

THE USE OF THE COMPUTER-ASSISTED MEASUREMENTS UTILITY

MARÍA R. ALONSO¹, MANUEL NOGALES² & MIGUEL IBÁÑEZ¹

¹Department of Animal Biology, La Laguna University, E-38206 La Laguna, Tenerife, Canary Islands, Spain.

²Island Ecology and Evolution Research Group (IPNA, CSIC), E-38206 La Laguna, Tenerife, Canary Islands, Spain.

Abstract We compare traditionally used straight linear shell measurements, with "surface" and "perimeter" measurements, obtained using image analysis software. A preliminary assessment was made on four species from the family Enidae. We evaluated the quality of all measurements used to identify those of greatest taxonomic value. The results obtained demonstrate that the use of computer-assisted measurements provide more reliable and less variable data than traditional ones.

Key words Mollusca measurements, surface, perimeter, morphometry, discriminant analyses.

INTRODUCTION

The amount of phylogenetic signal and homoplasy in gastropod shell characters has recently received considerable attention (Wagner 1999, 2001b; Haas 2000; Schander & Sundberg 2001; Papadopoulos, Todd & Michel 2004) and many neontological systematists express a general wariness over the utility of gastropod shell characters. As the shell is directly exposed to the external environment it has been suggested that shell morphology would tend to evolve faster than internal anatomy, resulting more often in homoplasy (Wagner 2001a), downgrading the perceived value of shell characters among some systematists. However, much of the systematics and phylogenetics of extant gastropods still relies on shell morphology and for fossil gastropods this will remain the case (Papadopoulos et al. 2004). For extant gastropods, shell morphology is increasingly used in concert with anatomical and molecular characters and as has been noted by Nützel, Erwin & Mapes (2000), the monophyly of many groups that were first recognized partly or wholly on shell characters is often being corroborated in the increasing numbers of molecular phylogenetic studies being undertaken. Papadopoulos et al. (2004) concluded that gastropod shells, when studied in sufficient detail and within an ontogenetic perspective, can provide entirely untapped sources of macroscopic characters valuable for phylogeny reconstruction. Among the shell characters, shell morphometric methods have undergone a renaissance in recent years with the advent of the application of multivariate approaches. The straight linear dimensions, mainly shell measurements and

related morphometric indexes (e.g. height - width plots) are easily available and can be used in different types of research not just for species discrimination. However, the information provided by these measurements is may be subject to variability even within a single population. Some of the recent examples of this variability are shown in the morphometric studies of Cabral (2003), Estebenet & Martín (2003) and Fehér (2003).

Although image analysis software measurements, such as molluscan shell plane view area, has been used to compare species (Stothard, Llewellyn-Hughes, Griffin, Hubbard, Kristensen & Rollinson 2002), we do not know of any statistical study which has evaluated the quality of these measurements against those from the more traditional methodology straight linear dimensions. Recently, we prepared a paper on using the taxonomy and phylogeny of some *Napaeus*-like species (Gastropoda, Enidae) from the Canary Islands, based on their anatomy and mitochondrial DNA data (Alonso, Goodacre, Emerson, Ibáñez, Hutterer & Groh, in press). Using the data in the former paper we undertake a comparative statistical analysis based on four of the species: *Napaeus rupicola* (Webb & Berthelot in Mousson 1872) and three new species.

