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Cladistic analysis of the Argentinian species of the genus *Bostryx* (Gastropoda, Stylommatophora) based on morphological evidence

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ABSTRACT

The genus Bostryx *Troschel, 1847 is found exclusively in South America, from Ecuador to Chile and mainly in the Andes mountain range. The phylogeny of Argentinian species of* Bostryx *was based on morphological characters of 19 species from Argentina, 5 from Peru and 7 from* Bostryx*-related genera, belonging to Bulimulidae and Odontostomidae. A matrix of 31 taxa and 78 morphological characters (19 of which were continuous and 59 discrete) from shell, pallial and reproductive systems was analysed under implied weighting with TNT. Argentinian species of the genus formed a monophyletic group together with the Peruvian species. The monophyly of this clade was supported by a set of 14 synapomorphies, principally related to continuous characters of the shell shape.*

Key-Word: Bulimulidae; TNT; Implied weighting; Continuous characters; Shell shape.

INTRODUCTION

The genus *Bostryx* Troschel, 1847 is found exclusively in South America, from Ecuador (0.6°S) to Chile (36°S) and mainly in the Andes mountain range. The highest species richness of this genus has been recorded in Peru, followed by Chile and Argentina (Miquel, 1993, 1995; Letelier *et al.,* 2003; Ramírez, R. *et al.,* 2003).

The first cladistic analysis of Bulimulinae (= Bulimulidae according to Breure *et al.,* 2010) was carried out by Breure (1979), based on shell morphology and internal anatomy. The author proposed that Bulimulinae and Odontostominae (= Odontostomidae according to Breure *et al.,* 2010) are closely related, with Orthalicinae+Amphibulinae as the sister clade. However, neither the monophyly of Bulimulinae nor that of Bulimulinae+Odontostominae could be corroborated with the characters analysed. Phylogenetic relations at genus level showed contradictory relationships, and hence the sister group of *Bostryx* could not be identified.

Ramírez, J. *et al.* (2009) studied the evolutionary position of the genera *Bostryx* and *Scutalus* Albers, 1850 within the suborder Stylommatophora. The authors included three Peruvian species: *Bostryx sordidus* (Lesson, 1826), *Bostryx scalariformis* (Broderip, 1832) and *Scutalus versicolor* (Broderip, 1832). In all phylogenetic hypotheses they obtained, *Bostryx, Scutalus* and *Placostylus* Beck, 1837 formed a monophyletic group that was well supported and with *Placostylus* as the most basal species.

Breure *et al.* (2010) performed a phylogenetic analysis of the superfamily Orthalicoidea, based on molecular sequences of 32 representative species of the main genera. That study included *Bostryx strobeli*

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(Parodiz, 1956) from Argentina and *Bostryx bilineatus* (Sowerby, 1833) from Ecuador. Five monophyletic clades were obtained and they were assigned the status of family: A, Bulimulidae; B, Odontostomidae; C, Bothriembryontidae; D1, Orthalicidae and D2, Amphibulimidae. *Bostryx, Bulimulus* Leach, 1814, *Naesiotus* Albers, 1850 and *Drymaeus* Albers, 1850 formed a highly supported monophyletic clade A. Breure & Romero (2012) performed a new molecular phylogenetic analysis of Orthalicoidea, with 74 species representing 30 genera. This analysis comprised ten *Bostryx* species, including *Bostryx torallyi* (d'Orbigny, 1835) and *B. strobeli* from Argentina, whereas the remaining species were from Peru and Ecuador. The hypotheses obtained recovered all monophyletic clades proposed by Breure *et al.* (2010) (A, B, C, D1 and D2). Three subclades were grouped within clade A and they were assigned the category of subfamily: A1, Bulimulinae (*Bulimulus, Naesiotus, Rabdotus* Albers, 1850 and several *Bostryx* species (*sensu lato,* Breure 1979)); A2, Peltellinae (*Drymaeus, Peltella* Gray, 1855, *Neopetraeus* Martens, 1885 and *Scutalus*) and A3, Bostrycinae, a new subfamily (*Bostryx*). With these results Breure & Romero (2012) claimed that *Bostryx* would be polyphyletic. The diagnostic characters proposed for Bostrycinae were a smooth protoconch and a relatively long penis sheath (Breure, 2012). The author also emphasized that, based on morphological characters and molecular data, this genus needed thorough revision.

Ramírez, J. & Ramírez, R. (2013) performed a molecular phylogenetic study to examine the evolutionary relationships within the genus *Bostryx* and its position in Bulimulidae. This analysis included 7 *Bostryx* species from Peru, 1 from Ecuador and Argentina (*B. strobeli*) and representatives of *Scutalus, Drymaeus, Naesiotus* and *Neopetraeus* as outgroups. The monophyly of *Bostryx* was not supported by any analysis carried out and conversely, the genus would be polyphyletic.

A new cladistic analysis based on new morphological data would be essential in order to examine which is the sister group of *Bostryx* and its possible relationships with other genera.

The aims of the present study are to establish the phylogenetic relationships among Argentine species of the genus and to determine the sister group of the genus.

MATERIALS AND METHODS

Extensive materials and type materials were studied from Argentinian malacology collections from the following institutes and museums: Instituto de Biodiversidad Neotropical, Tucumán (IBN); Instituto Fundación Miguel Lillo, Tucumán (IFML-Moll), Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN-In), Museo de La Plata, La Plata (MLP) and Museo de Ciencias Naturales Lorca, Mendoza (MCNL). A complete list of the material used for the study is presented in Table 1.

Shell morphology was observed under a Leica MZ6 stereoscopic microscope. Shell measurements, expressed in mm, were obtained using a caliper (characters 0, 3, 6, 9-12, 16-18) (Fig. 1). Measurements of the perimeter and area of the shell and parts of the shell (characters 1, 2, 4, 5, 7, 8, 13, 14) (Fig. 1) were obtained from digital images of specimens in standardised apertural view (axis of coiling and aperture parallel to the camera lens) with a Nikon D5000 digital camera. The images were used to construct Tps files with TpsUtil version 1.58 (Rohlf, 2013a) software. Shell outline drawings of all species were carried out with TpsDig2 version 2.17 (Rohlf, 2013b) directly on the shell photograph with the Draw Curves tool, using a line of dots around the outline. This outline allows computation of the enclosed area and the perimeter of the outline (expressed in cm and cm^2, respectively). Anatomical systems were dissected out from alcohol fixed specimens and inner wall sculpture of the reproductive organs was observed.

In the cladistic analysis the ingroup is composed of 19 Argentinian species of *Bostryx, Bostryx solutus* Troschel, 1847 (type species of the genus), *Bostryx hamiltoni* (Reeve, 1849), *Bostryx infundibulum* (Pfeiffer, 1853), *Bostryx conspersus* (Sowerby, 1833) and *Bostryx rhodolarynx* (Reeve, 1849), from Peru to assess the relationship between them. The outgroup includes species morphologically similar to the genus under study (Nixon & Carpenter, 1993). The taxa selected as outgroup are *Bulimulus apodemetes* (d'Orbigny, 1835), *Bulimulus bonariensis* (Rafinesque, 1833), *Bulimulus gracilis* Hylton Scott, 1948, *Naesiotus munsterii* (d'Orbigny, 1837) and *Drymaeus poecilus* (d'Orbigny, 1835), belonging to Bulimulidae and *Plagiodontes daedaleus* (Deshayes, 1851) and *Spixia tucumanensis* (Parodiz, 1941), belonging to Odontostomidae. The total number of taxa included in the matrix was 31. The trees were rooted indistinctly in *D. poecilus* or *N. munsterii,* closest species to *Bostryx,* based on previous phylogeny (Breure, 1979).

Characters used in the cladistic analysis were defined on the basis of anatomical studies and also taxonomical revisions previously published were consulted (Miquel, 1989a, b, 1991; Salas Oroño, 2007; Pizá & Cazzaniga, 2010). Information about internal anatomy was codified for 25 out of 31 species due to

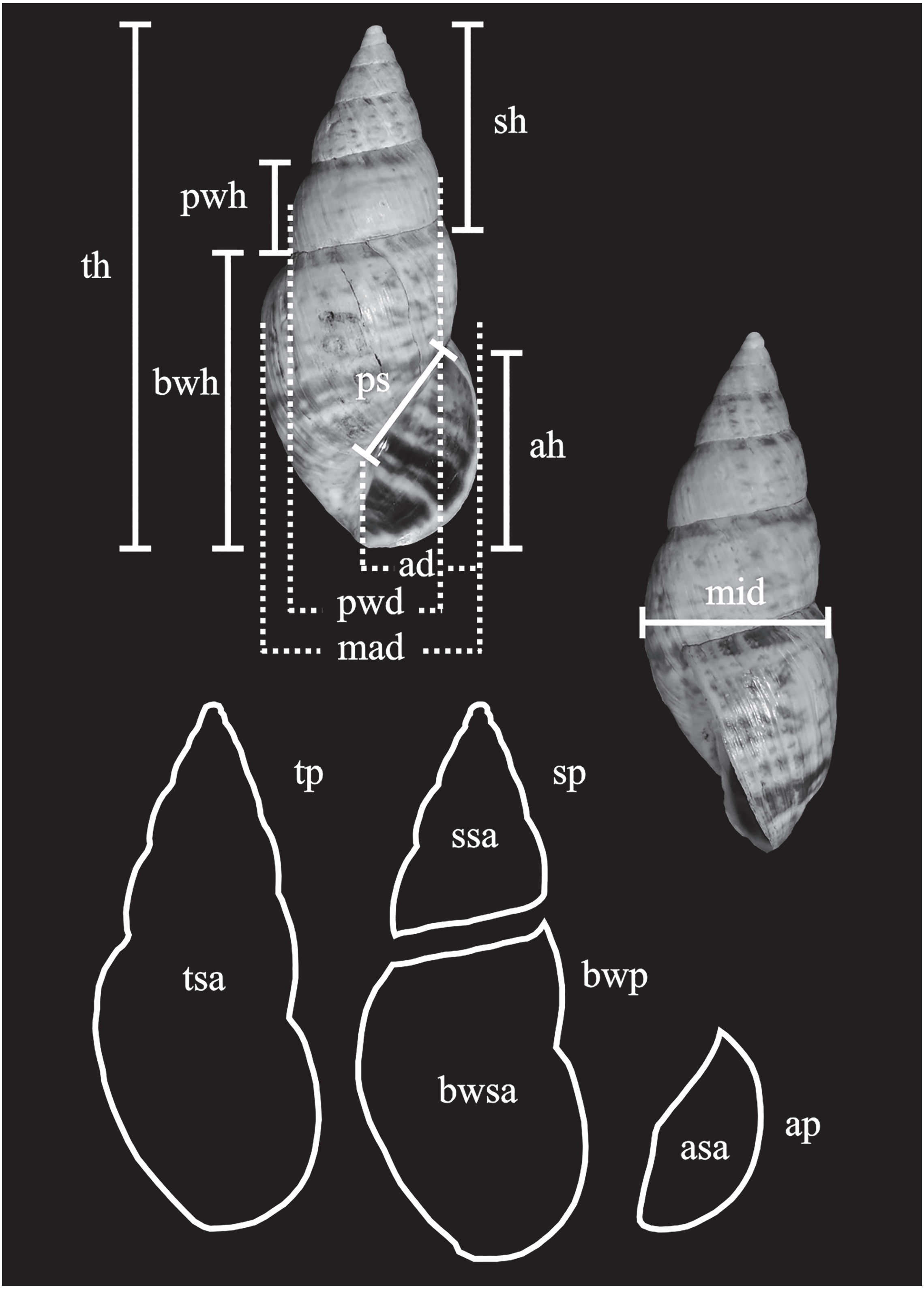

FIGURE 1: Continuous characters: Shell measurements. Abbreviations: **ad:** apertural diameter; **ah:** apertural height; **ap:** apertural perimeter; **asa:** apertural surface area; **bwh:** body whorl height; **bwp:** body whorl perimeter; **bwsa:** body whorl surface area; **mad:** major diameter; **mid:** minor diameter; **ps:** parietal space length; **pwd:** penultimate whorl diameter; **pwh:** penultimate whorl height; **sh:** spire height; **sp:** spire perimeter; **ssa:** spire surface area; **th:** total height; **tp:** total perimeter; **tsa:** total surface area.

TABLE 1: Material examined.Number of specimens examined is included in parentheses after acronym and collection number. Abbreviations: **IBN:** Instituto de Biodiversidad Neotropical; **IFML-Moll:** Instituto Fundación Miguel Lillo; **MACN-In:** Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"; **MLP:** Museo de La Plata; **MCNL:** Museo de Ciencias Naturales Lorca.

