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New distributional records of the exotic land snail *Bradybaena similaris* (Férussac, 1822) (Gastropoda, Bradybaenidae) in Argentina

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Abstract

Bradybaena similaris (Férussac, 1822) is an invasive land snail species native to East Asia, which is widely distributed in South America, including Argentina, Brazil, Colombia, Uruguay and Paraguay. In Argentina, very few previous records coming from the Tucumán and Misiones Provinces are available for the species. In this work, we report new occurrences of *B. similaris* in the country from Misiones Province, and include the southernmost record for the species in South America from Entre Ríos Province. We used shell morphology, morphological features of the reproductive system and radulae, and DNA sequences from 16S-rRNA gene for species-specific identification. These new distribution data reaffirm the need to monitor the southern dispersion of invasive snails in South America, especially those causing damage to several crop species.

Key words: distribution map, garden snail, intermediate host, invasive species, mitochondrial gene, South America, urban and rural habitats

Introduction

Bradybaena similaris (Férussac, 1822) is a land snail native to eastern Asia (Chang 2002), but has spread to all continents, except Antarctica, mainly in the tropical and subtropical areas (Araujo 1989; Picoral and Thomé 1989; De Winter et al. 2009; Ohlweiler et al. 2010; Capinera and White 2011). Like other specimens of the Bradybaenidae family this species comprises small to medium-sized heliciform snails (up to 15 mm in diameter), with different shell coloration patterns (Komai and Emura 1955; De Winter et al. 2009; Ohlweiler et al. 2010). Some *B. similaris* specimens may bear a narrow chestnut band on the perimeter of the whorl, with four

morphotypes characterized based on band and background pigmentation: unbanded yellowish, banded yellowish, unbanded brownish and banded brownish (Komai and Emura 1955; Neck 1976; Capinera and White 2011). *Bradybaena similaris* is nocturnal, usually inhabiting garden plants and herbaceous grounds covered with organic debris (Junqueira et al. 2003; Ohlweiler et al. 2010). The species has polyphagous habits, feeding on a wide variety of plants, and is known to cause damage to several crop species (e.g., citrus, mango, grapevines, cassava), being especially harmful to ornamental plants (Dundee and Cancienne 1978; Pena and Waddill 1982; Chang 2002; Robinson and Hollingsworth 2009; Herbert 2010; Matamoros 2014). The species typically occurs in urban and suburban areas, where it sometimes becomes dominant (Capinera and White 2011; Naranjo-García and Castillo-Rodríguez 2017). In relation to animal and human health, *B. similaris* can serve as intermediate host of the cecal fluke of poultry *Postharmostomum gallinum* Witenberg, 1923, and the pancreatic fluke of bovine, caprine and porcine animals, *Eurytrema coelomaticum* (Giard and Billet, 1892) (Amato and Bezerra 1989; Araujo 1989). Additionally, individuals of *B. similaris* have been recorded as the intermediate hosts of nematodes such as *Angiostrongylus costaricensis* Morera and Céspedes, 1971, the cause of human abdominal angiostrongyliasis, and the rat lungworm, *A. cantonesis* (Chen, 1935), which is a major cause of eosinophilic meningitis in humans (Caldeira et al. 2007; Ohlweiler et al. 2010; Cowie 2013).

In South America, an increase in the records of invasive mollusk species has been documented in recent decades, as the result from a variety of causes, ranging from deliberate introductions for food to inadvertent introductions in association with imported commodities (Cowie and Robinson 2003; Campos and Calvo 2006; Vogler et al. 2013; Letelier et al. 2014). For Argentina, about 50 exotic mollusk species have been recorded; over 20 of which are terrestrial gastropod species, including *B. similaris* (Rumi et al. 2010; Gutiérrez Gregoric et al. 2013a, b; Virgillito and Miquel 2013; Ferreira et al. 2017; Beltramino et al. 2018). *Bradybaena similaris* was reported for the first time in the Americas by d'Orbigny (1835) on the Brazilian coast (d'Orbigny 1835, 1838), and it was recorded in Argentina for the first time in Buenos Aires city by Doering (1875), although the species did not become established (Miquel et al. 2007; Virgillito and Miquel 2013). Almost a century later, the species was reintroduced in northeastern Argentina, a fact that went unnoticed for decades because of a taxonomic misidentification (Miquel et al. 2007). To date, few records of *B. similaris* have been reported in Argentina from the Tucumán and Misiones Provinces (Drahg 1999; Gutiérrez Gregoric et al. 2013a, b; Virgillito and Miquel 2013).