The general aim is to illustrate the utility of these computer-assisted measurements. Our particular objectives are: A) to show a comparison between the more frequently used straight linear shell measurements versus some computer-assisted measurements of plane views of shells, like those related to surface and perimeter in an elongated shell snail group, using the four *Napaeus*-like species; B) to statistically analyse these charac-

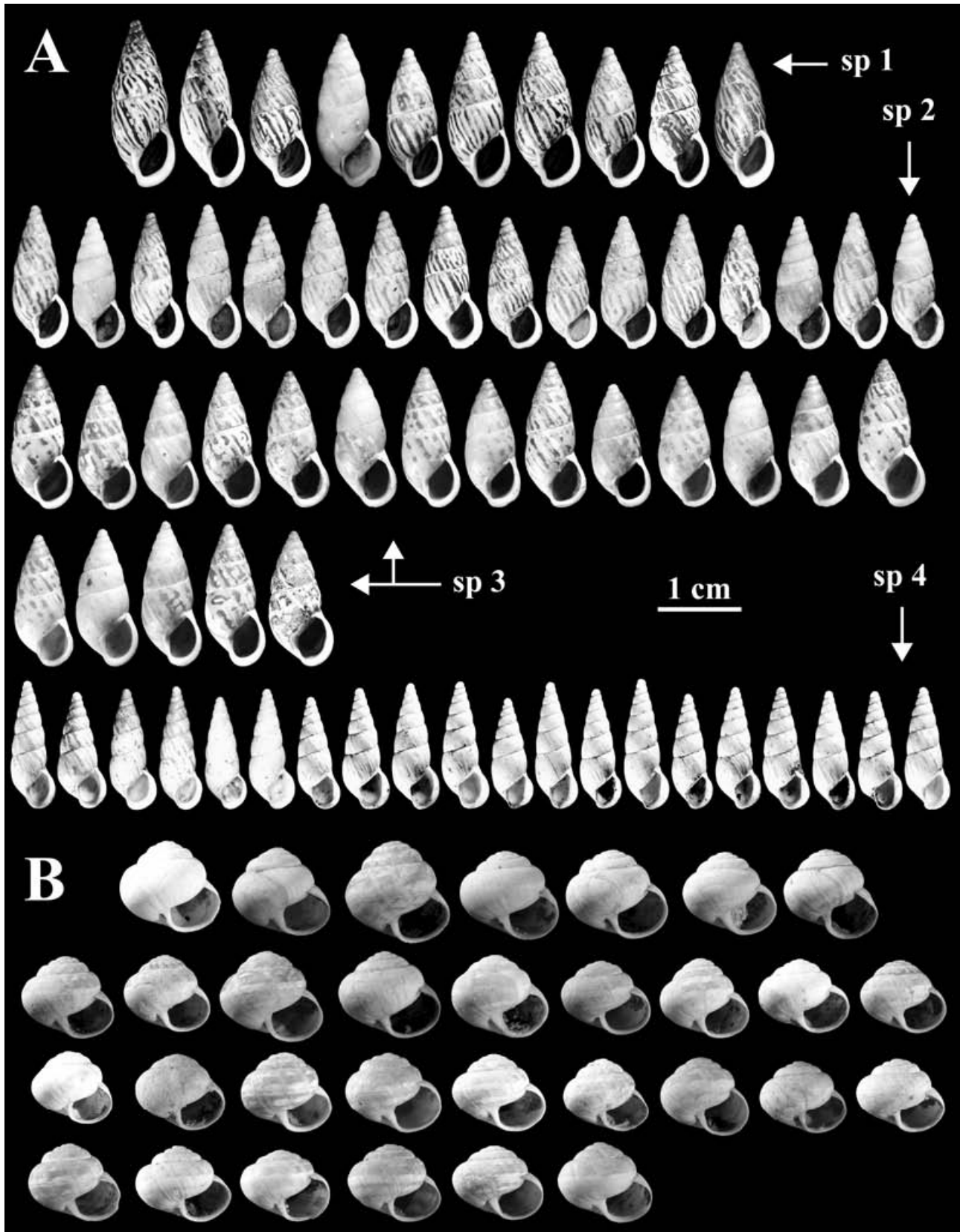


Figure 1 Shells studied. A. The four like-*Napaeus* species (sp1 – sp4). B. *Theba arinagae*.