Species	Dry Material	Alcohol Material
Bostryx birabenorum Weyrauch, 1965	IFML 985a (1), 985 (2); MLP 11620 (3)	—
Bostryx catamarcanus (Parodiz, 1956)	MACN-In 17778 (1), 17778-1 (10); MLP 8061 (5), 11440 (2)	—
Bostryx cordillerae (Strobel, 1874)	IFML-Moll 15580; MACN-In 1354 (1), 1906 (1), 3234 (3), 9849 (9), 9850 (1), 9592 (5), 10001 (1), 12114 (1), 19625 (3), 32867 (1), 36944 (1); MCNL 361 (5); MLP 897 (4), 8062 (10), 9660, 9669 (8)	MACN-In 10001-2 (2)
Bostryx costellatus (Hylton Scott, 1971)	MACN-In 4221-1 (1), 4221-5 (1)	MACN-In 4221-1 (1)
Bostryx cuyanus (Pfeiffer, 1867)	IFML-Moll 15581 (4); MACN-In 8845 (1), 9592-3 (11), 10000 (4), 12113 (8); MCNL 1117 (10); MLP 10980 (1), 10981 (1), 41801 (2), s/n (6)	MACN-In 12113 (1), 30498 (1)
Bostryx famatinus (Doering, 1879)	MACN-In 3233 (1)	—
Bostryx martinezi (Hylton Scott, 1965)	IFML-Moll 15281 (5), 15608 (1), 15609 (1), 15610 (1), 15611 (1); MACN-In 25870 (2), 27208 (3), 27281 (2), 27431 (1), 36892 (1); MLP 10995 (1), 10996 (1), 10997 (2), 11011 (1), 11012 (1), 11445 (1), 11446 (3), 11447 (3), 11448 (2)	IBN 826 (1), 829 (1); IFML-Moll 15506 (1), 15507 (3), 15508 (1), 15509 (1), 16358 (2), 16360 (1), 16361 (1); MACN-In 27431 (1), 36892 (2)
Bostryx mendozanus (Strobel, 1874)	MACN-In 9850 (3); MLP 8082 (1)	—
Bostyx pastorei (Holmberg, 1912)	IFML-Moll 15573 (1), 15582 (1); MACN-In 1316 (6), 1517 (1), 1517-1 (4), 4916 (10), 9834 (5), 9917 (1), 9917-1 (10), 28800 (5); MLP 9671 (1)	IFML-Moll 15573 (1)
Bostryx peristomatus (Doering, 1879)	IFML-Moll 10881 (5), 15471 (11), 15595 (20); MACN-In 31619 (10); MLP 9512 (10), 9530 (3), 9671 (1)	IFML-Moll 15471 (3); MACN-In 10666 (1), 36934 (1), 37032 (1)
Bostryx reedi (Parodiz, 1947)	MACN-In 10001-1 (1), 9592-1 (7); MCNL 1116 (5)	MACN-In 9592 (1), 10001-1 (1)
Bostryx roselleus Miranda & Cuezzo, 2014	IFML-Moll 928 (15), 15487 (10), 15488 (10), 15489 (6), 15490 (6); MLP 11017 (1)	IFML-Moll 15487 (2), 15488 (4), 15489 (2), 15490 (10), 15491 (2), 15531 (1), 15534 (5), 15535 (2); MACN-In 39122 (7); MLP 13740 (7)
Bostryx rudisculptus (Parodiz, 1956)	MACN-In 380 (1), 380-1 (5); MLP 10171 (4)	IFML-Moll 16460 (2)
Bostryx scaber (Parodiz, 1948)	IFML-Moll 15472 (12), 15517 (3); MACN-In 3217 (1), 4221 (1), 4221-1 (5)	IFML-Moll 15472 (13)
Bostryx stelzneri (Dohrn, 1875)	IFML-Moll 548 (16), 1616 (14), 11019 (10); MACN-In 306 (10), 433 (1), 433-1 (10), 1018 (10), 6243 (1), 6243-1 (10), 7810 (4), 8838 (10), 9159 (2), 9229 (8), 9834 (1), 9852 (8), 10505 (3), 12335 (3), 14086-1 (6), 15711 (2), 17591 (1), 17591-1 (7), 17613 (5), 17777 (10), 17850 (4), 18356 (10), 19061 (1), 19061-1 (6), 19506 (10), 19619 (10), 21116 (10), 24305 (10), 24398 (7), 26645 (1); MLP 1298 (1), 1307 (2), 9527 (2), 9533 (5), 9534 (2), 9535 (4), 9536 (3), 9539 (2), 11291 (2),	IFML-Moll 14443 (2), 14446 (4), 15455 (3), 15456 (1), 15459 (1), 15461 (1), 15462 (1), 15464 (2), 15465 (3), 15467 (3), 15468 (3), 15469 (4), 15470 (3), 15473 (1), 15475 (4), 15477 (2), 15478 (3), 15479 (2), 15480 (3), 15482 (2), 15484 (3), 15485 (3), 15486 (3), 15492 (1), 15542 (2), 15543 (3), 15544 (5), 15545 (3), 16357 (2); MACN-In 1018 (1), 2466 (1), 24308 (1), 32861 (1)
Bostryx strobeli (Parodiz, 1956)	MACN-In 9916 (1), 9916-1 (14), 27283 (1); MLP 11009 (1), 11010 (1), 11018 (3)	MACN-In 9916 (1)
Bostryx torallyi (d'Orbigny, 1835)	IFML-Moll 122 (10), 160 (10), 15493 (10), 15522 (10), 15601 (10); MACN-In 1349 (1), 1349-1 (1), 3236 (4), 6969 (2), 8848 (1), 8848-1 (5), 19167 (10), 25987 (9), 32639 (1); MLP 9667 (4), 9676 (5), 9677 (2), 9678 (2), 9679 (2), 9681 (2), 9966 (3)	IFML-Moll 15493 (3), 15494 (3), 15495 (4), 15496 (3), 15498 (2), 15499 (2), 15500 (1), 15547 (1)

Species	Dry Material	Alcohol Material
Bostryx tortoranus (Doering, 1879)	IFML-Moll 14877 (10), 15502 (8), 15505 (7), 15530; MACN-In 6516 (1), 6516-1 (4), 19461 (2), 19506 (1), 19590 (1), 27282 (4), 29450 (1), 30619 (1); 32686 (1); MLP 10995 (1), 10996 (1), 10997 (1), 13345 (1), 11443 (1), 11007 (1), 11149 (1), 11006 (1), 11442 (1), 11444 (1), 11014 (4), 11015 (1), 8072 (10)	IFML-Moll 15501 (2), 15502 (3), 15504 (1), 15505 (2), 15510 (1), 15574 (3), 16359 (1); MACN-In 30619 (1)
Bostryx willinki Weyrauch, 1964	IFML-Moll 121 (1), 121 (3); MACN-In 19062 (1)	—
Bostryx solutus Troschel, 1847	IFML-Moll 1132 (20), 1133 (40), 15382 (8), 3060 (20), 3134 (20), 15384 (10), 15386 (30), 15387 (34), 16587 (7), 16639 (7)	—
Bostryx conspersus (Sowerby, 1833)	IFML-Moll 1390 (10), 3115 (6), 3348 (20), 3392 (13), 3410 (4), 12150 (10), 16331 (6), 16332 (7), 16354 (5), 16355 (10)	—
Bostryx rhodolarynx (Reeve, 1849)	IFML-Moll 10208 (7), 10613 (2), 14040 (15), 16524 (1), 16525 (12), 16526 (2), 16528 (3), 16546 (19)	—
Bostryx infundibulum (Pfeiffer, 1853)	IFML-Moll 14057 (18), 16430 (100), 16432 (150), 16435 (20)	—
Bostryx hamiltoni (Reeve, 1849)	IFML-Moll 12143 (100), 16434 (18), 16442 (10), 16551 (20)	—
Spixia tucumanensis (Parodiz, 1941)	IFML-Moll 522 (6), 802 (10), 14769 (12), 14770 (20), 15204 (10), 15353 (5)	IFML-Moll 14774 (10), 15350 (5)
Plagiodontes daedaleus (Deshayes, 1851)	IFML-Moll 772 (16), 1065 (15), 10884 (5), 14048 (20)	IFML-Moll 14203 (4)
Drymaeus poecilus (d'Orbigny, 1835)	IFML-Moll 177 (10), 828 (18), 14256 (4), 14265 (4), 14899 (1), 14900 (2), 15689 (3)	IBN 478 (2), IFML-Moll 15696 (2)
Bulimulus apodemetes (d'Orbigny, 1835)	IFML-Moll 13524 (2), 14922 (30), 14971 (25), 15624 (11)	IBN 491 (4)
Bulimulus sporadicus (d'Orbigny, 1835)	IFML-Moll 172 (8), 15652 (7), 15653 (21)	IBN 547 (2), 599 (2)
Bulimulus gracilis Hylton Scott, 1948	MLP 11014 (4), 11015 (1)	IFML-Moll 15655 (4)
Naesiotus munsterii (d'Orbigny, 1837)	IBN 187 (2)	IBN 187 (2)

the lack of material with soft parts of the remaining species. Characters of *Bostryx cuyanus* (Pfeiffer, 1867), *B. solutus, B. hamiltoni, B. infundibulum, B. conspersus* and *B. rhodolarynx* were coded based on published information (Ramírez, R., 1988; Hylton Scott, 1954; Breure, 1978, 1979).

Character coding follows the methodology proposed by Sereno (2007), especially for morphological characters. A total of 78 morphological characters included shell (38 characters), pallial (5 characters) and reproductive systems (35 characters) were selected. From a total of characters, 19 were coded as continuous and analyzed such as, according to Goloboff *et al.* (2006). Of the 59 discrete characters, 9 were coded as multistate and the remaining 50 characters as binary characters. The multistate characters (32, 38, 40, 47, 48, 49, 57, 61 and 72) were treated as ordered. The character state unknown or not applicable were coded as "missing character" (?) (Strong & Lipscomb, 1999) and polymorphic characters have been coded as ($). The characters and characters states selectioned for the analysis are detail below.

Cladistic analyses of the character matrix (Table 2) carried out using the program TNT (Goloboff *et al.,* 2003b). Trees were searched with heuristic search strategies [Traditional Search, Tree Bisection Reconnection (TBR)]. The weighting method implemented explained in detail by Goloboff (1993).

Bremer support (absolute and relative), which evaluates the support for clades, was calculated (Bremer, 1988, 1994; Goloboff & Farris, 2001). Suboptimal trees were gathered in consecutive stages, saving at each stage 1000 trees and making the suboptimal trees worse up to 8 steps longer, obtained in 6 stages: 1, 2, 3, 4, 5 and 6 steps, saving 1000 additional trees on each. Frequency difference (GC, for "group present/contradicted") (Goloboff *et al.,* 2003a) was calculated with 200 replicates by symmetric resampling of the matrix (not distorted by weights/costs). Support is shown below of number of node, Symmetric resampling on the left side (Negative GC values are shown in square brackets), Absolute Bremer on the middle and Relative Bremer on the right side.

TABLE 2: Data matrix for the characters (78) and taxa (31) used in this study. Unknown condition indicated by '?', '-' for not applicable and polymorphic characters (01) as '*'.

Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Bulimulus bonariensis	27.32-32.58	6.43-6.55	2.25-2.49	17.24-20.26	5.17-5.43	1.72-2.00	10.66-14.10	2.90-3.04	0.41-0.49	10.94-13.26	10.06-12.30	11.85-14.39	6.75-8.27	3.16-3.30	0.70-0.72	7.00-8.26	3.48-4.0	6.14-6.60	6.94-7.48
Bulimulus apodemetes	29.05-34.67	6.93-8.227	3.05-4.49	23.55-26.57	6.28-7.78	2.75-4.21	6.86-10.32	2.27-2.67	0.27-0.33	15.01-18.01	14.02-16.1	16.85-19.65	10.4-12.06	4.09-5.07	1.15-1.83	5.0-7.0	2.54-3.32	6.90-7.56	9.14-10.16
Bulimulus gracilis	29.88-32.30	6.56-7.18	2.27-2.55	21.85-23.31	5.53-5.83	1.93-2.11	8.95-10.89	2.42-2.88	0.36-0.44	12.06-12.60	11.12-11.84	15.02-16.36	8.18-8.74	3.47-3.49	0.77-0.81	5.94-6.22	3.45-4.19	6.16-7.15	7.51-9.55
Naesiotus munsterii	19.19-21.75	7.707	3.813	13.42-15.62	6.881	3.363	5.69-6.55	2.647	0.417	10.34-11.66	14.13	14.6	4.79-6.49	4.536	1.482	6.93-7.49	5.18-6.08	2.86-3.62	8.56-9.4
Drymaeus poecilus	31.9-38.02	7.82-8.74	3.61-4.29	23.56-28.26	6.83-7.45	3.19-3.67	10.28-12.1	2.94-3.5	0.45-0.67	16.16-20.26	14.39-17.59	16.35-20.29	10.74-13.36	4.43-4.49	1.37-1.45	6.0-6.26	3.42-4.18	7.47-8.45	9.93-11.13
Spixia tucumanensis	20.5-25.5	4.73-5.61	1.02-1.44	10.29-12.13	3.24-3.8	0.6-0.88	10.25-14.13	2.91-3.35	0.42-0.58	7.2-9.0	6.24-7.54	7.0-8.3	5.2-6.0	1.9-2.28	0.25-0.35	9.5	3.46-4.28	7.55-8.19	5.2-6.0
Plagiodontes daedaleus	23.58-28.62	5.89-6.37	2.13-2.55	15.57-19.57	4.97-5.29	1.57-1.91	9.17-11.27	2.99-3.49	0.55-0.71	11.62-14.46	11.48-14.04	10.52-12.94	8.22-10.1	3.36-3.58	0.82-0.94	6.0-7.5	3.35-4.01	9.91-10.65	6.37-7.01
Bostryx solutus	8.65-9.67	2.52-2.56	0.29-0.31	6.18-6.7	2.21-2.27	0.244-0.245	2.69-3.43	0.91-1.01	0.04-0.06	3.71-4.57	3.98-4.62	2.9-3.78	2.54-3.04	1.11-1.15	0.089-0.095	5.0	1.98-2.4	3.74-4.38	?
Bostryx conspersus	20.35-23.03	4.98-5.14	1.6-1.64	14.57-17.51	4.46-4.58	1.42-1.44	5.85-7.53	1.84-1.94	0.18-0.2	10.55-12.07	10.16-11.42	10.26-12.02	6.88-7.9	2.85-2.93	0.58-0.62	4.79-6.21	2.85-3.69	5.18-6.04	5.06-6.42
Bostryx rhodolarynx	26.41-32.37	7.07-8.11	2.73-3.99	18.4-22.77	5.77-7.01	2.17-3.41	9.0-11.02	3.04-3.2	0.55-0.57	12.9-17.66	13.48-15.03	13.48-16.24	10.43-12.13	3.9-5.12	1.13-1.95	5.79-7.21	3.83-4.73	7.3-9.4	3.56-5.12
Bostryx infundibulum	18.51-21.07	4.4-4.8	1.09-1.13	10.31-11.41	3.18-3.52	0.64-0.82	8.7-10.1	2.15-2.91	0.26-0.46	6.55-7.45	6.31-7.09	7.42-8.26	3.51-4.19	1.58-2.04	0.01-0.022	8.79-10.21	2.66-3.38	5.43-6.17	2.05-2.67
Bostryx hamiltoni	16.79-19.23	4.05-4.77	0.81-1.13	8.76-9.8	2.73-3.27	0.47-0.69	8.28-9.6	0.656-0.777	0.024-0.033	5.92-6.96	5.54-6.44	5.83-6.63	3.52-4.38	1.44-1.76	0.13-0.21	6.79-8.21	2.79-3.53	4.78-5.44	2.4-3.46
Bostryx roselleus	17.9-30.3	4.7-6.52	1.17-2.15	10.9-18.4	3.81-5.09	0.94-1.66	6.37-12.11	2.08-2.88	0.22-0.44	8.6-13.6	7.4-11.4	9.2-14.0	6.0-9.2	2.33-3.07	0.39-0.69	5.5-6.5	2.48-3.26	5.41-6.69	4.45-5.65
Bostryx birabenorum	16.08-17.4	4.330	1.191	13.84-14.72	4.0	1.126	2.89-3.93	1.291	0.079	9.28-11.38	8.27-8.83	10.23-11.41	6.1-8.48	2.693	0.501	4.25-5.01	1.33-2.25	3.1-3.96	6.42-6.8
Bostryx catamarcanus	15.77-20.19	4.3-4.5	1.1-1.25	12.23-15.97	3.84-4.14	1.02-1.19	4.23-5.51	1.32-1.39	0.09-0.1	7.75-10.49	6.76-8.76	8.29-11.79	6.11-7.73	2.61-2.95	0.49-0.62	5.5	1.2-1.9	3.7	3.04-3.46

Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Bostryx cordillerae	12.25-16.45	2.87-3.25	0.48-0.58	9.05-11.47	2.47-2.71	0.4-0.48	3.68-5.74	1.2-1.48	0.07-0.11	5.27-7.51	5.23-7.03	5.92-7.76	3.89-5.79	1.47-1.57	0.15-0.17	5.0-6.0	1.62-2.04	3.31-3.79	2.82-2.98
Bostryx costellatus	11.36	2.817	0.501	9.26	2.524	0.451	3.32	0.986	0.05	7.44	6.66	6.97	5.42	1.672	0.182	4.5	1.33	2.8	3.1
Bostryx cuyanus	6.21-8.85	3.17-4.49	0.65-0.99	5.38-7.04	3.04-4.44	0.56-0.92	1.59-2.95	1.21-1.55	0.05-0.11	9.42-17.68	7.82-14.56	4.56-7.36	4.61-10.31	1.47-2.09	0.11-0.17	4.0-4.5	2.73-3.03	9.93-12.63	2.36-3.5
Bostryx famatinus	17.02	3.732	0.644	10.78	2.818	0.47	6.59	1.888	0.171	5.59	5.58	7.19	4.13	1.626	0.166	7.0	2.47	3.98	3.2
Bostryx martinezi	18.3-24.6	4.95-5.41	1.29-1.55	13.8-17.7	4.04-4.6	1.06-1.34	5.4-8.8	2.06-2.16	0.2-0.24	7.89-11.01	7.27-9.71	9.7-12.7	4.98-6.98	2.21-2.97	0.31-0.55	6.0-6.26	2.36-2.92	4.73-5.35	4.44-5.4
Bostryx mendozanus	16.28-21.92	4.5-5.04	1.18-1.56	13.68-18.16	4.04-4.74	1.06-1.48	4.11-5.65	1.32-1.42	0.09-0.11	8.18-11.1	7.33-9.43	10.62-13.24	5.91-7.65	2.77-3.21	0.52-0.7	4.25-5.01	1.81-1.91	4.16-4.66	4.03-6.57
Bostryx pastorei	15.57-23.75	4.48-5.08	1.04-1.36	11.7-17.12	3.61-4.25	0.85-1.17	3.40-8.42	1.87-2.11	0.17-0.21	7.48-10.0	6.88-8.6	8.74-14.12	4.72-7.2	2.21-2.69	0.32-0.5	6.0-6.26	2.48-2.82	4.44-5.52	3.98-5.58
Bostryx peristomatus	25.2-32.5	6.43-6.97	2.06-2.8	18.1-23.2	5.37-6.27	1.76-2.52	7.6-12.6	2.14-2.38	0.26-0.3	11.7-15.8	9.4-11.8	14.5-17.6	8.4-11.8	3.66-4.76	0.94-1.62	6.0-6.5	2.71-3.57	5.39-6.17	3.34-4.4
Bostryx reedi	13.33-15.05	3.35-3.95	0.67-0.91	8.83-10.17	2.85-3.29	0.54-0.74	4.74-5.86	1.45-1.83	0.12-0.18	6.87-8.05	6.47-7.39	5.9-6.84	4.08-5.02	1.66-2.0	0.21-0.29	6.0	1.81-2.29	4.43-5.17	2.97-4.09
Bostryx scaber	25.46-31.48	7.55-8.71	3.94-5.44	22.07-26.21	7.32-8.54	3.81-5.33	5.92-7.02	2.07-2.19	0.11-0.17	19.45-23.29	15.52-18.92	17.86-21.22	13.05-14.91	5.24-6.56	1.95-3.17	5.0-6.0	2.45-3.21	6.55-7.71	6.25-7.47
Bostryx stelzneri	19.31-34.01	6.32-6.8	2.62-2.66	15.3-26.0	5.77-5.85	2.28-2.38	4.57-11.41	2.23-2.53	0.26-0.36	10.69-21.51	9.21-17.13	10.8-22.9	7.9-15.5	3.77-3.87	0.95-1.13	5.0-6.5	2.61-3.25	6.41-7.95	5.65-7.31
Bostryx strobeli	15.11-19.85	4.31-5.55	1.07-1.79	11.86-15.62	3.84-4.9	0.97-1.63	3.5-6.42	1.38-1.96	0.09-0.19	7.4-10.12	6.6-8.84	8.32-11.36	4.82-7.22	2.46	0.0889	5.0-5.5	1.61-2.79	4.35-5.61	5.55-6.15
Bostryx torallyi	25.3-32.7	5.06-6.6	1.27-2.31	15.3-22.0	3.52-5.22	0.82-1.8	8.4-14.6	2.94-3.06	0.46-0.5	9.3-13.4	8.6-12.0	10.1-15.8	5.4-8.1	1.98-3.2	0.22-0.66	7.25-8.25	3.45-4.65	7.61-9.05	6.17-7.87
Bostryx tortoranus	18.8-29.88	5.63-5.87	1.83-1.99	14.7-22.12	4.95-5.05	1.69-1.71	5.6-8.9	1.82-2.38	0.16-0.28	8.93-11.99	8.7-11.0	10.0-15.36	6.1-8.9	2.89-3.31	0.53-0.71	5.5-6.5	2.08-2.72	4.46-5.14	6.46-7.44
Bostryx willinki	17.77-19.95	4.46	0.908	11.0-12.22	3.002	0.568	7.69-9.55	2.582	0.345	6.83-8.39	6.43-7.57	7.46-8.52	4.74-5.46	1.896	0.244	6.0-7.0	2.91-3.57	5.44-5.72	3.44-3.86
Bostryx rudisculptus	12.89-16.07	2.972	0.486	9.64-11.5	2.442	0.398	4.03-5.39	1.27	0.079	5.68-6.4	5.54-5.82	7.05-7.67	4.28-4.82	1.399	0.132	5.5	1.5	3.2	3.4

Species	1	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4
	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
Bulimulus bonariensis	0	0	0	0	0	0	0	0	0	1	0	1	0	2	0	—	0	0	—	1	0	—	0	1	1	0	0	1	1	0	2
Bulimulus apodemetes	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	—	1	0	—	0	1	1	0	1	1	2	0	2
Bulimulus gracilis	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	0	—	0	1	1	0	1	1	2	?	2
Naesiotus munsterii	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	—	0	0	—	2	0	—	0	1	1	0	0	1	1	0	1
Drymaeus poecilus	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	—	1	0	—	0	1	1	0	1	1	2	0	0
Spixia tucumanensis	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0	—	0	1	2	1	0	0	1	—	1	0	1	0
Plagiodontes daedaleus	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	0	1	0	—	1	1	0	1	0	0	1	—	0	2	1	1
Bostryx solutus	1	0	0	0	0	0	1	0	0	1	0	0	—	—	1	0	0	1	0	?	?	?	?	?	?	?	?	?	1	0	0
Bostryx conspersus	0	0	0	0	0	0	0	0	0	1	0	0	—	—	0	—	0	0	—	1	0	—	0	?	?	?	?	?	2	0	0
Bostryx rhodolarynx	0	0	0	0	0	0	1	1	0	1	1	0	—	—	1	0	0	0	—	?	?	?	?	?	?	?	?	?	2	0	0
Bostryx infundibulum	0	1	0	0	0	0	1	0	0	1	0	0	—	—	1	0	0	1	1	?	?	?	?	?	?	?	?	?	2	0	0
Bostryx hamiltoni	0	0	0	0	0	0	0	0	0	1	0	0	—	—	1	0	0	0	—	?	1	2	?	?	?	?	?	?	2	0	0
Bostryx roselleus	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	1	0	1	1	—	0	1	0	1	0
Bostryx birabenorum	1	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx catamarcanus	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx cordillerae	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	1	0	1	1	—	1	1	1	0	0
Bostryx costellatus	1	1	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx cuyanus	0	1	0	0	2	1	1	0	1	0	0	1	0	1	1	0	0	1	0	?	?	?	?	?	?	?	?	?	1	0	0
Bostryx famatinus	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx martinezi	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	0	0	1	1	—	0	1	1	0	0
Bostryx mendozanus	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx pastorei	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	0	0	1	1	—	0	1	0	0	0
Bostryx peristomatus	0	1	1	0	0	0	1	1	0	1	0	1	0	1	1	0	0	1	1	1	1	2	0	1	1	—	0	1	1	1	0
Bostryx reedi	1	0	0	0	0	1	1	0	0	1	1	1	0	1	1	0	0	0	—	1	1	2	0	1	1	—	0	1	1	1	0
Bostryx scaber	1	1	0	1	1	0	0	0	1	0	1	1	1	—	0	—	0	0	—	1	1	2	0	1	1	—	0	1	1	1	0
Bostryx stelzneri	0	[01]	0	[01]	0	0	0	0	0	1	1	1	0	1	1	0	0	0	—	1	1	[01]	0	1	1	—	[01]	1	1	1	0
Bostryx strobeli	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	—	1	1	2	0	1	1	—	1	1	0	1	0
Bostryx torallyi	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	2	0	1	1	—	0	1	0	0	0
Bostryx tortoranus	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	—	1	1	1	0	1	1	—	0	1	1	1	0
Bostryx willinki	1	1	0	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	—	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx rudisculptus	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	—	1	1	0	0	1	1	—	0	1	1	0	0

