Revising α-taxonomy in shelled gastropods: the case of *Rissoa panhormensis* Verduin, 1985 (Caenogastropoda: Rissoidae)

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ABSTRACT

In one of his landmark papers on the genus *Rissoa* (Caenogastropoda: Rissoidae), Verduin described the new species *Rissoa panhormensis*, based on a few empty shells. After its description, no new data on the species have been published and its taxonomic status has remained questionable. We studied the type material of *R. panhormensis* and several living specimens whose shells resembled those of *R. panhormensis*. Although we could differentiate *R. panhormensis* from *R. guerinii* on the basis of qualitative visual observations and geometric morphometric data, it was not possible to separate both taxa using body colour patterns and 16S + COI mitochondrial DNA sequence data. We therefore suggest that *R. panhormensis* may be a rare morphotype of *R. guerinii* and should be synonymized with this latter species.

Additional keywords: Gastropoda, mitochondrial DNA, geometric morphometry, variation, morphotype

INTRODUCTION

Most older gastropod species descriptions were limited to shell diagnoses, which for a long time were considered to be sufficient to justify specific taxa. However, more recent molecular systematic tools (e.g. Knowlton, 2000; Bickford et al., 2007) have often been used to invalidate species described purely on shell characters alone. On the other hand, cases in which genetic differentiation is hidden by similarity in shell morphology are not uncommon (e.g. references in Knowlton, 1993, 2000). Thus, the degree of shell morphological differentiation may not reflect genetic or anatomical differentiation even among congeneric species.

A purely conchological approach was followed by Verduin (1976, 1982, 1983, 1985, 1986) in his revision of the European species belonging to six subgenera of the genus *Rissoa* (Fréminville ms) Desmarest, 1814 (Gastropoda: Rissoidae). He took into account quantitative (shell measurements, number of sculptural elements) and qualitative shell features (colour pattern elements), assigning to the latter numerical values based on presence/absence or degree of intensity. For the species belonging to the subgenera *Loxostoma* Bivona-Bernardi, 1838, and *Rissoa* Verduin (1983, 1986) only provided narrative descriptions, but for the subgenera *Turboella* (Leach ms) Gray, 1847, *Rissostomia* Sars, 1878, *Goniostoma* (Megerle ms) Villa, 1841, and *Apicularia* Monterosato, 1884, Verduin (1976, 1982, 1985) presented more elaborate data. The shell characters examined in each paper were the same, except for some variation according to the main features of the group studied. In his papers, Verduin (1976, 1982, 1983, 1985, 1986) provided meticulous measurements which were summarized in classical representations (scatterplots or histograms) to facilitate the species comparisons. Verduin placed considerable importance on the dimensions of the shell apex. He observed that there was a larger and smaller type of apex, with no intermediates. According to Verduin (1986), shells sharing the same morphological features, but belonging to two different groups according to apex dimensions, must be considered members of separate species. This statement was supported by the observation that, in some *Rissoa* species (e.g. Rehfeldt, 1968), different types of apex are linked to different larval developmental strategy. A small apex is considered typical of a planktotrophic veliger and large apex is thought to be linked to a lecithotrophic larva (e.g. Thorson, 1950). It is commonly accepted for gastropods that there is no evidence of intraspecific polymorphism in developmental strategy (Bouchet, 1989). Verduin (1986) listed eight "pairs" of sibling species of *Rissoa* (*sensu* Mayr, 1963) that differed mainly in apex dimensions. In doing so, Verduin (1985) not only revised existing *Rissoa* species, but also described some as new. In his paper on the subgenera *Apicularia* and *Goniostoma* (Verduin, 1985),

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one of the new species, *R. panhormensis*, was based on seven empty shells in the Philippe Dautzenberg collection (now housed in the Royal Belgian Institute of Natural Sciences, RBINS).

Rissoa panhormensis, according to its original description (Verduin, 1985) was differentiated as follows: "The shells of *R. panhormensis* strongly recall the normal colour variety of *R. guerinii* (Dautzenberg and Durouchoux, 1914), but have the larger type of apex, i.e. $0.265 < D_0 + 0.72d < 0.290$ mm, and have only 5–6 ½ terminal ribs per whorl. The shells measure from 4.8 to 6.0 mm. All have punctate spiral striae on the lower part of the body whorl. For the remainder, the shells are covered with fine, dense spiral striae, which merge into the punctate spiral striae. There are 7.6–8.2 whorls and 3½–4¾ ribbed whorls. The ribs continue up to the labial rib. The labial rib is well developed, and of a whitish colour, as are the other ribs. As in the normal colour variety of *R. guerinii*, the uppermost whorls are of a remarkable greyish colour. The edge of the aperture is purplish." The type locality is Palermo (Sicily, Thyrrenian Sea).

Together with the description, Verduin (1985) provided a black and white photograph of the holotype, which until now is the only known picture of *Rissoa panhormensis*. After its description, the species slipped into obscurity with hardly a mention in the literature. Currently, *Rissoa panhormensis* is considered to be an endemic species of the Western-Central Mediterranean (Bodon et al., 1995). We examined the type material of *R. panhormensis* and other specimens of *Rissoa* in the Dautzenberg collection (RBINS). On the basis of that material we were able to critically revise the original description of the taxon and to verify shell measurements.

We also sampled living *Rissoa* spp., collecting some specimens whose shells closely resembled the description of *R. panhormensis*. This allowed us to study other features such as external soft body parts and mitochondrial DNA. Several empty shells corresponding to *R. panhormensis* were also found washed ashore. Based on this material we provide a morphological and molecular re-evaluation of the taxonomic status of *R. panhormensis*.

