Description of a new Late Cretaceous species of *Bulimulus* Leach, 1814 (Orthalicoidea: Bulimulidae) using geometric morphometric analysis

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ABSTRACT

In the Late Cretaceous fossil record of Uruguay, three morphotypes of *Bulimulus* (Bulimulidae) have been recorded: *Bulimulus klappenbachi* and two undescribed ones. A geometric morphometric analysis was conducted to show whether the three morphotypes represent one or more species. With this aim, the three fossils were compared with three extant species of the same genus: *Bulimulus bonariensis*, *B. guadalupensis* and *B. rushi*. Variability within and among these three well-known extant species was tested to evaluate differences in the fossil morphotypes. Twenty-eight landmarks were selected (9 permanent landmarks and 19 semi-landmarks) and principal component analysis and canonical variate analysis were performed. Results indicate that only one of the unnamed morphotypes can be described as a new species and that the other is a variant of *B. klappenbachi.* The latter fossil species is described as *Bulimulus frenguellii* n. sp.

INTRODUCTION

Bulimulidae Tryon, 1867, a diverse family of terrestrial stylommatophoran gastropods of the superfamily Orthalicoidea Albers, 1860 (Breure, Groenenberg & Schilthuizen, 2010; Breure & Romero, 2012), comprises several hundred species that are distributed all over the Neotropical region, in tropical and subtropical environments, with few taxa being found in South Africa, Australia, New Zealand and on some Australasian islands (Stanisic & Solem, 1998; Herbert, 2007; Neubert, Chérel-Mora & Bouchet, 2009; Delsaerdt, 2010; Breure & Romero, 2012). Bulimulids have been extensively studied because of their great capacity for adaptation and therefore colonization of new areas (Coppois & Glowacki, 1983). This characteristic allows them to occupy a wide range of ecological niches that show significant variation with respect to vegetation type, humidity and temperature. Many species within this genus can be found in island groups of volcanic origin (i.e. islands that have never been connected to the continent), such as the Galapagos or Lesser Antilles (Breure, 1974; Coppois & Glowacki, 1983; Parent & Crespi, 2006). The bulimulid fauna of these islands exhibits high species richness (possibly due to adaptive radiation) and high intraspecific variability, and it has been the focus of many studies on island diversification, speciation and morphological variation (Parent & Crespi, 2009; Parent, 2012; Triantis *et al.*, 2016; Kraemer *et al.*, 2019). The wide variation in morphology, which is evident not only in the bulimulids that inhabit islands but also in continental species (Miquel, 1991; Cuezzo, Miranda & Ovando, 2013), makes identification of *Bulimulus* species purely on the basis of the shell a difficult task. Where possible, extant species are identified by examination of the soft parts, such as the reproductive organs, or by DNA analysis (Breure, 1978, 1979, 2016). However, these analyses cannot be undertaken on fossils, since no soft parts are preserved, nor DNA available for extraction. Moreover, most of the time, fossils shells are altered chemically or physically by taphonomic processes.

In South America, the following fossil *Bulimulus* species are found: *Bulimulus klappenbachi* (Parodiz, 1969) (Late Cretaceous of Uruguay) (Fig. 1A, B; Frenguelli, 1930; Morton & Herbst, 1993; Martínez *et al.*, 1996; Martínez, Veroslavsky & Verde, 2001; Cabrera, 2015; Cabrera, Martínez & Verde, 2018; Salvador *et al.*, 2018); *B. fazendicus* (Maury, 1935) and *B. trindadeae* (Ferreira & Coelho, 1971) (both Early Palaeocene of Brazil) (Rodrigues & Da Fonseca, 2007; Salvador & Simone, 2013, 2014; Salvador *et al.*, 2018); two unidentified species from the Upper Cretaceous and Eocene of Argentina; and *Paleobulimulus eocenicus* (Parodiz, 1949) (Middle Eocene–Oligocene of Argentina) (Miquel, 1995; Melchor, Genise & Miquel, 2002; Miquel & Bellosi, 2010; Salvador *et al.*, 2018). Other reports of *Bulimulus* from the Neogene and Quaternary of Argentina, Brazil and Uruguay are assigned to extant species, such as *B. bonariensis* (Rafinesque, 1833) and *B. rushi* (Pilsbry in Pilsbry & Rush, 1896) (Simone & Mezzalira, 1994; Martínez & Rojas, 2004; Utida, 2009; Miquel & Aguirre, 2011).

The bulimulids from the Upper Cretaceous in Uruguay represent three different morphotypes, only one of which corresponds to the original description of *Lymnaea klappenbachi* (Fig. 1A, B) by

