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Morphometric evaluation of DNA-based cryptic taxa in the terrestrial decollate snail genus *Rumina*

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ABSTRACT

The land snail genus *Rumina* shows much intra- and interspecific variation in shell morphology. Three morphospecies are commonly recognized: *R. decollata*, *R. saharica* and *R. paivae*. The descriptions of these three morphospecies were based on differences in shell and genital characters for *R. saharica* and *R. decollata*, and shell size for *R. paivae*. However, recently DNA-sequence data have suggested that these morphospecies comprise at least seven phylogenetic species (*R. decollata* molecular operational taxonomic units (MOTUs) A–F and *R. saharica*). The present study explores to what extent these phylogenetic species are morphologically diagnosable and/or can be reconciled with the three currently recognized morphospecies. It shows that: (1) *R. saharica*, and to a lesser extent *R. decollata* MOTUs A and Eb, are significantly differentiated from the other phylogenetic species by both genital and shell characters, and can be regarded as three diagnosable biological and phylogenetic species; (2) there are no diagnostic genital features for *R. paivae*, so that this taxon should not be treated as a separate species; (3) none of the other DNA-based *R. decollata* MOTUs could be consistently differentiated by the shell and genital characters analysed in this study, so that their status needs further scrutiny.

INTRODUCTION

Rumina Risso, 1826 (family Subulinidae) is a genus of medium-sized, predatory land snails, indigenous to, and widely distributed in, the countries around the Mediterranean Sea (Pilsbry, 1905). It comprises several nominal species that were described on account of subtle differences in the shape and size of the shell and body coloration (e.g. Bourguignat, 1864; Pallary, 1901; Llabador, 1970; Bank & Gittenberger, 1993; Mienis, 2002). Currently, three species are recognized, viz. *R. decollata* (Linnaeus, 1758), *R. saharica* Pallary, 1901 and *R. paivae* (Lowe, 1860) (Fig. 1A) (Bank & Gittenberger, 1993; Mienis, 2008).

Rumina decollata has a rather thick and elongated shell with a light grey to dark brown colour (Fig. 1A) and reaches a height of up to 4 cm (Pallary, 1921). It can have a black to light grey body and the foot can be pale yellow to dull olive-grey (Giusti, Manganelli & Schembri, 1995; Prévot *et al.*, 2013a) (Fig. 1B, showing colour morphs 1–5, see Material and Methods). The shell suture is moderately deep and the external surface has weak growth lines. The species can be found in the entire Mediterranean region (Pilsbry, 1905; Singer & Mienis, 1993) and has been introduced in various other parts of the world (Matsukuma & Takeda, 2009). *Rumina saharica* has a slender shell with rather flat whorls. Its height varies from 2.4 to 2.8 cm (Pallary, 1921). It is more cylindrical and has a relatively smaller aperture than *R. decollata* (Fig. 1A). The body and shell are cream-coloured (Mienis, 2008) (Fig. 1B, colour morph 6). *Rumina saharica* is usually reported in the eastern part of the Mediterranean, but has probably been introduced by man at many places along the Turkish coast (Mienis, 1976, 1991, 2003; Singer & Mienis, 1993; Hausdorf & Hennig, 2005). Finally, *Rumina paivae* was described on the basis of two big (4.5 to 6.5 cm) empty shells from Rabat (Morocco) (Pallary, 1921). Its shell is wider, more convex, more opaque, thicker and has a deeper suture and distinctively larger aperture than *R. decollata*. The shell growth lines are also more pronounced (Fig. 1A). *Rumina paivae* is supposed to be distributed in Morocco, Algeria and Tunisia (Bank & Gittenberger, 1993; Mienis 2002, 2003, 2008).

Carr (2002) showed that *R. decollata* and *R. saharica* also differ in the shape and internal structure of the penis and vagina. The distal part of the penis in *R. decollata* contains transverse lamellae, which rapidly separate into abundant, prominent papillae towards the centre and proximal section of the penis. In contrast, *R. saharica* has a penis with a swollen, pear-shaped proximal end section, which usually does not occupy more than half the total length of the penis and contains transverse lamellae. These lamellae gradually separate into sparsely distributed, obscure papillae towards the proximal end (see Carr, 2002: figure 2). The vagina