Species	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Bulimulus bonariensis	0	1	0	0	0	1	1	1	1	0	0	0	0	—	0	0	1	0	0	0	0	0	1	1	0	1	0	1
Bulimulus apodemetes	0	1	1	0	0	1	1	1	0	0	0	0	0	—	0	0	1	0	0	0	0	1	[01]	1	0	0	0	1
Bulimulus gracilis	1	0	1	0	0	1	1	1	1	0	0	0	0	—	0	0	1	0	?	0	0	0	1	1	0	0	0	1
Naesiotus munsterii	1	0	0	0	0	1	1	2	0	0	0	0	0	—	0	0	1	0	0	0	0	0	1	1	0	0	0	1
Drymaeus poecilus	0	1	0	0	?	0	1	2	0	0	0	0	0	—	0	0	1	0	1	0	0	0	2	1	0	1	0	1
Spixia tucumanensis	1	0	0	0	1	0	1	0	0	0	0	2	0	—	0	1	1	1	0	0	1	0	2	1	1	1	1	0
Plagiodontes daedaleus	1	0	0	0	0	1	1	0	0	0	1	0	0	—	0	0	1	1	1	0	1	0	2	0	1	0	1	0
Bostryx solutus	?	?	0	?	1	?	1	2	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	1	0	2	0	1
Bostryx conspersus	?	?	1	0	1	1	0	—	—	?	?	?	?	?	?	?	?	—	?	?	0	0	1	1	0	1	0	1
Bostryx rhodolarynx	?	?	1	0	1	?	1	2	?	?	?	?	?	?	?	?	?	?	?	?	0	1	0	1	0	1	0	1
Bostryx infundibulum	?	?	0	0	1	?	1	2	?	?	?	?	?	?	?	?	?	0	?	?	0	0	2	1	0	1	0	1
Bostryx hamiltoni	?	?	0	0	1	?	1	2	?	?	?	?	?	?	?	?	?	0	?	?	0	?	?	1	0	1	0	1
Bostryx roselleus	0	1	0	1	1	1	1	2	1	1	0	1	1	2	1	0	0	0	1	1	0	1	0	1	0	2	0	1
Bostryx birabenorum	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx catamarcanus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx cordillerae	0	1	0	1	1	1	1	2	1	1	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx costellatus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx cuyanus	?	?	0	1	1	?	1	2	?	?	?	?	?	?	?	?	?	0	?	?	0	1	0	1	0	2	0	1
Bostryx famatinus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx martinezi	0	1	0	1	1	1	1	2	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx mendozanus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx pastorei	0	1	0	1	1	1	1	2	1	0	0	1	1	0	1	0	0	0	1	1	0	1	0	1	0	2	0	1
Bostryx peristomatus	0	1	0	1	1	1	1	2	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx reedi	0	1	1	1	1	1	1	2	1	0	0	1	1	1	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx scaber	0	1	1	1	1	1	1	2	1	0	0	1	1	[01]	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx stelzneri	0	1	1	1	1	1	1	2	1	1	0	1	1	[01]	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx strobeli	0	1	1	1	1	1	1	2	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx torallyi	0	1	0	1	1	1	1	2	1	0	0	1	1	1	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx tortoranus	0	1	1	1	1	1	1	2	1	0	0	1	1	1	1	0	0	0	0	1	0	1	0	1	0	2	0	1
Bostryx willinki	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Bostryx rudisculptus	0	1	1	1	1	1	1	2	1	0	0	1	1	0	1	0	0	0	1	1	0	1	0	1	0	2	0	1

Characters

Continuous (Fig. 1)

0. Total shell height. Measured from the apex of the shell to basal portion of aperture.
1. Shell perimeter.
2. Shell surface area.
3. Body whorl height. Measured from last suture to basal portion of aperture.
4. Body whorl perimeter.
5. Body whorl surface area.
6. Spire height. Measured from the apex to the lower suture.
7. Spire perimeter.
8. Spire surface area.
9. Major diameter of the shell. Measured including the peristome.
10. Minor diameter of the shell. Is a measurement perpendicular to the major diameter.
11. Shell apertural height. Measured from top of aperture to the outer lip of the peristome.
12. Shell apertural diameter. Measured including the peristome.
13. Aperture perimeter.
14. Aperture surface area.
15. Shell, number of whorls. Calculated following Kerney & Cameron (1979)' methodology.
16. Penultimate whorl height. Measured from the penultimate to the last suture comprises the spire.
17. Major diameter of the penultimate whorl. Measured including most outstanding of the whorl.
18. Parietal space length. Measured the portion of the body whorl bordering the upper part of the aperture.

Discrete

19. Shell, outline of spire: flat to slight convex (0); convex (1). In the state 0, the curvature in a central portion of the whorls is nonexistent or little pronounced, whereas in the state 1 the curvature is strongly marked (Fig. 2A).
20. Shell, teleoconch, body whorl sculpture: shallow ribs (0), ribs (1). The shallow ribs are considered small undulated of the surface and ribs are height well defined. In *Bostryx stelzneri* (Dohrn, 1875) the sculpture of the body whorl varies widely among specimens and was entered as polymorphic (Fig. 2B).
21. Shell, teleoconch, granulate appearance to the last whorls: absent (0), present (1). The state 1 is an autapomorphy of *Bostryx peristomatus* (Doering, 1879) and usually the granules are irregularly arranged on axial ribs. The granules are a kind uncommon of sculpture, however was described by Breure (2008) to some Peruvian species of the genus (Fig. 2C).
22. Shell, suture, shape in relation to whorls: simple (0), crenulated (1). The state 0 correspond to a spiral line plain of junction between adjacent whorls. In the state 1 the suture present small notches or wrinkles. Only *Spixia tucumanensis, Bostryx birabenorum* Weyrauch, 1965 and some specimens of *B. stelzneri* have a crenulated suture (Fig. 2D).
23. Shell, aperture, shape in relation to geometrics shape: elongate-ovate to subovate (0); rounded (1); subtriangular to subcircular (2). The shape elongate-ovate to subovate predominates over the other forms. The rounded shape is typical of *Bostryx scaber* (Parodiz, 1948) and a subtriangular to subcircular is present only in *Bostryx cuyanus* (Fig. 2E).
24. Shell, aperture, parietal callus: absent (0), present (1). The thickening on the parietal region of the shell, denomined parietal callus, is registered in *S. tucumanensis, B. cuyanus* and *Bostryx reedi* (Parodiz, 1947) (Fig. 2F).
25. Shell, umbilicus, opening degree: narrow (0), wide (1). The opening degree of the umbilicus is considered as a measure of its greatest diameter (Fig. 3A).
26. Shell, peristome, shape: simple to slight expanded (0); expanded (1). The peristome expanded is only present in *B. rhodolarynx* and *B. peristomatus* (Fig. 3B).
27. Shell, spire, shape according to spiral angle: acute (0); obtuse (1). In all species selected as outgroup, the spire is acute (angle of less than ninety degrees), whereas in the ingroup, only *B. cuyanus* and *B. scaber* have an obtuse spire (angle of more than ninety degrees) (Fig. 3C).
28. Shell, spire, shape: wider than tall (0); taller than wide (1). The spire taller than wide is a form more frequent in the species examined. Only *Bostryx birabenorum, Bostryx costellatus* (Hylton Scott, 1971), *Bostryx mendozanus* (Strobel, 1874), *B. cuyanus* and *B. scaber* have a spire wider than tall.
29. Shell, body whorl shape: similar diameter of spire (0); wider than the spire (1). In species with fusiform, ovalate-oblonge or turriteliform shell shape, the body whorl is similar in diameter respect to the spire. In shells with ovalate, globose or discoidal shape, the body whorl is wider than the spire.

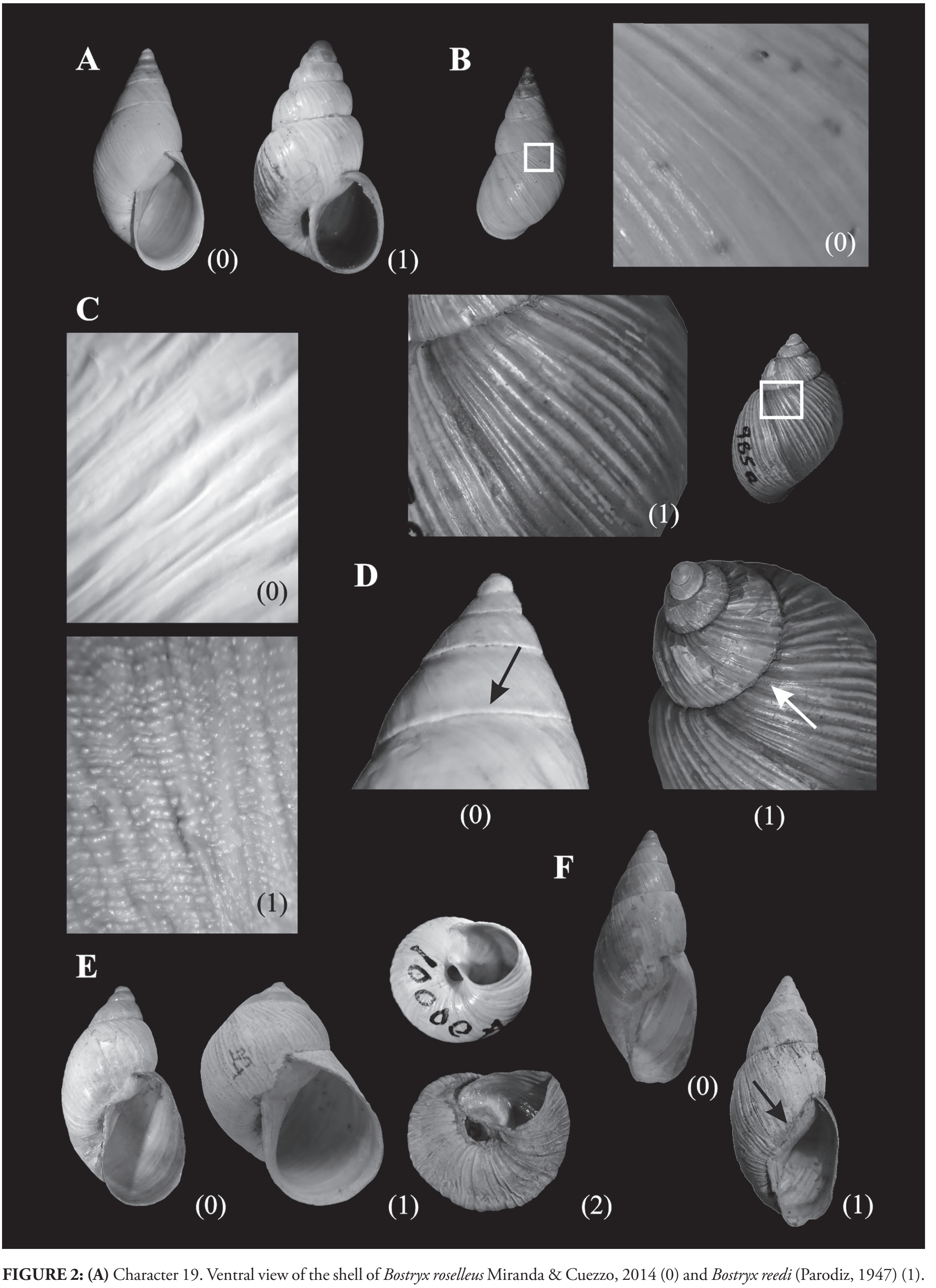

FIGURE 2: (A) Character 19. Ventral view of the shell of *Bostryx roselleus* Miranda & Cuezzo, 2014 (0) and *Bostryx reedi* (Parodiz, 1947) (1). **(B)** Character 20. Detail of body whorl sculpture in dorsal view of *B. roselleus* (0) and *Bostryx birabenorum* Weyrauch, 1965 (1). **(C)** Character 21. Detail of body whorl sculpture in dorsal view of *Bostryx stelzneri* (Dohrn, 1875) (0) and *Bostryx peristomatus* (Doering, 1879) (1). **(D)** Character 22. Suture simple in *B. stelzneri* (0) and crenulated in *B. birabenorum* (1) (arrows). **(E)** Character 23. Shell shape aperture in *Bostryx catamarcanus* (Parodiz, 1956) (0), *Bostryx scaber* (Parodiz, 1948) (1) and *Bostryx cuyanus* (Pfeiffer, 1867) (2). **(F)** Character 24. Ventral view of *Bostryx martinezi* (Hylton Scott, 1965) (0) and *Bostryx mendozanus* (Strobel, 1874) (1) showing a parietal callus (arrow).