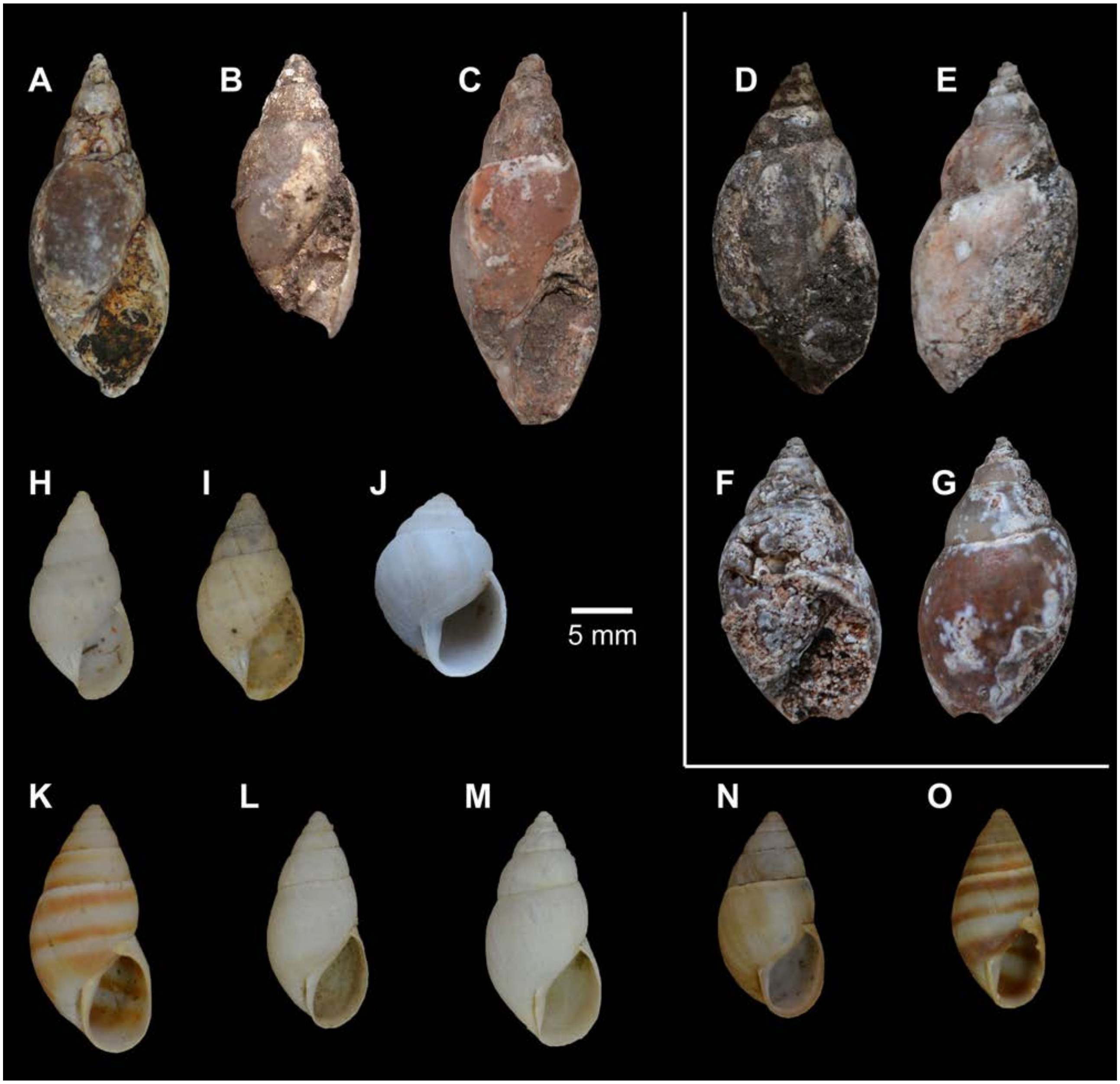

Figure 1. A, B. *Bulimulus klappenbachi* (Parodiz, 1969). **A.** A specimen from Quebracho Ville, Paysandú, Uruguay **(**FCDPI 6459). **B.** Holotype (CM 103840). **C.** *Bulimulus* sp. 1 (FCDPI 8256). **D–G** *Bulimulus frenguellii* n. sp. **D, E.** Holotype (FCDPI 8259; apertural and posterior views). **F, G.** Paratype (FCDPI 8260; apertural and posterior views). **H–O.** Extant *Bulimulus* species. **H.** *Bulimulus bonariensis* from Dolores (FCCI 213). **I.** *Bulimulus bonariensis* from Mercedes (FCCI 214). **J.** *Bulimulus rushi* (FCCI 222). **K–O.** *Bulimulus guadalupensis*. **K.** A specimen from Anguilla (RMNH.MOL.265443). **L.** A specimen from Friars Hill, Antigua (RMNH.MOL.265322). **M.** A specimen from Magueyes Island, Parguera, Puerto Rico (RMNH.MOL.265325). **N.** A specimen from St Lucia, Troumassa State, Uruguay (RMNH.MOL.265447). **O.** A specimen from Fort Willem ruins, St Martin, West Indies (RMNH.MOL.265456).

Parodiz (1969). Subsequent authors classified these fossils in *Bulimulus* (Frenguelli, 1930; Morton & Herbst, 1993), *Lymnaea* (Martínez *et al*., 1996, 2001), *Peronaeus* (Morton & Herbst, 1993) or *Physa* (Alonso-Zarza, Genise & Verde, 2011). Recently, Cabrera (2015) and Cabrera *et al.* (2018), after comparative study with specimens from several scientific collections (Museo Nacional de Historia Natural, Uruguay, Centro de Estudios Aplicados del Litoral, Argentina, and Carnegie Museum, USA), confirmed all of this material as *Bulimulus*.

Given that the three fossil morphotypes are morphologically similar (Fig. 1A–G) and most of the available specimens are preserved as silicified inner casts, a traditional delimitation of the species is not possible. Therefore, a geometric morphometric analysis was conducted to describe their morphology in detail and to evaluate whether the three morphotypes belong to the same morphologically variable species (Minton, Norwood & Hayes, 2008; Cruz, Pante & Rohlf, 2012; Smith & Hendricks, 2013).