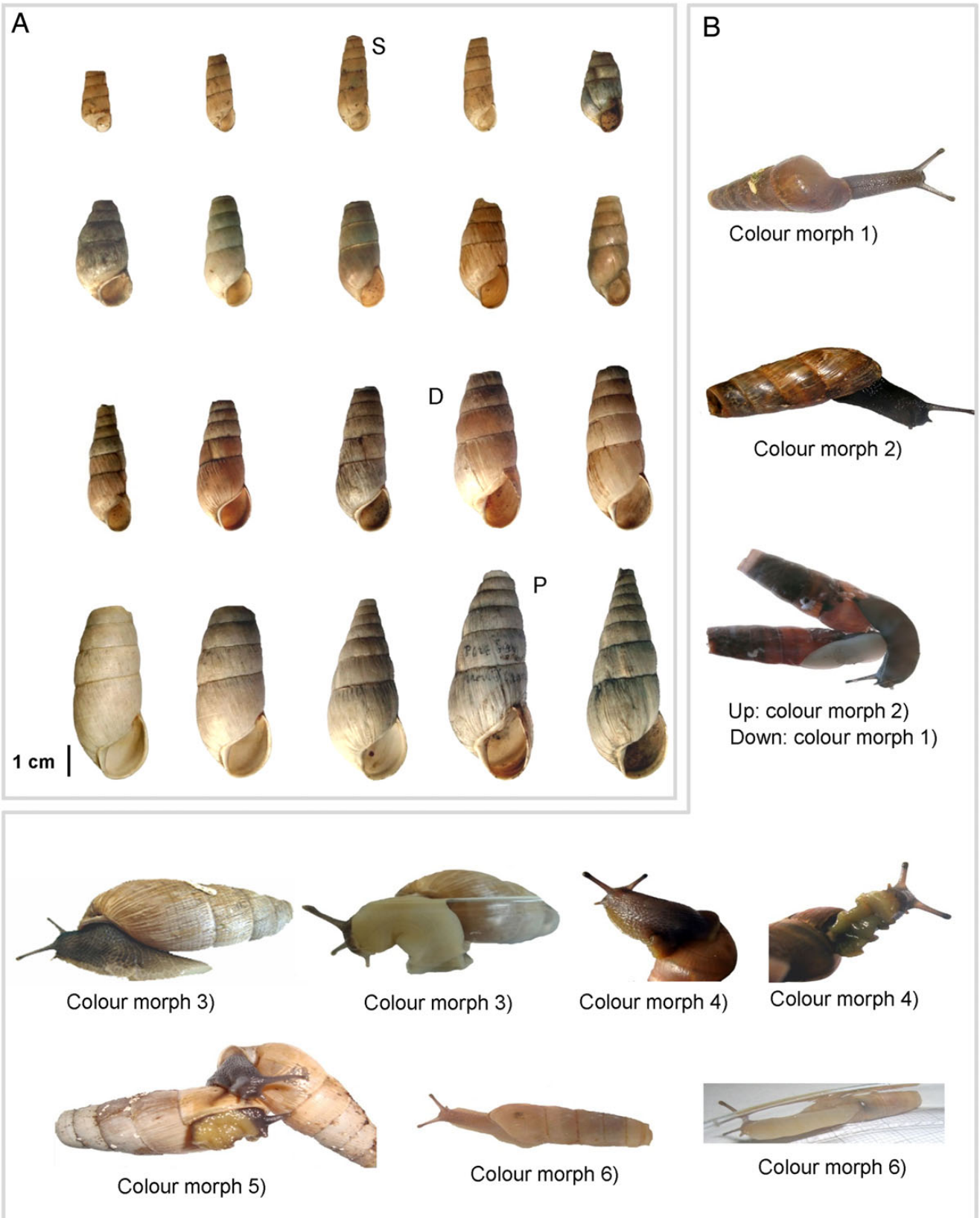

Figure 1. A. Shell variation in the genus *Rumina*. D: typical *Rumina decollata;* S: *R. saharica*; P: *R. paivae*. Scale bar = 1 cm. **B.** Colour morphs in *Rumina* species. Colour morph 1: light grey body with a medio-dorsal black line and pale yellowish foot; colour morph 2: black body and dull olive-grey foot; colour morph 3: olive-grey body and dark yellow foot; colour morph 4: olive-brown body and olive foot; colour morph 5: black body and olive foot; colour morph 6: beige body and foot.

Table 1. Summary of (A) shell height and shell width ranges found in the literature and (B) range of penis and vagina height and width (Carr, 2002) for *Rumina* species and the region from where individuals were studied. *n* is the number of specimens (if given by author). Values are given in cm.

		Region (*n*)	*R. saharica*	*R. decollata*	*R. paivae*
A					
Shell height (cm)	Pfeiffer (1848)			≤2.8	
	Lowe (1860)			2.1–3.2	3.9–4.4
	Bourguignat (1864)	Algeria	2.0–2.4	2.5–3.0	4.5–6.0
	Pallary (1921)	Atlas	2.4–2.8	3.0–4.0	4.5–6.5
	Giusti (1995)	Malta		1.8–4.0	
	Vicente & Bech (1999)			2.5–3.5	3.5–5.0
	Carr (2002)	(87 *R. decollata* and 134 *R. paivae*)[a]	1.77–3.37	1.93–5.22	
Shell width (cm)	Pfeiffer (1848)			≤1.0	
	Lowe (1860)			0.95–1.0	1.6
	Bourguignat (1864)	Algeria	0.7	1.0–1.1	1.5–1.8
	Giusti (1995)	Malta		0.65–1.65	
	Carr (2002)	(87 *R. decollata* and 134 *R. paivae*)[a]	0.71–0.94	0.89–1.97	
	Mienis (2008)		0.71–0.94	0.89–1.47	1.55–2.27
B					
Penis length	Carr (2002)	*R. decollata*: Canary Islands and	0.371–0.560	0.490–0.750	
Penis width		North Africa (9)*R. saharica*: Cyprus,	0.090–0.117	0.094–0.190	
Vagina length		Greece and Madeira (5)	0.384–0.800	0.507–0.940	
Vagina width			0.100–0.124	0.134–0.242	

[a]Canary Islands, Europe, Israel, Madeira, Turkey and North Africa. Numbers were extrapolated from Carr (2002: figs 4, 5).

