

Morphometric and Genetic Insights for Three Terrestrial Snails in Taif Province of Saudi Arabia

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Abstract: Terrestrial snails are widely distributed in Mediterranean region, Europe, North Africa and some parts of Asia. To date, there is no study regarding the record of these terrestrial snails in Saudi Arabia. The gastropod brown garden snails; *Helix aspersa*, *Eobania vermiculata* and *Rumina decollata* are collected for the first time from different regions in Taif province attacking some vegetables, ornamentals and other plants. The first two species are well known as a dangerous pest and the third is well known as a predator on the other snail pests. The present work is done to throw a light on the morphology and genetics of these aforementioned species. Morphological and biological aspects are mentioned. These notes are of a value in terrestrial snail control programs in Kingdom of Saudi Arabia. The obtained genetic data indicated that *Eobania vermiculata* and *Helix aspersa* were closely related to each other than the relationship between any of them and *Rumina decollata*.

Key words: Pulmonate snails • Morphology • Biology • Molecular relationship

INTRODUCTION

Terrestrial snails have been known to be destructive agricultural pests causing severe damage to vegetables, ornamentals and other plants [1-10].

The brown garden snail (European brown snail) *Helix aspersa* (Müller, 1774) was described by Müller in 1774 from specimens collected in Italy. This plant feeder has been disseminated into many parts of the world as a food delicacy and by hobbyists who collected snails and adapted in several countries [1, 11]. It is native to the shores of the Mediterranean and up the coast of Spain and France. It is found on most of the British Isles and was introduced into California by French immigrants and has become a serious pest. *Eobania vermiculata* (Müller, 1774) snails are widely distributed in Mediterranean region to East Bulgaria, Crimea and in all coastal parts of Turkey [12]. These two species which are belonging to the same family (Helicidae) were considered the most dangerous snail pests. Several investigations have been done to control these snails [1, 5, 10, 11, 13, 14]. So far there is no record for these snail species in the Kingdom of Saudi Arabia, especially in Taif Provinces.

The decollate snail *Rumina decollata* (Linnaeus, 1758) of the family Subulinidae was considered a minor

plant pest, although it was recognized as omnivorous [15, 16]. This snail is native to the Mediterranean region but it has been introduced into North America and other areas for the control of the brown garden snail (*Helix aspersa*), [1, 17]. The snail is a voracious predator and feeds readily upon common garden snails and their eggs. The snail eats plant matter as well but the damage it causes to plant is considered minor when compared to the benefit of its predation on garden snail pests [1]. These snails are ground dwellers, living among leaves and sometimes burrowing in the upper one inch of soil [17]. So far there is no study mentioned in the Kingdom of Saudi Arabia about this snail, especially in Taif province.

The present insights will be of a value for assessment of the possible success snail control program through the IPM programs. The first two snail species (*Helix aspersa* and *Eobania vermiculata*) are considered the most dangerous pests which attack several economic plants and vegetables and may transmit disease agents to human, domestic animals and to plants. The last species *Rumina decollata* is considered a voracious predator and feeds readily upon common garden snails and slug pests. We try herein to throw a light on biological and morphological aspects of these snail species.

Several molecular and genetic studies have investigated the genetic framework of pulmonate snails [18 - 20] however, there is no genetic study tackling the relationship among the taxa studied herein so far. We therefore, make an insight on the genetic relationship among these species.

MATERIALS AND METHODS

Morphology: Three snail species were collected from ornamental plants and citrus trees planted in gardens at Taif Province of Saudi Arabia during March 2007. Individuals of each species (20 snails) were placed in 2 liter glass jars tightly covered with cloth netting and rubber bands. Each jar was provided daily with fresh lettuce leaves and the snails were maintained under laboratory conditions at 25°C ±1.

The snails were identified morphologically by using the identification key of terrestrial gastropods [1] and the picture for each snail were taken by photocamera. Shell, soft body, sex and life span are described for each snail.