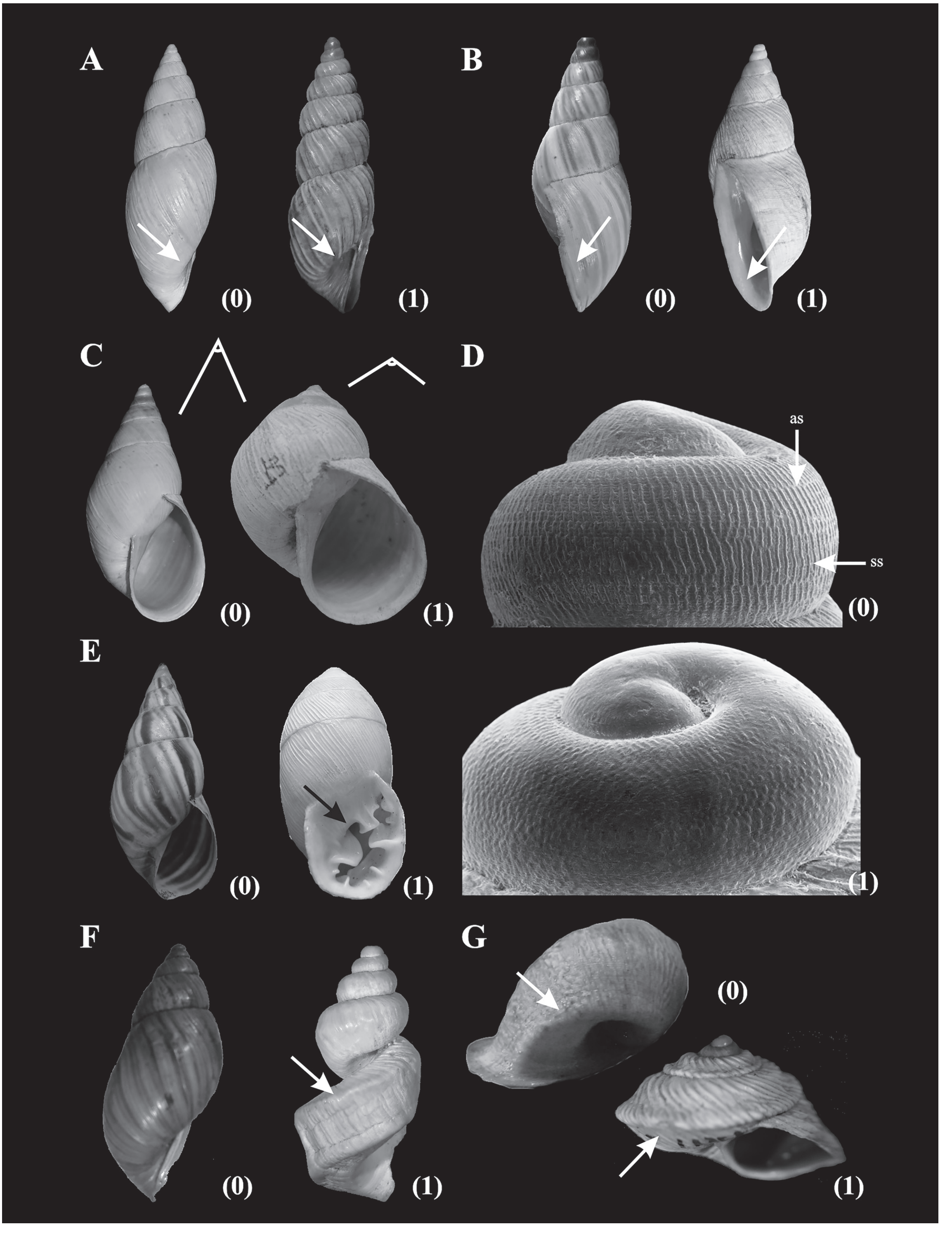

FIGURE 3: (A) Character 25. Lateral view of *Bostryx martinezi* (Hylton Scott, 1965) (0) and *Bostryx willinki* Weyrauch, 1964 (1). **(B)** Character 26. Lateral view of *Bostryx pastorei* (Holmberg, 1912) (0) and *Bostryx peristomatus* (Doering, 1879) (1). **(C)** Character 27. Ventral view of *Bostryx roselleus* Miranda & Cuezzo, 2014 (0) and *Bostryx scaber* (Parodiz, 1948) (1). **(D)** Characters 30-34. Microsculpture of protoconch of *B. peristomatus* (0) and *B. scaber* (1). **(E)** Character 35. Ventral view of the shell showing aperture in *Bostryx torallyi* (d'Orbigny, 1835) (0) and a presence of apertural teeth (arrow) in *Plagiodontes daedaleus* (Parodiz, 1946) (1). **(F)** Character 36. Lateral view of *Bostryx mendozanus* (Strobel, 1874) (0) and *Bostryx solutus* Troschel, 1847 (1), showing the presence of carina (arrow). **(G)** Character 37. Carina around of umbilicus in *B. peristomatus* (0) and around of whorl in *Bostryx cuyanus* (Pfeiffer, 1867) (1) (arrows). Abbreviations: **as:** axial sculpture; **ss:** spiral sculpture.

30. Shell, protoconch, axial sculpture: absent (0); present (1). Based on protoconch sculpture, Pilsbry (1895-1896, 1901-1902) made a generic framework of Bulimulidae and until now this character has a lot of importance for taxonomic identifications. Term axial refers to location in the direction of an axis, columella in this case. No axial sculpture in the protoconch is observed in Peruvian species of *Bostryx* examined; however Breure (1978) described wrinkles or riblets in protoconch of several species from Peru. This is a character highly variable within the genus *Bostryx* (Fig. 3D).
31. Shell, protoconch, axial sculpture: shallow ribs (0); elongates granules (1). Axial shallow ribs are present in the protoconch of all Argentinian species examined, except *B. scaber. Bostryx scaber* has elevated axial costules; crossed by dense number of spiral grooves giving the appearance of elongates granules. This rare type of sculpture was described to Peruvian species by Breure (1978) (Fig. 3D).
32. Shell, protoconch, axial sculpture with shallow ribs: straight (0); slightly wavy (1); in zigzag (2). The state 0 occurs in some species of the outgroup of the genera *Naesiotus* Albers, 1850, *Drymaeus* Albers, 1850 and *Spixia* Pilsbry & Vanatta, 1898. The state 1 occurs typically in all Argentinian species of *Bostryx,* whereas is considered inapplicable to Peruvian species. Such as proposed by Parodiz (1946), in *Bulimulus* Leach, 1814 shallow ribs vary from slightly wavy to zigzag (states 1 and 2) (Fig. 3D, Parodiz (1946)).
33. Shell, protoconch, spiral sculpture: absent (0); present (1). Term spiral refers to coiling around a fixed axis (columella), perpendicular to axial sculpture. The spiral sculpture in the protoconch is typically present in all species with axial sculpture observed, except in *Bulimulus bonariensis, Naesiotus munsterii* and *Bostryx conspersus* (Fig. 3D).
34. Shell, protoconch, spiral sculpture: thinner than axial shallow ribs (0); approximately equal thickness than axial shallow ribs (1). The state 1 is an autapomorphy of *Drymaeus poecilus* (Fig. 3D; Parodiz, 1946).
35. Shell, aperture, apertural teeth: absents (0), presents (1). The presence of teeth-like structures in the aperture of the shell is a typical character of Odontostomidae, represented in this analysis by *S. tucumanensis* and *P. daedaleus* (Fig. 3E).
36. Shell, carina: absent (0), present (1). A carination is defined by Pilsbry & Olsson (1949) as an angularity of the whorls. The presence of a carina is a relatively rare phenomenon in land snails. The carina has been registered within the Orthalicoidean genera *Bostryx, Scutalus* Albers, 1850, *Neopetraeus* Martens, 1885 and *Plagiodontes* Doering, 1877 (Breure, 2008; Pizá & Cazzaniga, 2012). Among the species included in the analysis, *B. solutus, B. infundibulum, B. peristomatus* and *B. cuyanus* have a carina (Fig. 3F).
37. Shell, carina, location: around of whorl (0), around of umbilicus (1). A carina around of whorl is present in *B. cuyanus* and *B. solutus* and Breure (2008) described it to several species from Peru. The presence of carina around of umbilicus is present in *B. peristomatus* and *B. infundibulum.* Pilsbry & Olsson (1949) described a carina circum-umbilical for Chilean species of *Bostryx* (Fig. 3G).
38. Pallial system, kidney, length in relation to total pulmonary roof length: ¼ of length (0); ⅓ of length (1); ½ of length (2). The kidney length is a constant character among *Bostryx* species analyzed. The state 1 is present in most species, whereas in *S. tucumanensis* there is a shorter kidney (0) and in *N. musnterii* is larger (2) (Fig. 4A).
39. Pallial system, secondary ureter, degree of occlusion: closed (0); open (1) (Fig. 4B).
40. Pallial system, secondary ureter, position of opening, in relation to pulmonary roof: ⅓ proximal (0); half of length (1); ⅓ distal (2). Terms proximal and distal refers to position of opening in relation to proximity with a kidney or a collar mantle, respectively. In the pallial system, the position of the secondary ureter opening is the only character varying between Argentinian species of *Bostryx* studied, with all states of this character represented. Cases where the secondary ureter is closed, are considered inapplicable (Fig. 4C. State 2 corresponds to state 1 of Fig. 4B).
41. Pallial system, pallial gland: absent (0), present (1). The pallial gland is present next to collar mantle only in species of Odontostomidae (Ilustrated in Pizá & Cazzaniga, 2010 and Salas Oroño, 2007).
42. Pallial system, interramus area, shape: triangular (0); rectangular (1). The triangular interramus area is observed exclusively in Odontostomidae (Fig. 4B. State 0 illustrated in Salas Oroño, 2007).
43. Reproductive system, vagina, inner wall sculpture, longitudinal folds: absents (0); presents (1).

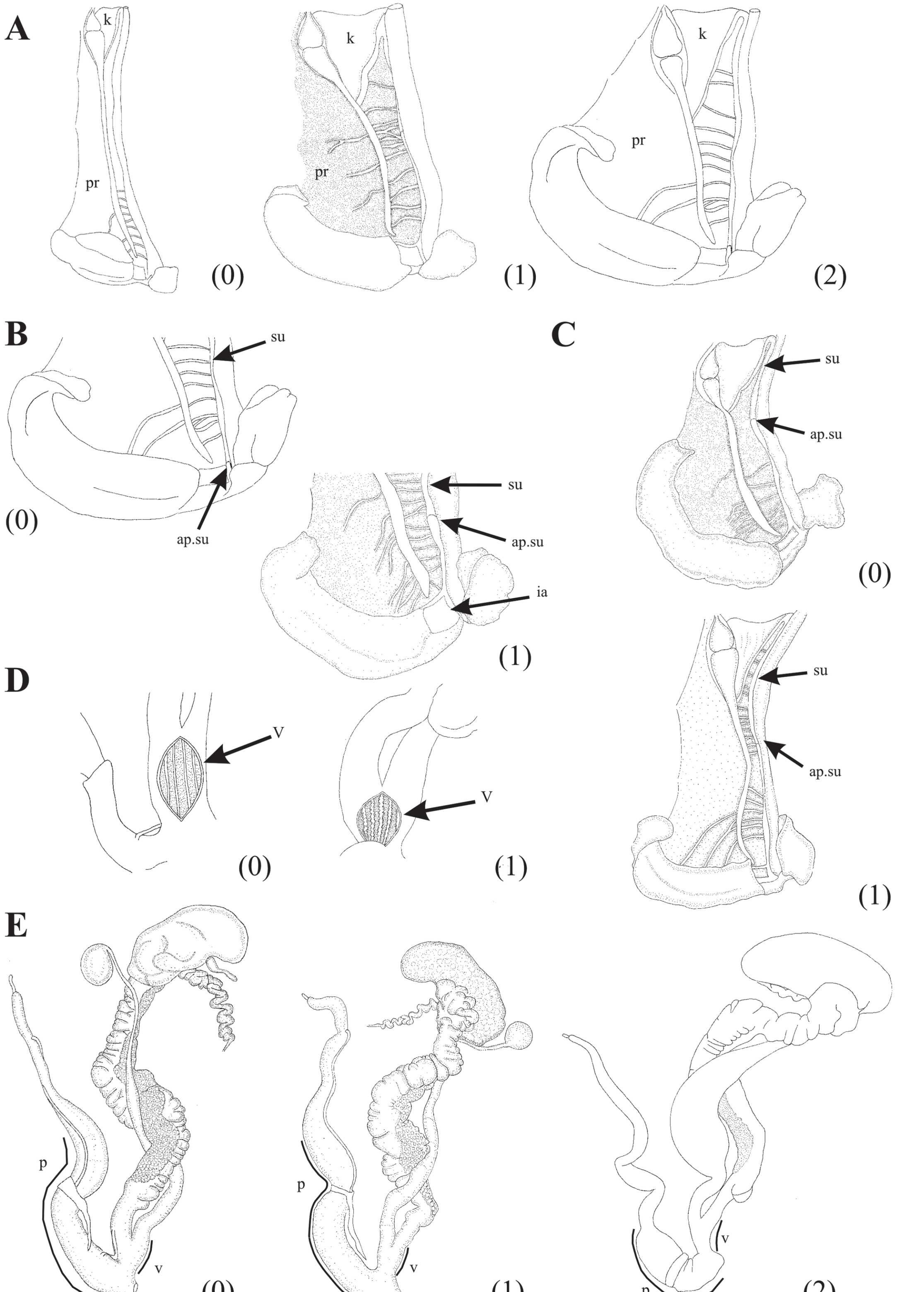

FIGURE 4: Pallial system dissected out. **(A)** Character 38. Length of kidney in relation to total pulmonary roof length. **(B)** Characters 39, 40, 42. Degree of occlusion of secondary ureter. **(C)** Characters 40. Position of secondary ureter opening. Reproductive system dissected out. **(D)** Characters 43, 45, 46. Vagina inner wall sculpture. **(E)** Characters 47, 74. Shape and length of vagina in relation to penis length. Abbreviations: **ap.su:** aperture of secondary ureter; **ia:** interramus area; **k:** kidney; **p:** penis; **pr:** pulmonary roof; **su:** secondary ureter; **v:** vagina.

