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# New record and range extension of *Bradybaena similaris* (Férussac, 1822) (Gastropoda, Camaenidae) in Argentina

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**Abstract**

The Asian tramp snail *Bradybaena similaris* (Férussac, 1822) is an exotic mollusk native to Southeast Asia. The species has been catalogued as invasive in several countries and is important to human health, animal health, and agriculture. We report for the first time the presence of *B. similaris* in Córdoba Province, Argentina, extending the southern distribution of the species in this country and in South America. Anatomical, conchological, and molecular information obtained here represent the second contribution for this species in Argentina.

**Keywords**

16S-rRNA, Asian tramp snail, exotic land snail, invasive species, reproductive system, shell morphotypes, South America.

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## Introduction

The genus *Bradybaena* Beck, 1837 is rather speciose with more than 100 species only in China (De Winter et al. 2009; Wu and Asami 2018). Although the native range of this genus is Southeast Asia, the Asian tramp snail, *Bradybaena similaris* (Férussac, 1822), has reached all continents except Antarctica probably due to the plant trade, which is considered the main cause of its introduction (Carvalho et al. 2008; Schileyko 2011). This species has invaded many countries; it is well known to cause damage to several crop and ornamental species and to host parasites of medical and veterinary importance (Alicata 1940; Cowie et al. 2009; Robinson and Hollingsworth 2009; Capinera and White 2011; Matamoros 2014). In particular, this species has been found to be the intermediate host of the digenetic trematodes *Postharmostomum gallinum* Witenberg, 1923, a cecal fluke of poultry, and *Eurytrema coelomaticum* (Giard & Billet, 1892), a pancreatic fluke of bovine, caprine, and porcine animals (Amato and Bezerra 1989; Araujo 1989). Additionally, individuals of *B. similaris* can serve as the intermediate hosts of human parasitic nematodes such as *Angiostrongylus costaricensis* Morera & Céspedes, 1971, the cause of abdominal angiostrongyliasis, and the rat lungworm,

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*Angiostrongylus cantonensis* (Chen, 1935), the etiologic agent of eosinophilic meningitis (Caldeira et al. 2007; Ohlweiler et al. 2010; Cowie 2013). *Bradybaena similaris* exhibits four shell morphotypes based on ground color of periostracum and banding pattern of the ostracum and periostracum: dark brown and banded, dark brown and unbanded, light brown and banded, and light brown and unbanded (Komai and Emura 1955; Asami and Asami 2008). Anatomically, the reproductive system of *B. similaris* is broadly characterized by the presence of two mucous glands inserting on a dart sac with a single dart, and particularly by having about six pilasters with rhomboidal pustules on the inner penis surface (Wu 2004; Wu and Asami 2018; Serniotti et al. 2019). Together with molecular markers, the latter feature is also the most important character to discriminate *B. similaris* from the sibling species *B. pellucida* Kuroda & Habe, 1953, which is difficult to distinguish by shell color and gross morphology of the reproductive system (Asami and Asami 2008; Seki et al. 2008).

In South America, specimens of *B. similaris* were first reported by d'Orbigny (1835, 1838) on the Brazilian coast and by Doering (1875) in Argentina from Buenos Aires city, where they did not become established (Miquel et al. 2007; Virgillito and Miquel 2013). In the mid-1950s, the species was recorded in Tucumán and Misiones provinces without further information other than the general statement as occurring in those provinces (Drahg 1999; Miquel et al. 2007; Gutiérrez Gregoric et al. 2013a; Virgillito and Miquel 2013). Recently, Serniotti et al. (2019) reported eight new occurrences of *B. similaris* in the Misiones Province and the southernmost record for the species in South America from a locality of Entre Ríos Province, providing the first molecular and anatomical data for the species in Argentina.

In this work, we report for the first time the presence of *B. similaris* in Córdoba Province, central Argentina, extending the southern distribution range of the species in this country, as well as in South America. The molecular and anatomical data obtained here represent the first information of this species from Córdoba Province and the second one for Argentina.

