa*) show many variations in size and shape. *E. vermiculata* is known as a chocolate-band snail with shell variability in colour, ranging from whitish to greenish yellow (Desouky & Busais, 2012), while *E. desertorum* is known as a desert snail with well-marked dark brown spiral bands and can rarely be Ali et al., 2016 white (Nrubert, 2017). *H. aspersa* is a large-bodied snail with a brown or yellowish variable number of dark spiral bands (Neubert, 2014).

The wide range of conchological and anatomical characteristics of land snails lead them to be great models for the molecular assessment of taxonomic positions. However, the evolutionary labile characteristics of land snail shells frequently cause some inconsistency between phylogeny and taxonomy (Gajera *et al*., 2022), The morphology of land snails is remarkably varied as they exhibit significant diversity in shell colour and variances in shell size (Santos, 2014). Previously, the primary basis for phylogenetic analyses was morphological characteristics, like conchological parameters (Fiorentino *et al*., 2008), genital gross anatomy (Giusti *et al*., 2011), and/or radular morphology (Marimuthu, 2016). Love darts are characteristic features of the reproductive tract for the hermaphrodite land snail family Helicidae. These darts are assembled within a thick-walled dart sac and have now been described in a variety of species, each with their structural architecture (Shimizu *et al*., 2018). The dart is a little white fragile construction with a variable number of blades, a flare, and an extended shaft flanked by lateral vanes (El-Sherief *et al*., 1995). Interestingly, love darts vary greatly in number and shape between species, ranging from multiple basic cone-shaped structures to one elegantly bladed structure (Chapman *et al*., 2003). Therefore, it can be used as a taxonomic tool to differentiate between different species of snails. Radula morphology has traditionally been used for molluscan systematics (Fernandes *et al*., 2013). Molluscan radular teeth are characterized by their distinct shape and form, which makes them unique within a species or genus. Certain characteristics of the radula, including tooth number, have been utilized to explore the upper levels of molluscan taxonomic relationships (Gajera *et al*., 2022). Scanning Electron Microscope is widely used to identify the radula and dart differentiation among species of Mollusca (Marimuthu, 2016).

Molecular markers can be used to identify organisms and estimate genetic distances (Palasio *et al*., 2019). Random amplified polymorphic DNA (RAPD) has been used for a variety of applications, ranging from individual-level research (e.g., genetic identification) to studies involving closely related species (Ansari *et al*., 2021). The small subunit 18 S rRNA gene is a highly used gene in phylogenetic investigations and a key marker for random target PCR in environmental biodiversity screening (de Jonge *et al*., 2021). The present work aimed to analyze the taxonomic position of species, *E. vermiculata*, *E. desertorum*, and *H. aspersa* snails within the Helicidae family in Egypt using the morphological, anatomical, and molecular tools including 18 s RNA and RAPD.

List of abbreviations

PCR	polymerase chain reaction
RAPD-PCR	random amplified polymorphic DNA
H.aspersa	*Helix Aspersa*
E.vermiculata	*Eobonia Vermiculata*
E. desertorum	*Eremina Desertorum*
SW	shell width
SH	shell height
AH	aperature height
AW	aperature width
18 s rRNA	18 s ribosomal RNA
Ec	ectocones
M	mesocones
En	endocones
PCA plot	principal component analysis

2. Patients and methods

2.1. Snails' collection

A total of 30 snails of *E. vermiculata* and *H. aspersa* were collected from the Kerdasa region (Giza Governorate), while 30 of *E. desertorum* snails were collected from Western Sahara on the road from Marsa-Matruh to Al-Alamein. The collection period was from November 2022 to February 2023.

The collected snails were transferred and reared in the laboratory of Invertebrates and Parasitology, Zoology Department, Faculty of Science, Helwan University, Egypt. All individuals had been wiped clean with dechlorinated water to eliminate excretions on their mantles and shells. Snails have been stored in plastic boxes, at temperatures of 26–28 °C below excessive soil moisture (80 % relative humidity) and ate up clean leaves of lettuce.

2.2. Morphometric study

After the collection of samples, the measurements of each species were taken, using Vernier caliper as

Fig. 1. Egypt Map showing the sites of collection. 1: Kerdasa region (Giza Governorate), 2: The Western Sahara on the road from Marsa-Matruh to Al-Alamein.

shown in Figs. 1 and 2, all measurements were given in centimeters (cm) unless otherwise indicated. The measurements were measured and abbreviated as follows: shell height (SH): represents the vertical measurement from the top to the bottom of the shell; shell width (SW) represents the distance between points on one side of the shell to the opposite side through the apex, aperture height (AH): it represents the height of shell opening, aperture width (AW) represents the width of shell opening.