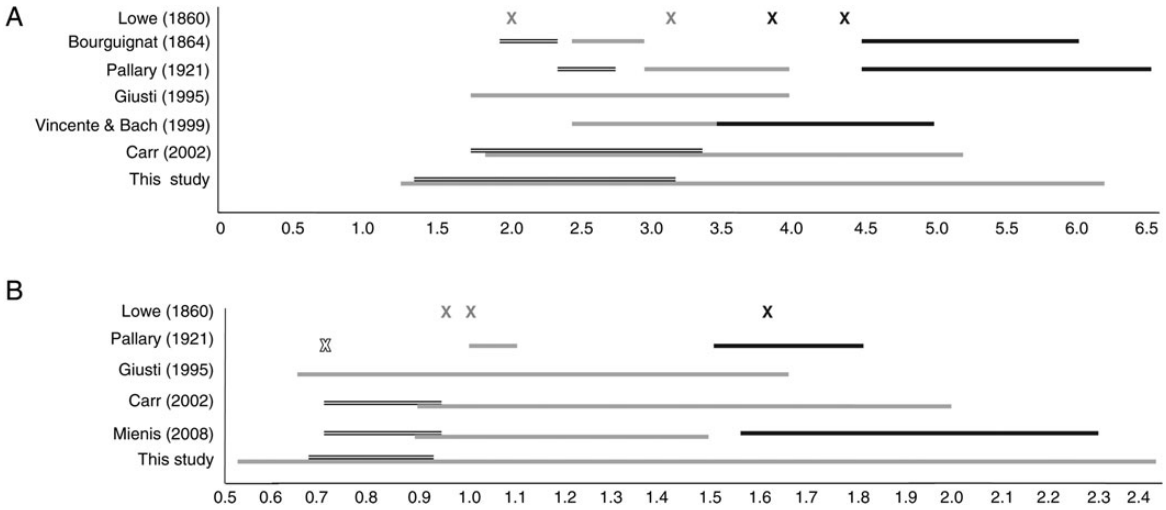

Figure 2. Range of shell height (**A**) and shell width (**B**) reported by various authors, and in this study, in *Rumina saharica* (double line), *R. decollata* (grey) and *R. paivae* (black) and for the different MOTUs (black). Lowe (1860) and Pallary (1920) gave measurements from one or two specimens of *R. saharica* so these have been indicated with crosses.

of *R. decollata* is wide and flattened. Internally it has longitudinal, crenulated lamellae. Conversely, the vagina of *R. saharica* is narrow and tubular, with straight internal lamellae (Carr, 2002).

The taxonomy of *Rumina* is still confused. *Rumina decollata* and *R. saharica* show a high degree of shell size and shape variation (Figs 1A and 2). Previous morphological studies have shown that *R. decollata*, *R. saharica* and *R. paivae* cannot be discriminated unambiguously on the basis of shell size, since size ranges overlap and vary strongly between studies (Table 1; Fig. 2). Only Bourguignat (1864), Pallary (1921) (for both shell height and width) and Mienis (2008) (for shell width) have documented the morphometric ranges for the three morphospecies. In addition, most of the studies mentioned in Table 1 were based on small sample sizes of specimens from a limited part of the species' distribution. Moreover, except for Carr (2002), none of the previous morphological studies implemented statistical analyses.

Rumina decollata and *R. saharica* seem to maintain their morphological differences in sympatry (Bank & Gittenberger, 1993). Conversely, the conchological distinction between *R. decollata* and *R. paivae* is not straightforward, while the reproductive organs of *R. paivae* have never been described.

Against this background, Prévot *et al.* (2013a) re-evaluated the taxonomy of *Rumina* using DNA sequence data. On this basis, several molecular operational taxonomic units (MOTUs) were proposed, one of which corresponded to *R. saharica* (MOTU S) while six others (A to F) represented *R. decollata* and *R. paivae*. However, only four specimens of *R. paivae* were included and these belonged to MOTUs C, D and E. Hence,

Prévot *et al.* (2013a) rejected the species status of *R. paivae*, instead provisionally interpreting the seven MOTUs as phylogenetic species (PS). PS are defined as diagnosable clusters of organisms within which there is a parental pattern of ancestry and descent (Cracraft, 1983, 1989). Subsequently, Prévot *et al.* (2013b) used allozymes and microsatellites to assess the population genetic structure of MOTUs A and Eb (a specific clade within MOTU E), two groups that differ by their body colour (black body and a dull olive-grey food in MOTU A, *vs* light grey body with a black medio-dorsal line and a pale yellowish foot in MOTU Eb), which were extensively studied in the 1970s with allozyme analyses (Selander & Hudson, 1976; Selander & Ochman, 1983). These previous data, combined with the new analyses of Prévot *et al.* (2013b), suggested that MOTUs A and Eb are not only well-defined PS, but that they may also be interpreted as biological species.

The present study implements uni- and multivariate statistics of shell and genital characters to explore whether the three *Rumina* species, their colour morphs and the DNA-based MOTUs of Prévot *et al.* (2013a) are morphologically differentiated and diagnosable.

MATERIAL AND METHODS

A total of 524 specimens (body and shell) and 346 empty shells of *Rumina* sp. were studied (Supplementary Material S1). Specimens were morphologically identified from the work of Mienis (2008) (see also Table 1). As such, the material comprised 450 specimens and 297 empty shells of *R. decollata*, 70 specimens and 49 empty shells of *R. saharica* and 4 specimens of *R. paivae*. Given the small sample size of *R. paivae*, this species was not included in the statistical analyses. Only adult specimens were taken into account. Specimens were judged to be adult following Kat (1981), who considered specimens to be adult when they decollate. Specimens dissected for genital analyses were first relaxed in a closed receptacle filled with water for 24 h to elongate and then fixed in 70% ethanol.

Morphometric measurements

Six shell characteristics were measured: height (SH, maximum length), width (SW, maximum width), diagonal length of aperture (AT, greatest transverse measurement of aperture), apertural width (AW, maximum horizontal width of aperture), height of body whorl (FwH, length of body whorl at middle of shell and drawing a line vertically from the whorl to external border of aperture) and number of whorls (WN, number of whorls at middle of shell, to nearest half whorl) (Fig. 3). The following genital measurements were taken from 54 *R. decollata*, 15 *R. saharica* and 3 *R. paivae*: penis length (PL), penis width (PW), vagina length (VL) and vagina width (VW) (Fig. 4). Measurements were taken with digital callipers to a precision of 0.01 mm. The general appearance and internal structure of the penis and vagina were assessed qualitatively. Supplementary Material S1 and S2 summarize the collection information and raw measurements.