## Methods

Specimens of *Bradybaena similaris* were collected by hand in a residential garden from the locality of Río Tercero, Córdoba Province, Argentina. Individuals were relaxed in water with menthol crystals for 4–10 h, then immersed in hot water (80 °C) and finally preserved in 96 % ethanol. Voucher material was deposited in the malacological collection of the Instituto de Biología Subtropical (IBS-Ma), CONICET–UNaM, Misiones Province, Argentina. For the morpho-anatomical studies, shells of four adult individuals (IBS-Ma 385-2, 385-3, 385-5, 385-7) were separated from the soft parts, cleaned in an ultrasonic bath Codyson CD4810 and photographed in dorsal-lateral, apertural, apical and umbilical views. The soft parts were dissected using a Labomed Luxeo 4D stereomicroscope for the study of the reproductive system. Genomic DNA was isolated from a portion of pedal muscle of the same morpho-anatomical analyzed specimens by means of a cetyltrimethylammonium bromide protocol (Beltramino et al. 2018). Partial sequences of the 16S-rRNA mitochondrial marker were amplified by polymerase chain reaction (PCR) using the primers 16SF-104 and 16SR-472 (Ramirez and Ramírez 2010). PCR reaction master mix and thermal profile were performed as in Serniotti et al. (2019). Due to the co-amplification of nonspecific fragments, PCR products were purified from 1.5 % (w/v) agarose gel using an ADN PuriPrep-GP Kit (Inbio Highway, Argentina), and bidirectionally sequenced by Macrogen Inc. (Seoul, Korea). The resulting sequences were trimmed to remove the primers and assembled using BIOEDIT v. 7.2.5 (Hall 1999). To confirm the identity of the species, consensus sequences were compared with reference sequences in GenBank through BLASTn algorithm (Altschul et al. 1990). Partial DNA sequences of the 16S-rRNA marker were deposited in GenBank under the accession numbers MN158200 to MN158203.

## Results

### Taxonomic account

Gastropoda Cuvier, 1795
Stylommatophora A. Schmidt, 1855
Camaenidae Pilsbry, 1895
*Bradybaena* H. Beck, 1837

#### *Bradybaena similaris* (Férussac, 1822)

**New record.** Argentina: Córdoba Province: Río Tercero city (32°09.83′S, 064°06.6′W; 377 m a.s.l.), collected by D. Sequeira, P. Depetris and R.E. Vogler, 4 January 2019 (81 specimens and 10 dried shells, IBS-Ma 385) (Table 1, Fig. 1).

**Identification.** Snails were firstly identified as *B. similaris* based on the size and color of the shells, which were found to be light brown banded and unbanded (Fig. 2A, B). Morphology of the reproductive system was consistent with those described by Araujo (1989), Picoral and Thomé (1989), and Wu (2004) and fits particularly well with that shown by Serniotti et al. (2019) for Argentine specimens (Fig. 2C). All individuals analyzed exhibited the inner penial wall with six to eight branched and crenulated pilasters anastomosing towards the atrium, a diagnostic feature for the species and the only character capable of discriminating *B. similaris* from *B. pellucida* (Fig. 2D). The length of the amplified fragments was 265–266 bp for all individuals. When compared to GenBank sequences, 16S-rRNA sequences obtained here showed top coverage and high similarity scores of 95–100 % with specimens of *B. similaris* (Table 2).

**Table 1.** Records of *Bradybaena similaris* in Argentina. Shell morphotypes and genetic data are provided if available. IBS-Ma: malacological collection of the Instituto de Biología Subtropical (CONICET–UNaM), Misiones, Argentina. MACN-In: malacological collection at Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina. IFML: malacological collection at Instituto Fundación Miguel Lillo, Tucumán, Argentina. LB: light brown and banded. LU: light brown and unbanded.