MATERIALS AND METHODS

Dautzenberg Collection Material: The type material of *Rissoa panhormensis* in the Dautzenberg collection comprises the holotype and six paratypes from Palermo (Sicily, Tyrrhenian Sea). Four other lots were selected, which, according to present taxonomy belonged to *R. guerinii* Recluz, 1843. The specimens of these latter lots closely resembled *R. panhormensis* type material. Table 1 summarises the data of the four lots and the acronyms used in this study to identify them. All the shells of the five lots were examined using an Olympus® SZX10 stereoscopic microscope. Damaged shells and misclassified specimens (e.g. *R. violaeea* Desmarest, 1814) were not further considered. Only five shells from lot Rpt were considered. These five shells of *R. panhormensis* and 20 randomly chosen shells of *R. guerinii* (five from each of the four lots, Rca, Rcb, Rcm and Rsm), were selected for this study. The presence of a well-formed labial rib in all the shells indicated that they were all at terminal growth (Warén, 1996).

Table 1. The composition of the studied lots of *Rissoa* spp., with the inscription of the original label and their acronym. No further data (exact locality, date, etc.) regarding these lots were available.

Acronym	Content	Label
Rpt	6 *R. panhormensis* 1 *R. violacea*	*Rissoa panhormensis* Verduin/Det.: Verduin, 1983
Rca	51 *R. guerinii* 2 *R. violacea*	*Rissoa costulata*
Rcb	92 *R. guerinii*	*Rissoa costulata*, Alder
Rcm	15 *R. guerinii*	*Rissoa costulata*, Medit. Monts.
Rsm	12 *R. guerinii*	*Rissoa subcostulata*, Schwarz Mediterranée

Each shell was placed in vertical position under the microscope by fixing the shell base with plasticine. Total numbers of whorls, ribbed whorls and terminal ribs per whorl, were counted according to Verduin's (1982) methods. Then each protoconch was photographed from above using an Olympus® CAMEDIA C-7070 WZ digital camera. Shells were then positioned, with the help of plasticine, with their vertical axes parallel to the observation plane and pictures were taken of the teleoconchs. Digitized images were opened in Adobe® Photoshop CS2 image editor software. Using measurement tools provided by the software (appropriately calibrated), total shell length (L), the diameter of the protoconch nucleus (d) and the diameter of the first half whorl (D_0) were measured, according to Verduin's (1985) methods. Furthermore, the apex type ($=D_0 + 0.72d$) was calculated according to Verduin (1985). Using the statistical software SPSS v.15 (© SPSS Inc., 2006), a discriminant function analysis was performed on the data matrix obtained, in order to detect significant differences between the type specimens of *R. panhormensis* and shells of *R. guerinii* (treating the four lots as a single group).

Live-collected Material: Living material was sampled on the rocky shore of Santa Tecla, Sicily (Mediterranean, Ionian Sea) at 1–5 m depth in Apr 2006. About 0.03 m^3 of the red alga *Pteroeladiella capillacea* (Gmelin) Santelices and Hommersand was collected by SCUBA diving during each sampling. Collected material was immersed in seawater and transferred to the laboratory. The total amount of sampled material was partitioned into 20 subsamples that were washed for no more than 5 min in a tank containing 5 l of 50% seawater. The osmotic shock provided forced all vagile fauna to detach from the algal thalli and to fall on the bottom of the tank, from where specimens were easily collected and returned to seawater. After recovering from the osmotic shock, live

mollusks were sorted and identified under a Wild Makroskop M420 stereoscopic mieroscope. Specimens of *Rissoa guerinii* and five specimens referable to *R.* cf. *panhormensis* were picked up from the sorted material. Some of them were placed in running seawater at 18°C and provided with fresh *P. capillacea* talli; others were preserved in 80% ethanol. After eaeh sampling, beached detritus was also eollected on the beaeh faeing the sampling site. This yielded 18 empty shells belonging to *R.* cf. *panhormensis*. The relative proportion of specimens of *R.* cf. *panhormensis* and speeimens of *R. guerinii* was about 3/100 in samplings of both living and dry material.

Head-Foot: Adult living speeimens of both *R.* cf. *panhormensis* and *R. guerinii* were plaeed in a Petri dish with seawater under a Leica Z16 APO stereoscopie mieroseope. Shells were held with forceps and the snails attempted to crawl extending their foot eompletely, enabling the head-foot to be observed in detail. Images of shells and head-foot were taken and digitized using a Leiea DFC 300 FX video eamera and Leica Application Suite version 2.4.0 software. Color drawings were also made to better represent the color pattern of the head-foot.

Geometric Morphometry: We randomly choose 15 adult shells from ethanol preserved specimens of *R. guerinii* and 15 adult shells of *R.* cf. *panhormensis* (three from ethanol-stored material and the remaining 12 from eollected empty shells). Shells were observed using a Leica Z16 APO stereoseopic microscope, and eolor images were taken and digitized using a Leica DFC 300 FX video camera and Leica Application Suite version 2.4.0 software. The shells were always plaeed in the same position, with the coiling axis in vertieal position and the aperture on the same plane as the objective (Carvájal-Rodríguez et al., 2005). Using the software tpsDIG2 v. 2.10 (Rohlf, 2007a), 19 landmarks (LM) were established (Figure 1). LM1 is the apex of the shell; LM2, LM4 and LM6 are placed on the right border of the profile at the beginning of the three last complete whorls. LM15, LM17 and LM19 are the corresponding landmarks on the left border of the profile. LM3, LM5, LM16 and LM18 mark the intermediate position respectively between LM2 and LM4, LM4 and LM6, LM15 and LM17, LM17 and LM19 along the curvature of the whorl; LM8 is at the lower suture of the last complete whorl and LM7 marks the intermediate position between LM6 and LM8 along the eurvature of the whorl. LM9 is the most external position in the upper part of the outer lip; LM10 and LM12 are the most external positions respectively in the external right and left part of the outer lip; LM11 is the lowest point at the base; LM14 is the most external point in the last whorl at the left profile of the shell; LM13 is the profile point between LM12 and LM14 (closest to LM7). As deseribed in Carvájal-Rodríguez et al. (2005) the matrix of raw coordinates generated by tpsDIG2 was used in tpsRelw v.1.45 (Rohlf, 2007b) to compute shell size (CS), uniform (U1 and U2) and non-uniform (several relative warps, RWs) shape components for eaeh specimen. Classical parametrie tests were performed on the obtained variables by the SPSS/PC package v. 15.0.