Although *B. similaris* has become widespread throughout several South American countries including Argentina, Brazil, Colombia, Uruguay and Paraguay (Quintana 1982; Hausdorf 2002; Scarabino 2003; Agudo-Padrón

2008; Ohlweiler et al. 2010; Virgillito and Miquel 2013), at the present time only two DNA sequences are available in GenBank for representatives from the Americas, which includes partial DNA sequences from Brazil for the mitochondrial 16S-rRNA gene and the nuclear 28S-rRNA locus (Sei et al. 2017). In this study, we report nine new records for *B. similaris* in Argentina, eight from Misiones Province and the first one from Entre Ríos Province, which also constitutes the southernmost record for the species in South America. Newly generated 16S-rRNA sequences for *B. similaris* presented here represent the first molecular data available for Argentina and are expected to contribute to further genetic reconstructions aimed to elucidate invasion pathways of this snail in South America.

Materials and methods

Individuals of *Bradybaena similaris* were collected across the Misiones Province as part of mollusk surveys conducted in urban and rural areas, including residential gardens, waterfalls and agricultural plantations. In addition, one location in Entre Ríos Province was specifically surveyed based on an unconfirmed record of *B. similaris* (N. R. Meichtry de Zaburlín, *personal communication*). Geographic coordinates were recorded with GPS Garmin eTrex Legend®. Specimens were captured by hand, photographed, relaxed in water with menthol crystals for 4–10 h, and subsequently immersed in hot water (80 °C). Voucher specimens were preserved in 96% ethanol. A small piece of muscular tissue was taken from representatives of each lot for further genetic analyses. Voucher specimens were deposited at the malacological collection at the Instituto de Biología Subtropical (IBS-Ma), UNaM-CONICET, Misiones Province, Argentina.

Specimens were firstly identified based on external shell morphology following Ohlweiler et al. (2010). Given that shell morphology of *Bradybaena similaris* is very similar to that of other members of the genus *Bradybaena*, e.g., *B. pellucida* Kuroda and Habe, 1953, *B. transbaicalia* (Schileyko, 1978) (Seki et al. 2008; Hirano et al. 2014; Snegin et al. 2017), we used morphological characteristics of the reproductive system and radulae, and DNA sequences for species-specific identification. We examined the genital anatomy from one specimen from each of the new localities (voucher specimens: IBS-Ma Nos. 96/1, 102/2, 122/3, 142/1, 165/7, 242/2, 243/1, 247/1, 253/3) following Emura (1932), Araujo (1989), and Picoral and Thomé (1989). Specimens were dissected using a Labomed Luxeo 4D stereomicroscope. The radulae were separated from the buccal mass and cleaned following the non-destructive method described by Holznagel (1998). Radular features were examined with a scanning electron microscope (SEM-JEOL 6360) in the Museum of La Plata, Argentina.

Genomic DNA was extracted from muscle tissue of the same anatomically dissected specimens using a cetyltrimethylammonium bromide protocol (Beltramino et al. 2018). Partial sequences of the mitochondrial 16S-rRNA

marker were amplified by polymerase chain reaction (PCR) through the use of the primers 16SF-104 and 16SR-472 (Ramirez and Ramírez 2010). The PCR reaction was performed in a total volume of 30 µl containing 30–50 ng of template DNA, each primer at 0.2 µM, 1× reaction buffer, 2 mM $MgCl_2$, 200 µM dNTPs and 1.25 U *Taq* Pegasus DNA polymerase (Productos Bio-Lógicos, Argentina, #EA0101). Amplification was performed in a T18 thermocycler (Ivema Desarrollos) as follows: 35 cycles of 30 sec at 94 °C, 30 sec at 48 °C, 1 min at 72 °C; followed by a final extension at 72 °C for 1 min. Successful PCR reactions were verified by agarose gel electrophoresis, purified using an AccuPrep PCR Purification Kit (Bioneer, Korea), and bidirectionally sequenced by Macrogen Inc. (Seoul, Korea). Sequences were trimmed to remove the primers and assembled by means of the BIOEDIT 7.2.5 software (Hall 1999). For molecular confirmation of the species, all newly generated DNA sequences were compared with those available in the GenBank database using the BLASTN algorithm (Altschul et al. 1990). DNA sequences were deposited in GenBank under accession numbers MH428039 to MH428047.