2.3. SEM analysis for radula morphology and dart sac

For radula analysis, a single snail from each species was taken out of its shell, and the dorsal surface of the head was lightly incised until the radula was exposed. The extracted radulae were cleaned in 10 % KOH for 24 h. Also, the dart sac was extracted from the reproductive system of each species. Then, the radulae and dart sac were transferred into separated tubes containing 2.5 % glutaraldehyde in 1 × phosphate buffer solution (pH 7.4). After that, samples were transferred to be processed as described by (El-Bakary & Abumandour, 2017) then the samples were put on a copper plate (1 × 1 cm) coated by carbon tape and examined using a Thermo Fisher Scientific (FEI) scanning electron microscope (Quanta FEG 250), at the National Research Center, Giza, Egypt.

2.4. DNA isolation, molecular identification, and genetic variation

The three samples under investigation, *E. vermiculata*, *E. desertorum,* and *H. aspersa* were dissected and shell parts were removed then their feet were preserved in absolute ethanol at −20 °C until used. Using FavorPrep Tissue Genomic DNA Extraction Mini Kit (FATGK 001), total genomic DNA was extracted from frozen ethanol-preserved soft parts by the instructions provided by the manufacturer. The isolated genomic DNA was checked in 1 % agarose gel (Sutcharit *et al.*, 2020).

2.5. 18 s rRNA amplification

PCR amplification of the nuclear gene, 18 s rRNA for snail species under study, were carried out from the purified genomic DNA using the primer sets, EUK1.f, 5′−AGCGGAGGAAAAGAAACTA−′3; andEUK2.r,5′−
TACTAGAAGGTTCGATTAGTC−′3. The total volume of the PCR reaction mixture was 25 μl; contained 12.5 μl of master mix, 1 μl of each primer, and 50 ng DNA template. Amplifications were performed using BioRad Thermocycler, programmed for 35 cycles of 94 °C for 30 s, the annealing temperature was 54 °C for 30 s, and 1 min extension at 72 °C. The final extension was turned into 72 °C for 10 min for one cycle. The purified 18 S rRNA gene amplicons were analyzed directly by sequencing, using an ABI 3730xl DNA sequencer by using forward and reverse sequences, by combining the

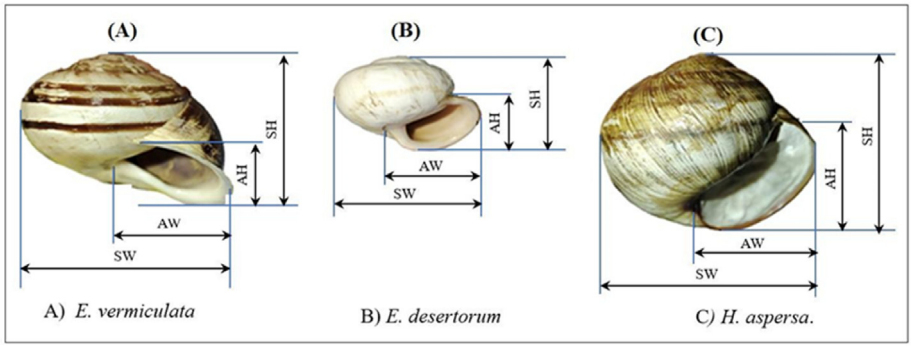

Fig. 2. Shells of collected snails showing the measured morphological parameters. (a) E. vermiculata, (b) E. desertorum and (c) H. aspersa.

Traditional Sanger with the new 454 technology (GATC Company, Germany) (Mokhtar *et al.*, 2023).

2.6. RAPD-PCR

A RAPD molecular marker was used to estimate the genetic variation within snail species under investigation. The entire reaction required is a total of 25 µl consisting of 12.5 µl of PCR master mix, 2 µl of DNA sample, and 2 µl of RAPD primers sequence (CGTTGGCCCG, ATCGGCTGGG, CTTGCCCACG, and GTGGCAAGCC). The PCR (BioRad thermocycler) condition was as follows: '95 °C for 5 min, then 35 cycles of 95 °C for 30 s, annealing temperature for 30 s, and 72 °C for 1 min'. The resulting product underwent analysis by 1.7 % agarose gel electrophoresis, and its genetic polymorphism and variation were quantified using BioRad Quantity One software.

2.7. Statistical analysis

For the shells morphometrics, statistical parameters were calculated and principal component analyses performed (using a correlation matrix). The gel electrophoresis pictures were analyzed, and bands that were detected were assigned a score of 1, while those that were not detected were assigned a score of 0. A similarity matrix was generated using Jaccard's coefficient of similarity, and cluster analysis was performed using the unweighted pair group method with the arithmetic averaging algorithm (UPGMA) to construct the dendrogram. The software version is Bio-Rad Quantity One 4.6.2 (Abd El Hamid *et al.*, 2023) was used to make these calculations. Also, Jalview (2.11.2.7) software was applied to obtain the phylogenetic relationship among species.