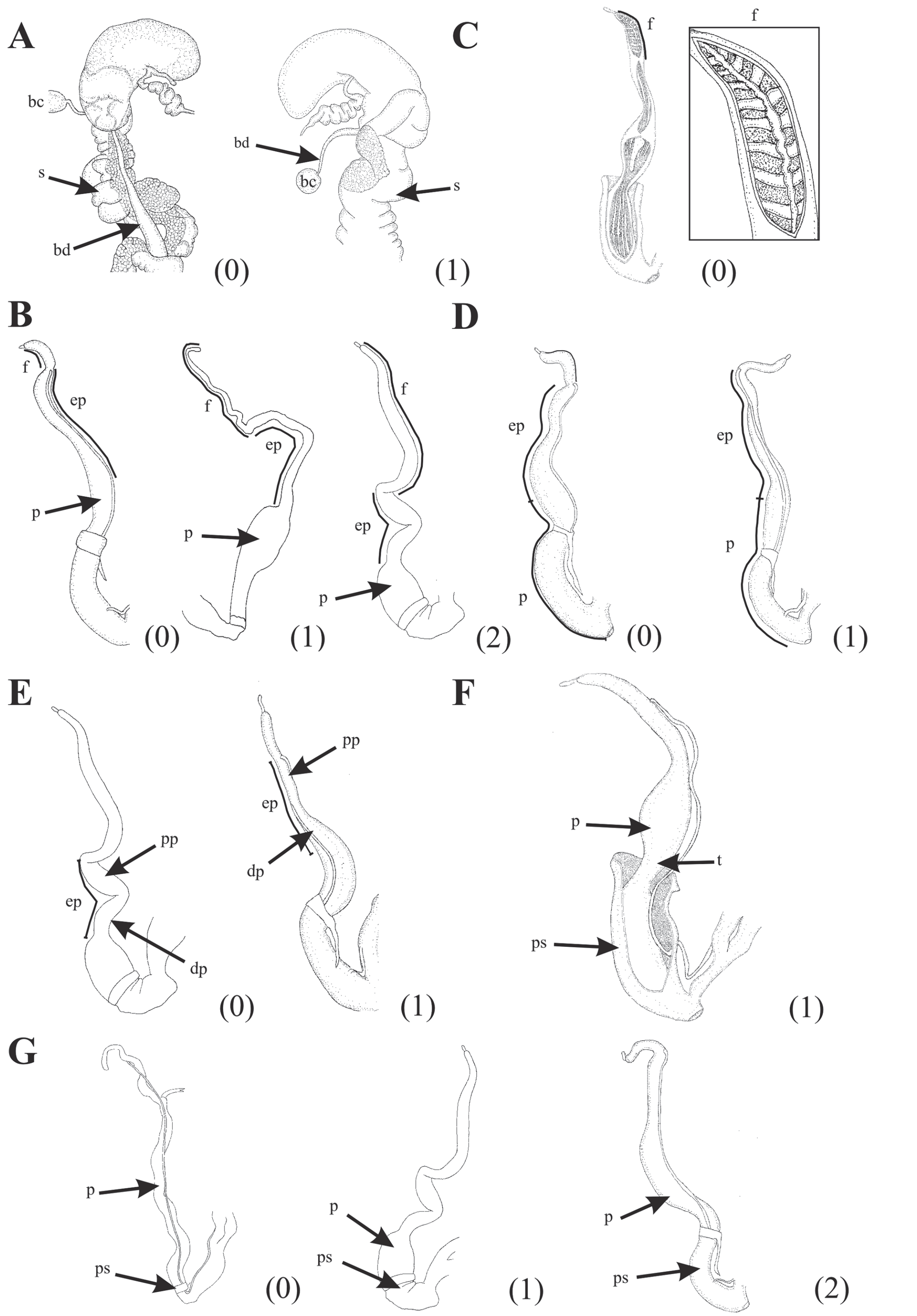

FIGURE 5: Reproductive system dissected out. **(A)** Character 48. Length of bursa copulatrix duct in relation to spermoviduct length. **(B)** Characters 49, 54. Length of flagellum in relation to epiphallus length and external transition from epiphallus to penis. **(C)** Characters 50, 51. Inner wall sculpture of flagellum. **(D)** Character 52. Length of epiphallus in relation to penis length. **(E)** Character 53. Shape of epiphallus. **(F)** Characters 55, 56, 67. Phallic complex showing the thinning in the middle portion of penis and a vas deferens in relation to penis sheath. **(G)** Character 57. Length of penis sheath in relation to penis length. Abbreviations: **bc:** bursa copulatrix; **bd:** bursa copulatrix duct; **dp:** distal portion; **ep:** epiphallus; **f:** flagellum; **p:** penis; **pp:** proximal portion; **ps:** penis sheath; **s:** spermoviduct; **t:** thinner middle section.

Terminology for all reproductive organs follows Tompa (1984). In the description of all remaining characters, terms proximal and distal refer to the position of an organ in relation to the direction of gamete flow, from ovotestis (proximal) towards genital atrium (distal). The folds are thin and relatively flat bends of the inner wall of the organs. The presence of longitudinal folds is a typical character of all genera of Bulimulidae considered (Fig. 4D).

44. Reproductive system, vagina, inner wall sculpture, pilasters: absents (0); presents (1). Pilasters are solids elements projecting from a wall, usually imitating the form of a column. Character state 1 is typical of genera of Odontostomidae examined (Illustrated in Pizá & Cazzaniga, 2010).
45. Reproductive system, vagina, inner wall sculpture, type of longitudinal folds: straight (0); in zigzag (1). The presence of straight or zigzag folds in the inner wall of vagina is a variable character among species of the same genus. Straight folds are the sculpture more frequent in *Bostryx* and a polymorphic character in *B. stelzneri* (Fig. 4D).
46. Reproductive system, vagina, inner wall sculpture, longitudinal folds or pilasters, disposition: in contact between them (0), no in contact between them (1). The state 0 is an autapomorphy of *P. daedaleus* (pilasters are in contact with a reticulate aspect). In other species included in the analysis, the folds or pilasters are parallel to each other (1) (Fig. 4D. State 0 illustrated in Pizá & Cazzaniga, 2010).
47. Reproductive system, vagina, length in relation to penis length: ¼ (0); ⅓ (1); ½ (2) (Fig. 4E).
48. Reproductive system, bursa copulatrix duct, length in relation to spermoviduct length: approximately equal or slightly longer (0), longer (1). The bursa copulatrix duct is considered equal or slightly longer (0) when their proximal end reach to distal portion of the albumen gland. In the state 1, proximal portion of bursa copulatrix duct can reach to half or proximal portion of the albumen gland as in *Bostryx strobeli* (Parodiz, 1956) or surpasses as in *P. daedaleus* (Fig. 5A).
49. Reproductive system, flagellum, length in relation to epiphallus length: minor (0); approximately equal (1); longer (2). The flagellum is present in all species examined, with cylindrical shape and variable longitude. The shorter flagellum is present in all *Bostryx* species as well as *D. poecilus* and *S. tucumanensis.* In *Bulimulus'* species examined is longer than epiphallus (Fig. 5B).
50. Reproductive system, flagellum, inner wall sculpture, arrangement of the folds: diagonal (0); longitudinal (1). Folds are defined just as was described for the vagina, in all following characters. According to Breure & Eskens (1978), longitudinal folds have a function in the formation of the spermatophore. Diagonal folds are presents in all Argentinian species of *Bostryx, Drymaeus poecilus, Bulimulus bonariensis* and *Bulimulus apodemetes* (Fig. 5C. Character 1 illustrated in Salas Oroño, 2007).
51. Reproductive system, flagellum, inner wall sculpture, longitudinal central fold: absent (0); present (1). The presence of a longitudinal, thin fold extending towards epiphallus, is a character typical of all Argentinian species of *Bostryx.* Also is present in *D. poecilus, Bulimulus bonariensis* and *Bulimulus apodemetes* (Fig. 5C).
52. Reproductive system, epiphallus, length in relation to penis length: shorter (0); approximately equal (1). According to Cuezzo (1997, 2006), the distinction between epiphallus and penis is based on the internal sculpture differences of these organs (Fig. 5D).
53. Reproductive system, epiphallus, diameter: uniform (0); proximal portion thinner than distal portion (1). In Argentinian species of *Bostryx* the epiphallus progressively increasing its width towards penis, with a thinner proximal portion and wider distal portion. In other species the shape of epiphallus is subcylindrical, with uniform diameter (Fig. 5E).
54. Reproductive system, epiphallus, external transition to penis: marked (0); gradual (1). This transition can be evidenced clearly by a change of shape as in the case of species of *Bulimulus, Naesiotus* and *Plagiodontes* (0). Two types of gradual transition are observed, in *S. tucumanensis* both penis as epiphallus have cylindrical shape due to which differences between them are not evident externally. In species of *Bostryx* both organs progressively increase its width towards their junction (Fig. 5B).
55. Reproductive system, penis, thinner middle section: absent (0); present (1). The state 0 is present only in *D. poecilus* and *Spixia tucumanensis.* In these species the penis has subcylindrical shape, with constant width in all it longitude (Fig. 5F. State 0 illustrated in Salas Oroño, 2007).
56. Reproductive system, penis, penis sheath: absent (0); present (1). In all species analyzed, the

penis sheath is muscular and thick, with a proximal border well defined. Typically in *Bostryx* the penis sheath is folded upon itself in its proximal portion, overlapping the distal portion of penis. The absence of penis sheath is an autapomorphy of *B. conspersus.* Breure (1978) described a penis sheath to several species of *Bostryx* from Peru and Chile (Fig. 5F. State 0 illustrated in Ramírez, R., 1988).

57. Reproductive system, penis, penis sheath, length in relation to total penis length: basal (0), overlapping ½ (1), overlapping ¾ (2). The basal penis sheath is characteristic of Odontostomidae. Within Bulimulidae, species of *Bulimulus* present a penis sheath overlapping at half of penis, whereas in *Naesiotus munsterii, Drymaeus poecilus* and all species of *Bostryx,* overlapping more than half of penis (Fig. 5G).
58. Reproductive system, penis, retractor muscle of penis sheath: absent (0), present (1). When present, a retractor muscle is inserting on upper end of penis sheath (proximal portion). The state 1 is shared by all Argentinian species of *Bostryx, B. bonariensis* and *B. gracilis* (Fig. 6A).
59. Reproductive system, penis, inner wall sculpture, number of areas of different sculpture in distal portion (covered by penis sheath): one (0); two (1). The existence of two types of sculptures in the inner wall of the distal portion of the penis is a character present in some species of *Bostryx* from Argentina. In *Bostryx roselleus* Miranda & Cuezzo, 2014 there are relaxed zigzag folds, followed by straight longitudinal folds area. In *B. stelzneri* there are straight or relaxed zigzag folds, followed by straight longitudinal folds area. Finally, in *Bostryx cordillerae* (Strobel, 1874) there are stightly in zigzag folds followed by straight longitudinal folds (Fig. 6B).
60. Reproductive system, penis, inner wall sculpture, type of sculpture in distal portion (covered by penis sheath): folds (0), pilasters (1). According to Gómez (2001), the folds and pilasters can function as stimulator or hold-fast surfaces during copulation. The presence of pilasters is an autapomorphy of *P. daedaleus* (Fig. 6B. State 1 illustrated in Pizá & Cazzaniga, 2010).
61. Reproductive system, penis, inner wall sculpture, number of areas of different sculpture in proximal portion (no covered by penis sheath): one (0); two (1); five (2). The presence of two types of inner sculpture in the proximal portion of penis is a character shared by all Argentinian species of *Bostryx.* A presence of five different types is an autapomorphy of *S. tucumanensis* (Fig. 6C. State 2 illustrated in Salas Oroño, 2007).
62. Reproductive system, penis, type of inner wall sculpture in proximal portion (no covered by penis sheath), folds: absents (0); presents (1). The inner sculpture of penis consisting in folds is typical of species of *Bostryx* (Fig. 6D).
63. Penis, inner wall sculpture in proximal portion (no covered by penis sheath), type of folds: straight (0); in zigzag (1). This character establishes difference among *Bostryx* species and is a polymorphic character in *B. scaber* and *B. stelzneri* (Fig. 6D).
64. Reproductive system, penis, inner wall without sculpture in proximal portion (no covered by penis sheath): absent (0); present (1). The presence of a portion of inner wall of penis without sculpture is observed only in Argentinian species of *Bostryx* (Fig. 6D).
65. Reproductive system, penis, inner wall sculpture, in proximal portion (no covered by penis sheath), pustules: absents (0); presents (1). Pustules are a sculpture blister-like shape. The presence of pustules is an autapomorphy of *S. tucumanensis* (Illustrated in Salas Oroño, 2007).
66. Reproductive system, penis, inner wall sculpture, in proximal portion (no covered by penis sheath), pilasters: absents (0); presents (1). Pilasters are defined just as was described for the vagina. According to Barker (2001), the pilasters on eversion of the penis function as stimulatory surfaces during courtship. The pilasters in the inner wall of penis are present in Odontostomidae and all species of Bulimulidae examined except *Bostryx* (Illustrated in Pizá & Cazzaniga, 2010).
67. Reproductive system, vas deferens in relation to penis sheath: insert (0), no insert (1). In species of Bulimulidae a vas deferens inserting at basal penis sheath and emerging at proximal portion of penis. In species of Odontostomidae a vas deferens passes under the penis sheath without inserted therein (Fig. 5F. State 1 illustrated in Salas Oroño, 2007).
68. Reproductive system, bursa copulatrix duct, inner wall sculpture, number of areas: one (0), two (1). The inner wall sculpture of the bursa copulatrix duct is very variable a species level. The state 0 predominate in the species examined (Fig. 6E).
69. Reproductive system, epiphallus, inner wall sculpture, number of areas: one (0), two (1). The internal folds of the epiphallus mould the