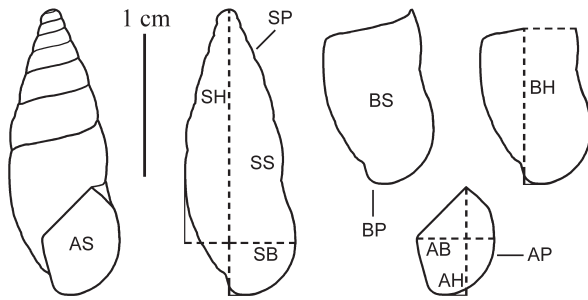


Figure 2 Drawings of an Enidae shell showing the placement of the measurements analyzed.

ters in order to evaluate the quality level of all measurements used and to find those of greatest taxonomic value; and C) to repeat the comparisons of the measurements previously described in the first aim ("A") but on a globular shell snail species, *Theba arinagae* Gittenberger & Ripken, 1987, to find out if the new type of measurement is more widely applicable.

MATERIAL AND METHODS

The shells of 65 specimens belonging to four conchologically similar *-Napaesus-like* species, and 31 specimens of *Theba arinagae*, were photographed with a digital camera (Olympus DP70) coupled to a stereomicroscope (Olympus SZX12). All the shells were oriented with the shell axis (columella) to Y axis of coordinates and the maximum shell breadth accurately in plane view (Fig. 1A). All *-Napaesus-like* specimens used are deposited in the Collection of Department of Animal Biology, La Laguna University, with the exception of the holotypes of the new species, which are in the Museo de Ciencias Naturales de Tenerife (catalogue numbers TFMC MT 0382, 0384 and 0386, respectively). The *Theba arinagae* material is in the K. Groh private collection, Hackenheim, Germany.

The shell measurements have been obtained with the program *analySIS*®, of the Soft Imaging System GmbH (2002). The image preparation and placement of measurements are shown in Fig. 2, the straight linear shell measurements being obtained by the *analySIS*® program as the projections on the X and Y axis of the respective structures. In this study we have not obtained the first whorl measurements, but they are also equally accessible. With this methodology, all the

measurements are easily made and are repeatable.

ABBREVIATIONS:

AB	aperture breadth (mm)
AH	aperture height (mm)
AP	aperture perimeter (mm)
AS	aperture surface (plane view; mm ²)
BH	body whorl height (at columella level; mm)
BP	body whorl perimeter (mm)
BS	body whorl surface (plane view; mm ²)
SB	shell breadth (mm)
SH	shell height (mm)
SP	shell perimeter (mm)
sp1	- The four <i>Napaesus-like</i> species studied
sp4	
SS	shell surface (plane view; mm ²)

A preliminary comparison between the different measurements was made by scatter plots (Fig. 3) to evaluate their relative variability and possible taxonomical importance.

Descriptive statistics and data exploration of the variables (normality and variance homogeneity) were also carried out initially. The correlations between variables were previously calculated in order to have a general overview of their respective relationships and for helping in the interpretation of the obtained results. One-way ANOVA's (with posterior Scheffé tests) were performed to test the statistical significance of differences in means for the eleven characters measured for each of the four species. Data were log-transformed in order to attain the assumptions of normality. A discriminant function analysis (DFA) combined with multivariate analysis of variance (MANOVA), considering all the eleven shell characters, were performed in order to determine which of the measurements contribute to the discrimination between the four *-Napaesus-like* species treated in the present study. Furthermore, three-discriminant analyses were made in order to evaluate the importance of the characters traditionally used in taxonomical studies (SH, SB, AH, AB and BH) against those new ones which relate shell height + breadth, aperture height + breadth and height of body whorl to surface and perimeter, and proposed in this contribution (SS, SP, BS,

Table 1 Data (in mm or mm²) for the shell characters measured and univariate statistical comparison of the eleven shell characters among the four like-*Napaeus* species.