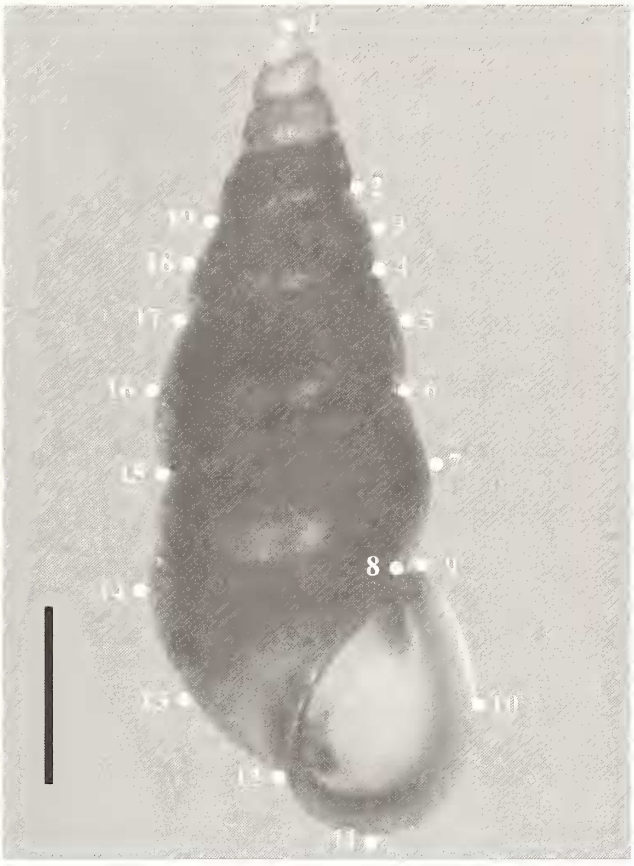


Figure 1. A shell of a living *Rissoa* cf. *panhormensis* from S. Teela showing the placement of the 19 landmarks used for geometric morphometric analysis. Scale bar = 1 mm.

Molecular Systematics: Thirty-five live *R. guerinii* and five live *R.* cf. *panhormensis*, from the Santa Teela samples, were used for DNA analysis. The color pattern of each specimen was recorded before processing. The shell of each specimen was broken in a mortar and the entire organism was homogenized in a 1.5 ml eppendorf tube using 150 µl 2x CTAB extraetion buffer (50 mM Tris HCl [pH 8.0], 0.7 M NaCl, 10 mM EDTA, 1% CTAB, 0.4% β-mercaptoethanol) with the addition of 10 µl of Proteinase K. DNA was extracted using standard CTAB protocol (Doyle and Doyle 1987) with one extra wash in phenol:ehloroform:isoamylalcohol (25:24:1) and one in chloroform:isoamylalcohol (24:1) in order to eliminate polysaccharides. Two mitochondrial DNA markers were amplified by PCR: (1) a 337 bp fragment of 16S rRNA was amplified in a 20 µl final volume containing 1 µl template DNA, 2 µl of 10X Roche diagnostic PCR reaction buffer, 2 µl dNTPs 10X (2 mM), 0.8 µl of eaeh primer (20 pmol/µl), 1 µl Biogem Taq polymerase (3 u/µl), 0.2 µl BSA. The primers were (designed with Oligo v. 6.71 software), U37 (5'-AGAGAATTACGCTGTTATCC

CTGT-3') and L373 (5'-AGAGAATTACGCTGTTATCC CTGT-3') with a target length of 360 bp; PCR conditions were: 94°C for 5 min, 40 cycles of 94°C for 1 min, 50.1°C for 1 min and 72°C for 1 min and a final elongation step of 7 min at 72°C; (2) a 372 bp fragment of COI was amplified in a in 20 µl final volume containing 20 to 50 ng template DNA, 2 µl of 10X Roche diagnostic PCR reaction buffer, 2 µl dNTPs 10X (2 mM), 1 µl of each primer (20 pmol/ µl), 1 µl Biogem Taq polymerase (2.5 u/µl), 0.2 µl BSA; the primers were, 59R-COI (forward: 5'-ATTGGTGGCTTTGGAAATTG-3') and 59L-COI (reverse: 5'-GATAGGGTCACCACCTCCTG-3'; Panico and Patti, 2005) with a target length of 450 bp; PCR conditions were: 94°C for 5 min, 40 cycles of 94°C for 1 min, 45°C for 30 sec and 72°C for 45 sec and a final elongation step of 7 min at 72°C.

PCR products were separated by gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions. Purified products were sequenced on a Beckman Ceq 2000 automatic sequencer, using a Dye-terminator cycle sequencing kit (Beckman) according to manufacturer's instructions. Sequences were assembled using the DNASTAR computer package (Lasergene), supplied with the Beckman sequencer. Sequences obtained for different marker from the same individual were concatenated in Bioedit v. 5.0.6 (Hall, 1999), treated as single sequence and aligned with CodonCode Aligner v. 1.6.3 (CodonCode Corporation, Dedham, MA), using ClustalW (Thompson et al., 1994) alignment method. The alignment was refined by eye. For all samples, both forward and reverse strands were analysed. Genbank accession numbers range from GU177879 to GU177963 for the 16S gene and from GU177964 to GU178011 for the COI gene.