Results

A total of 168 living specimens and 174 empty shells were collected at eight localities in the Misiones Province and at Villaguay city in the Entre Ríos Province (Figure 1, Supplementary material Table S1). All Argentinian specimens appear to be referable to the banded and unbanded yellowish morphotypes of *Bradybaena similaris* (Figure 2). Anatomically, the specimens exhibited the morphological characters of the reproductive system defined for *B. similaris* as described by Emura (1932), Araujo (1989), and Picoral and Thomé (1989). The following distinctive characters are emphasized (Figure 3A–D): ovotestis with at least six distinct lobes. Hermaphroditic duct nearly straight towards proximal and distal portions; seminal vesicle long, convoluted, at the middle portion of hermaphroditic duct. Distal hermaphroditic duct ending in a small fertilization pouch-spermathecal complex at the end of albumen gland (Figure 3C); fertilization pouch-spermathecal complex thin, finger-shaped. Albumen gland thick, long, curved. Spermoviduct long, consisting of uterus and prostate gland (Figure 3A, B); length of prostate not corresponding to that of uterus. Penis cylindrical, long, slightly curved. Penial sheath short, thickened, at distal end of penis. Major portion of penial internal surface covered by branched and largely crenulated longitudinal pilasters, anastomosing into a few major pilasters near the atrium (Figure 3D). Epiphallus shorter than penis, a little longer than penial sheath; slight constriction separating epiphallus from penis; inner epiphallus wall with rhomboidal pustules and crenulated thin pilasters (Figure 3D). Penial retractor muscle ribbon-shaped, inserting at proximal end of epiphallus. Vas deferens thin, long. Bursa copulatrix sacculiform.