Shell character	Statistical descriptive			ANOVA test		Differences among species
	sp.	mean	sd	<i>F</i>	<i>p</i>	
SH	1	17.92	1.17	42.86	< 0.001	sp1 vs. sp2, sp3, sp4 sp2 vs. sp4 sp3 vs. sp4
	2	16.33	0.76			
	3	16.28	0.87			
	4	14.47	0.65			
SB	1	7.48	0.38	166.42	"	sp1 vs. sp2, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	6.57	0.26			
	3	7.29	0.34			
	4	5.43	0.28			
SS	1	87.14	9.30	164.82	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	68.07	5.17			
	3	76.88	5.68			
	4	48.35	3.50			
SP	1	41.56	2.48	51.06	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp4 sp3 vs. sp4
	2	37.83	1.68			
	3	38.57	1.68			
	4	33.65	1.46			
BH	1	9.76	0.76	135.48	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	8.26	0.50			
	3	9.07	0.44			
	4	6.68	0.33			
BS	1	56.73	6.74	220.38	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	41.46	3.70			
	3	51.10	3.50			
	4	27.90	2.31			
BP	1	30.10	1.79	188.59	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	25.93	1.20			
	3	28.52	1.10			
	4	21.46	0.84			
AH	1	6.85	0.56	100.35	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp4 sp3 vs. sp4
	2	5.92	0.49			
	3	6.16	0.38			
	4	4.57	0.27			
AB	1	4.96	0.28	106.83	"	sp1 vs. sp2, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	4.37	0.19			
	3	4.74	0.26			
	4	3.63	0.23			
AS	1	23.69	3.22	108.62	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	17.54	2.13			
	3	20.42	2.30			
	4	11.73	1.35			
AP	1	18.39	1.27	120.22	"	sp1 vs. sp2, sp3, sp4 sp2 vs. sp3, sp4 sp3 vs. sp4
	2	15.79	0.96			
	3	16.94	0.87			
	4	12.76	0.72			