The concatenated sequences were subjected to Maximum Parsimony and Maximum Likelihood tree reconstruction using PAUP* v. 4.04 (Swofford, 2003). *Rissoa labiosa* (Montagu, 1803) was used as the outgroup (Genbank accession numbers: AY676128 for COI and AY676117 for mt16SrRNA). The program Modeltest version 3.06 (Posada and Crandall, 1998) was employed to selected HKY + I model for ML analysis. Trees were computed with 1000 bootstrap replicates. Bremer support values (Bremer, 1994) were used in conjunction with bootstrap. A reduced median joining network (MJ) (Bandelt et al., 1999) was obtained with the software Network v. 4.5 (Fluxus Technology).

RESULTS

Dautzenberg Collection Material: **Visual Observation:** The type specimens of *Rissoa panhormensis* were kept in a glass tube in a small cardboard box. The holotype was isolated from the paratypes and enclosed in a small plastic case. The bad state of preservation of the periostracum and the consistent presence of mineral concretions, visible on the surface and in the inside of some shells, indicated some degree of shell degradation. The types appeared to be very similar to specimens identified as *R. guerinii* from the lots Rca, Rcb, Rsm and Rcm (see Table 1). Paratypes were rather slender with a reduced number of ribs often showing a high degree of bluntness towards the earlier whorls. This feature of the ribs was exacerbated on the spire of the holotype, giving this specimen the most peculiar aspect of the shells of type series. The shell pigmentation was typical of *R. guerinii:* white with brown spaces between ribs and a gray-violet apex. The tube with the holotype contained a label with two different handwritings: Verduin's original note: "*Rissoa panhormensis* Verduin/ Det.: Verduin, 1983" and an additional indication added in handwriting: "7 paratypes". Unfortunately, the staff of the malacological section of RBINS was unable to identify this handwriting. The box contained a larger label with the text: "*Rissoa panhormensis* VERDUIN/Palerme/Lemoro, Monts./PARATYPES". We were not able to find the original label with the inscription "*Rissoa guerinii*, Recl./ Palerme, Lemoro Monts." mentioned by Verduin (1985). This has probably been lost.

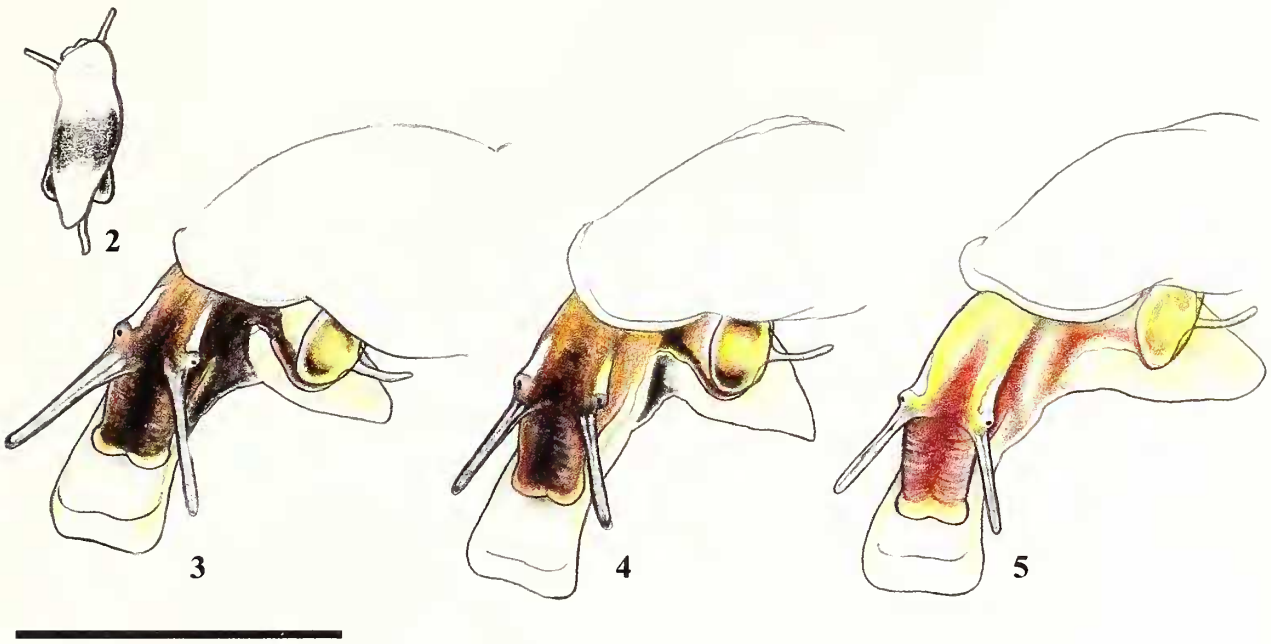
Morphometry: The eigenvalue of the discriminant function between the two species was 0.683, the canonical correlation 0.673 and Wilks' lambda (0.594) was not significant ($p > 0.05$), indicating a lack of any statistically valid separation between the two groups. As shown by Table 2, of the variables employed, the number of ribbed whorls was the most important one in distinguishing the two groups. Moreover, it was the only one revealing a consistent degree of correlation with the discriminant function (0.772).

Using the values of the discriminant function of each individual to predict its *a posteriori* species membership, 21 (84%) individuals, out of the total 25 used in the analysis, were attributed to the correct species and only four (16%) were erroneously *a posteriori* classified. Looking at species statistics, all shells of *R. panhormensis* were correctly assigned to this species. Only four (20%) specimens of *R. guerinii* were erroneously assigned to *R. panhormensis*, whereas 16 (80%) were assigned to the correct taxon.

Live-collected Material: **Head-foot:** The intensity of the pigmentation of the shell and head-foot enabled two color types of *Rissoa guerinii* to be distinguished, viz. typical *R. guerinii* and *R. guerinii* "var. *conspersa*" (Dautzenberg and Durouchoux, 1914; Figure 4 and 5). In both

Table 2. Shell variables in *Rissoa* spp. and their coefficients and correlation with the discriminant function.