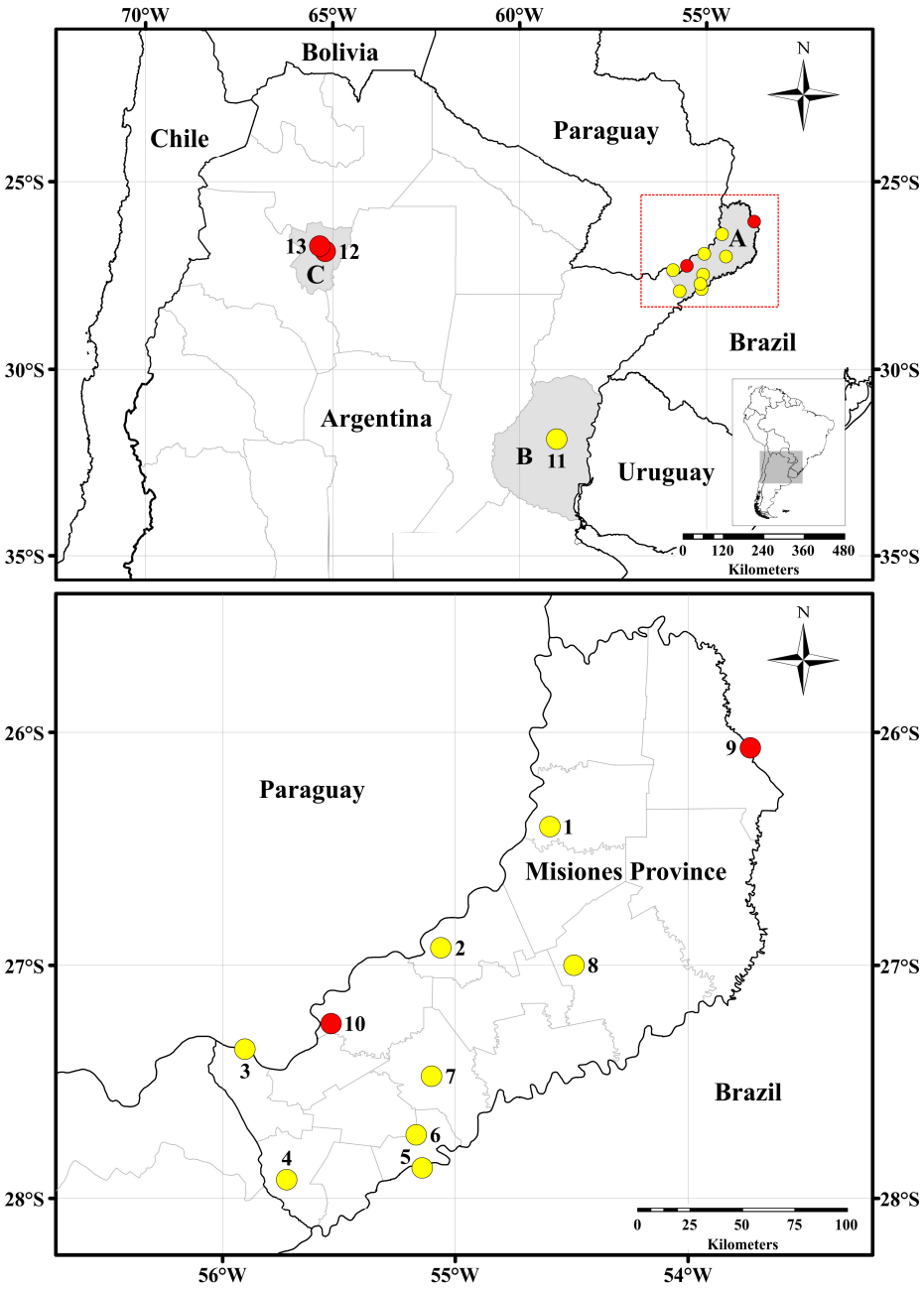

Figure 1. Distribution of the exotic land snail *Bradybaena similaris* in Argentina (upper map), and detailed distribution of the species in Misiones Province (lower map). Locations in red correspond to literature records and the yellow circles indicate the new localities where the specimens of *B. similaris* were found (A: Misiones Province, B: Entre Ríos Province, C: Tucumán Province). Location numbers correspond to the numbers in Supplementary material Table S1.

Bursa copulatrix duct long, inserting at junction of free oviduct and vagina (Figure 3B). Free oviduct medium in length, extending from spermoviduct to vagina; proximal portion with inner sculpture consisting of thick anastomosing pilasters; distal portion with thin longitudinal pilasters on inner wall, parallel to each other (Figure 3B). Vagina short, cylindrical; inner wall with thin longitudinal pilasters extending from the distal portion of free oviduct (Figure 3B). Dart sac well developed, elongated oval. No accessory dart sac present. Two mucous glands inserting laterally on dart sac; both glands with multiple, long, thick branches; one of the glands

Figure 2. Yellow morphotypes of *Bradybaena similaris* from Misiones Province, Argentina. A, unbanded (San Vicente, Misiones, IBS-Ma 096/5). B, banded (San Javier, Misiones, IBS-Ma 242/4). Shell views, shown from left to right: apertural, dorsal-lateral, apical, and umbilical. Photographs by A.A. Beltramino.

dichotomizing in all individuals analyzed. Penis, vagina and dart sac inserting independently in a genital atrium.

The examined radulae of *Bradybaena similaris* exhibited the following features (Figure 3E–G): radular teeth arranged in almost straight lines, with 125–146 rows, and 32–35 teeth per half-row (n = 4). Radular formula: 20–16 + 5–4 + 11 + C + 11 + 4–5 + 16–20. Central tooth somewhat smaller than the first laterals; central tooth basal plate symmetrical, trapezoidal, with raised lateral basal extensions at a sharp angle (almost a right angle) on both sides (Figure 3E). Mesocone triangular, pointed, generally exceeding 80% of the height of tooth base; cusp base rounded, indented medially, of about 80% of tooth base width; mesocone flanked by a short ectocone on either side, recessed back on tooth (Figure 3E). Lateral teeth tricuspid, asymmetrical; lateral tooth basal plate with a raised basal extension on the outer edge; cusp base rounded, larger than that of central tooth, regularly indented; mesocone triangular elongate; endocone very tiny; ectocone pointed, triangular, recessed back on tooth (Figure 3E, F). Transition between lateral and marginal teeth not clearly defined (Figure 3F). Marginal teeth tricuspid; mesocone large, triangular, with cusp somewhat rounded; endocone small, close to apex of mesocone, with a triangular blunt cusp, becoming prominent to outermost marginals; ectocone located basally, triangular, somewhat pointed, frequently split into bifid or even trifid denticles towards outer edge (Figure 3G). From innermost marginal teeth to the outer edge, teeth progressively smaller with basal plates shorter; outermost marginals bearing larger endocones; rows more widely spaced (Figure 3F, G).