3. Results

3.1. Morphological characteristics of shells

The shell of *E. vermiculata* has a cream-whitish background, compressed, spherical, and thick-walled. It contains four mainly reddish to dark brown spiral bands with a thick and erratic pattern of white dots. The aperture is broad and oblique, and it has a moderately strong lip Fig. 3a and b while the shell of *E. desertorum* is sub-globose, opaque with five convex whorls with shoulder whorls. The colour ranges from white to yellowish-corneous, with irregular radial striation and reddish or yellowish bands. The aperture has a thickened, straight, or slightly reflexed edge and is generally rounded

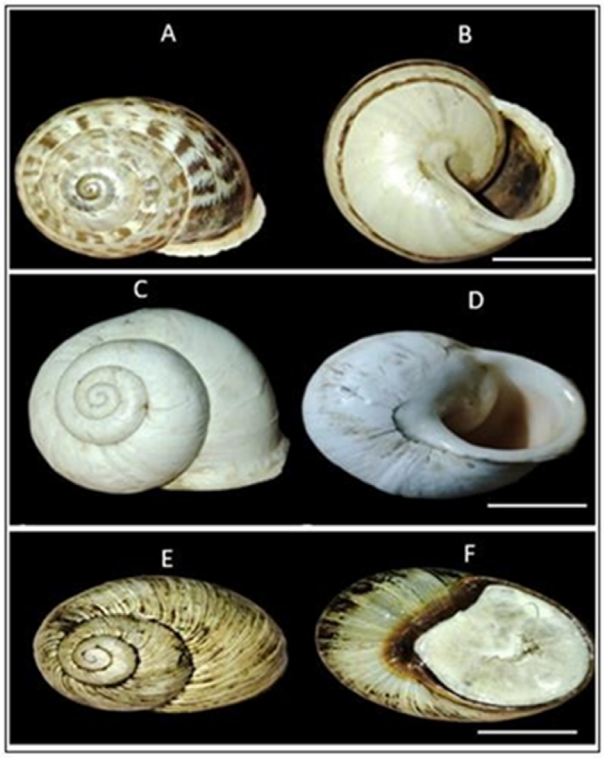

Fig. 3. The shell morphology of species under study. (a, b) Dorsal and ventral view of E. vermiculata shell. (c, d) Dorsal and ventral view of E. desertorum shell. (e, f) Dorsal and ventral view of H. aspersa shell. Scale bar 1 cm.

Fig. 3c and d. The shell of *H. aspersa* has a conical shape. The growth lines are noticeable, and the surface is wrinkled, which appears to have faint, interrupted spiral lines. The aperture is large, with a brown lip that is somewhat enlarged Fig. 3e and f.

3.2. Morphometric study

3.1.1. Shell measurements

Measurements of the shell length, width, AH, and AW indices of the species under investigation were taken using a Vernier caliper. As shown in Table 1, and Fig. 4. The SH, SW, and AW of *E. vermiculata*

Table 1. Shell measurements of E. vermiculata, E. desertorum, and H. aspersa. The values are in (cm) and represented as (mean ± standard deviation).

Species parameter	Shell height	Shell width	Aperture height	Aperture width
E. vermiculata	2.5 ± 0.4^{b}	2.4 ± 0.4^{b}	1.8 ± 0.4^{b}	1.9 ± 0.4^{b}
E. desertorum	2.8 ± 0.3^{a}	3.1 ± 0.4^{a}	1.8 ± 0.4^{b}	1.7 ± 0.4^{a}
H. aspersa	2.5 ± 0.4^{b}	2.4 ± 0.4^{b}	1.8 ± 0.4^{b}	2 ± 0.4^{b}

The small letters represent the significance where *E.desertorum* measurements are more significant than the two snails, *P* value less than 0.05. a represents significance, where b is no significance.

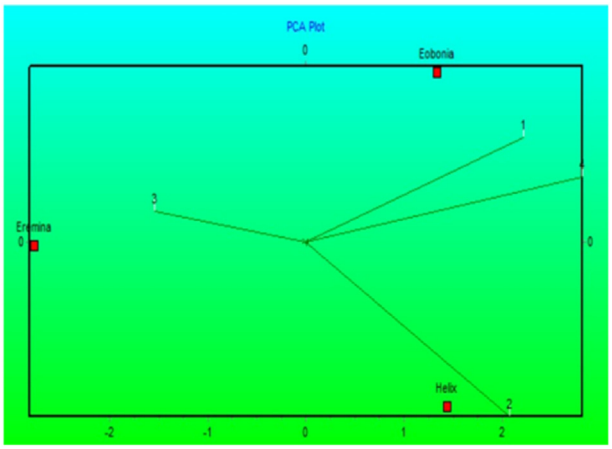

Fig. 4. Principle component analysis (PCA) of Morphometric measurements of E.desertorum, E.vermiculata, and H.aspersa shells.