Scoring of colour variation

Body and foot colour were scored by eye for 421 specimens (the remaining 103 specimens had lost their colour after having been kept too long in ethanol). Body colour was scored as: light grey with a medio-dorsal black line, black, olive-grey, olive-brown or beige. Foot colour was scored as: pale yellowish, dull olive-grey, dark yellow, olive or beige.

Statistical analysis

All statistical analyses were performed using the software package STATISTICA v. 6.1 (StatSoft, 2003).

Two sets of operational taxonomic units (OTUs) were considered for statistical analysis: (1) the morphospecies (*Rumina decollata* and *R. saharica*) and (2) the MOTUs A–F, S, Ea and Eb (Prévot *et al.*, 2013a).

Descriptive statistics (range, mean and standard deviation) were calculated for the shell and genital characters of *R. decollata* and *R. saharica*. A plot showing shell width in relation to shell height was drawn in order to check for differentiation between the morphospecies.

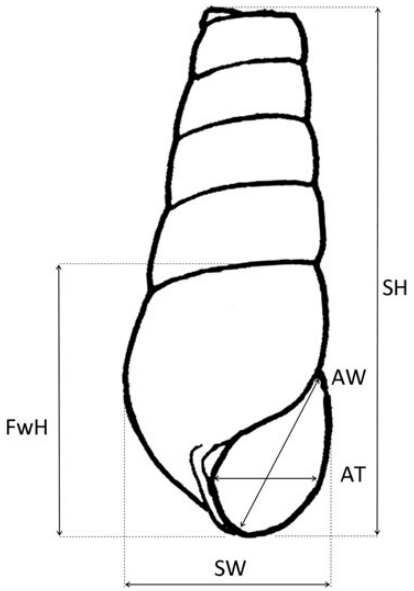

Figure 3. Camera lucida drawing of the frontal view of a shell of *Rumina* sp. showing the measured characters. Abbreviations: SH, shell height; SW, shell width; AT, aperture transversal; AW, aperture width; FwH, height of body whorl.

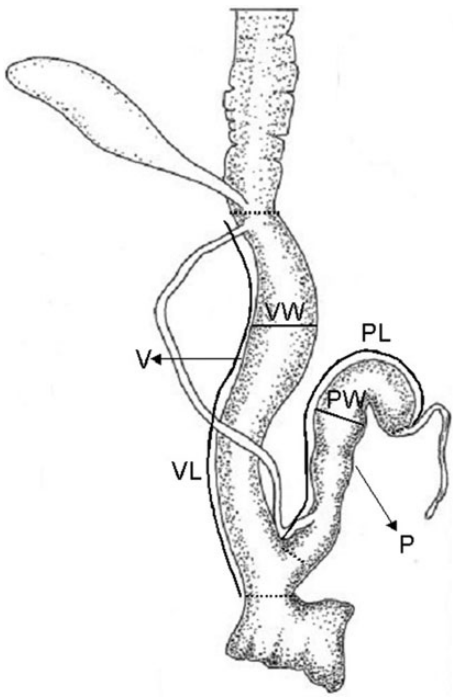

Figure 4. Camera lucida drawing of the proximal genital parts of *Rumina decollata* (adapted from Carr, 2002) showing the characters of the penis (P) and vagina (V) that were measured. Abbreviations: LP, penis length; PW, penis width; VL, vagina height; VW, vagina width.

Measurements were $\log_{10}$-transformed and the numbers of whorls were square-root transformed to minimize allometric effects (Hui *et al.*, 2010). Pearson's product moment correlations were calculated and *P*-values were adjusted with the sequential Bonferroni procedure (Rice, 1989). Since a principal component analysis (PCA) showed that $\pm 76\%$ of the variation in shell and genital variables was due to variation in size (results not shown), shell and genital measurements were regressed against shell height to reduce the effects of size. Differences in shell and genital variables between *R. decollata* and *R. saharica* were tested with a *t*-test. Normality and homogeneity of variance were checked with the Shapiro–Wilk (Shapiro, Wilk & Chen, 1968) and Levene's test (Levene, 1960), respectively. The differences in SH among MOTUs were tested on log-transformed measurements with a one-way analysis of variance (ANOVA), while differences in the other shell and genital variables among MOTUs were tested on log-transformed measurements with an analysis of covariance (ANCOVA) with shell height as the covariate. Pairwise differences between MOTUs were evaluated with a post-hoc Scheffé test (Armitage & Berry, 1994). The ANCOVA method is preferred over a PCA, because PCA may generate systematic statistical artefacts (Berner, 2011).

RESULTS

Shell morphometrics

Descriptive statistics of the shell measurements for the species and for the MOTUs are given in Tables 2 and 3. *Rumina* showed much and continuous variation in shell size (Fig. 5). All shell measurements were strongly, positively and significantly ($P < 0.05$) correlated (all $r \geq 0.85$), even after sequential Bonferroni correction, except for WN and AT ($r = -0.05$; $P = 0.146$), WN and AW ($r = -0.04$; $P = 0.286$), and WN and FwH ($r = -0.05$; $P = 0.143$). *Rumina decollata* was significantly larger than *R. saharica* for all shell measurements (*t*-tests: all $P < 0.001$), whereas the number of whorls (WN) was significantly larger in *R. saharica* (*t*-test: $P < 0.001$). This means that *R. saharica* is smaller, but has more whorls, than *R. decollata*.

MOTUs differed significantly for all shell measurements (ANOVA and ANCOVA: all $P < 0.001$; covariate: all $P < 0.001$). Scheffé's test for SH showed that MOTU A and MOTU Eb were significantly smaller than MOTUs B, C, D and Ea (Fig. 6A). The results of Scheffé's test for all other measurements are shown in Figure 6B–E. In general: (1) MOTUs S (*R. saharica*) and Eb were significantly smaller than all other MOTUs for all other shell measurements; (2) MOTUs B and D were significantly larger than the other MOTUs; (3) MOTUs A, C, F, Ea and Eb had mean values that were intermediate between those of MOTU S and those of MOTUs B and D and (4) MOTU Ea was significantly larger than Eb.