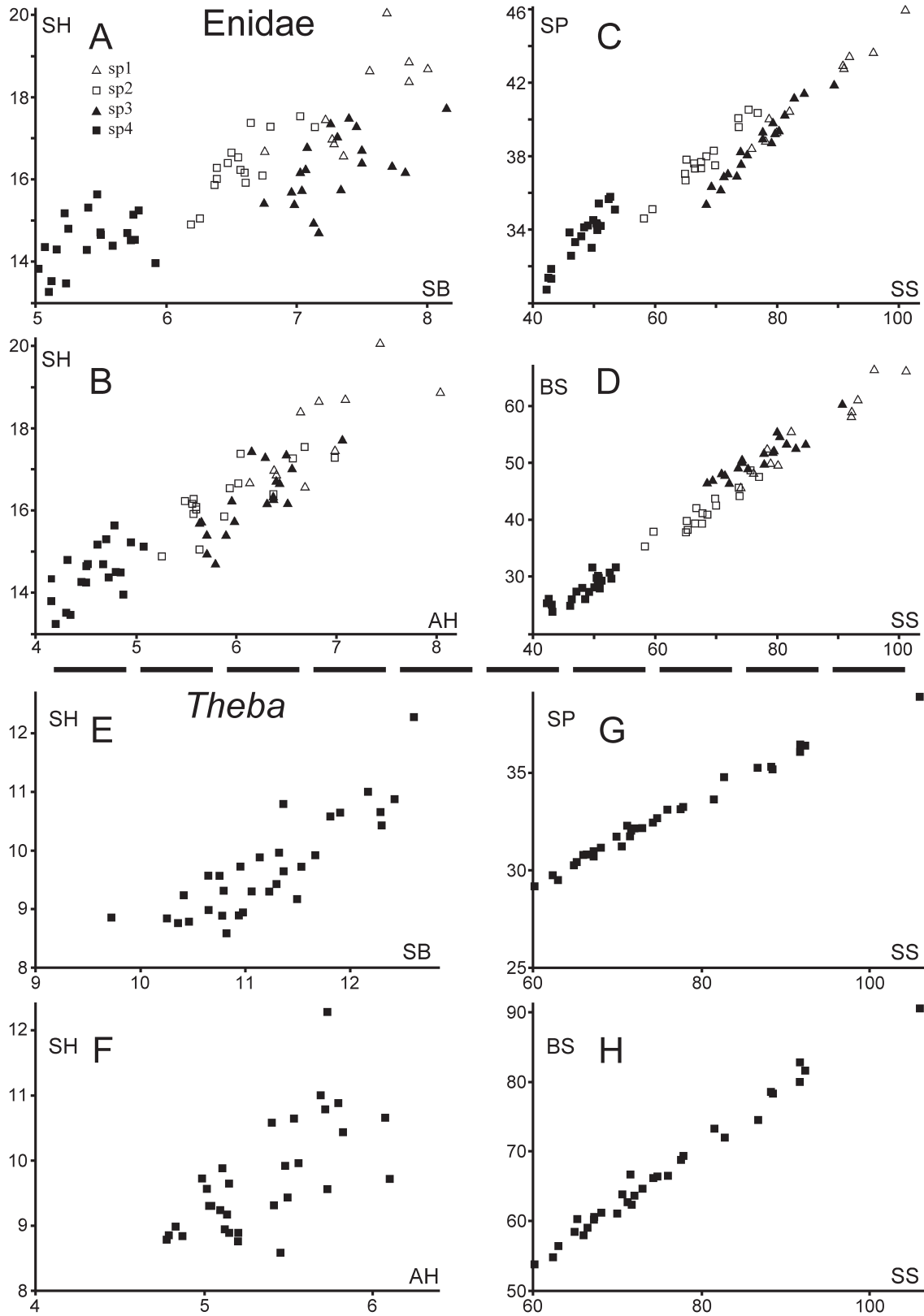


Figure 3 Comparison between the scatter plot of some of the most common shell measurements used in the gastropod bibliography (A, B, E, F) and some of the surface and perimeter measurements (C, D, G, H). A-D, for the four like-*Napaeus* species; E-H, for *Theba arinagae*.

TABLE 2 Canonical structure for discriminant analysis of the four species, considering the eleven shell characters included in the present study. Variables with the highest loadings are indicated in bold face.

Shell character	Canonical variable		
	1st	2nd	3rd
BP	0.497	- 0.022	0.367
BS	0.496	- 0.113	0.441
SB	0.481	- 0.064	0.149
SS	0.436	0.083	0.487
BH	0.416	0.031	0.382
AP	0.395	0.128	0.403
AB	0.389	0.032	0.234
AS	0.349	0.037	0.422
AH	0.345	0.262	0.385
SP	0.257	0.190	0.422
SH	0.224	0.285	0.493

BP, AS and AP). Therefore, these three analyses were performed grouping the same data matrix according to: 1) the measurements of all the characters together, 2) straight linear measurements only, and 3) those measurements related to surface and perimeter. Individual canonical scores of each specimen and centroids of each species were calculated, then plotted in order to predict which species a particular individual belonged (Fig. 4). Statistical analyses were performed using the SPSS statistical package (version 11.0; 2003).

Finally, we repeated the shell measurement process and the preliminary comparison between the different measurements, by scatter plots, for a population of *Theba arinagae* (Fig. 1B).

RESULTS AND DISCUSSION

The preliminary comparison between the different variables studied in the *-Napaesus-*like species (Fig. 3A-D) shows the value of surface and perimeter measurements compared to linear ones which have more variability. The same results are also obtained consistently when a globular snail shell, *Theba arinagae*, is studied (Fig. 3E-H).