Partial 16S-rRNA sequences consisted of 266 base pairs (bp) for all individuals, and a single novel haplotype was identified in all analyzed samples. The identification of the specimens by DNA-data analysis through

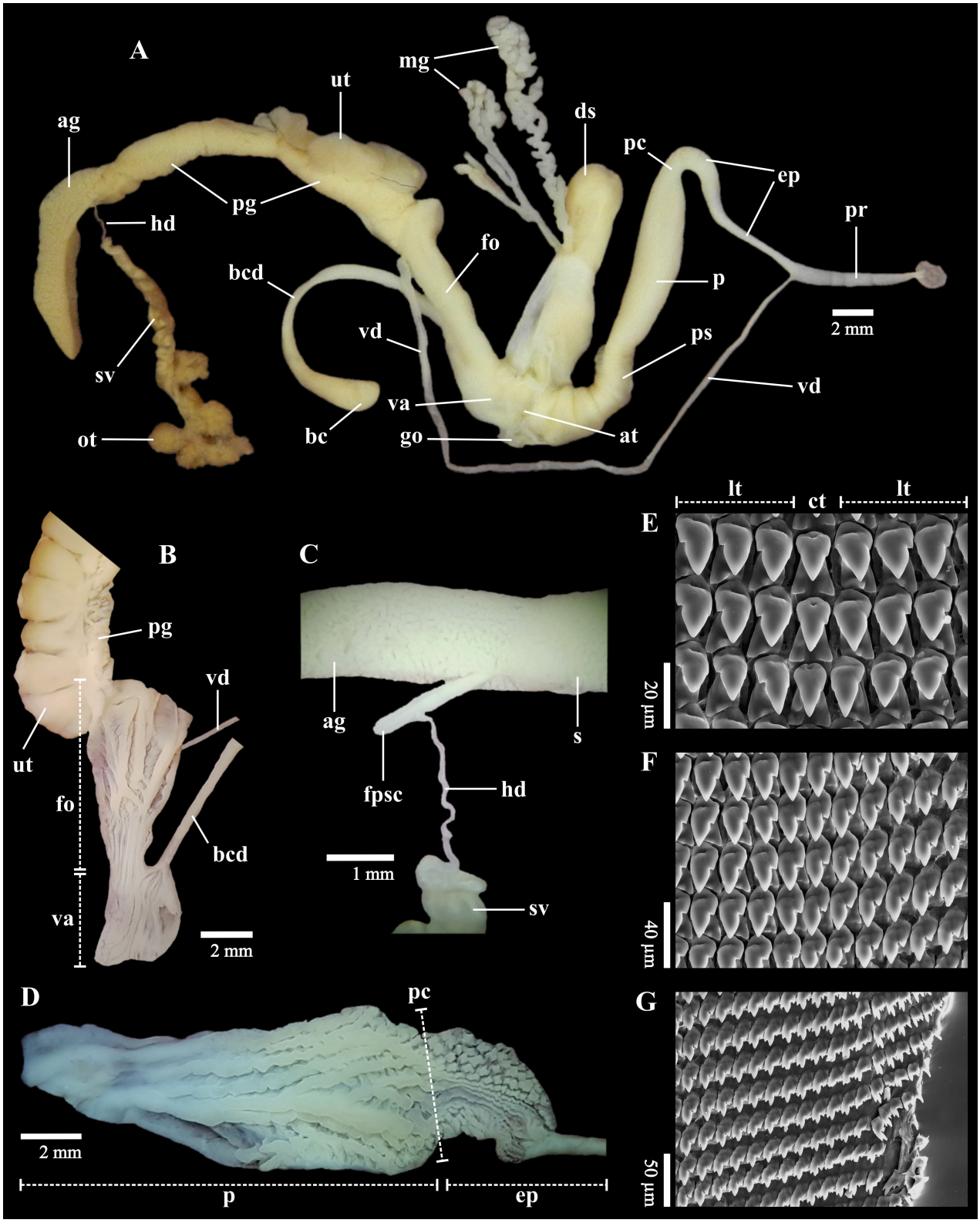

Figure 3. *Bradybaena similaris* from Argentina, reproductive system (A–D) and scanning electron micrographs of radula (E–G). A, general view of the reproductive system. B, detail of inner surfaces of vagina and free oviduct. C, detail of the fertilization pouch-spermathecal complex. D, inner sculpture of epiphallus-penis wall. E, detail showing central and first lateral teeth. F, transition between lateral and marginal teeth. G, marginal teeth. Abbreviations: ag, albumen gland; at, atrium; bc, bursa copulatrix; bcd, bursa copulatrix duct; ct, central teeth; ds, dart sac; ep, epiphallus; fo, free oviduct; fpsc, fertilization pouch-spermathecal complex; go, genital opening; hd, hermaphroditic duct; lt, lateral teeth; mg, mucous glands; ot, ovotestis; p, penis; pc, penial constriction; pg, prostate gland; pr, penial retractor muscle; ps, penial sheath; s, spermoviduct; sv, seminal vesicle; ut, uterus; va, vagina; vd, vas deferens. Photomicrographs by A.A. Beltramino (A–D) and A. Rumi (E–G).

Table 1. Top five BLASTN search results for the 16S-rRNA haplotype of *Bradybaena similaris* from Argentina.

Most significant alignment	GenBank #	Score	E-value	Coverage (%)	Identity (%)	Geographical origin	References
Bradybaena similaris	GQ851001	462	2e-126	100	98	Brisbane, Queensland, Australia	Hugall and Stanisic (2011)
Bradybaena similaris	GQ851164	459	2e-125	100	98	Mount Kinabalu, Borneo, Malaysia	Hugall and Stanisic (2011)
Bradybaena similaris	HQ245444	459	2e-125	100	98	–	Köhler and Criscione (2013)
Bradybaena similaris	KF247037	450	1e-122	100	98	Rio Grande do Sul, Brazil	Sei et al. (2017)
Bradybaena sequiniana (Heude, 1885)	KU586458	236	5e-58	100	80	Badong, Hubei, China	Ding et al. (2016)

the BLASTN algorithm confirmed their specific identity as *B. similaris*, given that the search results exhibited top-ranking scores (100% coverage, 98% similarity) with the 16S-rRNA sequences available for the species in the GenBank database (Table 1).

Discussion