| No. | Location | Voucher # | Latitude | Longitude | Habitat | Morph | GenBank # | References |
|---|---|---|---|---|---|---|---|---|
| 1 | Río Tercero city, Córdoba | IBS-Ma 385-2 | 32°09.83′S | 064°06.6′ W | Residential garden | LB | MN158200 | Present study |
| 2 | Río Tercero city, Córdoba | IBS-Ma 385-3 | 32°09.83′S | 064°06.6′W | Residential garden | LB | MN158201 | Present study |
| 3 | Río Tercero city, Córdoba | IBS-Ma 385-5 | 32°09.83′S | 064°06.6′W | Residential garden | LB | MN158202 | Present study |
| 4 | Río Tercero city, Córdoba | IBS-Ma 385-7 | 32°09.83′S | 064°06.6′W | Residential garden | LU | MN158203 | Present study |
| 5 | Eldorado, Misiones | IBS-Ma 165-7 | 26°24.27′S | 054°35.65′W | Residential garden | LU | MH428043 | Serniotti et al. 2019 |
| 6 | Salto Capioví, Misiones | IBS-Ma 247-1 | 26°55.48′S | 055°03.72′W | Waterfall environment in urban area | LU | MH428046 | Serniotti et al. 2019 |
| 7 | Posadas, Misiones | IBS-Ma 122-3 | 27°21.54′S | 055°54.2′W | Residential garden | LU | MH428041 | Serniotti et al. 2019 |
| 8 | Apóstoles, Misiones | IBS-Ma 253-3 | 27°55.18′S | 055°43.48′W | Close to agricultural plantations | LU | MH428047 | Serniotti et al. 2019 |
| 9 | San Javier, Misiones | IBS-Ma 242-2 | 27°52.13′S | 055°08.52′W | Residential garden | LU | MH428044 | Serniotti et al. 2019 |
| 10 | Salto Las Mujeres, Misiones | IBS-Ma 243-1 | 27°43.66′S | 055°10.13′W | Waterfall environment in rural area | LB | MH428045 | Serniotti et al. 2019 |
| 11 | Oberá, Misiones | IBS-Ma 102-2 | 27°28.54′S | 055°06.12′W | Residential garden | LU | MH428040 | Serniotti et al. 2019 |
| 12 | San Vicente, Misiones | IBS-Ma 096-1 | 26°59.98′S | 054°29.43′W | Residential garden | LU | MH428039 | Serniotti et al. 2019 |
| 13 | San Antonio, Misiones | MACN-In 30444 | 26°04′S | 053°44′W | — | — | — | Miquel et al. 2007; Virgillito and Miquel 2013 |
| 14 | San Ignacio, Misiones | MACN-In 35896 | 27°15′S | 055°32′W | — | — | — | Virgillito and Miquel 2013 |
| 15 | Villaguay, Entre Ríos | IBS-Ma 142-1 | 31°52.22′S | 059°01.84′W | Residential garden | LU | MH428042 | Serniotti et al. 2019 |
| 16 | San Miguel de Tucumán, Tucumán | IFML 14990 | 26°48.6′S | 065°14.81′W | Plant nursery | — | — | Virgillito 2012; Virgillito and Miquel 2013 |
| 17 | Tafí Viejo, Tucumán | IFML 15419 | 26°44′S | 065°16′W | Residential garden | — | — | Virgillito 2012; Virgillito and Miquel 2013 |