We adopted characters of the shell that were practical for use in identification [21]. A total of 7 continuous shell measurements were taken with calipers made along imaginary straight lines (Figure 1). Shell length (SL) was measured along an axis passing through the apex (a) to the bottom (i) of the shell. Shell width (SW) is the maximum width perpendicular to the shell length distance (d'-g'). Aperture length (AL) is the length from the beginning of the 1st suture (e) to the bottom of the aperture (i). Aperture width (AW) is the maximum diameter perpendicular to the aperture length (f-h). The body whorl length (WL) was measured from the intersection of the axis passing through the apex (b) to the bottom of the shell (i). The penultimate whorl length (P1) is the length between the beginning of the first suture (e) to the beginning of the 2nd suture (c). Spire height (PL) was measured from the beginning of the 1st suture (e) to the apex of the shell (a). The ratios, AL/SW and SL/SW, representing different proportions of growth rates in shells, were analyzed against aperture length (AL) (representing the size category) using linear regression. If allometric shell growth exists in this species, the correlation coefficient, R^2 , of both regressions will significantly differ. Furthermore, the minimum shell aperture length for each snail can be obtained from the intersection of the 2 linear regression equations of SL/SW vs. AL. The general linear regression option was utilized in SPSS 10 [22].

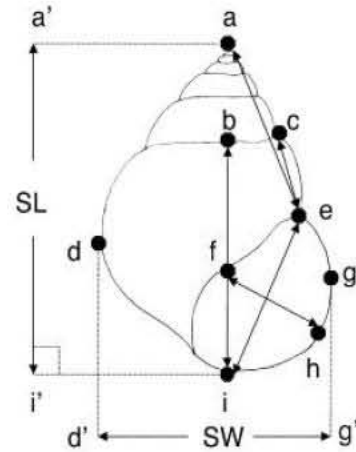


Fig. 1: Measurements made on shells of the studied snails. This diagram was designed by Chiu *et al.* [22].

Genetics: Partial sequences of 490 nucleotides of cytochrome oxidase subunit 1 mitochondrial gene for *Helix aspersa* (accession number AY345052), *Fumina decollata* (accession number AY345050) and *Eobania vermiculata* (accession number AY546277) were obtained from the GenBank and were used to show the relationship among these pulmonate snails. DNASIS program was used to handle these sequences and to convert it into their corresponding amino acids. The obtained sequences were aligned separately and manually using MacClade v.4. The genetic analysis were done with PAUP* 4.0b10 [23] to calculate the pairwise genetic differences.

RESULTS

Morphology: The morphometric measurements for the different snails are shown as means and standard errors (Table 1). As the studied snails represent different genera and families, the obtained allometric data were only descriptive for each snail independently. The ratios of SL/SW and AL/SW are the only characters used for differentiation among the snails

***Helix aspersa* (Müller, 1774):** The shell is large, generally spherical in shape with a short spine. It is rather thin, moderately glossy and is sculptured with fine wrinkles. It is yellow of horn-colored with chestnut brown spiral bands which are interrupted by yellow flecks or streaks. The turned-back tip is thickened and white. Adult shells have four to five whorls and measure up to 28-32 mm in diameter (Figure 2). These snails have pale grey moist skin. At the front end there are four tentacles,

Table 1: Mean \pm SE measurements of 9 shell characters studied. Unit for measurements is in centimeters. Number of samples was 13 for each species.

Character	<i>H. aspersa</i>	<i>E. vermiculata</i>	<i>R. decollata</i>
SL	2.82 \pm 0.041	2.33 \pm 0.039	2.29 \pm 0.041
SW	3.01 \pm 0.051	2.96 \pm 0.045	0.99 \pm 0.018
AL	2.09 \pm 0.037	1.75 \pm 0.086	0.81 \pm 0.014
WL	1.75 \pm 0.034	1.48 \pm 0.024	0.57 \pm 0.013
WL	2.81 \pm 0.056	2.48 \pm 0.044	1.31 \pm 0.019
P1	0.97 \pm 0.034	0.86 \pm 0.027	0.51 \pm 0.017
PL	1.42 \pm 0.036	1.37 \pm 0.028	1.52 \pm 0.035
SL/SW	0.94 \pm 0.008	0.79 \pm 0.006	2.29 \pm 0.040
AL/SW	0.81 \pm 0.017	0.56 \pm 0.008	0.81 \pm 0.007

Fig. 2: *Helix aspersa* (Müller, 1774) snails

the shorter two are for feeling and the longer pair is eye stalks. The mouth is located beneath the tentacles and contains a chitinous radula which is used to scrape and manipulate food particles. The snail secretes mucus to facilitate locomotion by reducing friction against the substrate (Figure 2).