In this work, we report new occurrences of *Bradybaena similaris* in Argentina including eight location records from Misiones Province. Previous records of *B. similaris* were limited to two locations in Tucumán Province, northwestern Argentina (i.e., San Miguel de Tucumán and Tafí Viejo; Drahg 1999; Virgillito 2012; Virgillito and Miquel 2013), and two separate sites in Misiones Province, northeastern Argentina (i.e., San Ignacio and San Antonio; Miquel et al. 2007; Gutiérrez Gregoric et al. 2013b; Virgillito and Miquel 2013). The new records in the Misiones Province are distributed in the south and central regions of the province, covering a distance of about 200 km. This suggests the species is established in northeastern Argentina and implies that *B. similaris* is becoming widespread. This distribution pattern may reflect a historical dispersal of the species over the last 60 years, which went unnoticed due to the lack of sampling effort across the study region. However, this scenario seems highly unlikely since the Misiones Province is one of the most explored areas at the malacological level in Argentina (Rumi et al. 2006; Núñez et al. 2010; Gutiérrez Gregoric et al. 2013b). An alternative explanation could be linked to a range expansion in recent years, probably together with plants, as a direct result of an increased movement of humans and intense commercial trade (Beltramino et al. 2018). This hypothesis seems to be more plausible but needs further investigation.

Furthermore, the presence of this species in the Entre Ríos Province is reported here for the first time. To our knowledge, that location corresponds to the southernmost record in South America, which suggests that the southern distribution area of the species would be larger than previously thought. The record in Villaguay, Entre Ríos Province, is located in a temperate region at about 350 km southwest of the nearest and previous southernmost record from Rivera city in Uruguay, extending the austral

distribution range of *B. similaris* over approximately one degree of latitude from 30°S to 31°S (Scarabino 2003; Campos and Calvo 2006). To our understanding, the southernmost records of the species in other southern hemisphere continents are limited to subtropical regions, such as Durban, South Africa (Herbert 2010), and Brisbane, Australia (Hugall and Stanisic 2011). These environmental conditions are similar to those found in the Misiones Province, which is characterized by a subtropical climate. In this scenario, further fieldwork is required to assess the distribution area and southern spread of *B. similaris* in Argentina, as it seems probable that temperate regions may contain the minimal environmental conditions able to support an invasion of the species, which was shown to be greatly resistant to adverse conditions including low temperatures (Komai and Emura 1955; Neck 1976).