MATERIAL AND METHODS

Geological and paleontological setting

The Queguay limestones crop out in southern Uruguay in the Santa Lucía Basin, and along the northern coast of Uruguay River in the North Basin. In fact, this group of limestone units can

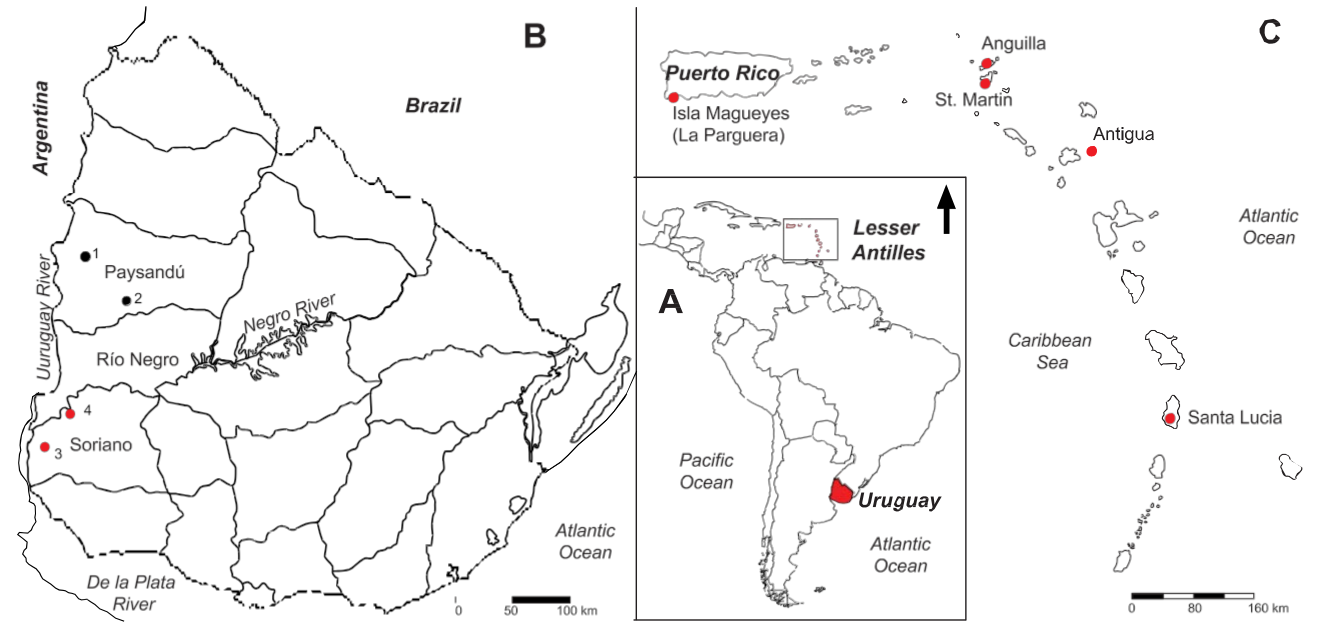

Figure 2. Map showing the localities from which the specimens studied were collected. **A.** Location of study area (red shading) in South America and the Caribbean. **B.** Localities in Uruguay: 1, Quebracho, Paysandú; 2, Piedras Coloradas, Paysandú; 3, near Algorta Town, Río Negro; 4, near Trinidad City, Flores; 5, Palmitas Town, Soriano; 6, Mercedes, Soriano; and 7, Dolores, Soriano. **C.** Localities in the Lesser Antilles. In **B** and **C**, black dots indicate fossil species and red dots denote extant species.

be found in three different Upper Cretaceous formations, namely Mercedes, Asencio and Queguay (Veroslavsky *et al.*, 2019). The southern limestones correspond to thick groundwater calcrete beds with variable amounts of carbonate and a second, upper level of fossiliferous calcareous palaeosols of varying thicknesses. In northern Uruguay, the carbonate facies vary in character, including calcareous and fossiliferous palaeosols (like those mentioned above), calcareous lenses of groundwater calcretes, palustrine calcretes with rhizomes, and secondarily silicified zones (silcretes) (Martínez & Veroslavsky, 2004; Tófalo & Morrás, 2009; Alonso-Zarza *et al.*, 2011; Veroslavsky *et al.*, 2019).

The fossil content is mainly composed of terrestrial and freshwater gastropods, and ichnofossils (hymenopterans nests and coleopteran pupal chambers). The terrestrial gastropods include *Bulimulus*, *Bahiensis*, *Eoborus*, *Pupoides*, *Succinea* and Clausiliidae, whereas the freshwater taxa comprise *Biomphalaria* and *Physa*. The non-molluscan fossils include freshwater characean gyrogonites and ostracods and terrestrial fossils, such as *Celtis* endocarps, rhizoliths and dinosaur eggshells (Neosauropoda) (Martínez *et al.*, 2001; Alonso-Zarza *et al.*, 2011; Verde, 2012; Cabrera *et al.*, 2018). The co-occurrence of arid-associated biotic elements, such as hymenopteran nests and coleopteran pupal chambers (Genise *et al.*, 2010; Alonso-Zarza *et al.*, 2011), along with the occurrence of freshwater taxa (mostly those associated with temporary water bodies; e.g. gastropods, characean gyrogonites and ostracods), indicates an alternation of arid and highly humid conditions in a short span of time (see Cabrera *et al.*, 2018; Veroslavsky *et al.*, 2019).