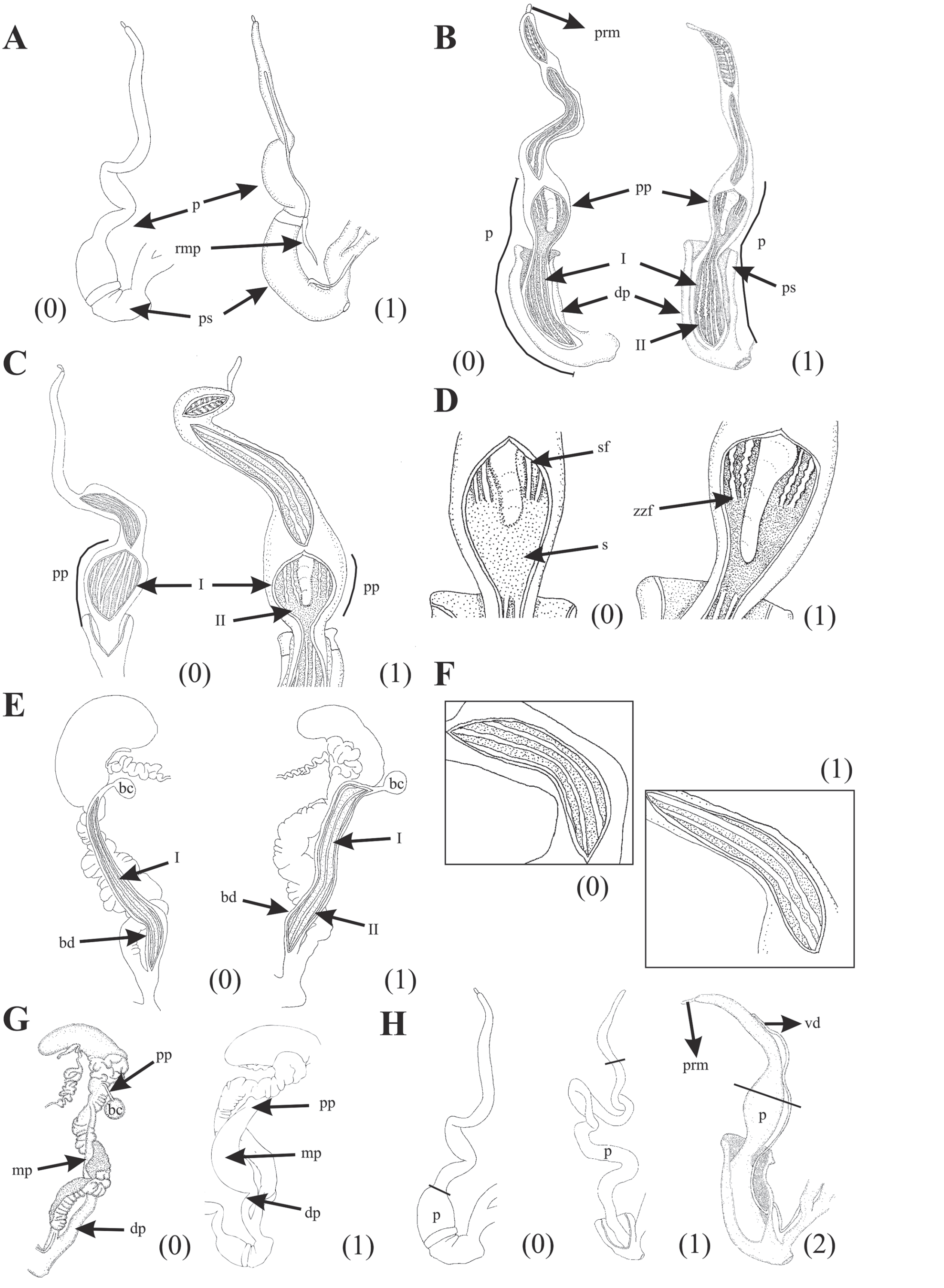

FIGURE 6: (A) Character 58. Penis sheath and retractor penis muscle (arrow). **(B)** Characters 59, 60. Inner wall sculpture in distal portion of the penis. **(C)** Character 61. Inner wall sculpture in proximal portion of the penis. **(D)** Characters 62-64. Inner wall sculpture in proximal portion of the penis. **(E)** Character 68. Inner wall sculpture in duct of bursa copulatrix. **(F)** Character 69. Inner wall sculpture in epiphallus. **(G)** Characters 71-73. Shape of bursa copulatrix duct in proximal, middle and distal portion. **(H)** Characters 70, 75-77. Shape of penis, position of penis retractor muscle and thickness and position of vas deferens. Abbreviations: **I:** number of area; **II:** number of area; **bc:** bursa copulatrix; **bd:** bursa copulatrix duct; **dp:** distal portion; **mp:** middle portion; **p:** penis; **pp:** proximal portion; **ps:** penis sheath; **prm:** penis retractor muscle; **s:** smooth inner wall; **sf:** straight folds; **vd:** vas deferent; **zzf:** zigzag folds.

spermatophores into species-specific structures (Barker, 2001). The presence of two types of sculpture in the inner wall of epiphallus is a typical character of species of *Bostryx* dissected out. In this genus, the change of shape externally visible of epiphallus is accompanied by a change of inner sculpture, from straight parallel folds in proximal (thinner) portion to zigzag folds in distal (wider) portion (Fig. 6F).

70. Reproductive system, penis, penis retractor muscle, location in flagellum: proximal portion (0); medium to distal portion (1). The position of the penis retractor muscle differs among Bulimulidae (0) and Odontostomidae (1) (Fig. 6H. State 1 illustrated in Salas Oroño, 2007).
71. Reproductive system, bursa copulatrix duct, distal portion: approximately equal or thinner than proximal portion (0); wider than proximal portion (1). This is an invariable character among Argentinian species of *Bostryx* (1), in contrast in Peruvian species both states can be present, as well as the outgroup (Fig. 6G).
72. Reproductive system, bursa copulatrix duct, middle portion in relation to distal portion: slightly thinner (0); wider (1); approximately equal (2). Character invariable among Argentinian species of *Bostryx* (0). In *Bulimulus apodemetes* is a polymorphic character (Fig. 6G).
73. Reproductive system, bursa copulatrix duct, proximal portion: no slimmed in binding with bursa copulatrix (0); slimmed in binding with bursa copulatrix (1). The state 0 is an autapomorphy of *P. daedaleus* (Fig. 6G. State 0 illustrated in Pizá & Cazzaniga, 2010).
74. Reproductive system, vagina, shape: cylindrical (0), notably wider in proximal portion respect to distal portion (1). The cylindrical shape of the vagina is found in all species of Bulimulidae examined (0) and differs from Odontostomidae (1) (Fig. 4E. State 1 illustrated in Pizá & Cazzaniga, 2010).
75. Reproductive system, penis, shape: clava (0); subcylindrical (1); cup (2). The shape of penis is a variable character among species. All Argentinian species of *Bostryx* have a penis with cup shape, as in *B. solutus.* Subcylindrical shape of penis is present in all other species of *Bostryx* from Peru considered in the present study. In other Peruvian species not included in this phylogeny, however the cup shape of the penis was observed too (Breure, 1978). In *Bulimulus apodemetes, B. gracilis* and *Plagiodontes,* the penis has clava shape, whereas subcylindrical shape is present in *Bulimulus bonariensis, Drymaeus poecilus* and *S. tucumanensis* (Fig. 6H).
76. Reproductive system, vas deferens in relation to penis retractor muscle: not adhered or related (0); adhered or related (1). In Bulimulidae' species examined, the penis retractor muscle is not in relation with vas deferens, contrary to Odontostomidae' species (Fig. 6H. State 1 illustrated in Pizá & Cazzaniga, 2010).
77. Reproductive system, penis retractor muscle, thickness: thick (0), thin (1). Thickness of penis retractor muscle is characteristic of each family examined: Odontostomidae (0), Bulimulidae (1) (Fig. 6H. State 1 illustrated in Pizá & Cazzaniga, 2010).

RESULTS

Phylogenetic analysis resulted in a single optimal tree (total fit = 47.40; score = 27.59) with a length of 603, 742 steps (Figs. 7, 8). Synapomorphies of each node of the phylogenetic hypothesis are in Figs. 7 and 8. Rooting the tree in *Drymaeus poecilus* (d'Orbigny, 1835) or *Naesiotus munsterii* (d'Orbigny, 1837) did not change the results in the ingroup. According to the optimal tree obtained, there are two main monophyletic clades; the first comprises *Plagiodontes daedaleus* (Deshayes, 1851) and *Spixia tucumanensis* (Parodiz, 1941) (Odontostomidae) (node 37) and the second contains all Bulimulidae species (node 34) (Fig. 7).

The genus *Bostryx* Troschel, 1847 resulted monophyletic (node 50) and the relationships among the species were fully resolved in the tree obtained. Node 50 is defined by 14 synapomorphies of the shell (characters 0, 1, 2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 18 and 30: 0) (Fig. 8). *Bostryx conspersus* (Sowerby, 1833) is the most basal species of the genus (node 50) and the sister species of the remaining *Bostryx* species. Node 47 includes all Argentine *Bostryx* species plus *Bostryx solutus* Troschel, 1847. Synapomorphies supporting this node are presence of axial sculpture on the protoconch (character 30:1), vagina length is ⅓ of penis length (character 47:1), and proximal portion of epiphallus is thinner than distal portion (character 53:1) and penis with cup shape (character 75:2). Two major clades can be distinguished: the first clade (node 54) is integrated by *Bostryx willinki* Weyrauch, 1964, *B. reedi* (Parodiz, 1947), *B. tortoranus* (Doering, 1879), *B. stelzneri* (Dohrn, 1875) and *B. roselleus* Miranda & Cuezzo, 2014 (Fig. 8). The monophyly of this clade (node 54) is sustained by only one synapomorphy: the epiphallus and the penis have about the same length (character 52:1). The second subclade (node 45) is composed of *Bostryx peristomatus* (Doering, 1879), *B. catamarcanus* (Parodiz, 1956), *B. strobeli* (Parodiz, 1956), *B. mendozanus* (Strobel, 1874), *B. scaber* (Parodiz, 1948), *B. birabenorum* Weyrauch, 1965, *B. cordillerae* (Strobel, 1874), *B. martinezi* (Hylton Scott, 1965), *B. pastorei* (Holmberg, 1912), *B. famatinus* (Doering, 1879), *B. rudisculptus* (Parodiz, 1956), *B. solutus, B. cuyanus* (Pfeiffer, 1867) and *B. costellatus* (Hylton Scott, 1971) (Fig. 8). This subclade (node 45) is also based on a single synapomorphy: the proximal portion of the penis inner wall has straight folds (63:0). Four sister clades were obtained: *Bostryx willinki+B. reedi* (node 60, supported by characters 1, 2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 19:1 and 25:1); *B. stelzneri+B. roselleus* (node 52, character 59:1); *B. scaber+B. birabenorum* (node 55, characters 19:1, 20:1 and 22:1) and *B. cuyanus+B. costellatus* (node 59, characters 9, 10, 15 and 28:0) (Fig. 8).

Bostryx peristomatus, B. roselleus, B. scaber and *B. stelzneri* that comprise the *Bostryx stelzneri* species complex were located in different clades. *Bostryx roselleus* and *B. stelzneri* resulted to be sister species (node 52). *Bostryx peristomatus* occupies the most basal position within the clade integrated by *B. catamarcanus, B. strobeli, B. mendozanus, B. scaber, B. birabenorum, B. cordillerae, B. martinezi, B. pastorei, B. famatinus, B. rudisculptus, B. solutus, B. cuyanus* and *B. costellatus* (node 45). *Bostryx scaber* turned out to be the sister species of *B. birabenorum* (node 55). With respect to the *B. tortoranus* species complex (composed of *B. martinezi, B. rudisculptus* and *B. tortoranus*), *B. tortoranus* (node 53) was located in another clade than *B. martinezi* (node 42) and *B. rudisculptus* (node 39) (Fig. 8).

The measures of support (absolute and relative Bremer support and frequency differences) were low across the entire topology of the tree obtained (Figs. 7, 8).

DISCUSSION

The first cladistics study and the first hypothesis regarding a relationship between Argentinian species of the genus *Bostryx* Troschel, 1847 was proposed using shell and anatomical characters. While it is common that taxa with high proportion of missing data cells are excluded to phylogenetic analysis, this study includes 20 species (38% of the total) with these characteristics. These taxa however offer the potential to address and resolve the relationships of all of the taxa of interest (Wiens, 2003). According to Wiens (2003) the solution to the problem of including highly incomplete taxa is increasing the number of characters scored. For this reason the variations observed in shell characters were expressed mainly as continuous changes in size as was proposed by Wiens (2001) and González-José *et al.* (2008). The Argentinian and Peruvian species of *Bostryx* resulted to be a monophyletic clade. The synapomorphies supporting the clade mainly corresponded to continuous characters such as length, perimeter and area of the shell. According to the results obtained, *Bostryx* has smaller dimensions compared with other genera of Bulimulidae regarding height, diameter, perimeter and area of the shell and parts of the shell.