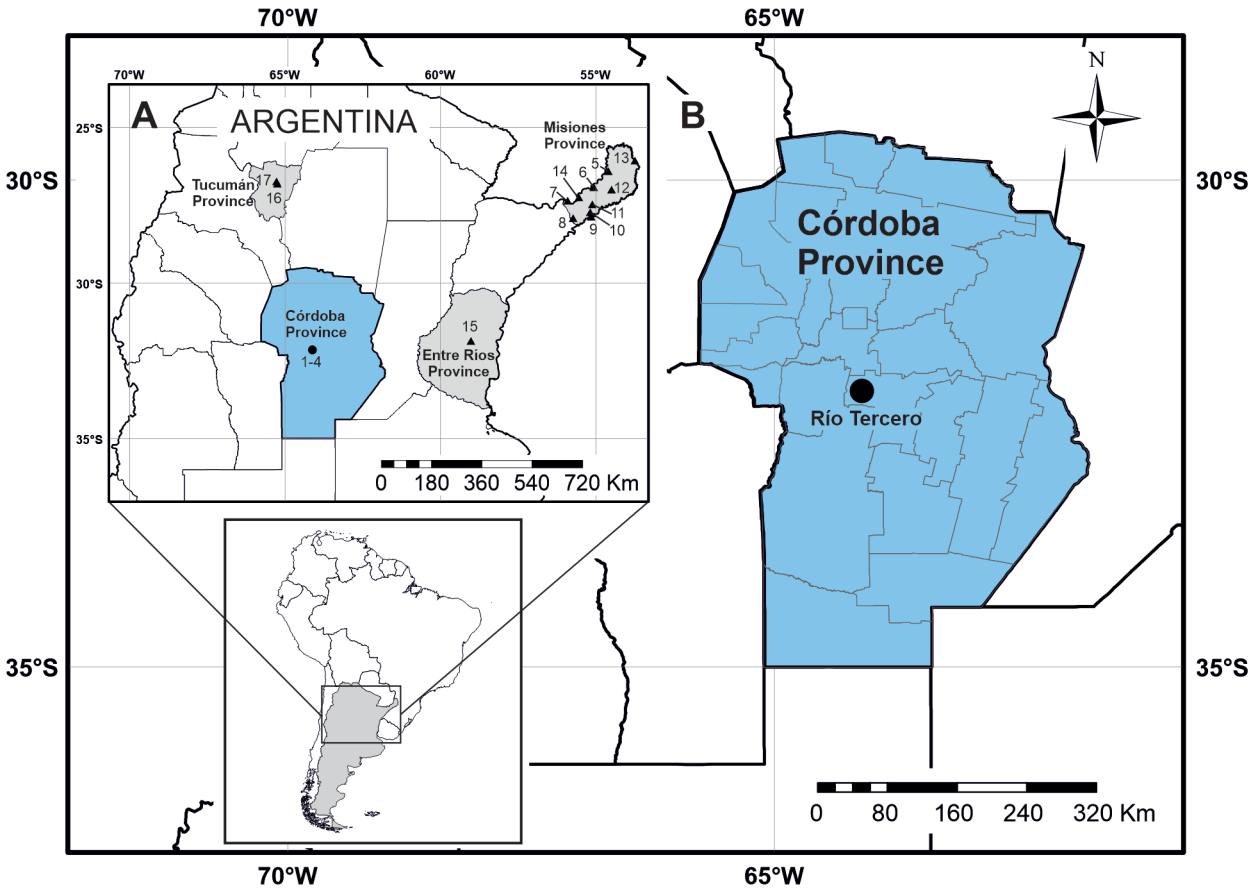


**Figure 1.** Distribution map of the exotic land snail *Bradybaena similaris*. **A.** Locality records of the species in Argentina. **B.** Detail of the new record in Córdoba Province. Locations indicated with triangles correspond to literature records and the circles correspond to the new locality where the specimens of *B. similaris* were found. Location numbers correspond to the numbers in Table 1.

**Table 2.** Top five results of the BLASTn search for each haplotype of *Bradybaena similaris* found in this study.

| Sequence accesion # | Most significant alignment | GenBank # | E-value | Coverage (%) | Identity (%) | Geographical origin | References |
|---|---|---|---|---|---|---|---|
| MN158200 (266 bp) | *B. similaris* | MH428047 | 8e-132 | 100 | 100 | Misiones, Argentina | Serniotti et al. 2019 |
| | *B. similaris* | MH428046 | 8e-132 | 100 | 100 | Misiones, Argentina | Serniotti et al. 2019 |
| | *B. similaris* | MH428045 | 8e-132 | 100 | 100 | Misiones, Argentina | Serniotti et al. 2019 |
| | *B. similaris* | MH428044 | 8e-132 | 100 | 100 | Misiones, Argentina | Serniotti et al. 2019 |
| | *B. similaris* | MH428043 | 8e-132 | 100 | 100 | Misiones, Argentina | Serniotti et al. 2019 |
| MN158201 MN158202 MN158203 (265 bp) | *B. similaris* | HQ245444 | 1e-117 | 100 | 96.23 | — | Köhler and Criscione 2013 |
| | *B. similaris* | GQ851164 | 1e-117 | 100 | 96.23 | Sabah, Malaysia | Hugall and Stanisic 2011 |
| | *B. similaris* | GQ851001 | 2e-115 | 100 | 95.86 | Queensland, Australia | Hugall and Stanisic 2011 |
| | *B. similaris* | KF247037 | 6e-115 | 100 | 95.47 | Rio Grande do Sul, Brazil | Sei et al. 2017 |
| | *B. similaris* | MH428047 | 1e-111 | 100 | 94.74 | Misiones, Argentina | Serniotti et al. 2019 |

# Discussion

In this work we report a new record of *Bradybaena similaris* in Argentina, the first one of the species from Córdoba Province. This new locality record expands the southern distribution of *B. similaris* from the previous nearest records in Argentina, which are located at about 480 km northeast on the Entre Ríos Province and 600 km northwest on the Tucumán Province. With reference to Serniotti et al. (2019), specimens found in this study also constitute the new southernmost record of South America. This finding indicates the species is more widespread than previously thought and suggests it is spreading rapidly through the country. On the other hand, residents of the new locality where *B. similaris* was found reported that these snails have been present in Río Tercero city since for about 10 years, which suggests the species is already established and reproducing successfully. During this time, *B. similaris* could have spread to nearby cities and towns, although the species has not been documented in recent studies on invasive exotic mollusk species from Argentina, and particularly from Córdoba Province (Rumi et al. 2010; Gordillo et al. 2013; Reyna et al. 2018). Further surveys are required to gain insights into the distribution of the species in this area.