Specimens used for analyses

Completely intact specimens of the three fossil morphotypes of *Bulimulus* were used for the geometric morphometric analysis. All material derives from the Paleontological Collection of the Facultad de Ciencias, Montevideo, Uruguay (collection nos FCDPI 6454, 6455, 6456, 6459, 6460 and 6461). Extant specimens of *Bulimulus bonariensis* and *B. rushi* are stored at the Invertebrate Comparative Collection of the Department of Palaeontology, Facultad de Ciencias, Montevideo (collection nos FCCI 213 and 214 for *B. bonariensis*, and FCCI 222 for *B. rushi*). The two populations of *B. bonariensis* are referred to as '*B. bonariensis* Dolores' and '*B. bonariensis* Mercedes', following the localities where they were collected (Fig. 2). The specimens of *B. guadalupensis* examined belong to the Naturalis Biodiversity Center, Leiden, The Netherlands, and were collected from five localities in the West Indies, Lesser Antilles. The localities and associated registration nos were as follows: Troumassa Estate, Saint Lucia (RMNH.MOL.265447); Friars Hill, Antigua (RMNH.MOL.265322); Parguera, Isla Magueyes, Puerto Rico (RMNH.MOL.265325); Anguilla (no further details; RMNH.MOL.265443); and Fort Willem ruins, St Martin (RMNH.MOL.265456). The analysis was performed with 281 adult shells (Table 1). Other examples of the fossil morphotypes were used only for the systematic analysis and were as follows: FCDPI 2339, 4664, 4670, 6473, 7211, 7213, 7221, 7222, 7243, 7244, 7246, 7248, 7251; PZ CTES 5341, 5342, 5343 (Paleontological Collection, Universidad del Nordeste, Corrientes, Argentina); MNHN 5286 (Museo Nacional de Historia Natural, Montevideo, Uruguay); CM 103839 (holotype of *B. klappenbachi*, Molluscan Collection, Carnegie Museum, Carnegie, Pittsburgh, PA, USA) (Fig. 1B).

Geometric morphometrics and statistical analyses

To perform the geometric morphometric analysis, intact specimens assigned to the three fossil morphotypes were compared with three extant species of *Bulimulus*: *B. bonariensis* (Rafinesque, 1833), *B. rushi* (Pilsbry in Pilsbry & Rush, 1896) and *B. guadalupensis* (Bruguière, 1789) (Fig. 1H–O). The extant species were selected to test the interspecific similarities/dissimilarities of well-known *Bulimulus* species, particularly in the highly variable taxon *B. guadalupensis* (Breure, 1974). To explore the intraspecific variability, two alternatives hypotheses were tested. For the first hypothesis, shells from known localities of *B. bonariensis* and *B. guadalupensis* were selected (Figs 1H–O, 2), with the aim of evaluating whether there are morphological differences among localities for individuals of the same species. The second hypothesis involved picking a pool of shells from random localities of *B. rushi*, in order to emulate the time

Table 1. Specimens of *Bulimulus* used for the geometric morphometric analysis.

Species	Number of specimens	Locality	Collection number
B. klappenbachi	26	Quebracho Ville, Paysandú, Uruguay	FCDP-I 6454, 6459
Bulimulus sp. 1	23	Quebracho Ville, Paysandú, Uruguay	FCDP-I 6456, 6461
B. frenguellii n. sp.	13	Quebracho Ville, Paysandú, Uruguay	FCDP-I 6455, 6460
B. rushi	21	Several locations in northern Uruguay	FCCI 222
B. bonariensis Dolores	18	Near Dolores City, Soriano, Uruguay	FCCI 213
B. bonariensis Mercedes	30	Near Mercedes City, Soriano, Uruguay	FCCI 214
B. guadalupensis Antigua	30	Friars Hill, Antigua, Lesser Antilles	RMNH.MOL.265322
B. guadalupensis Magueyes	30	Magueyes Island, Parguera, Puerto Rico	RMNH.MOL.265325
B. guadalupensis Anguilla	30	Anguilla, West Indies, Lesser Antilles	RMNH.MOL.265443
B. guadalupensis St Lucia	30	Troumassa Estate, Santa Lucía, Lesser Antilles	RMNH.MOL.265447
B. guadalupensis St Martin	30	Fort Willem ruins, St Martin, Lesser Antilles	RMNH.MOL.265456

averaging (mixing of populations of different age) that is present in most fossil assemblages (Walker & Bambach, 1971; Kidwell & Bosence, 1991; Kowalewski, 2014).

All the specimens were photographed in apertural view. Twenty-eight landmarks were selected of which 9 were permanent (type I and type II) landmarks and 19 semi-landmarks. The spire of the shell was delimited by five landmarks: one for the apex and four for the sutures on the penultimate whorl (usually the fourth whorl), since this whorl had observable differences in one of the morphotypes (landmarks 2–5).