The pathway for the introduction of *B. similaris* into Argentina remains unknown, but the historical record of 1956 from San Antonio, Misiones Province, a location that borders Brazil, suggests that it could be linked to a dispersion from that country, probably related to human inadvertent transport with plant material, as suggested for other exotic gastropod species recorded in the Misiones Province (Gutiérrez Gregoric et al. 2013a; Beltramino et al. 2018). The habitats in which *B. similaris* was collected in this study include urban and rural settings, ranging from residential gardens to waterfall environments, and are similar to the habitats described in the literature (e.g., disturbed places, forests, plantations, greenhouses, gardens; De Winter et al. 2009; Virgillito and Miquel 2013; Naranjo-García and Castillo-Rodríguez 2017). Given the species' adaptability to a wide range of environmental conditions, and because of its high colonization capabilities, it is probable that new colonized habitats will be reported for the country in the future. In this context, the importance of *B. similaris* as a pest to crops and ornamental plants in Argentina needs to be carefully surveyed, as the species is known to be a crop pest in several countries such as Cuba, Taiwan, the southern states of the USA and pacific American islands (Dundee and Cancienne 1978; Pena and Waddill 1982; Chang 2002; Robinson and Hollingsworth 2009; Herbert 2010; Matamoros 2014).

Bradybaena similaris has already been described as a natural intermediate host in the life cycle of parasites of humans and domesticated animals, and it was found infected with *Angiostrongylus cantonensis* in Brazil (Caldeira et al. 2007). This pathogen is widespread in Brazil including the southern States surrounding the Misiones Province, although the parasite has not yet been reported in Argentina (Morassutti et al. 2014; Valente et al. 2018). Because of the role of *B. similaris* as a vector for parasitic nematodes relevant to public health, and its occurrence in peridomiciliary areas, further epidemiological monitoring and parasitological investigations need to be undertaken.

Bradybaena similaris is known to be polymorphic for banding and background color. Following Komai and Emura (1955), most of the shells

found in Misiones Province belonged to the yellow-unbanded morphotype, except for specimens from Salto Las Mujeres where all specimens were yellow-banded, and from San Javier where the coexistence of both morphotypes was observed. The presence of brownish morphotypes in the study area is not disregarded, and new surveys in northeastern Argentina could reveal the presence of further morphotypes. Anatomically, the general scheme of the reproductive system of *B. similaris* from Argentina was consistent with the typology defined for the species by Emura (1932), and fits particularly well with that reported by Araujo (1989) and Picoral and Thomé (1989) for specimens from Brazil. All the individuals analyzed in this study presented the microsculpture on the inner penial wall with branched and largely crenulated longitudinal pilasters, anastomosing into a few major pilasters near the atrium, the most robust diagnostic taxonomic character for diagnosing *B. similaris*. This feature allowed distinguishing the individuals from Argentina from the similar species *B. pellucida*, whose pilasters on penial internal surface are not branched nor fused to each other (Seki et al. 2008; Hirano et al. 2014). The gross radular features of the individuals from Argentina were consistent with the description and schemes made by Araujo (1989). However, the Argentinian specimens differ from their Brazilian conspecifics in having tricuspid teeth instead of unicuspid ones, a finding which needs to be confirmed by SEM observations based on specimens from Brazil.

On the other hand, a single novel haplotype was identified for the molecular marker examined, with no genetic differences between both morphotypes. These results conform well to the low genetic diversity typically exhibited by introduced molluscan species as the result of genetic bottlenecks (e.g., Meunier et al. 2001; Facon et al. 2003; Chuong et al. 2008; Fontanilla et al. 2014), and may reflect a single introduction event of *Bradybaena similaris* in Argentina. To date, only one 16S-rRNA sequence of *B. similaris* from the Americas (i.e., Brazil) was available in GenBank for comparison (Sei et al. 2017). Further research involving more populations and molecular markers is needed to explore the genetic background of the South American populations in order to provide new hypotheses about the invasion pathways and the number of introductions of *B. similaris* in Argentina. This information is essential for invasive species management to strategically allocate surveillance and control efforts aimed at limiting the spread and impacts of this land invasive species.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Records of *Bradybaena similaris* in Argentina and habitat information.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Serniotti_etal_Table_S1.xlsx