and *H. aspersa* were more closely to each other than *E. desertorum* where the SH of *H. aspersa and E. vermiculata* was 2.5 ± 0.4 while the SW was 2.4 ± 0.4 and for AW ranged from 1.9 ± 0.4 to 2.0 ± 0.4, while for *E. desertorum,* they were 2.8 ± 0.33, 3.1 ± 0.4, 1.7 ± 0.4 for SH, SW and AW, respectively. The AH of the three measured samples was the same (1.8 ± 0.4).

3.3. Dart morphology

The dart has a funnel-shaped corona, an extended cylindrical shaft, and a short flare surrounded by lateral vanes Fig. 5a–c. The dart of the three species shows some differences in size and shape. It measures 3.9 mm, 4.5, and 5.87 mm for *E. vermiculata, E. desetrorum,* and *H. aspersa,* respectively.

3.4. Scanning electron microscopy of radula

The three species under study showed the pulmonate pattern of radulae that are distinguished with one central tooth, and many laterals and marginal teeth per each row. The central and lateral teeth of *E. vermiculata*, *E. desertorum,* and *H. aspersa* showed close similarity in appearance and size (Fig. 6a–c. The central tooth is tricuspid,

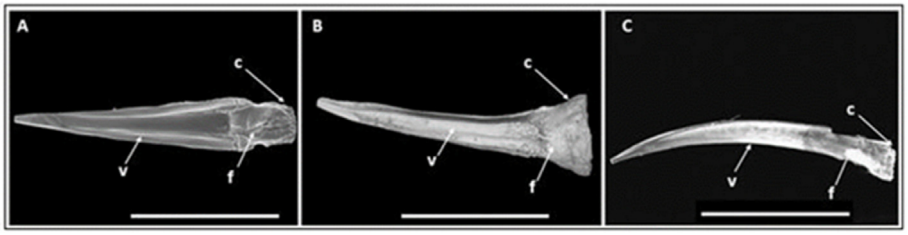

Fig. 5. Scanning electron microscope showing the morphological characteristics of the love darts. (A) Represents the dart of E. vermiculata, and (B) represents the dart of E. desertorum. (C) Represents the dart of H. aspersa. Abbreviations, (C) Corona, (F) flare, (V) vane. All scales are 1 mm.

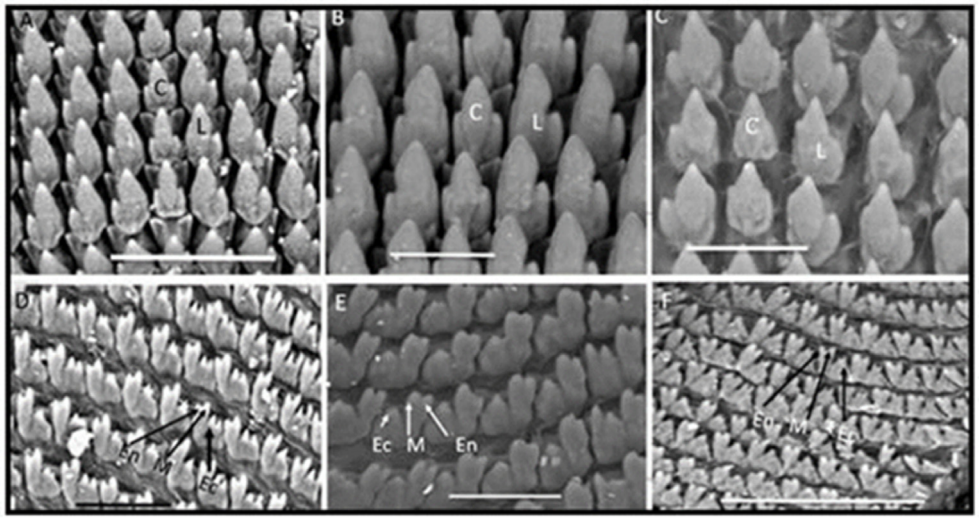

Fig. 6. Scanning electron microscope showing the radulae of E. vermiculata, E. desertorum, and H. aspersa. Scanning electron microscope of the central (C) and lateral (L) teeth and marginal teeth of E. vermiculata (Figure A, D), E. desertorum (Figure B, E), and H. aspersa (Figure C, F). The scale bar is 50 μm for B, C, D, and E, while A is 40 μm and F is 30 μm. Abbreviations. (Ec) ectocones, (M) mesocones, and (En) endocones.

symmetrical with mesocone lanceolate, ectocones are small and recessed far back on the tooth, while the lateral teeth are bicuspid, symmetrical elongate mesocone, and small triangular endocone. Marginal teeth are tricuspid, with long mesocone and endocone while ectocone is shorter and narrower, split into extra denticles toward the outer edge. The marginal teeth of radulae showed a close relationship between *E. vermiculata* and *Helix* Fig. 6d–f while it showed a different pattern for the *E. desertorum* radula Fig. 6e.