The most obvious difference among all the species is the relative size and shape of the last whorl, but this structure has only three type I landmarks (a mathematical point whose claimed homology from case to case is supported by the strongest evidence) and one type II landmark (a mathematical point whose claimed homology from case to case is supported only by geometric evidence) (Viscosi & Cardini, 2011; Cardini & Loy, 2013; Cooke & Terhune, 2015). Therefore, to accurately quantify the shape of the shell, several semi-landmarks (type III landmarks) were positioned on the curves delimiting the contour of the last whorl and the aperture. A consensus configuration was obtained using a generalized Procrustes analysis (GPA) in order to align the landmarks and to remove differences in the position, orientation and scaling of the specimens, and to relax the distribution of the semi-landmarks. In addition, the centroid size of each specimen was obtained for further analysis (Zelditch *et al*., 2004; Cardini & Loy, 2013). Landmark setting and consensus were done using the tps software family: tpsdig v. 2.0, tpsutil v. 1.64 and tpsrelw v. 1.6 (Rohlf, 2015).

Since our aim was to study shape variation in *Bulimulus* species independently of size (using centroid size), a regression analysis was performed to discard any allometric effect. When allometry was detected, the remaining analyses were performed using the residuals of the regression instead of the raw aligned data. A principal component analysis (PCA), based on a Euclidean distance matrix, was performed in order to observe variation between the individuals without taking into account the group to which they belong. Following this, canonical variate analysis (CVA) with a permutation test (Brockhoff, 2000; Monrozier & Danzart, 2001; Peltier, Visalli & Schlich, 2015) was performed, the goal being to compare individuals between species groups. To test whether there were no significant differences between *Bulimulus* species, a one-way permutation multivariate analysis of variance (PERMANOVA) test based on Euclidean distances and 9,999 permutations (Anderson, 2001; Legendre & Legendre, 2012) was calculated. If significant differences were found between *Bulimulus* species, pairwise PERMANOVAs between all pairs of groups were used as post-hoc tests (Bonferroni-corrected). For the regression analysis, PCA and CVA, we used the software MorphoJ v. 1.07a (Klingenberg, 2011). For one-way PERMANOVA, PAST v. 4.03 was used (Hammer & Harper, 2001).

RESULTS

The regression analysis indicates an allometric effect in shape variation (5.0991% predicted, $P < 0.0001$). The values of permutation tests for the GPA and CVA (Mahalanobis distances) were highly significant ($P < 0.0001$). A consensus configuration was obtained from the results of the GPA (Fig. 3A). For the PCA (Fig. 3B), the first two axes explained 80.4% of the total variability (PC1 = 48.4% and PC2 = 32.0%). The shape variability along PC1 shows a transition from short shells with elongated aperture and short, wide spire (negative values) to elongated shells with prominent spire and somewhat rounded aperture (positive values). Thin-plate splines for PC2 show wide, rounded shell, with a rounded aperture (positive values) and thin elongated shells with an elongated aperture (negative values). In the PCA plot, the three fossil species are positioned in the part with negative values for both PCs; *Bulimulus klappenbachi* and *Bulimulus* sp. 1 are more or less clustered together, with *B. frenguellii* n. sp. on its own but close to *B. klappenbachi*. The distributions of *Bulimulus bonariensis* from Dolores and Mercedes partly overlap each other and that of *B. guadalupensis*; all the *B. guadalupensis* specimens are tightly clustered. *Bulimulus rushi* is isolated from the other species.

The CVA (Fig. 3C) results show that the first two axes explain 80.8% of the total variability (CV1 = 57.4% and CV2 = 23.4%). We calculated Mahalanobis distances for all pairs of *Bulimulus* groups studied (Table 2) and found that all species are significantly different from each other ($P < 0.0001$ in all cases). The permutation tests for Procrustes distances among groups show a similar result (Table 3; $P < 0.0001$ in all cases). Both Mahalanobis and Procrustes distances indicate that there are significant intraspecific differences between *B. bonariensis* and *B. guadalupensis*. Along CV1, thin-plate splines with negative values indicate elongated shells with a wide spiral and semi-rounded aperture, while positive values correspond to compressed shells with short compressed spires and elongately oval apertures. For CV2, positive values were associated with wide, compressed shells with a compressed spire and wide aperture, while negative values were associated with slender elongated shells with an elongated spire and elongated aperture.

The CVA plot shows three distinct groups. One group is large and is composed of the two samples of *B. bonariensis* and the five samples of *B. guadalupensis*. The latter are associated mostly with negatives values of both CV1 and CV2, and can be subdivided into