Descriptive statistics and univariate statistical comparison for the eleven shell characters of the four *-Napaesus-*like species studied in this

contribution are listed in Table 1. Univariate one-way ANOVA's demonstrates that there are significant variations for all the variables studied ($p < 0.001$). The MANOVA revealed that at least one of the four previously defined species differs significantly from the others (Wilks' $\lambda = 0.005$, $df = 33$, $p < 0.001$). The most important variables differentiating these species are body whorl surface [$F(3,61) = 167.4$, $p < 0.001$], body whorl perimeter [$F(3,61) = 166.0$, $p < 0.001$] and shell breadth [$F(3,61) = 153.2$, $p < 0.001$], although the effects of the other variables were also significant ($p < 0.001$). Significant correlations ($p < 0.001$) were generally found between the different shell dimensions, the strongest ones being: 1) between two of the characters related with the body whorl (body whorl surface and body whorl perimeter: 0.99), 2) between these two characters with respect to another body whorl character (body whorl height: 0.98 for both cases), and shell height versus shell perimeter: 0.98.

Results from the first discriminant analysis (measurements of all characters together) are displayed in Table 2. The first canonical variable accounted for the great majority of the variation between species (92.1%), while the other two canonical variables accounted only 4.7% and 3.2%, respectively. The variables with the highest loading which show a higher absolute correlation with respect to every discriminant function were body whorl perimeter, body whorl surface, shell breadth, shell surface and body whorl height (Table 2). The 1st discriminate function clearly separates three groups: sp2, sp4 and a complex sp1 - sp3, while the 2nd function separates rather well the species sp1 and sp3 (Fig. 4C). Sequential Chi-square test revealed that the 1st discriminate function contributes to the discrimination of the groups to a large extent ($\chi^2 = 296.0$, $df = 33$, $p < 0.001$); the contribution of the 2nd discriminant function is less significant ($\chi^2 = 97.6$, $df = 20$, $p < 0.001$). Structure coefficients (correlations between the original variables and the discriminant functions) showed that body whorl perimeter, body whorl surface, shell breadth, shell surface and body whorl height determine the 1st function and the 2nd is defined by shell height and aperture height (Table 2). These results show that in the 1st function the characters belonging to the last whorl and the entire shell surface stand out while in the 2nd acquire importance some of those related with the height of the shell.