	DF Coefficients	Correlation with DF
Ribbed whorls	1.020	0.772
Terminal ribs per whorl	–0.052	–0.278
D_0	–0.075	–0.236
d	–0.292	0.151
Length	–0.404	0.031



Figures 2–5. Pigmentation of the soft body parts of living specimens. **2.** Dark smudge of the middle part of the sole. **3.** *Rissoa* cf. *panhormensis*. **4.** *R. guerinii* "var. *conspersa*." **5.** *R. guerinii* (typical pigmentation). Scale bar = 1 mm. Drawings by Danilo Scuderi.

types, the foot was whitish and the middle part of the sole was stained brown (Figure 2), this latter feature being lighter in typical *R. guerinii* than in "var. *conspersa*." The snout was light brown in *R. guerinii* and darker brown in "var. *conspersa*." The margin of the distal portion of the snout and the rest of the head was yellowish in *R. guerinii* and light brown in "var. *conspersa*." The cephalic tentacles were whitish, but sometimes dark brown in "var. *conspersa*." A whitish spot behind the base of cephalic tentacles was always present. The body pigmentation of *R.* cf. *panhormensis* was similar to that of *R. guerinii* "var. *conspersa*," with a slight tendency to be darker (Figure 3). Table 3 summarizes the comparison among the different pigmentation patterns.

Geometric Morphometry: Table 4 shows the percentages and a descriptive statistical summary of the relative score for CS, the two uniform components and the first 8 RWs, explaining more than the 91% of the overall variation. Table 5 shows the results of the allometric analysis for shell shape measurements conducted by stepwise multiple regression analysis for centroid size (as dependent variable) and two uniform and 29 non-uniform measurements, as independent variables. The F-test of the regression analysis was significant ($p < 0.05$) and only one relative warp, RW3, contributed significantly to the regression model on the centroid size (Beta = –0.406). Centroid size, uniform components and only the first eight relative warps were considered in the analysis of variance (ANOVA) performed to evaluate the significance of differences in size and shape variables. Shells of *R. guerinii* and *R.* cf. *panhormensis* differed significantly in U1 ($p < 0.001$), RW2 ($p < 0.001$ and RW6 ($p < 0.05$). The cumulative results of the analysis are shown in Table 4. The significance level obtained for the corrected analysis (ANCOVA) with centroid size as covariate was not maintained for the difference in RW6, but was only slightly affected for RW2 and for U1 (both with $p < 0.05$). In addition, a significant difference was found between the two groups also for RW3 ($p < 0.05$). Table 4 shows these results.

Table 3. Summary of the observation on the pigmentation features in the specimens of *Rissoa* spp. considered.

Feature	*R. guerinii*	*R. g.* "var. *conspersa*"	*R.* cf. *panhormensis*
Foot colour	whitish	whitish	whitish
Sole stain colour	light brown	dark brown	dark brown
Snout colour	light brown	dark brown	dark brown
	yellowish distally	light brown distally	light brown distally
Head colour	yellowish	light brown	light brown
Cephalic tentacles colour	whitish	whitish or dark brown	whitish or dark brown
Tentacular spot colour	whitish	whitish	whitish

Table 4. Descriptive statistical summary and results of ANOVA and ANCOVA for the main shell size and shape variables between *Rissoa guerinii* and *R.* cf. *panhormensis*. $^{**}p < 0.05$, $^{***}p < 0.001$, ns = non significant.

Measure		CS	U1	U2	RW1	RW2	RW3	RW4	RW5	RW6	RW7	RW8
Variance explained					39.3%	27.5%	7.3%	5.1%	4.1%	3.2%	2.5%	2.0%
R. guerinii	Mean	1021	–0.010	–0.002	0.002	–0.017	–0.005	0.000	–0.002	0.004	0.001	–0.001
	St. dev.	122	0.014	0.010	0.039	0.025	0.017	0.014	0.010	0.011	0.011	0.009
R. cf. *panhormensis*	Mean	996	0.010	0.002	–0.002	0.017	0.005	0.000	0.002	–0.004	–0.001	0.001
	St. dev.	89	0.013	0.007	0.038	0.029	0.014	0.014	0.014	0.008	0.008	0.008
ANOVA		0.41^{ns}	18.26^{***}	1.47^{ns}	0.06^{ns}	11.80^{***}	2.49^{ns}	0.04^{ns}	0.64^{ns}	5.52^{**}	0.38^{ns}	0.34^{ns}
ANCOVA			9.09^{**}	3.14^{ns}	0.56^{ns}	5.69^{**}	3.84^{**}	0.50^{ns}	0.89^{ns}	3.31^{ns}	0.30^{ns}	1.65^{ns}

The eigenvalue of the stepwise discriminant function between the two species, calculated for all 29 RWs, was 4.033, the canonical correlation 0.895 and Wilks' lambda (0.199) was highly significant ($p < 0.001$), indicating a good separation between groups. Seven shape variables contributed to the discriminant function (RW2, RW6, RW13, RW3, RW27, RW16, and RW25). The standardized coefficient matrix (Table 6) shows the relative importance of the independent variables in determining the standardized canonical discriminant function. The mean values of the discriminant function for the two groups were –1.940 for *R. guerinii* and 1.940 for *R.* cf. *panhormensis*. Using the individual values of the discriminant functions to predict *a posteriori* species memberships, 26 (86.7%) individuals out of 30, were assigned to the correct species, leaving only 4 (13.3%) that were erroneously classified. Looking at species statistics, 13 (86.7%) specimens of *R. guerinii* were correctly assigned to this species and only two (13.3%) were assigned to *R.* cf. *panhormensis*. The same percentages of correctly/erroneously classified specimens of *R.* cf. *panhormensis* were observed. In Figure 3 the thin plate spline representation allowed us to interpret in geometric terms the positive (characteristic of *R.* cf. *panhormensis*) and negative deviations (characteristic of *R. guerinii*) values for the most significant non uniform shape variable, RW2, between the two species.