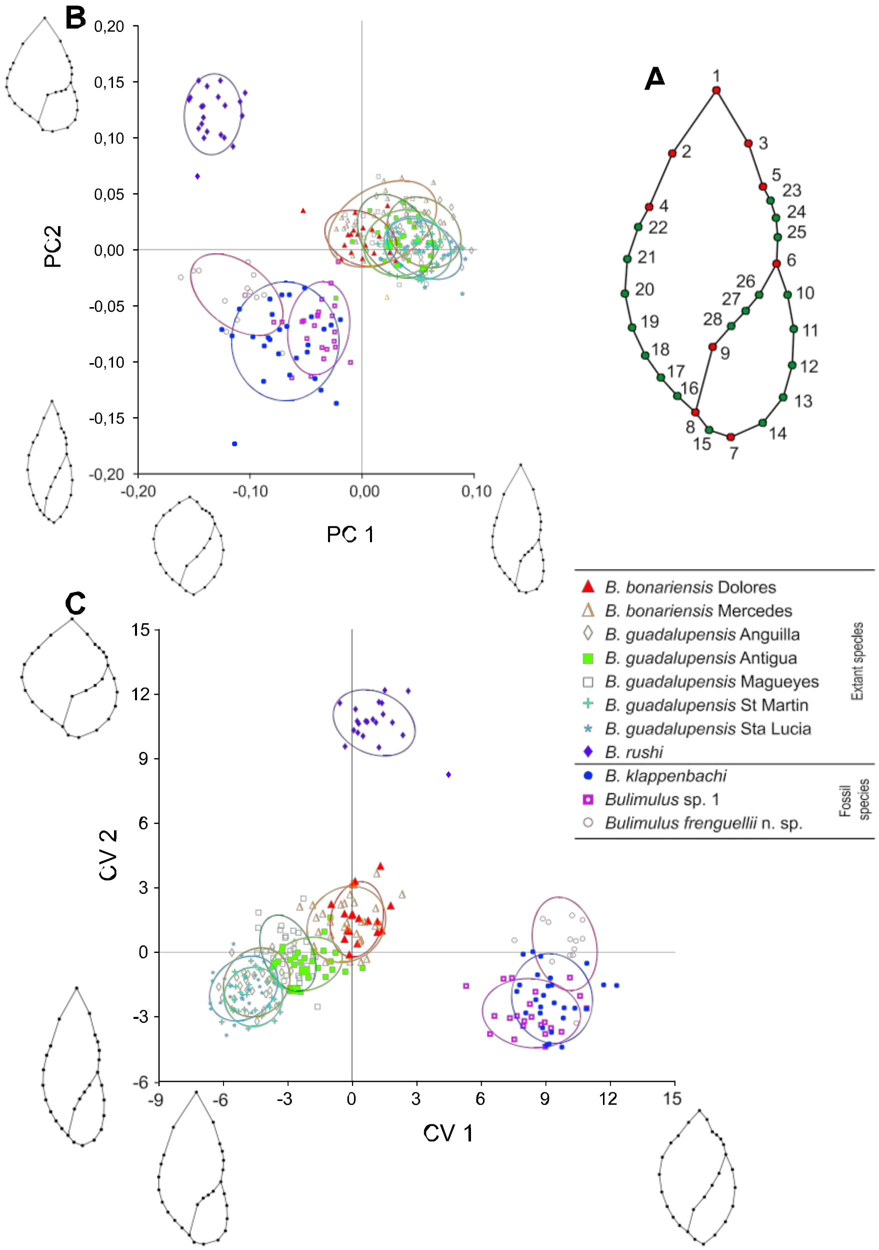

Figure 3. **A.** Consensus configuration from the GPA showing the 28 landmarks. Permanent landmark types I and II are shown in red (landmarks 1–9) and semi-landmarks are shown in green (landmarks 10–28). **B.** PCA plot for showing PC1 and PC2 with the thin-plate spline configuration for the negative and positive values of each of the PCs. **C.** CVA plot showing CV1 and CV2, with the thin-plate spline configuration for the negative and positive values of each of the CVs.

two groups, one containing samples from Antigua and Magueyes, and the other with samples from Anguilla, St Martin and St Lucia. The second group is composed only of *B. rushi*. The third group consists of the three fossil morphotypes.

The one-way PERMANOVA indicates that interspecific differences in shell shape are significant ($P = 0.0001$). Bonferroni-corrected post-hoc tests (Table 4) show that the only nonsignificant differences were between samples of *B. bonariensis* ($P = 0.8195$), and between *B. guadalupensis* from Anguilla and St Lucia ($P = 0.0605$), from Anguilla and St Martin ($P = 0.8635$) and from St Lucia and St Martin ($P = 0.561$).

SYSTEMATIC DESCRIPTIONS

Superfamily ORTHALICOIDEA Albers, 1860
Family BULIMULIDAE Tryon, 1867
Subfamily BULIMULINAE Tryon, 1867

Genus *Bulimulus* Leach, 1814

Type species: *Bulimulus trifasciatus* Leach, 1814 (= *Bulimus guadalupensis* Bruguière, 1789) (by subsequent designation, Pilsbry (1896: 125)).

Table 2. Mahalanobis distances among groups for all *Bulimulus* species.

	1. *B. bonariensis* Dolores	2. *B. bonariensis* Mercedes	3. *B. guadalupensis* Anguilla	4. *B. guadalupensis* Antigua	5. *B. guadalupensis* Magueyes	6. *B. guadalupensis* St Martin	7. *B. guadalupensis* St Lucia	8. *B. klappenbachi*	9. *B. rushi*	10. *Bulimulus* sp. 1
2. *B. bonariensis* Mercedes	2.5639									
3. *B. guadalupensis* Anguilla	7.7055	7.1512								
4. *B. guadalupensis* Antigua	4.7826	4.4729	4.1097							
5. *B. guadalupensis* Magueyes	6.9072	6.4449	4.0168	4.4329						
6. *B. guadalupensis* St Martin	8.6008	8.0285	2.7593	4.9097	4.9638					
7. *B. guadalupensis* St Lucia	8.5035	8.0292	3.5806	5.1330	5.3967	3.2580				
8. *B. klappenbachi*	11.2788	11.5021	14.0294	12.1208	13.0957	14.3015	14.7876			
9. *B. rushi*	11.2702	11.4008	13.4696	12.3587	12.1185	14.0801	14.0337	15.5570		
10. *Bulimulus* sp. 1	10.8479	11.0715	13.3892	11.4599	12.4849	13.5181	14.0603	5.4467	15.7241	
11. *B. frenguellii* n. sp.	11.9683	12.2024	15.1813	13.2067	13.9074	15.2813	15.6666	6.1892	14.3638	7.0428

Table 3. Procrustes distances among groups for all *Bulimulus* species.