Specimens of *B. similaris* were found in an urban habitat, specifically on pot plants located on a residential garden. According to the literature, this is the habitat where populations of *B. similaris* usually live and in turn one of the most common introduction vectors for terrestrial mollusk species (Cowie and Robinson 2003; Gutiérrez Gregoric et al. 2013b, 2013c). When consulted, residents stated that it is common practice to bring ornamental plants and/or organic compost from Chaco, Formosa, and Misiones provinces, pointing out these regions as probable sources of *B. similaris*. However, due to the lack of records of the species in Chaco and Formosa provinces, Misiones becomes the most probable geographic source for the introduction in Córdoba Province. In this sense, additional studies comprising more populations from different provinces are needed to elucidate the introduction history of *B. similaris* in Córdoba Province and dispersal routes in Argentina.

The new record from Córdoba Province, as well as the previous southernmost one from Entre Ríos Province, were both found in temperate climates. Together with the evidence gathered on cold resistance of *B. similaris* (Komai and Emura 1955; Asami and Asami 2008), this finding reinforces the hypothesis that temperature may be limiting factor for spread of this species (Serniotti et al. 2019). Nonetheless, studies using bioclimatic modeling are needed to effectively assess those areas of the country where the species could invade or where the species is already present but not detected.

Four shell morphotypes of *B. similaris* have been described so far and are either lighter or darker shells with or without a single chestnut spiral band. Since Komai and Emura (1955), various authors have referred to the lighter shells as yellowish and to the darker ones as brownish (e.g. Neck 1976; Ohlweiler et al. 2010; Capinera and White 2011; Serniotti et al. 2019). However, Asami and Asami (2008) stated that there is no yellow pigmentation either in the body or the shell of this species (e.g. as it is in the sibling species *B. pellucida*) and proposed a more accurate way to refer to the shell morphotypes of *B. similaris* based on ground color of periostracum and banding pattern of the ostracum and periostracum. Following Asami and Asami (2008), in this study the lighter shells are referred to as light brown and the darker ones are referred to as dark brown. As found by Serniotti et al. (2019) for Argentine specimens from Misiones and Entre Ríos provinces, the two lighter shell morphotypes were found among the 91 shells analyzed here. Although all the shells were found to be light brown, we suggest there is some variation in the ground color of shells classified as "light" and "dark"; this hypothesis needs further research.

Anatomically, the individuals analyzed in this study exhibited the gross morphology and inner penial structure as defined for *B. similaris* (see Araujo 1989; Picoral and Thomé 1989; Wu 2004; Serniotti et al. 2019). Specimens dissected here were different from those of *B. pellucida* in having long, thick, anastomosed pilasters running from near the atrium to the epiphallus instead of short, neither branched nor crenulated pilasters