Molecular Phylogeny: After combining the COI and 16S rRNA sequences, a concatenated sequence of 709 bp was obtained, yielding 20 different haplotypes 18 of which involved exclusively *R. guerinii*, while the two remaining ones were shared by both *R. guerinii* and *R.* cf. *panhormensis*. The topologies of the MP and ML trees (Figure 4) were comparable: four haplotypes of *R. guerinii* occupy nested basal positions in the tree, while the remaining 16 haplotypes, belonging to *R. guerinii* and *R.* cf. *panhormensis*, form a terminal clade supported by bootstrap values of 70–75 (MP and ML respectively). The MJ network (Figure 5) confirmed the presence of a common haplotype occurring in 19 specimens (n = 19) of both *R. guerinii* (n = 15) and *R.* cf. *panhormensis* (n = 4), one haplotype occurring in two specimens of *R. guerinii*, 17 unique haplotypes, and one haplotype that occurred in one specimen of each species. 14 haplotypes are separated by 1–5 differences from the main one, but four showed larger distances (27, 25, 23 and 15 respectively).

Table 5. Multiple regression model to test allometry for the non-uniform shell shape variables in *Rissoa* spp.

Multiple regression		Variables in the model	
r^2	F	Name	Beta
0.165	5.6^{**}	RW3	-0.406^{**}

$^{**}P < 0.05$, $^{***}P < 0.001$.

DISCUSSION

Dautzenberg Collection Material: **Visual Observation:** Monterosato (1884) considered *R. costulata* Alder, 1844, and *R. subcostulata* Schwarz, 1864, as synonyms of *R. guerinii*, so we checked if the original label for *R. panhormensis* might be found accompanying a lot of those two taxa. Only two labels were found to have these characteristics: that of the already mentioned sample Rcm (*Rissoa costulata/Medit./Monts.*) and one found in the bottom of a box containing lots of *R. guerinii* coming mainly from French coasts, whose inscription was: "*Rissoa costulata, Alder*/Palerme/Lemoro Monts.". While it is possible that one of these could be the original label accompanying the type lot of *R. panhormensis*, it is unlikely as it does not correspond exactly to the wording given by Verduin. We are unsure if the type material originally constituted a single separated sample or whether it was a part of a larger sample from which Verduin isolated seven specimens. However, there was a strong resemblance between *R. panhormensis* type material and the four lots referable to *R. guerinii* (Rca, Rcb, Rcm and Rsm) suggesting that these lots may at least share a common geographic origin.

Table 6. Standardized coefficient matrix showing the relative importance of the shape variables in *Rissoa* spp.

	Discriminant function
RW2	1.145
RW6	–0.929
RW13	–0.702
RW3	0.686
RW27	0.683
RW16	0.581
RW25	0.460

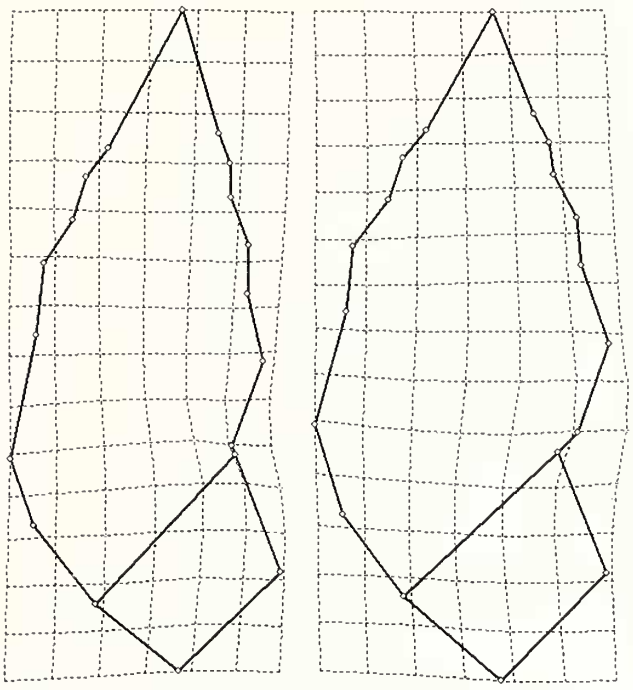


Figure 6. Thin plate spline representations for RW2, showing the deformation of the grid for the average values of *Rissoa guerinii* (left) and *R.* cf. *panhormensis* (right).

Another important observation was the unrepresentative nature of the holotype with respect to the paratype series. This shell, rather than summarizing the average characteristics of the type series, represents instead the most extreme variant with a nearly total lack of ribs. The illustration provided by Verduin for this shell is not of good quality and this has contributed to perpetuating the idea that *R. panhormensis* is a ribless species. This characteristic is reflected in the shells of our sampled specimens, here referred to as *R.* cf. *panhormensis*.

Morphometry: Verduin (1985) stated that the samples containing the type specimens were probably dredged, because Monterosato used to obtain detritus from fishing nets and then pick out and classify the interesting shells. Using this method he selected the samples that he retained for his collection. In view of this possible lack of randomness in the samples obtained from Monterosato, we could not use them to reliably infer interpopulational or interspecific differences between samples. Our morphometric investigation arose from the observation that the type material of *Rissoa panhormensis* strongly resembles *R. guerinii*, despite the claim of a morphological (and morphometric) distinction between both taxa (Verduin, 1985). This claim was mainly based on alleged differences in the size of the apex and differences in the number of shell ribs. However, our discriminant analysis of three protoconch variables (d, D_0, and At) did not support significant differences between the size of the apex of *R. panhormensis* and *R. guerinii*, and both belong to the larger apex category (At > 0.235 mm). Moreover, we