	1. *B. bonariensis* Dolores	2. *B. bonariensis* Mercedes	3. *B. guadalupensis* Anguilla	4. *B. guadalupensis* Antigua	5. *B. guadalupensis* Magueyes	6. *B. guadalupensis* St Martin	7. *B. guadalupensis* St Lucia	8. *B. klappenbachi*	9. *B. rushi*	10. *Bulimulus* sp. 1
2. *B. bonariensis* Mercedes	0.0267									
3. *B. guadalupensis* Anguilla	0.0645	0.0474								
4. *B. guadalupensis* Antigua	0.0422	0.0315	0.0293							
5. *B. guadalupensis* Magueyes	0.0470	0.0386	0.0366	0.0288						
6. *B. guadalupensis* St Martin	0.0629	0.0511	0.0188	0.0295	0.0346					
7. *B. guadalupensis* St Lucia	0.0663	0.0521	0.0236	0.0324	0.0368	0.0179				
8. *B. klappenbachi*	0.1143	0.1356	0.1567	0.1361	0.1361	0.1449	0.1483			
9. *B. rushi*	0.1754	0.1833	0.2170	0.2054	0.1965	0.2188	0.2240	0.2101		
10. *Bulimulus* sp. 1	0.0938	0.1143	0.1313	0.1103	0.1137	0.1191	0.1238	0.0406	0.2151	
11. *B. frenguellii* n. sp.	0.1234	0.1453	0.1748	0.1547	0.1508	0.1655	0.1705	0.0578	0.1658	0.0821

Table 4. Bonferroni-corrected *P*-values for one-way PERMANOVA.

	B. klappenbachi	*B. rushi*	*Bulimulus* sp. 1	*B. frenguellii* n. sp.	*B. bonariensis* A	*B. bonariensis* B	*B. guadalupensis* Antigua	*B. guadalupensis* Magueyes	*B. guadalupensis* Anguilla	*B. guadalupensis* St Lucia
B. klappenbachi										
B. rushi	**0.0055**									
Bulimulus sp. 1	**0.0055**	**0.0055**								
B. frenguellii n. sp.	**0.0055**	**0.0055**	**0.0055**							
B. bonariensis A	**0.0055**	**0.0055**	**0.0055**	**0.0055**						
B. bonariensis B	**0.0055**	**0.0055**	**0.0055**	**0.0055**	0.8195					
B. guadalupensis Antigua	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0165**				
B. guadalupensis Magueyes	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**			
B. guadalupensis Anguilla	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**		
B. guadalupensis St Lucia	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	0.0605	
B. quadalupensis St Martin	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	**0.0055**	0.8635	0.561

Significant differences ($P < 0.05$) are shown in bold font.

Bulimulus klappenbachi **(**Parodiz, 1969**)**

(Fig. 1A–C)

Lymnaea klappenbachi Parodiz, 1969: 163, pl. 11, fig. 5 (Mercedes Formation, near Palmitas Town, Soriano Department, Uruguay; Upper Cretaceous; holotype CM 103839).

Bulimulus aff. *sporadicus* Morton & Herbst, 1993: 448, pl. 1, figs 1, 2.

Bulumulus aff. *Bulimulus gorritiensis* Morton & Herbst, 1993: 450, pl. 1, fig. 11.

Other material examined: 288 specimens from four localities in Uruguay: near Palmitas Town, Soriano Department; near Trinidad City, Flores Department; route 25, near Algorta Town, Río Negro Department; and Quebracho and Piedras Coloradas, Paysandú Department (FCDPI 2339, 4664, 6454, 6459, 6473, 7211, 7221, 7243, 7244, 7246, 7248, 7251; PZ-CTES 5342; MNHN 5286).

Distribution: Upper Cretaceous of Queguay, Mercedes and Asencio Formations, Uruguay: near Palmitas Town, Soriano Department; near Trinidad City, Flores Department; route 25, near Algorta Town, Río Negro Department; and Quebracho and Piedras Coloradas, Paysandú Department (Fig. 2).

Original description from Parodiz (1969: 163): "The shell was metamorphosed into chalcedony, well preserved except for the basal portion of the lip, which appears to have been twisted to the left (a feature common in *Stagnicola*). The lip is sharply expanded toward the front at the superior angle, where it is very lightly detached from the body whorl. The spire is about one-third of the length of the last whorl and is slender. Last whorl rounded at the base. Columella with a weak twist at the middle. L. 22 mm., d. 10 mm., aperture 11 mm. × 14 mm. Complete, with the lip entire, it must have been 24 mm. long and the last whorl 16 mm."

Revised description: Medium-sized, oval, dextral shell; elongated, conical spire with six slightly convex whorls, the last one slender and barely expanded and two-thirds of total shell length; clearly defined, slightly oblique suture; elongate-ovate, narrow aperture with unexpanded lip. A variant morphotype (*Bulimulus* sp. 1 in morphological analysis) has penultimate whorl larger, more convex than preceding spire and accounts for 50% of spire length.