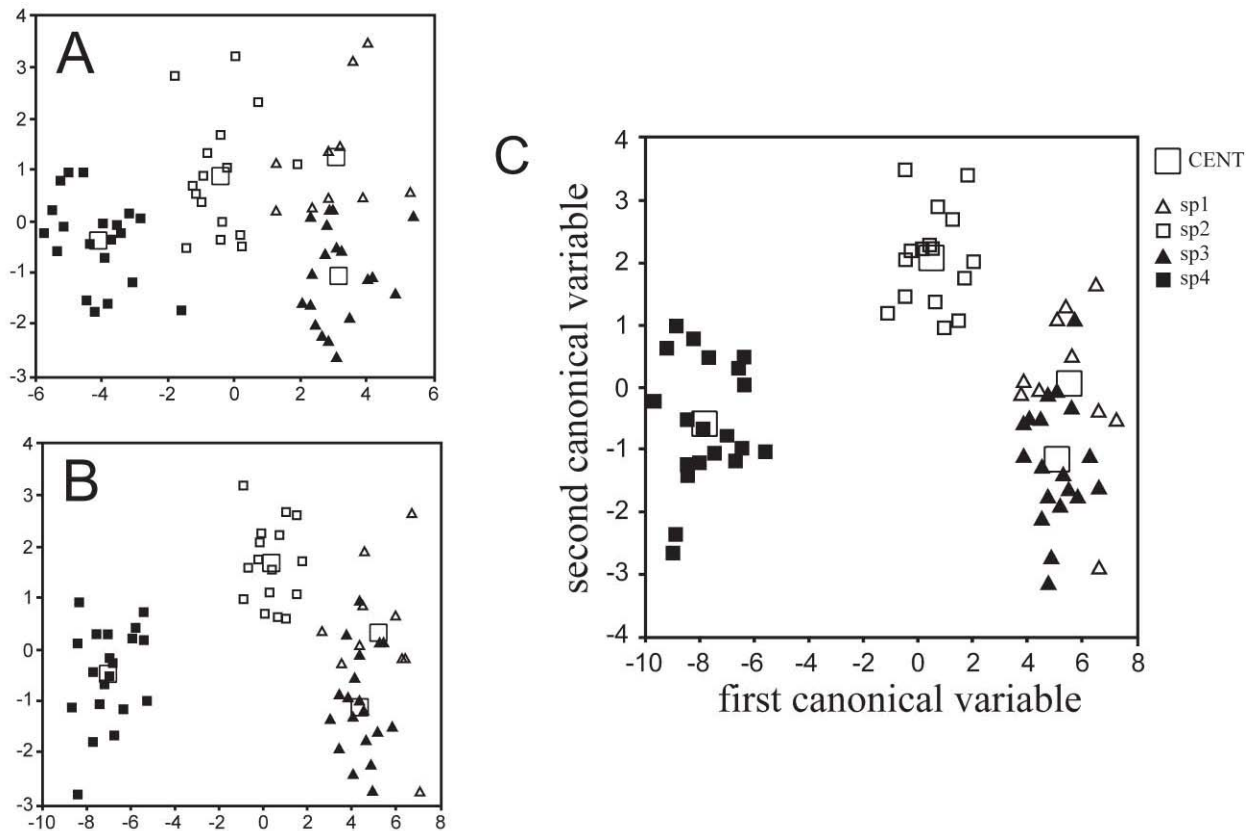


Figure 4 Scatter plot of the first and second canonical variables in the discriminate analyses for the four like-*Napaeus* species included in the present study using: **A**, the more frequently straight linear shell measurements usually used in taxonomical studies (SH, SB, AH, AB and BH); **B**, the shell measurements related to surface and perimeter (SS, SP, BS, BP, AS and AP), and **C**, all the shell measurements studied (the two canonical variables accounted for a total of 96.8% of the total variance).

From the three discriminant analyses performed for the same data set, the best canonical functions were obtained when all the variables were included (Fig. 4C). This analysis indicates a high discrimination among the four species, with 97% of the specimens *a posteriori* correctly identified to each species group. The only two individuals wrongly identified in the scatter plot are interchanged between the species sp1 and sp3.

When a comparison is made between the discriminant functions from the traditional shell measurements only (Fig. 4A) versus those which use shell surface and perimeter (Fig. 4B), it is clear that the latter mentioned new characters separate the species better than the traditional ones, which only differentiate the species slightly.

These results demonstrate that the use of some computer-assisted measurements are useful for discriminating shells of similar species, not only for gastropod shells but also where the straight linear dimensions of the samples have great variability.

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A new genus and species of Enidae (Gastropoda: Pulmonata) from the Quaternary of the Balearic Islands (Western Mediterranean)

CRISTIAN R. ALTABA

Laboratory of Human Systematics, University of the Balearic Islands, 07071 Palma de Mallorca, Illes Balears, Spain.

E-mail: cristianr.altaba@uib.es

Abstract

Balearena gymnesica **gen. nov., spec. nov.**, is described from late Upper Pleistocene shore sediments in Mallorca, Balearic Islands, Spain. The new species may be closely related to enids living in the eastern Mediterranean, North-west Africa, and Macaronesia, but geometric morphometrics shows it differs in having a distinctly slender shape; it also differs in the embryonic whorls with a deep suture and a sculpture consisting of shallow collabral grooves on top and delicate incised spiral lines laterally, a teleoconch sculpture formed only by low, simple, irregular collabral wrinkles, and the aperture with a low columellar fold and a blunt angular tooth. Such affinities point to a vicariant origin for *Balearena* in the formation of the Mediterranean during the Miocene. This species, or closely related forms, are widespread in terrigenous Upper Pleistocene sites in the northern Balearics, but have never been found alive. Its extinction predated the arrival of humans, and may have been coeval with a mass extinction in the southern Balearics putatively caused by a submarine volcanic explosion in the Valencia Trough.