3.5. Molecular identification using 18 S rRNA technique

After the DNA was extracted, eukaryote-specific primers were utilized on a molecular basis to identify each species by using the 18 S rRNA-PCR method. To identify the snail species, the product was sequenced (deposited in the supplementary materials), and the output sequence was compared with NCBI data. The results showed that *E. desertorum* and *E. vermiculata* snails have 97,93 % and 97,53 % identity rates with *Cepaea nemoralis* snails found in the same family, respectively. On the other hand, *H. aspersa* showed a 98,56 % identity rate with *C. nemoralis* snail. Based on results obtained from 'Jalview'. Figs. 7–10) illustrate the phylogenetic relationship among the variable species.

3.6. Estimating the genetic variations using RAPD analysis

The variation in the morphological and physiological responses is reflected in the genetic variation within the genetic material of each snail species.

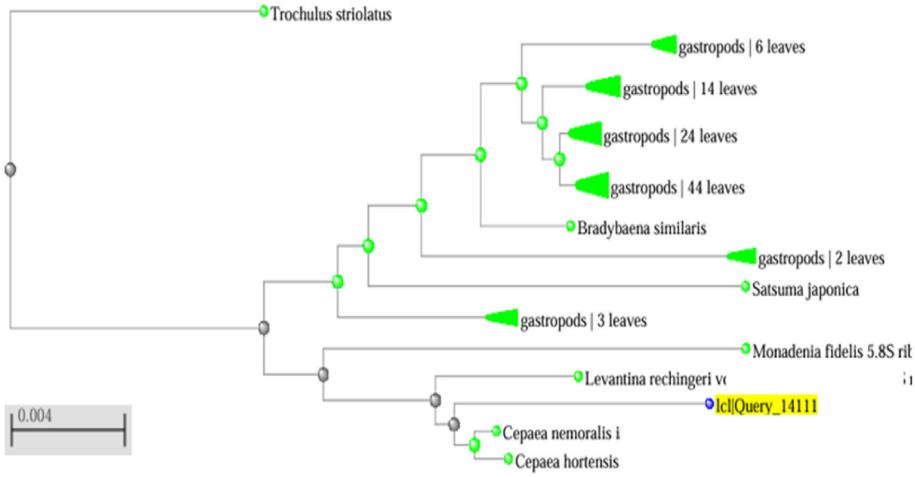

Fig. 7. The phylogenetic tree of alignment E. desertorum sequence based on NCBI data.

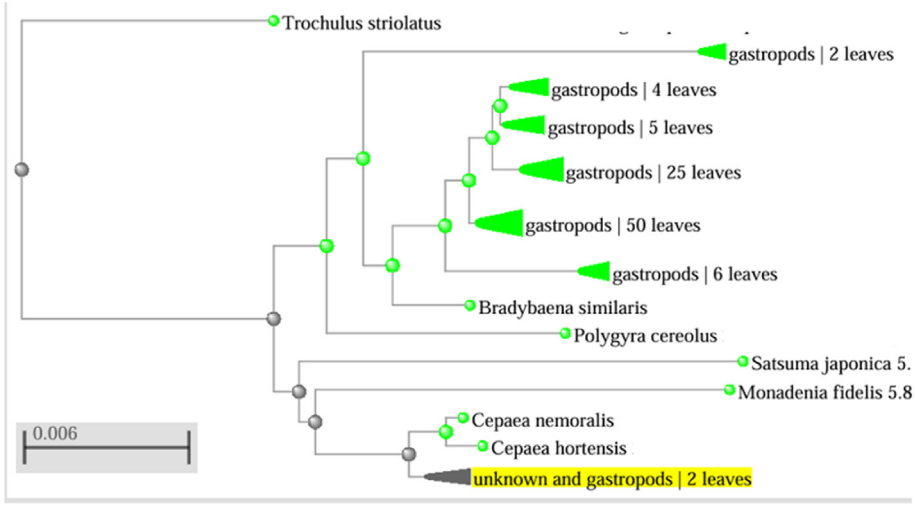

Fig. 8. The phylogenetic tree of alignment E. vermiculata sequence based on NCBI data.