The snails are hermaphrodites and in spite, they require a partner for reproduction. In optimal conditions, they have a life span of 2-5 years. Mating prolongs for several hours and during it, the snails exchange sperms. After few days each will dig a nest in the soil and deposit fertilized eggs in it. The young emerges from the eggs after two weeks and take one to two years to reach maturity.

***Eobania vermiculata* (Müller, 1774):** The shell is moderate in size (26 \pm 4 mm, to 35 mm in diameter). Its shape is compressed spherical, thick-walled and with covered umbilicus. Its surface is opaque or translucent. The remaining shell surface is somewhat bright with fine wrinkled to grooved sculpture. The background color is between pure white and yellow to light gray brown. It is mostly with 5 reddish to dark brown bands, the first 2 often are merged and the 3rd is widest just above the periphery line. Bands are continuous and regularly cut and all of which end at a wide light zone next to peristome.

Fig. 3: *Eobania vermiculata* (Müller, 1774) snails

Locally, albinistic populations may be encountered. Last turn is descending at the peristome broadened with sharp edges and a poor lip. Insertations are widely separated from each other and there is no callus. (Fig. 3).

These snails have pale grey or light brown moist skin. At the front end there are four tentacles, the shorter two are for feeling and the longer pair carry eyes. The mouth is located beneath the tentacles and contains a chitinous radula. The snail secretes mucus to facilitate locomotion on the rough matters.

The snails are hermaphrodite and have life span of 1-2.5 years. Both *E. Vermiculata* and *H. aspersa* are edible species that have been introduced to places away from their original homelands. It is easy to distinguish between the shells of the two snail species. The most significant difference is the width of the body whorl which is relatively larger in *H. aspersa* than it is in *E. vermiculata*. The aperture of *H. aspersa* is also relatively wider than that of *E. vermiculata*.

***Rumina decollata* (Linnaeus, 1758):** The adult shell is large (up to 45 mm in height, 14 mm in diameter) but only retains four to seven whorls in adulthood and the other eight to 10 whorls being lost. The shell is perforate, glossy and sculptured with prominent axial growth lines and fine spiral strain. The columella is straight;

Fig. 4: *Rumina decollata* (Linnaeus, 1758) snails

	1				50
<i>Helix aspersa</i>	IRFELGTSGY	LTDDHFYNVI	VTAHAFVMIF	FMVMPIMIGG	FGNWMVPLLI
<i>Rumina decollata</i>	IRLELGTA ^A GV	LTDDHF ^F NVV	VTAHAFVMIF	FMVMPIMIGG	FGNWMVPLLI
<i>Eobania vermiculata</i>	IRLELGTS ^G GV	LTDDHFYNVI	VTAHAFVMIF	FMVMPIMIGG	^S GNWMVPLLI
	51				100
<i>Helix aspersa</i>	GAPDMSFPRM	NNMSFWLLPP	SFLLLISSSL	VEGGAGTGWT	VYPPLSSLSG
<i>Rumina decollata</i>	GAPDMSFPRM	NNMSFWLLPP	SF ^I LLI ^M SS ^M	VEGGAGTGWT	VYPPLS ^G LI ^M G
<i>Eobania vermiculata</i>	GAPDMSFPRM	NNMSFWLLPP	SFLLLISSSL	VEGGAGTGWT	VYPPLSSLSG
	101				150
<i>Helix aspersa</i>	HSGASVDLAI	FSLHLA ^G MS	ILGAINFITT	IFNMRS ^P GL ^S	LERMSLFVWS
<i>Rumina decollata</i>	HSGASVDLAI	FSLHLA ^G MS	ILGAINFITT	IFNMRS ^P GL ^S	^M ERVSLFVWS
<i>Eobania vermiculata</i>	HSGASVDLAI	FSLHLA ^G MS	ILGAINFITT	IFNMRS ^P GL ^S	LERMSLFVWS
	151	163			
<i>Helix aspersa</i>	ILVTVFLLLL	SLPV			
<i>Rumina decollata</i>	ILVTVFLLLL	SLPV			
<i>Eobania vermicularis</i>	ILVAVFLPLS	SLPV			