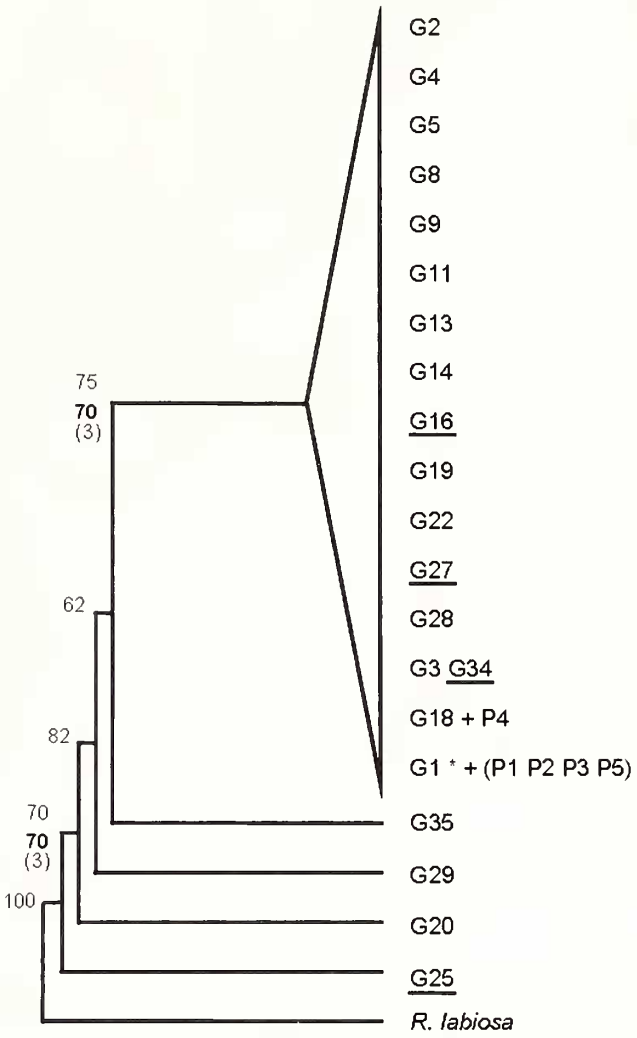


Figure 7. Maximum Likelihood tree obtained from concatenated sequences of 16S and COI genes obtained for *Rissoa guerinii* and *R.* cf. *panhormensis*. A number of 1000 bootstrap replicates were performed and its values (if above 50%) are shown on the nodes. Bootstrap values for Parsimony are in bold; in brackets the Bremer index values. Letter G refers to *R. guerinii* haplotypes, letter P to *R.* cf. *panhormensis* haplotypes. G1* = G1, G6, G7, G10, G12, G15, G17, G21, G23, G24, G26, G30, G31, G32, G33. Specimens of *R. guerinii* "var. *conspersa*" are underlined.

observed apex types exceeding Verduin's arbitrary, species specific "cut-off values" within *R. guerinii* (Criscione and Patti, submitted.). Hence, if only the dimensions of the apex were used to distinguish *R. panhormensis* (type material) from Dautzenberg's *R. guerinii* samples, then this would not result in a clear separation of both taxa. In other words: the *R. panhormensis* types were not the only specimens among Dautzenberg's *R. guerinii* material to have the larger type of apex.

Although number of whorls (N) and shell length (L) show considerable intraspecific variation, they nevertheless often reveal consistent interspecific differences (F.C., pers. observ.). However, our statistical analysis revealed

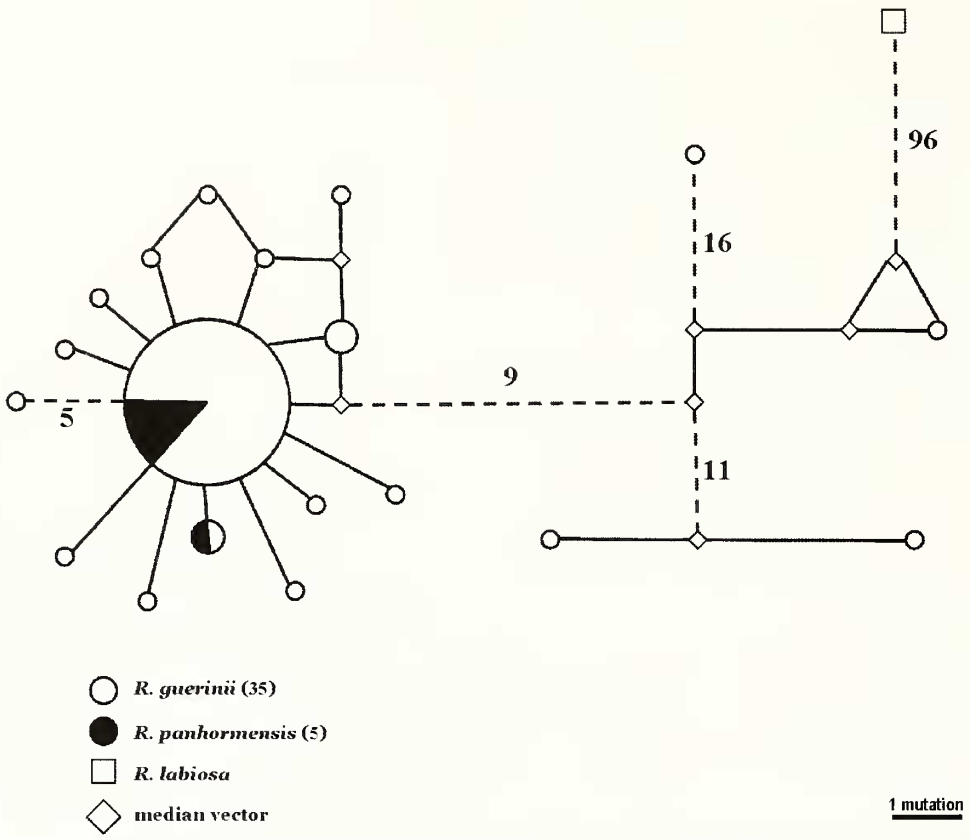


Figure 8. Median Joining Network drawn from concatenated sequences of 16S and COI genes obtained for *Rissoa guerinii* and *R.* cf. *panhormensis*. In round brackets the number of sequences employed. Dashed lines represent higher number of mutations (values reported nearby).