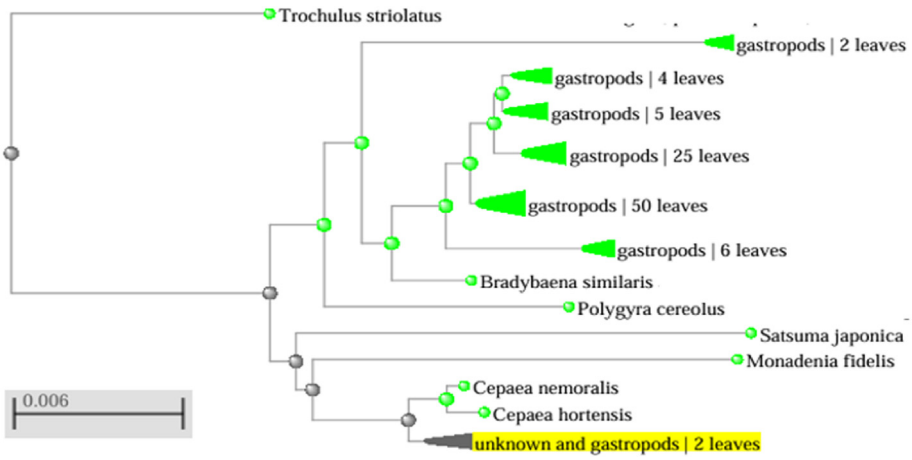

Fig. 9. The phylogenetic tree of alignment H. aspersa sequence based on NCBI data.

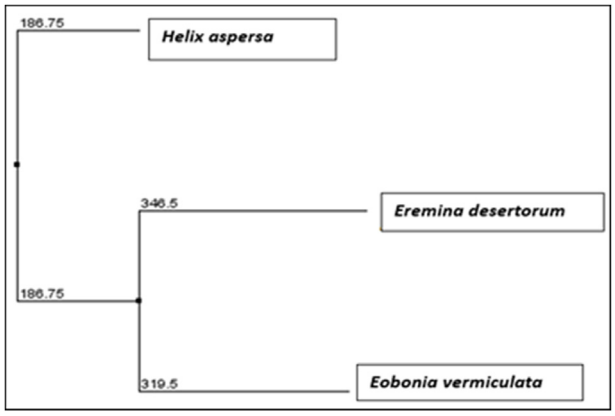

Fig. 10. The total phylogenetic tree is based on the identification sequences of the three species.

Only four primers gave reproducible bands of all the seven primers that were used. The agarose gel banding pattern is depicted in Fig. 11, while the polymorphism data are presented in Table 2. The

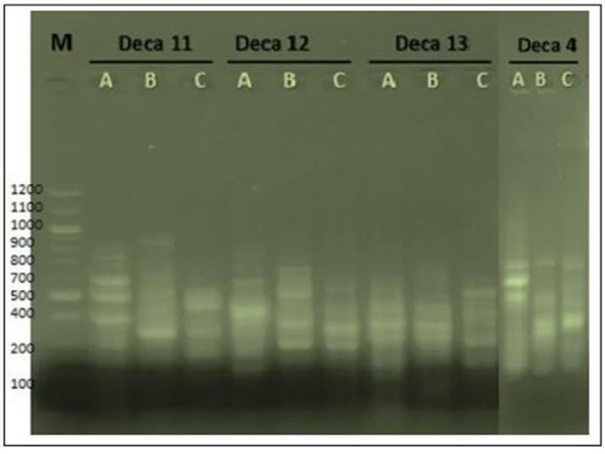

Fig. 11. Gel banding pattern based on RAPD-PCR for the three snail species. (A: E. desertorum, B: E. vermiculata, C: H. aspersa).

overall genetic variation among snails, as measured by polymorphism, was 58.18 %. The dendrogram in Fig. 12 based on RAPD data confirmed the similarities among these species and confirmed via the principal component analyses plot in Fig. 13. The total similarity matrix was explained in Table 3 and indicated that the similarity ranged from 49 to 37 %. All these data concluded that *E. desertorum* snail is more closely related to *E. vermiculata* snail than *H.aspersa* snail.

4. Discussion

Land snails exhibit remarkable diversity in their colour, banding pattern, and shell structure so they can be used for taxonomic and phylogenetic purposes (Korábek et al., 2021). The largest and best-known land snails is the family Helicidae. However, until recently, most of our understanding of its variety was based on morphology-based taxonomy studies. The identification of snails was performed through the morphological study of thirty specimens from each species. The SH, SW, and AW of *E. vermiculata* and *H. aspersa* were very similar to each other than of *E. desertorum*. Similar results were obtained by (Ali, 2017) who collected *E. desertorum* and *E. vermiculata* at different plots on the road to Marsa Matrouh and measured different parameters for the shell and the results were very close in ranges for the four measured parameters. The morphological studies in the present study were very different compared with other values estimated for the same species in different habitats. The polymorphism in shell shape and size of *H. aspersa* species correlates with the ecosystem where they live and that may result in genetic diversity in some alleles (Kougiagka *et al.*, 2022).