Table 2. Mean (standard deviation, SD) and range of six shell and four genital characters, and number of specimens for each colour type (in parentheses), in *Rumina decollata* and *R. saharica*.

			Species	
			R. decollata	*R. saharica*
Shell	*n*		751	119
	SH	Range	1.310–6.168	1.409–3.187
		Mean (SD)	2.725 (0.794)	2.440 (0.293)
	SW	Range	0.525–2.369	0.66–0.918
		Mean (SD)	1.098 (0.281)	0.821 (0.051)
	AT	Range	0.263–2.263	0.549–0.876
		Mean (SD)	0.997 (0.289)	0.750 (0.066)
	AW	Range	0.309–1.616	0.355–0.673
		Mean (SD)	0.708 (0.211)	0.533 (0.060)
	FwH	Range	0.649–3.257	0.784–1.399
		Mean (SD)	1.504 (0.431)	1.170 (0.105)
	WN	Range	2.000–7.5000	3.000–6.000
		Mean (SD)	4.065 (0.783)	4.613 (0.609)
Genital	*n*		57	15
	PL	Range	0.034–0.096	0.041–0.046
		Mean (SD)	0.058 (0.011)	0.043 (0.002)
	PW	Range	0.009–0.018	0.009–0.013
		Mean (SD)	0.013 (0.002)	0.011 (0.001)
	VL	Range	0.050–0.119	0.046–0.060
		Mean (SD)	0.072 (0.014)	0.053 (0.003)
	VW	Range	0.012–0.024	0.009–0.014
		Mean (SD)	0.019 (0.003)	0.012 (0.002)

Abbreviations: *n*, number of specimens; n.a., not assessed; Int, individual with intermediate colour. Shell variables: SH, shell height; SW, shell width; AT, length of aperture transversal; AW, aperture width; FwH, height of body whorl; WN, number of whorls. Genital variables: PL, penis length; PW, penis width; VL, vagina height; VW, vagina width. Measurements are given in cm.

Genital morphometrics

Descriptive statistics of the genital measurements for both the species and the MOTUs are given in Tables 2 and 3. All genital measurements were significantly correlated with each other, even after sequential Bonferroni correction (all $r \geq 0.48$; all $P < 0.001$). *Rumina decollata* had a significantly longer penis and vagina than *R. saharica* (*t*-test: PW: $P = 0.015$; all other $P < 0.001$). The ANCOVA showed that: (1) MOTU S (*R. saharica*) had significantly smaller proximal genitalia than the other MOTUs (Fig. 6F, H, I), except for PW for which MOTU S was only significantly smaller than MOTUs B, C and Ea (Fig. 6G) and for VL for which MOTU S was only significantly smaller than MOTUs A, B, C, D and Ea (Fig. 6H); (2) MOTU D had a significantly higher mean value for PL and VL than the other MOTUs except for Ea (Fig. 6F, H); (3) MOTU A had a significant lower mean value for PW than the MOTUs B, C and Ea (Fig. 6G) and (4) MOTU Ea was significantly larger than MOTU Eb except for VW.

The internal structure of the penis and vagina of *R. decollata* and of *R. saharica* corresponded with the description of Carr (2002). The internal penis and vagina structures of the *R. paivae* specimens were indistinguishable from those of *R. decollata*.

Colour variation

Colour morphs are described in Table 4 shown in Figure 1B. Colour morphs 1 and 2 corresponded to the light and dark morph of Selander & Kaufman (1976), respectively.

Except for two specimens, MOTU A corresponded to colour morph 2 (99%), one specimen corresponded to colour morph 1 (0.5%) and one other (0.5%) had a body colour that was typical for colour morph 2 and a foot colour that was typical for colour morph 1. All specimens of MOTU B corresponded to colour morph 4 (100%). The specimens from MOTU C could not be scored for colour since they were received in ethanol. All individuals from MOTU D were of colour morph 3 (100%). Eighty-six specimens (91.5%) of MOTU E corresponded to colour morph 1 and eight (8.5%) corresponded to colour morph 2. MOTUs Ea and Eb had a majority of specimens from colour morph 1 (95% for Ea and 91% for Eb), while the remaining specimens were from colour morph 2 (5% for Ea and 9% for Eb). Specimens of MOTU F were all of colour morph 5 (100%). All *R. saharica* specimens were of colour morph 6 (100%). Finally, two specimens of *R. paivae* belonged to colour morph 3.

Table 3. Mean (standard deviation, SD) and range of six shell and four genital characters, and number of specimens for each colour type (in parentheses), in the phylogenetic species of *Rumina* recognized by Prévot *et al*. (2013a).