that the differences between the samples for these two characters were not significant. Finally, although Verduin (1985) emphasized the low number of radial ribs of *R. panhormensis*, it is unclear to us as to whether this related to a smaller number of ribbed whorls or a smaller number of ribs on the last whorl. In this study only the total number of ribbed whorls (RW) can be used to discriminate the two groups and this may be what Verduin really meant as it appears to be the only difference by which *R. panhormensis* and *R. guerinii* can be separated.

Living Material: **Geometric Morphometry:** The ANOVA did not reveal any significant difference in size (CS) between the two groups, confirming our non-casual observations of the Dautzenberg collection samples. A high level of significance was instead observed for the first of the uniform shape components, U1, which, accounting for compression-dilation deformations, may be interpreted as indicating that *Rissoa* cf. *panhormensis* has a more slender shell compared to *R. guerinii*. Analysis of variance showed a highly significant difference ($p < 0.001$) between the two groups in the second non uniform shape variables (RW2) and a significant difference ($p < 0.05$) for the sixth relative warp (RW6). The significance for U1 remained unaltered when correcting the analysis for CS (ANCOVA), while that of RW2 decreased to significant ($p < 0.05$) and that of RW6 was not significant, indicating that the shape difference explained by those variables was dependent on size. The difference in RW3, was not significant in the ANOVA, but became significant ($p < 0.05$) in the ANCOVA. Despite these variations, RW2 always showed the lower p value, which means that the two groups mostly differ on this shape variable independently from the correlation between shape and size.

The discriminant function calculated from all the non uniform shape variables, was successful in morphometrically discriminating the two groups. RW2 was the most important variable in determining the distinction between *Rissoa guerinii* and *R.* cf. *panhormensis*. The mean values of this variable for each of the two groups (positive for *R.* cf. *panhormensis* and negative for *R. guerinii*) were plotted in a tps representation (Figure 3). The plot showed that that variable RW2 is a reflection of the most obvious discernable shell shape difference. This comprised the slenderer aspect of *R.* cf. *panhormensis*, contributed by a consistently narrower penultimate whorl (represented by the contraction of the corresponding zone of the grid) than that of *R. guerinii*. A similar interpretation of a single relative warp

resulting in shell slenderness has also been reported by Carvájal-Rodríguez et al. (2006) in *Nassarius*. In this case, however, the absenee or reduction of axial ribs may have affected the representation of horizontal dimensions, resulting in this visual difference. The flatter aspeet of the whorls of *R.* ef. *panhormensis* is also linked to the lack of ribs. Also notieeable is the opposite relative displaeement of landmarks 8 and 9 (Figure 6) and the subsequent modifieations of the grid. In *R.* cf. *panhormensis*, LM 9 is overlapped to a greater extent by LM 8 than in *R. guerinii*. This is beeause of the presence of ribs on the penultimate whorl of *R. guerinii*, whieh partly overhang the posterior part of the outer lip, giving the impression of a less protruding peristome. In contrast, the laek of ribs in *R.* ef. *panhormensis* aecounts for the more protruding peristome of this morph.

Head-foot: In the genus *Rissoa*, the pigmentation of head-foot is often an important speeies-speeifie eharacter (Fretter and Graham, 1978). In addition, *Rissoa* speeies also often differ in the relative proportions of head-foot eomponents (eephalic tentacles, snout and anterior part of the foot) (D.S. pers. observ.). One cannot use these characters to distinguish *R.* ef. *panhormensis* from *R. guerinii* "var. *conspersa.*"

Molecular Systematics: The combined 16S and COI sequenee data showed that *R. guerinii* and *R.* cf. *panhormensis* cannot be separated.

Radial Ornamentation and Species Distinction: Our results indieate that reduced radial ornamentation is the only distinguishing feature between *R. panhormensis* and *R. guerinii*. Problems with using the number of ribs for speeies distinction in the genus *Rissoa* are not new. There is evidence to show that rib number can be influeneed by environmental conditions. For example, in fluetuating salinity, *R. parva* (Da Costa, 1778) ean show reduction in radial ornamentation (Wigham, 1975; Verduin, 1976; Warén, 1996). Moreover, several unpublished field observations made by the authors on other species of *Rissoa*, living in low salinity environments (sheltered bays, eoastal seagrass meädows), revealed the tendeney in entire populations (*R. similis*) or a small number of individuals in a population (*R. auriscalpium*, *R. labiosa*) to laek axial ribs. It appears that this latter situation is the case with *R. guerinii*. There is an abundant inflow of freshwater in our sampling site as well as other parts of the Sicilian Ionian coast. Where those conditions oceur, it seems possible that *R. guerinii* might develop "smooth" morphotypes, as in our sample loeality.

This view is in agreement with the idea of *R. guerinii* as a highly polymorphie and/or plastic taxon. Indeed, besides *R. panhormensis*, there are other Mediterranean speeies of *Rissoa* (e.g. *R. decorata* Philippi, 1846, *R. torquilla*, Pallary, 1912, and *R. frauenfeldiana* Brusina, 1868) whose shells are quite similar to *R. guerinii*. It would not be surprising if further studies show that those species are also ecophenotypes of *R. guerinii*.

CONCLUSIONS

We find that *Rissoa* cf. *panhormensis* is a rare morphotype of *R. guerinii* charaeterized by reduced development of radial ornamentation, and that, as such, it should be eonsidered to be a junior synonym of this latter speeies.

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