Fig. 5: The aligned amino acid sequences translated from the mitochondrial CO1 gene for the three studied snails.

its lip margin is reflexed but the outer lip is simple. The shell color is pinkish brown. It is not confused with any other snail (Figure 4).

These snails have pale grey moist skin. At the front end there are four tentacles, the shorter two are for feeling and the longer pair carry eyes. The mouth is located beneath the tentacles and contains a chitinous radula. The snail secretes mucus to facilitate locomotion on the rough matters. It is a hermaphrodite and has life span of 1-3 years.

Genetics: Figure 5 shows the aligned amino acids for the cytochrome C gene. The calculated genetic distances were as follow: 0.16 between *Eobania vermiculata* and *Helix aspersa*, 0.24 between *Eobania vermiculata* and *Rumina decollata* and 0.23 between *Helix aspersa* and *Rumina decollata*. Approximately 140 nucleotide differences were counted among the three species (data not shown). Among these differences, 16 were non-synonymous giving rise to different amino acids.

DISCUSSION

Shells of gastropods contain a rich source of taxonomic information that can be used to interpret relationships among taxa. Shell morphological characters are used as primary guidelines for species identification in general handbooks and the taxonomic literature [22]. Linear regression analyses of the AL/SW ratio vs. AL and the SL/SW ratio vs. AL indicated that allometric shell growth exists only in *E. vermiculata* (Table 2). SL/SW ratios decreased insignificantly ($p > 0.05$) in the three snails as AL increased (r for *H. aspersa*, *E. vermiculata* and *R. decollata* = 0.37, 0.10, 0.22, respectively), but AL/SW ratios increased insignificantly in *H. aspersa* and *R. decollata* as AL increased ($r = 0.15$ and 0.21, respectively). Only in *E. Vermiculata* AL/SW ratios increased significantly ($r = 0.72$, $p < 0.05$) as AL increased. The slopes of both linear regression lines significantly suggested that elongation of shell length and enlargement of shell width do not probably contribute equally to the growth of *E. vermiculata*.

Table 2: Regression equations indicating the allometric growth of the three studied snails

Species	Regression Equation	r-value	n	p-value
<i>H. aspersa</i>	SL/SW=1.09 - 0.07AL	0.37	13	$p > 0.05$
	AL/SW=0.68 + 0.07AL	0.15	13	$p > 0.05$
<i>E. vermiculata</i>	SL/SW=0.82 - 0.02AL	0.10	13	$p > 0.05$
	AL/SW=0.25 + 0.19AL	0.76	13	$p > 0.05$
<i>R. decollata</i>	SL/SW=2.8 - 0.65AL	0.22	13	$p > 0.05$
	AL/SW=0.72 + 0.17AL	0.21	13	$p < 0.05^*$

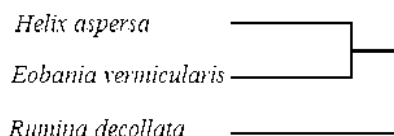


Fig. 6: The constructed dendrogram based on the genetic differences among the studied pulmonate snails.

The mutations that were estimated from the aligned CO1 gene were higher in *Rumina decollata* than in other snails. The subulinid snail showed 12 out of 16 non-synonymous substitutions (that are giving rise to different amino acids). The estimated genetic distance between this species and either of the other two species were comparable with this finding. Based on the obtained results, *Eobania vermiculata* and *Helix aspersa* were closely related to each other than the kinship between any of them and *Rumina decollata*.

The dendrogram (Figure 6) that is constructed based on the genetic variability among the studied species showed concordance to the genetic data. The two helicid species (*Helix aspersa* and *Eobania vermiculata*) clustered together and the third subulinid snail (*Rumina decollata*) came out.

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