			MOTU								
			A	B	C	D	E	Ea	Eb	F	S
Shell	*n*		270	13	35	4	103	27	76	29	71
	SH	Range	1.310–4.114	2.270–3.382	1.474–3.731	2.275–4.034	1.344–4.355	1.651–4.355	1.344–3.297	1.546–3.424	1.409–3.187
		Mean (SD)	2.250 (0.504)	2.824 (0.352)	2.717 (0.495)	3.190 (0.843)	2.330 (0.604)	2.658 (0.746)	2.213 (0.501)	2.530 (0.418)	2.408 (0.330)
	SW	Range	0.561–1.323	1.123–1.364	0.746–1.654	0.951–1.694	0.525–1.598	0.918–1.598	0.525–1.147	0.779–1.430	0.669–0.918
		Mean (SD)	0.934 (0.139)	1.231 (0.074)	1.127 (0.166)	1.304 (0.393)	0.952 (0.201)	1.185 (0.167)	0.869 (0.137)	0.998 (0.115)	0.824 (0.057)
	AT	Range	0.501–1.318	1.062–1.346	0.619–1.361	0.971–1.680	0.426–1.539	0.690–1.539	0.426–1.212	0.742–1.153	0.549–0.876
		Mean (SD)	0.846 (0.159)	1.187 (0.091)	1.024 (0.174)	1.305 (0.385)	0.845 (0.233)	1.071 (0.222)	0.765 (0.179)	0.882 (0.090)	0.760 (0.070)
	AW	Range	0.349–0.896	0.534–0.931	0.474–1.240	0.581–1.140	0.309–1.065	0.542–1.065	0.309–0.878	0.543–0.932	0.355–0.673
		Mean (SD)	0.589 (0.106)	0.747 (0.110)	0.743 (0.142)	0.853 (0.253)	0.608 (0.167)	0.768 (0.157)	0.551 (0.130)	0.641 (0.083)	0.543 (0.064)
	FwH	Range	0.649–2.081	1.588–1.988	0.747–2.056	1.311–2.452	0.671–2.309	1.59–2.309	0.671–1.823	1.024–2.010	0.784–1.368
		Mean (SD)	1.267 (0.265)	1.732 (0.142)	1.533 (0.298)	1.891 (0.575)	1.288 (0.332)	1.594 (0.325)	1.179 (0.261)	1.288 (0.171)	1.178 (0.112)
	WN	Range	2–7	3–5	2.5–5	4–4.5	2.5–7.5	2.5–5	3–7.5	3–7	3.5–6
		Mean (SD)	3.974 (0.812)	3.923 (0.534)	4.057 (0.639)	4.125 (0.250)	4.203 (0.920)	3.741 (0.739)	4.367 (0.926)	4.207 (1.005)	4.570 (0.662)
Colour	Type (*n*)	2 (222), 1 (1), int (1)	4 (13)	n.a.	3 (4)	1 (86), 2 (8)	1 (18), 2 (1)	1 (68), 2 (7)	5 (15)	6 (71)	
Genital	*n*		10	10	12	3	12	3	9	10	15
	PL	Range	0.052–0.060	0.058–0.072	0.034–0.064	0.074–0.096	0.042–0.073	0.058–0.073	0.042–0.060	0.050–0.062	0.041–0.046
		Mean (SD)	0.055 (0.003)	0.066 (0.006)	0.052 (0.011)	0.085 (0.011)	0.054 (0.009)	0.067 (0.008)	0.050 (0.005)	0.057 (0.004)	0.043 (0.002)
	PW	Range	0.009–0.013	0.011–0.016	0.012–0.017	0.012–0.015	0.010–0.018	0.014–0.018	0.010–0.014	0.010–0.013	0.009–0.013
		Mean (SD)	0.011 (0.001)	0.014 (0.001)	0.014 (0.002)	0.014 (0.001)	0.013 (0.002)	0.017 (0.002)	0.012 (0.001)	0.012 (0.001)	0.011 (0.001)
	VL	Range	0.062–0.076	0.068–0.080	0.062–0.096	0.089–0.119	0.050–0.102	0.0593–0.102	0.050–0.077	0.058–0.068	0.046–0.060
		Mean (SD)	0.065 (0.004)	0.073 (0.004)	0.075 (0.012)	0.104 (0.015)	0.070 (0.018)	0.097 (0.005)	0.061 (0.010)	0.064 (0.004)	0.053 (0.003)
	VW	Range	0.016–0.023	0.016–0.022	0.013–0.024	0.015–0.023	0.012–0.022	0.016–0.022	0.012–0.019	0.015–0.021	0.009–0.014
		Mean (SD)	0.019 (0.002)	0.018 (0.002)	0.020 (0.003)	0.018 (0.004)	0.017 (0.003)	0.019 (0.003)	0.016 (0.002)	0.019 (0.002)	0.012 (0.002)

Abbreviations same as Table 2. Measurements are given in cm.