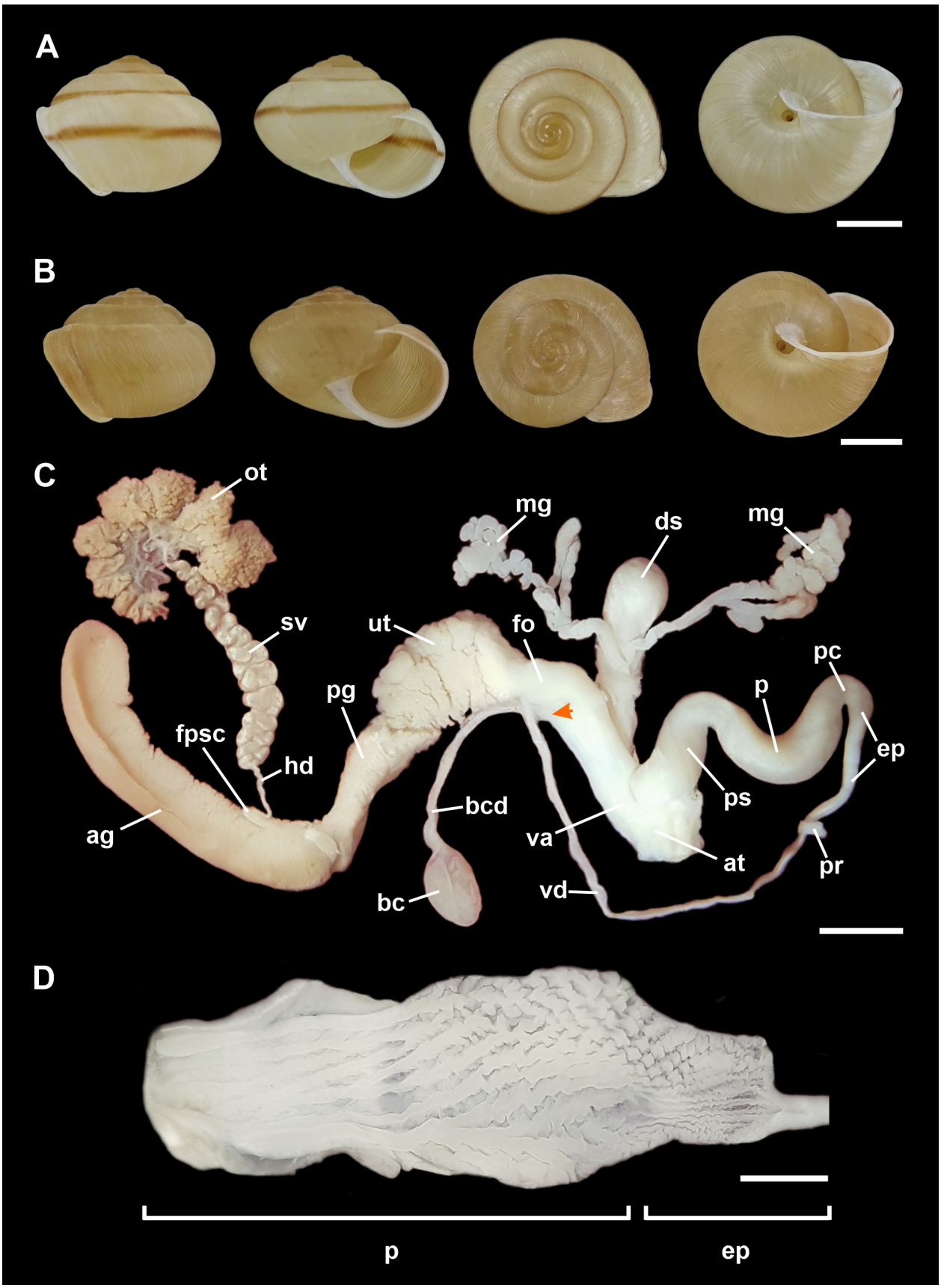


**Figure 2.** Shells and reproductive system of *Bradybaena similaris* from Córdoba Province, Argentina. **A.** Light brown and banded shell morphotype. Scale bar = 5 mm. **B.** Light brown and unbanded shell morphotype. Scale bar = 5 mm. **C.** General view of the reproductive system. The arrow indicates the insertion of the bursa copulatrix duct into the vagina. Scale bar = 2.5 mm. **D.** Detail of the inner penial wall. Scale bar = 1.25 mm. Abbreviations: ag, albumen gland; at, atrium; bc, bursa copulatrix; bcd, bursa copulatrix duct; ds, dart sac; ep, epiphallus; fo, free oviduct; fpsc, fertilization pouch-spermathecal complex; hd, hermaphroditic duct; mg, mucous glands; ot, ovotestis; p, penis; pc, penial constriction; pg, prostate gland; pr, penial retractor muscle; ps, penial sheath; sv, seminal vesicle; ut, uterus; va, vagina; vd, vas deferens.

occupying one-third of the penial wall. Additionally, individuals from Córdoba Province showed fine, very compressed, and crenulated pilasters on the inner wall of epiphallus rather than thin and partly crenulated ones as in *B. pellucida* (Seki et al. 2008).

Molecularly, two different haplotypes were found among the sequences analyzed for the specimens from Córdoba Province. One of these haplotypes was already reported by Serniotti et al. (2019) for eight localities of Misiones Province and one locality from Entre Ríos Province, suggesting the carriers could have been introduced from this region. The other haplotype detected here is a novel one and was found in three of the four analyzed specimens. A major study with more sequences from Tucumán, Misiones, Entre Ríos, and Córdoba provinces is needed to clarify the invasion pathways and number of introductions of *B. similaris* in Argentina.

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## Authors' Contributions

ENS and AAB conceived the research question. ENS, LBG, REV, AR, and JGP collected data and prepared voucher materials. ENS and LBG performed molecular and morpho-anatomical analyses and confirmed the taxonomic identity of specimens. LBG took all the photographs. ENS, LBG, REV, and AAB wrote the first draft of the manuscript. All authors reviewed the final version of the manuscript.

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