Bulimulus frenguellii **new species**

(Fig. 1D–G)

?*Peronaeus* sp.—Morton & Herbst, 1993: 450, pl. 1, figs 3, 4.

Type material: Holotype (shell height 24.7 mm, shell width 13.8 mm, height of last whorl 19.3 mm, aperture height 14.45 mm, aperture width 8.05 mm), Quebracho, Paysandú Department, Uruguay 31°55.226 S, 57°54.360 W. Queguay Formation, Upper Cretaceous, FCDPI 8259. Paratype, same locality as holotype, FCDPI 8260.

Other material examined: 35 specimens (FCDPI 4670, 6455, 6460, 8261; PZ-CTES 5343).

ZooBank registration: F7501E38-D18A-48A9-B144-F26792949833.

Etymology: After Joaquim Frenguelli, an Italian–Argentinean naturalist and medical doctor, who was born in 1883 at Rome, Italy, and died in 1958 at Santa Fe, Argentina. He was the first worker to suggest that some gastropods from the Queguay Formation were in fact bulimulids (Frenguelli, 1930).

Distribution: Upper Cretaceous of Queguay and Asencio Formations, Uruguay: Quebracho and Piedras Coloradas, Paysandú Department (Fig. 2).

Diagnosis: Medium-sized, elongately ovate, nonumbilicate shell with five convex whorls. Thin, elongately ovate aperture, devoid of teeth and with simple peristome.

Description: Medium-sized shell (mean length *c.* 23 mm; $n = 15$ complete specimens). Smooth, elongately ovate, nonumbilicate shell with five convex whorls. Suture well defined; barely visible growth lines. Spire short; last whorl accounts 80% of shell height. Elongate-ovate aperture without internal barriers and with simple peristome.

Remarks: The shell of *B. frenguellii* n. sp. is similar to *B. klappenbachi* in size and the shape of the spire. However, the shell as well as the last whorl and aperture are more ovate in the former. *Bulimulus frenguellii* n. sp. differs from *B. bonariensis* and *B. guadalupensis* in the shape of the shell. *Bulimulus frenguellii* n. sp. has an elongately ovate shell and *B. bonariensis* has an elongate shell. Whereas the shell whorls are distinctly convex in *B. frenguellii* n. sp., they are only slightly convex in *B. bonariensis*. *Bulimulus frenguellii* n. sp. can be distinguished from *B. rushi* by shell size, the latter being slightly smaller. Also, the spire in *B. rushi* is a bit longer than that in *B. frenguellii* n. sp.; it represents 30% of shell height in the former and 20% in the latter.

DISCUSSION

Among the extant species, the samples of *Bulimulus rushi* are well differentiated from the other species in both multivariate analyses and the spread of points, overall, in both plots is similar; the separation of this species from the others was expected, since it has the most distinct shell (Fig. 1J). Examining the data for *B. bonariensis*, both analyses do not have any difficulties in recognizing both samples as the same species. Although the specimens of *B. bonariensis* from Mercedes are more variable than the specimens from Dolores, both analyses unambiguously recognize both samples as the same species. *Bulimulus guadalupensis* is traditionally considered as a hypervariable species (Breure, 1974, 2016). However, despite the fact that it has a relatively wide spread in both plots, all the specimens of *B. guadalupensis* are clustered together. In the CVA, the localities Anguilla, St Martin and St Lucia cluster together in the plot with the specimens from Antigua and Magueyes. In the PCA, *B. bonariensis* and *B. guadalupensis* cluster together and could not be differentiated from each other. However, in the CVA only a few specimens of *B. guadalupensis* cluster with *B. bonariensis*. These two species are very similar in shell shape (Fig. 1H, I, K, O). The main differences are in the shape of the aperture (more oblong in *B. bonariensis*) and the overall shape of the shell (slightly more fusiform in *B. guadalupensis*). In addition, *B. bonariensis* has a thicker, mostly whitish shell (the colour pattern is infrequently present) in comparison to *B. guadalupensis*.

Our analyses consistently showed that the three fossil morphotypes are well differentiated from the extant *Bulimulus* species. Neither the PCA nor the CVA was able to separate *B. klappenbachi* from *Bulimulus* sp. 1, with *B. frenguellii* n. sp. being apart from the *B. klappenbachi*–*Bulimulus* sp. 1 cluster. Moreover, the distribution of the fossil specimens in both PCA and CVA plots is similar to the distribution in the plots of the extant species. In sum, *B. frenguellii* n. sp. can be considered a distinct (new) species, whereas *Bulimulus* sp. 1 simply falls with the natural variation of *B. klappenbachi*.

As with the extant species, morphological variation in the fossil species *B. klappenbachi* could be explained in terms of environmental adaptation. The ‘Queguay limestones’ are known to vary with climate (i.e. both temperature and humidity; see Cabrera *et al*., 2018; Veroslavsky *et al*., 2019). Extant bulimulid species (e.g. *B. guadalupensis*) show substantial variation in shell shape in relation to environmental differences (Breure, 1974; Coppois & Glowacki, 1983; Parent & Crespi, 2006, 2009; Parent, 2012; Triantis *et al*., 2016; Kraemer *et al*., 2019), suggesting a similar scenario for fossil bulimulids.

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