Key words: Mollusca, land snail, Mediterranean, islands, *Balearena gymnesica*, fossil, geometric morphometrics, vicariance, extinction

Introduction

Since the early stages of research on the Quaternary molluscs of Mallorca, an extinct, previously unrecorded land snail was often found in paleosoils, fossil beaches and aeolianites at the top of coastal stratigraphic sequences. This snail was identified (Cuerda 1959) as an elongated form of *Mastus pupa* (Linnaeus, 1758), a species of Enidae that is widespread in southern and eastern Mediterranean countries (Arrébola 1990). This identification, even though suspect from the onset, was never challenged and has been used in postulating biogeographic scenarios (Gasull 1966, 1972; Cuerda 1989; Giusti *et al.* 1995; Vicens *et al.* 2001).

Extensive collecting in numerous fossil sites in the Balearic archipelago during the last 15 years (Paul & Altaba 1992; Altaba 1993, 2003) has yielded well-preserved specimens of this enigmatic snail. In the light of this evidence, it is postulated that the extinct Balearic enid represents an undescribed endemic taxon. The following description of the new genus and species is based on specimens from a single locality and stratum, a well-known site where it was first reported. Many additional specimens have been studied from various localities throughout the northern Balearics, yet the slight variations among sites is not considered here and will be the subject of further research. In the absence of anatomical data, the taxonomic position of the Balearic fossil enid may be assessed only through comparison with shell characters of living taxa, taking as a yardstick the variability within extant genera and species. Such analysis shows a unique combination of traits in the new taxon and supports its recognition as a monotypic genus.

Materials and methods in morphometrics

The diversity of enid landnails is masked by a fairly uniform shell shape –i.e., aside from apertural denticles present in some taxa, conchological differences are far from conspicuous. Thus, the literature is plagued with vague adjectives, under the widespread assumption that enid shells are rather useless as characters for phylogenetic inference, or even for identification. Subtle differences in overall shape can be objectively judged and tested as diagnostic traits through a geometric morphometric analysis (Roth and Mercer 2000). This approach is most appropriate to distinguish

shapes, especially in cases where distance measurements are similar –such as occurs when comparing an oval and a teardrop (Adams *et al.* 2004). Landmarks set at points considered homologous among specimens provide an optimal dataset, and only those hypotheses that can be distinguished on the basis of information available from landmark data should be considered, using the results not as an end by themselves but to understand and further explore the processes working to produce the differences revealed (Richtsmeier *et al.* 2002). Actually, when homologous landmarks can be identified, even if few and sparsely located, geometric morphometrics performs more efficiently than traditional or curve-fitting approaches in distinguishing among closely similar species (Ponton 2006). Indeed, landmark-based methods have proven useful to discriminate among related gastropod species possessing rather featureless shells (Urra *et al.* 2007). Even when applied to simulated coiling shells, landmark analysis successfully recovered the groupings established by dynamic parameters such as whorl expansion (Stone 1998).

A geometric morphometric analysis was performed to compare *Mastus pupa* and the Balearic fossils.

First, outlines of 5 adult shells of each species were drawn from digital photographs taken at a fixed distance, with the coiling axis and the axial aperture plane parallel to the camera plane (Fig. 3). Then landmarks were located on these outlines (Fig. 4) and digitally recorded. The PAST software (Hammer *et al.* 2001, 2007; Hammer & Harper 2005) was used to perform a Procrustes principal component analysis. This involved the following steps. The coordinates of the apex landmark were set to (0,0) and the maximum length of all specimens set to a uniform size; this yielded shape variables resulting from alignment, translation and scaling of landmarks (Procrustes coordinates). These were then transformed to Procrustes residuals through the “subtract mean” option. Finally, a principal component analysis was performed on these residuals.