Table 2. Data of bands scoring of RAPD-PCR of variable snails' species.

No.	Primer code	Primer sequence (5′–3′)	^{T}m (^{O}C)	GC%	Total number of bands	No. of polymorphic bands	Polymorphism percentage (%)
1	Deca-04	CGTTGGCCCG	44	80	5	3	60
2	Deca-11	ATCGGCTGGG	39.3	70	7	5	71.42
3	Deca-12	CTTGCCCACG	38.5	70	7	3	42.85
4	Deca-13	GTGGCAAGCC	39	70	4	2	50
Total					23	13	56.067

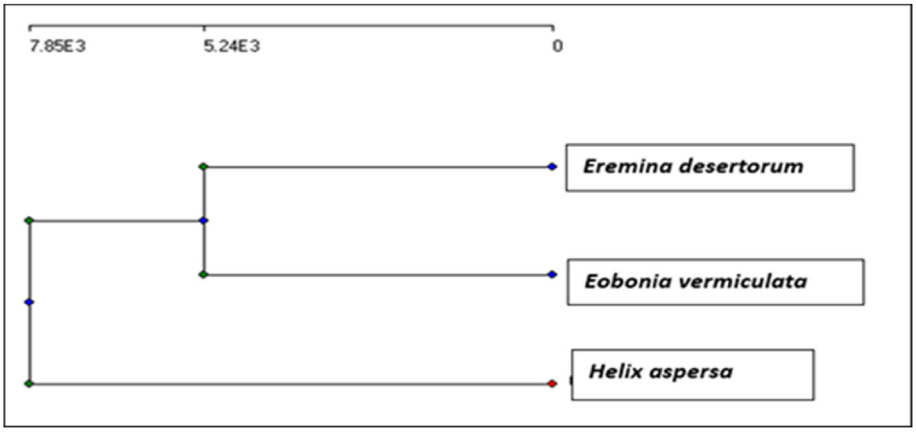

Fig. 12. Dendrogram of complete linkage clustering of total similarity matrices of variable snail's species obtained from RAPD-PCR gel analysis.

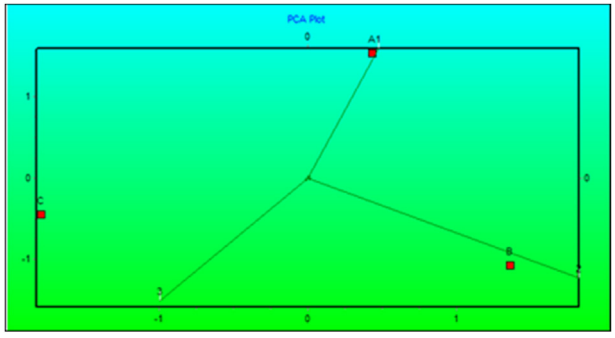

Fig. 13. PCA blot of total similarity matrices of variable snail's species obtained from RAPD-PCR gel analysis. (a) E. desertorum, (b) E. vermiculata, (c) H.aspersa.

Table 3. Total similarity matrices of variable snail species obtained from RAPD-PCR gel analysis.

Similarity matrix	*E. desertorum*	*E. vermiculata*	*H. aspersa*
E. desertorum	100	49.08	44.98
E. vermiculata	49.08	100	37.42
H. aspersa	44.98	37.42	100

Few studies discussed the shape of gastropod darts and its relation to their taxonomic positions (Lodi & Koene, 2015), however many studies showed the use of darts in reproduction physiology and its benefit between the two mates (Shimizu *et al.*, 2018). In the present study, the length of *E. vermiculata* and *E. desetrorum* darts was similar, while for *H. aspersa*, it was longer in length and slightly curved, Also the structure of each dart was very similar in shape for *E. desertorum* and *E. vermiculata*. Similarly (El-Sherief *et al.*, 1995), found that *Eremina ehrenbergi* dart is a small white structure that consists of a corona with two blades; a short flare, and an extended shaft which is surrounded by two lateral vanes which is very close in shape to *E. desertorum* dart as they are the same genus. Moreover (Hasse *et al.*, 2002) found the shape of *Helix pomatia* dart is a four-bladed, hollow spicule of calcium carbonate and was very similar to the shape of *H. aspersa* dart detected in this study. In addition (Jirapatrasilp *et al.*, 2022), suggested that dart complex found in the land snail *Amphidromus* could correct their taxonomic position for some of these East Asian arboreal snails *Amphidromus* under the subfamily Camaeninae.