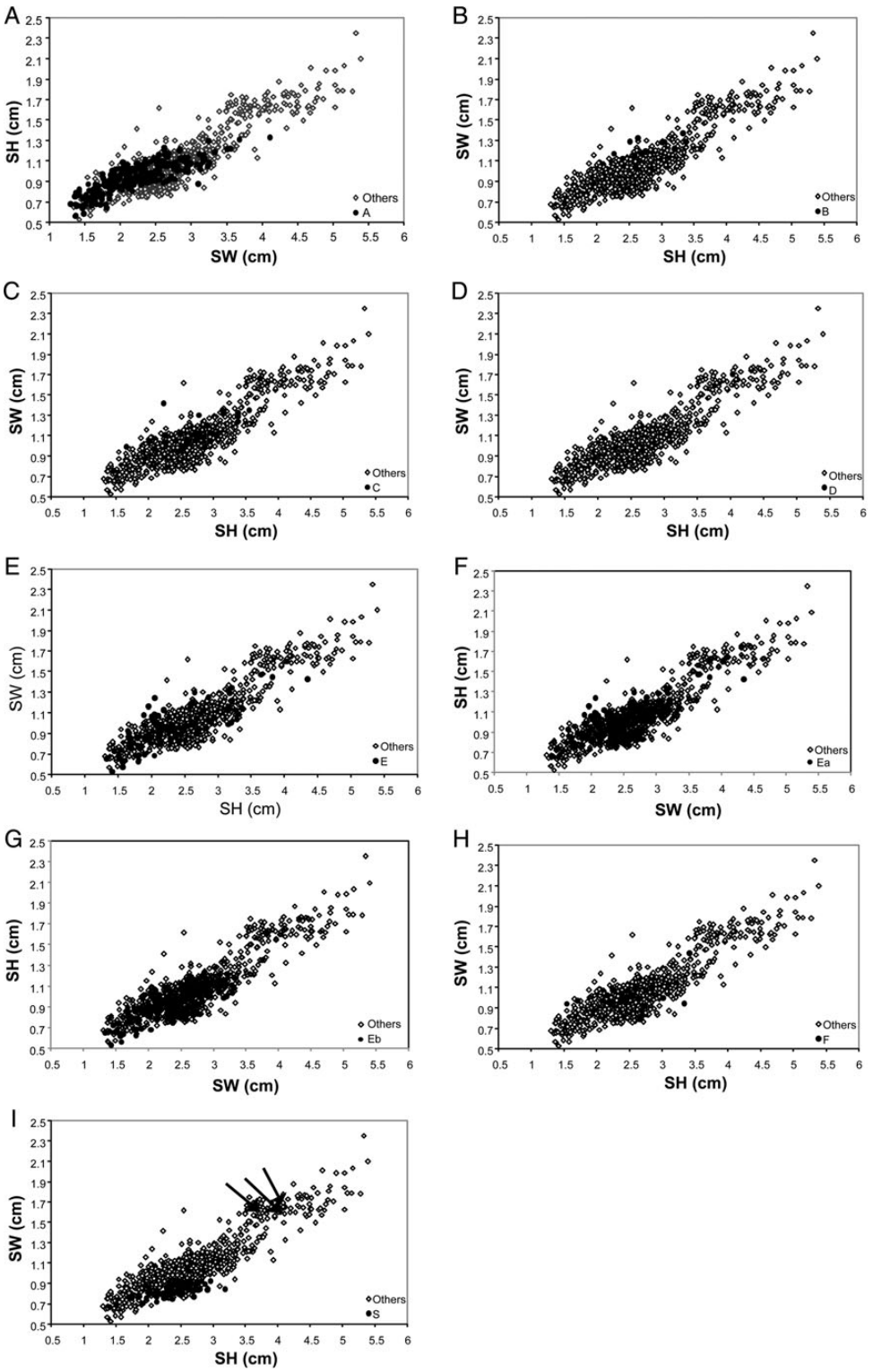

Figure 5. A-H. Plot of shell height *vs* shell width in *Rumina* species. Closed circles indicate different MOTUs, open diamonds the remaining specimens. **I.** Plot of shell height *vs* shell width in *Rumina* species. Closed circles indicate *R. saharica*, open diamonds *R. decollata*/*R. paivae*. Black arrows point to *R. paivae* specimens analysed for genital anatomy.

DISCUSSION

The three *Rumina* species that are currently recognized in the literature were described on a morphological basis only. Since size is often influenced by age, diet or ecological conditions, multivariate statistical techniques often use a size correction method for biological interpretations (Berner, 2011). The present study showed that shell size alone is not a good character to discriminate between *Rumina* species. The size ranges in the literature for *Rumina* species largely overlap and there is no