The absence of sculpture in the protoconch has traditionally been considered as a character of high taxonomic value and it has been widely used to characterise this genus (Pilsbry, 1895-1896, 1901-1902;

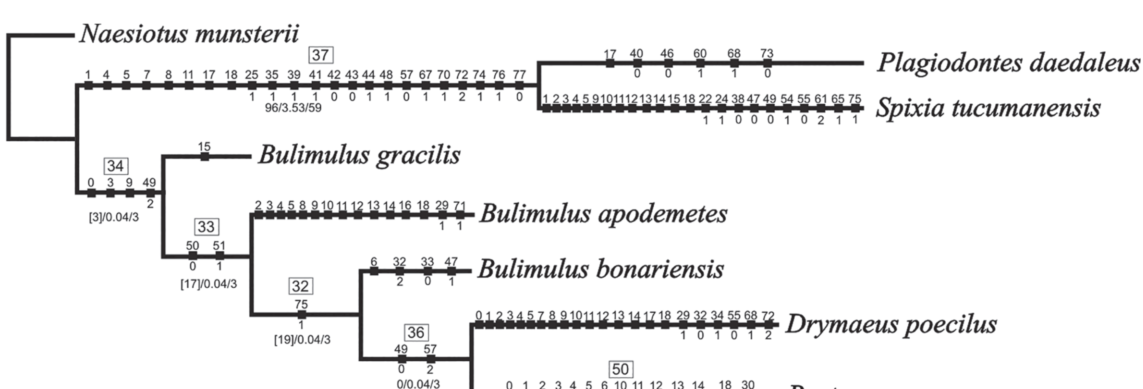

FIGURE 7: Cladogram showing relationships of the *Bostryx* with Odontostomidae and remaining Bulimulidae. Numbers within empty squares are node numbers. Symmetric Resampling support (left), Absolute (middle) and Relative (right) Bremer support scores are the numbers in below nodes. Black squares are synapomorphies.

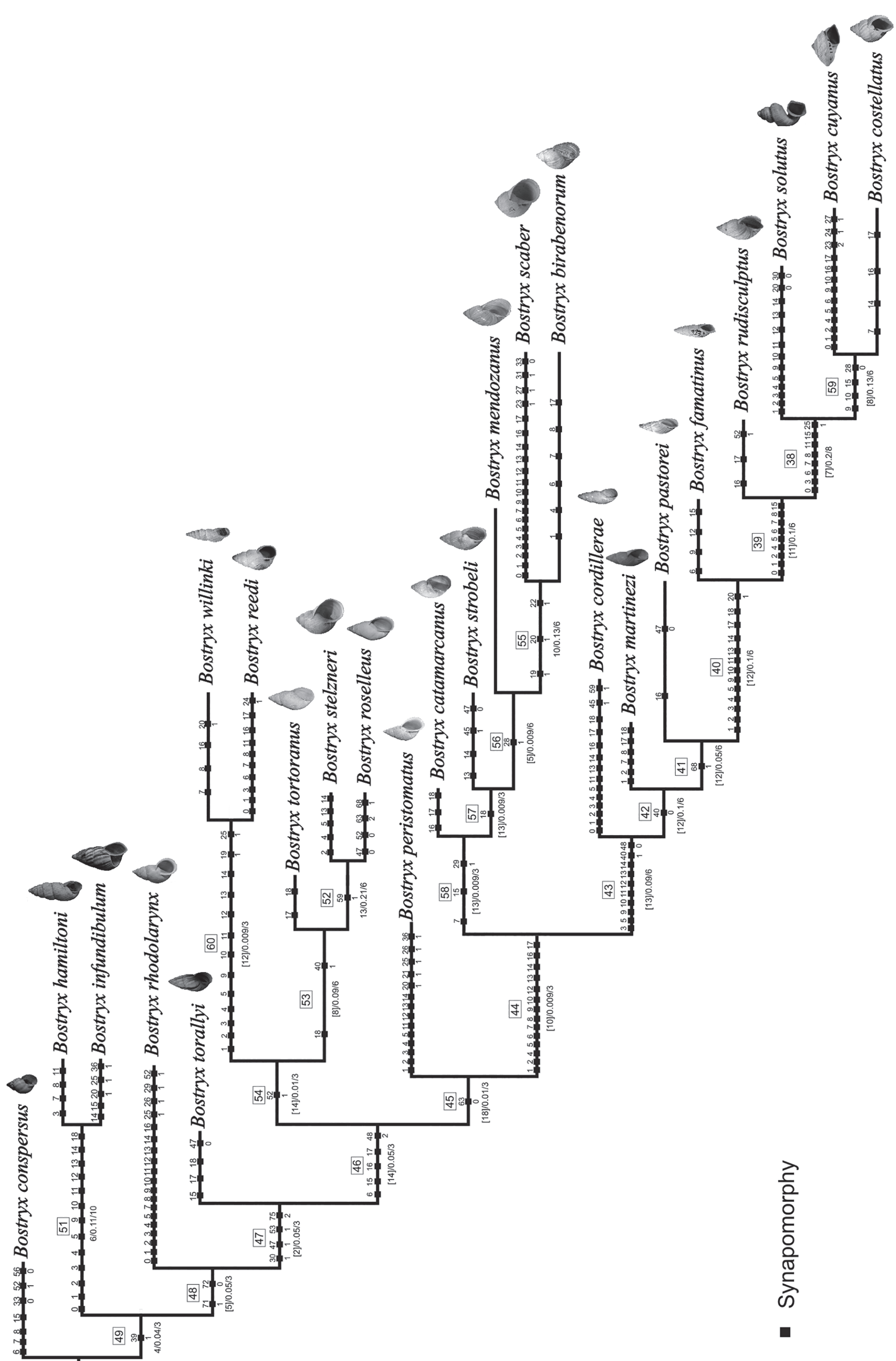

FIGURE 8: Optimal tree obtained from the matrix under implied weighting. Numbers within empty squares are node numbers. Symmetric Resampling support (left), Absolute (middle) and Relative (right) Bremer support scores are the numbers in below nodes. Black squares are synapomorphies.

Parodiz, 1946). However, based on observations of species from Peru, Ecuador, Chile and Argentina, some years later Weyrauch (1958, 1964, 1965, 1967) proposed that the sculpture of the protoconch within *Bostryx* is variable: it can be smooth or have spiral lines or even present spiral lines plus axial folds. This variability present in the genus was also observed by Breure (1979). The present cladistic analysis establishes that the absence of axial sculptures in the protoconch (character 30:0, apomorphic) is a synapomorphy of the genus. Respect to spiral sculpture, in all species examined, except for *Bostryx conspersus* (Sowerby, 1833), spiral lines were examined in the protoconch.

The Argentinian *Bostryx* species have been grouped as *Bostryx solutus* Troschel, 1847, the type species of the genus (node 47). This clade is supported by the following synapomorphies: presence of axial sculpture in the protoconch (character 30:1, which reverts to a plesiomorphic state in this clade); vagina of ⅓ of the penis length (character 47:1); proximal and distal portion of the epiphallus with different diameter (character 53:1) and penis with cup shape (character 75:2). Characters such as two types of inner wall sculptures in the epiphallus (character 69:1), two areas with different sculptures in the proximal portion of the penis (character 61:1) with folds (character 62:1) and area without sculpture (character 64:1) are shared only among species of node 47 (Argentinian species plus *B. solutus*). While these characters have a lot of taxonomic importance none of them becomes a synapomorphy of the clade.

The phylogenetic results obtained show that the *Bostryx stelzneri* (Dohrn, 1875) and *Bostryx tortoranus* (Doering, 1879) species complexes were not monophyletic, which supports the statement by Cuezzo *et al.* (2013), Miranda & Cuezzo (2014) and Miranda (2015).

The low support for the clades obtained from three support measurements is remarkable. According to Goloboff *et al.* (2003a), support values obtained with resampling methods and Bremer indices depend on the analysis and only serve to compare the support of one node with that of others. *I.e.,* they do not indicate the probability that a node is correct. The values of support obtained show that favourable evidence for a node is less dominant than adverse evidence.

Although molecular data are widely used in phylogenetic studies, they are oriented to obtain phylogenetic trees and should not be interpreted for the origin and diversification of complex characters, or to find out homologies and synapomorphies of the groups (Wheeler, 2008). Frequently in land snails the relationships between species stablished with molecular phylogenies are different from previous hypothesis based on morphological characters (Ketmaier *et al.,* 2006; Wade *et al.,* 2007; Stankowski & Johnson, 2014). In a similar manner, the results presented here do not agree with previously published phylogenetic hypothesis. Breure & Romero (2012) employed six of the 31 species included in the present study for their molecular phylogeny. The authors proposed that *Bostryx* could be a polyphyletic genus. *Bulimulus sporadicus* (Rafinesque, 1833) (= *B. bonariensis*), *Bostryx apodemetes* (d'Orbigny, 1835) (here considered as *Bulimulus apodemetes* based on anatomical characters) and *Bostryx strobeli* (Parodiz, 1956) belong to Bulimulinae, whereas *Bostryx torallyi* (d'Orbigny, 1835) and *B. solutus* belong to Bostrycinae. Molecular and morphological phylogenies group *B. torallyi* and *B. solutus,* but not *B. strobeli,* which is grouped with *Bulimulus* Leach, 1814 species using molecular phylogeny and with *Bostryx* species applying morphological phylogeny. Morphological observations revealed clear differences between *B. strobeli* and *Bulimulus* species, especially in the phallic complex. According to the synapomorphies obtained in the present study, the protoconch of the genus is not considered smooth as mentioned before and hence the long penis sheath is not a synapomorphy of *Bostryx* and can also be found in *N. munsterii* (d'Orbigny, 1837) and *D. poecilus* (d'Orbigny, 1835), closely related species. Consequently, the present cladistic analysis does not support the creation of Bostrycinae as a monophyletic group as previously defined by Breure (2012).

In the molecular phylogeny proposed by Ramírez, J. & Ramírez, R. (2013), who employed 4 species [*B. solutus, B. conspersus, B. strobeli* and *B. bonariensis* (= *B. sporadicus*)] that were also used in the present analysis, the genus *Bostryx* resulted polyphyletic. The authors proposed that only the clade composed of *B. solutus* and other *Bostryx* species like *B. conspersus* should be considered as *Bostryx.* In addition, they suggested that further studies are necessary to establish the position of other species traditionally classified as *Bostryx* such as *B. strobeli.*

Traditionally there has been a debate about the use of shell characters in phylogenetic analysis. It has been proposed that shell characters are less important to establish phylogenetic relationships because their morphology is plastic in response to environmental factors and they are targets of intense selection pressure (Breure, 1979; Kool, 1993). In contrast, Schander & Sundberg (2001) and Collin (2003) concluded that there is no reason to exclude shell characters because they would be phylogenetically misleading factors. Similarly, Smith & Hendricks (2013) proposed that while the shell characters are more homoplasious than anatomical characters, they could still contribute to develop a phylogenetic hypothesis. It should be emphasised that the shell characters in the present morphological hypothesis provided valuable information; especially continuous characters generated from analysis of the shell shape. Continuous characters contributed efficiently to infer the phylogenetic hypothesis and to resolve the relationships between *Bostryx* species. Whereas in land snails the majority of species are recognized based on differences in genital characters, compared to other orthalicoidean species, the genital system of the Argentinian *Bostryx* species is simple and has high similarity between their species. The Peruvian species studied in change show a more variable anatomy, however characters of inner wall sculpture of the reproductive system, important to Argentinian species recognition, could not be analysed due to the lack of soft parts for dissection. In the phylogeny performed, the anatomical characters resulted important to establish differences with other genera. Based on observations of the specimens, the character of taxonomic importance in the species of Bulimulidae considered is the inner wall sculpture of the phallic complex whereas diagnosis of shell characters is not unequivocal. However, the results conclude that a combination of both characters, morphology of the shell and internal anatomy, is necessary to obtain a well-resolved morphological hypothesis.

Further analyses are needed to confirm monophyly of the genus, including more species from other parts of the world.

CONCLUSIONS

— Based on the present analysis with cladistic methodology, *Bostryx* is a monophyletic genus and the monophily is supported by shell synapomorphies.
— Argentinian *Bostryx* species and *Bostryx solutus* Troschel, 1847, the type species of the genus, conformed a monophyletic clade supported by shell and genital characters.
— Shell continuous characters contributed efficiently to infer the phylogenetic hypothesis and to resolve the relationships between *Bostryx* species. Genital characters, especially concerning the phallic complex, have been found to be the most informative to establish phylogenetic relationships among the species of Bulimulidae examined. A combination of morphology of the shell and internal anatomy is necessary to obtain a well-resolved morphological hypothesis.
— The morphological evidence analyzed here does not include all the anatomical variation present in the genus *Bostryx*. More contributions in this area are still possible and further analyses are needed to confirm monophyly of the genus.

RESUMEN

El género Bostryx *Troschel, 1847 es encontrado exclusivamente en Sudamérica, desde Ecuador hasta Chile, principalmente en relación a la cordillera de los Andes. La filogenia de* Bostryx *está basada en caracteres morfológicos de 24 especies de este género, 19 de las cuales correspoden a especies de Argentina, 5 son procedentes de Perú, además de 7 especies representantes de otros géneros, perenecientes a Bulimulidae y Odontostomidae. Una matriz de 31 taxa y 78 caracteres morfológicos (19 de los cuales son continuos y 59 son discretos) de conchilla, sistemas paleal y reproductor, fue analizada bajo pesos implicados con TNT. Las especies argentinas y peruanas del género se agruparon en un clado monofilético soportado por 14 caracteres de conchilla, los cuales son principalmente caracteres continuos de forma de la conchilla.*

Palabras-Clave: Bulimulidae; TNT; Pesos implicados; Caracteres continuos; Forma de la conchilla.

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