The radula morphology has been widely used as a tool in the classification of gastropods (Vortsepneva *et al.*, 2023). In the present study, *E. vermiculata, E. desertorum, and H. aspersa* showed the pulmonate radula pattern, which is distinguished by the presence of one central tooth and many laterals and marginals teeth (Krings & Gorb, 2021). The central and lateral teeth of radulae of the three species under study were very similar to each other while, the marginal teeth of radulae of *E. vermiculata* and *H.aspersa* showed close similarity in the structure

while it was different in the radulae of *E.desertorum.* Similarly (Krings *et al.*, 2019), found that the radula of *Cornu aspersum* snail (Gastropoda, Helicidae) followed the pulmonated pattern like the radulae of the species under study. Unlike the present study (Marimuthu, 2016), found that the radula of *Pila virens* (Gastrpoda, Ampullariidae) had the taenioglossate type that was distinguished by seven teeth in the transverse row, one central (rachidian), one lateral, and two marginals, following the general radular formula 2M+1L+1R+1L+2M. In addition (Gajera *et al.*, 2022), found the radula of *Turbo intercostalis* snail (Gastropoda, Turbinidae) followed the rhipidoglossan pattern, which is characterized by a single central tooth with 5 lateral teeth and many marginal teeth. Many studies have suggested that the radula morphology might be affected by many factors such as seasonal changes, sexual differences, and age (De los Ríos *et al.*, 2020). As *H. aspersa* and *E. vermiculata* feed on the same vegetation and they occur in similar habitats, it was expected that they show a similar and close pattern of the radula than *E. desertorun* that lives in different habitats and feed on wild plants and shrubs found in deserts.

Using shell morphometry or any single analysis as taxonomic markers is insufficient (Caro *et al.*, 2019). So, species delimitation for several snails has been addressed using a variety of genomic approaches, including DNA sequencing (Young *et al.*, 2021). In the current study, 18S rDNA and RAPD-PCR molecular markers showed that *E. desertorum* was more related to *E. vermiculata* than *H. aspersa* which contradicts some morphological results. Environmental influences and adaptive convergence tend to modify phenotypic traits, to provide potentially misleading results. Some of the examples are that certain environmental factors such as climate, soil, and habitat conditions may lead, through convergence, to the development of similar morphological characters as size or different colour shells in genetically unrelated species (Pfenninger & Schwenk, 2005). The appeal of genetic analysis, in other words, is this dismissal of ambiguity arising from being able to measure the DNA sequences, This method is thus particularly important in biodiversity studies, as it enables the recognition of cryptic species and the understanding of the mechanisms by which such species may arise, particularly for land snails, wherein the degree of environmental control can influence phenotypic variation (Schilthuizen, 2000).

The molecular results in the present study were similar to the studies of (Neiber *et al.*, 2021), which showed that *E. desertorum* and *E. vermiculata* were related to each other by placing them in the same tribe (Thebini) under subfamily Helicinae and *H. aspersa* belongs to tribe Helicini suggesting that the dependance on morphological results can lead to wrong taxonomy as their remarkable phenotypic diversity in shell colour, banding pattern has occasionally resulted in disagreeable taxonomy (Kornilios *et al.*, 2009). Similarly (Hirano *et al.*, 2019), used the molecular phylogenetic analyses 18 S rRNA to explore phylogeographic differences between land snails *Bradybaena circulus* and *Bradybaena phaeogramma* found in continental islands of Japan. In addition (Sutcharit *et al.*, 2020), concluded that using genital organ morphology and genetic divergence is consistent and useful in delimiting species while using shell characters only showed little overall taxonomic utility in some species such as the land snail *Sophina Benson* (Eupulmonata: Helicarionidae). In these cases, sequencing data has frequently allowed researchers to develop and assess alternatives to the morphological one-species 'hypothesis' and support or contradict the taxonomy based solely on morphology.

4.1. Conclusion

The present results indicated the importance of using molecular techniques besides morphological studies. The morphological and anatomical studies revealed the presence of a close relationship between *E. vermiculata* and *H. aspersa*. On the other hand, the molecular techniques used as 18 s rRNA and RAPD showed that *E. vermiculata*, and *E. desertorum* are more closely related species than *H. aspersa*. The findings of this study will be valuable for future research into the evolutionary relationships of land snails, as well as for the conservation status of these snails in Egypt.

Ethics information

This study has been approved by university animal ethics committee and recieved ethics number [HU-IACUC/Z/SE1703-27].

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Author contributions

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I.S.G., E.T.H., and M.Y.M; data curation, S.E.A, I.S.G., R.M.M, A.M.I, E.T.H., and M.Y.M; funding acquisition, R.A.-G; writing original draft preparation S.E.A, I.S.G., R.M.M, A.M.I, E.T.H., and M.Y.; writing review and editing, S.E.A, I.S.G., R.M.M, A.M.I, E.T.H., and M.Y.M. All authors have studied and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts of interest.

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