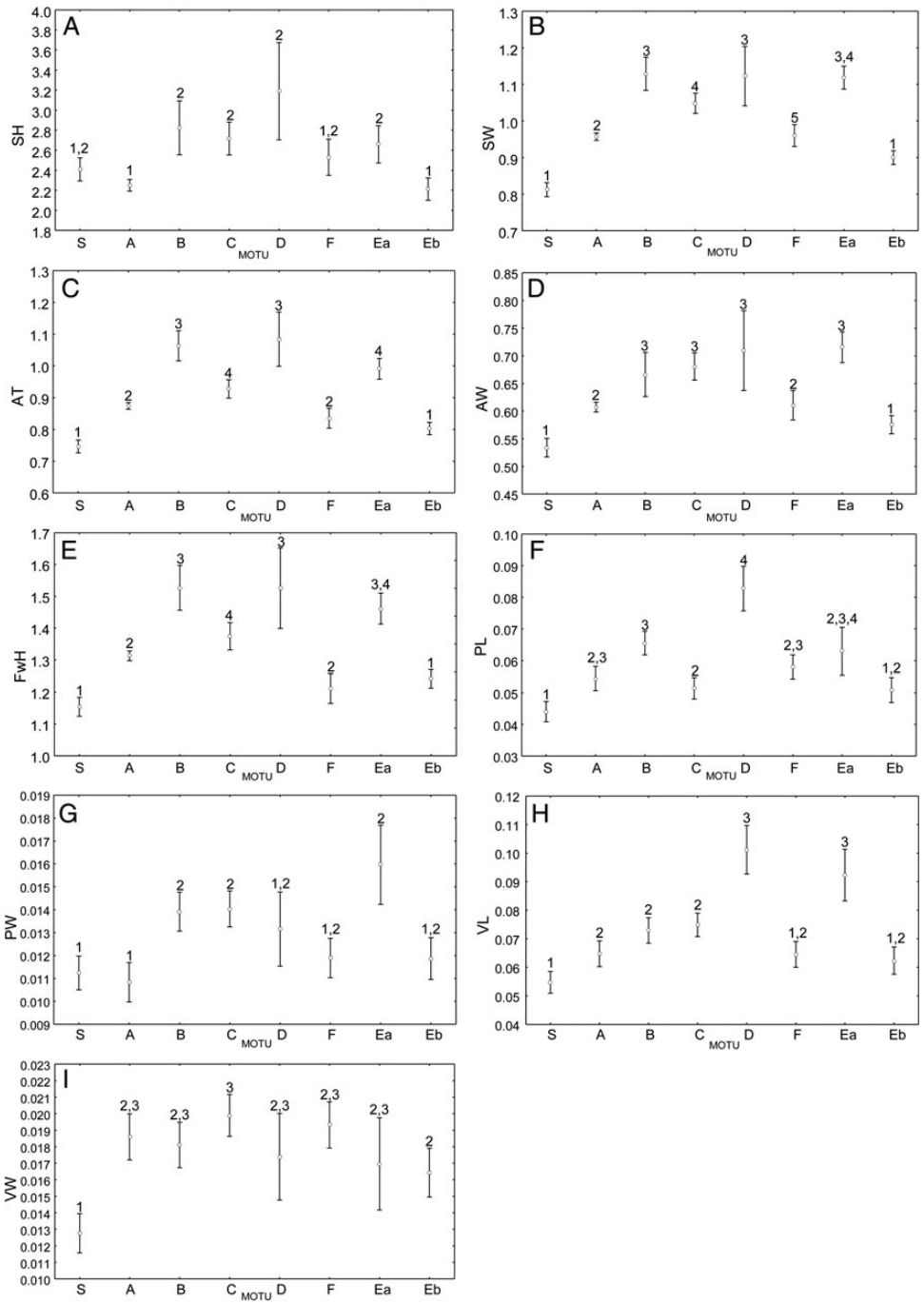

Figure 6. Mean and confidence intervals for each of the shell and genital characters in the seven phylogenetic species within *Rumina decollata* as defined by Prévot *et al.* (2013a). Abbreviations: S, *R. saharica*; A–F, the six MOTUs of *R. decollata*/*R. paivae*. MOTUs that did not differ significantly from each other with the Scheffé test are given the same figure.

consistency among studies (Table 1, Fig. 2). Our data also showed a large overlap in shell size between the species and that the size range of *R. decollata* has smaller minimum values, for both SH and SW, than *R. saharica*.

All in all, *R. saharica* can easily be distinguished from *R. decollata* by its slender and more cylindrical shell (Mienis, 2008), the lighter colour of the shell and of the body (Mienis, 2008) and by its genital anatomy (Carr, 2002). Conversely, the fact that there are no genital characters that distinguish *R. paivae* from *R. decollata* and *R. saharica* may further question the taxonomic validity of *R. paivae*. MOTUs A–F of Prévot *et al.* (2013a) cannot be unambiguously differentiated from each other by the internal structure of the penis and the vagina or by the morphometric data from the shell (except for the general observation that specimens of MOTUs B, D and Ea are on average larger than the others). So, although shell and genital characters may differ significantly between some of these MOTUs, these morphological characters do not allow diagnosis of MOTUs A–F of Prévot *et al.* (2013a). Nevertheless, specimens of MOTU Ea are significantly larger than the specimens from MOTU Eb for all shell and genital

Table 4. Description of the six colour morphs found in *Rumina* species.

Colour		Colour morph						Species
		1	2	3	4	5	6	
Body	Light grey with a medio-dorsal black line	+						D
	Black		+			+		D
	Olive-grey			+				D, P
	Olive-brown				+			D
	Beige						+	S
Foot	Pale yellowish	+						D
	Dull olive-grey		+					D
	Dark yellow			+				D,P
	Olive				+	+		D
	Beige						+	S

The last column gives the morphospecies of *Rumina* in which the colour morph was found. Abbreviations: D, *R. decollata*; S, *R. saharica*; P, *R. paivae*.

measurements (except for the width of the vagina). In addition to their morphometric differentiation, MOTUs Ea and Eb are also separated by their geographic distribution. MOTU Ea occurs in North Africa, whereas MOTU Eb occurs in southern France and eastern Spain (Supplementary Material S1). MOTUs B and D occur in Morocco, while MOTU Ea occurs in Tunisia and Algeria. A somewhat similar geographic and taxonomic break has been observed in the land snail *Cornu aspersum*, of which the subspecies *C. aspersum maxima* occurs in Morocco, while the nominal subspecies occurs in Algeria and Tunisia (Guiller, Madec & Daguzan, 1994).

Although there is some continuity in the colour variation of the body and the foot of *Rumina* species, there are six different colour morphs that tend to be diagnostic for the MOTUs (Table 3, Fig. 1B). However, colour morphs 1 and 2 were both observed in MOTUs A and Eb. Still, 99% of the specimens from MOTU A belonged to colour morph 2 and 91% of the specimens from MOTU Eb belonged to colour morph 1. These two colour morphs are sympatric in southern France and were studied by Selander & Hudson (1976), Selander & Ochman (1983) and Prévot *et al.* (2013b). Colour morph 2 corresponds to the dark phenotype described by Selander & Hudson (1976) and morph 1 to the light phenotype. Selander & Hudson (1976) described their two colour phenotypes as fixed homozygous multilocus genotypes, differing at 13 out of 26 allozyme loci. Later, Prévot *et al.* (2013b) suggested that both phenotypes could be biological species, since they maintain a strong multimarker differentiation (body and foot colour, allozymes, mitochondrial and nuclear DNA), even in microsympatry. Therefore, the colour differentiation between MOTUs A and Eb likely reflects an ancient divergence that can be used as a taxonomically diagnostic marker. Moreover, the two morphs (MOTU A and MOTU Eb) showed significant morphometric differences for all shell variables, such that MOTU A (dark morph) is significantly larger than MOTU Eb (light morph).

In conclusion, although molecular data suggest that *Rumina* comprises at least seven phylogenetic species (Prévot *et al.*, 2013a), the current morphometric data do not allow unambiguous diagnosis of these species, except for *R. saharica*, and to a lesser extent MOTUs A and Eb. Conversely, the status of *R. paivae* is questioned by the morphological data, since no significant differentiation is found in the genital characters of this species. Therefore, and considering that *R. paivae* could not be differentiated with molecular data either, we suggest that it is not a separate species, but a large phenotype of *R. decollata*. We currently regard *R. saharica*, *R. decollata* MOTU A and *R. decollata* MOTU Eb as three diagnosable phylogenetic and biological species. The formal nomenclatural consequences for the latter two taxa will be explored in a future study of nominal *Rumina* taxa in the old literature and of type material in museum collections, in addition to a further comparative analysis of other MOTUs in *R. decollata*.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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