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First record of the semi-slug *Omalonyx unguis* (d'Orbigny, 1837) (Gastropoda, Succineidae) in the Misiones Province, Argentina

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Abstract
Omalonyx unguis (d'Orbigny, 1837) is a semi-slug inhabiting the Paraná river basin. This species belongs to Succineidae, a family comprising a few representatives in South America. In this work, we provide the first record for the species from Misiones Province, Argentina. Previous records available for *Omalonyx* in Misiones were identified to the genus level. We examined morphological characteristics of the reproductive system and used DNA sequences from cytochrome oxidase subunit I (COI) gene for species-specific identification. These new distributional data contribute to consolidate the knowledge of the molluscan fauna in northeastern Argentina.

Key words
Aquatic vegetation fauna; High Paraná River; mitochondrial marker; native species; Panpulmonata.

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Introduction

Omalonyx (d'Orbigny, 1837) is a genus of the family Succineidae, which includes semi-slugs that live generally in backwater areas, on floating macrophytes and on riparian vegetation (Arruda and Thomé 2011, Garcia et al. 2012). This genus has a reduced fingernail-shaped shell with specimens usually exhibiting a light to medium-brown coloration, dark scattered spots, and 2 dark dorsal longitudinal bands (Arruda et al. 2006, Cuezzo 2009). *Omalonyx* are herbivorous and have been reported as pests of several plant species such as *Nymphoides indica* (L.) Kuntze, *Hydrocleys nymphoides* Willd. Buchenau and *Nymphaea prolifera* Wiersema without further indication of the species implicated (Martínez and Franceschini 2018). In addition, *O. pattersonae* Tillier, 1981 and *Omalonyx* sp. were reported as pests of *Pennisetum purpureum* Schum. (Garcia et al. 2012), while *O. unguis* was found causing damage to *Eichornia crassipes* (Mart.) and *Nasturtium officinale* R. Br. (Poi de Neiff et al. 1977, Olazarri 1979, Cazzaniga 1985). *Omalonyx* spp. also act as intermediate hosts of nematodes and trematodes, with recorded cases in Brazil of specimens of *Omalonyx* sp. infected with bird flukes of the genus *Leucochloridium*

Carus, 1835 (Lutz 1921, Pinto and Melo 2013, Vidigal et al. 2018). In South America, these semi-slugs can also serve as potential intermediate hosts of *Angiostrongylus vasorum* (Baillet, 1866), which has been experimentally proven for *O. matheroni* (Pontiez & Michaud, 1835), and the parasite *A. costaricensis* Morera & Céspedes, 1971, whose definitive hosts are canids and rodents respectively, and where humans may act as accidental hosts (Incani et al. 2007, Montresor et al. 2008, Mozzer et al. 2011, Rebello et al. 2011).

According to potential distribution models for *Omalonyx* species in South America, the Argentine province of Misiones represents a suitable area for the occurrence of *O. unguis* and *O. convexus* (Heynemann, 1868) (Coscarelli et al. 2018). Both species occur in the Paraná river basin and are typical inhabitants of hygrophilous environments (Coscarelli and Vidigal 2011, Coscarelli et al. 2018). Although the genus has been recorded in the Misiones Province, these records have not been identified at a specific level (Gutiérrez Gregoric et al. 2013). To date, specific-identity of the *Omalonyx* species in Misiones Province is unknown. Recently, the first molecular data for *Omalonyx* in South America became available, which comprises DNA sequences for the mitochondrial COI gene, and the nuclear internal transcribed spacer 2 (ITS2) region (Vidigal et al. 2018). Nonetheless, DNA sequences for *Omalonyx* from Argentina are still scarce, and are only available for *O. convexus*.

In this study, we report new records for the genus *Omalonyx* in Argentina, with that finding also constituting the first anatomically and molecularly confirmed records for *O. unguis* in the Misiones Province. Moreover, DNA sequences obtained here constitute the first genetic data generated for this species in Argentina.

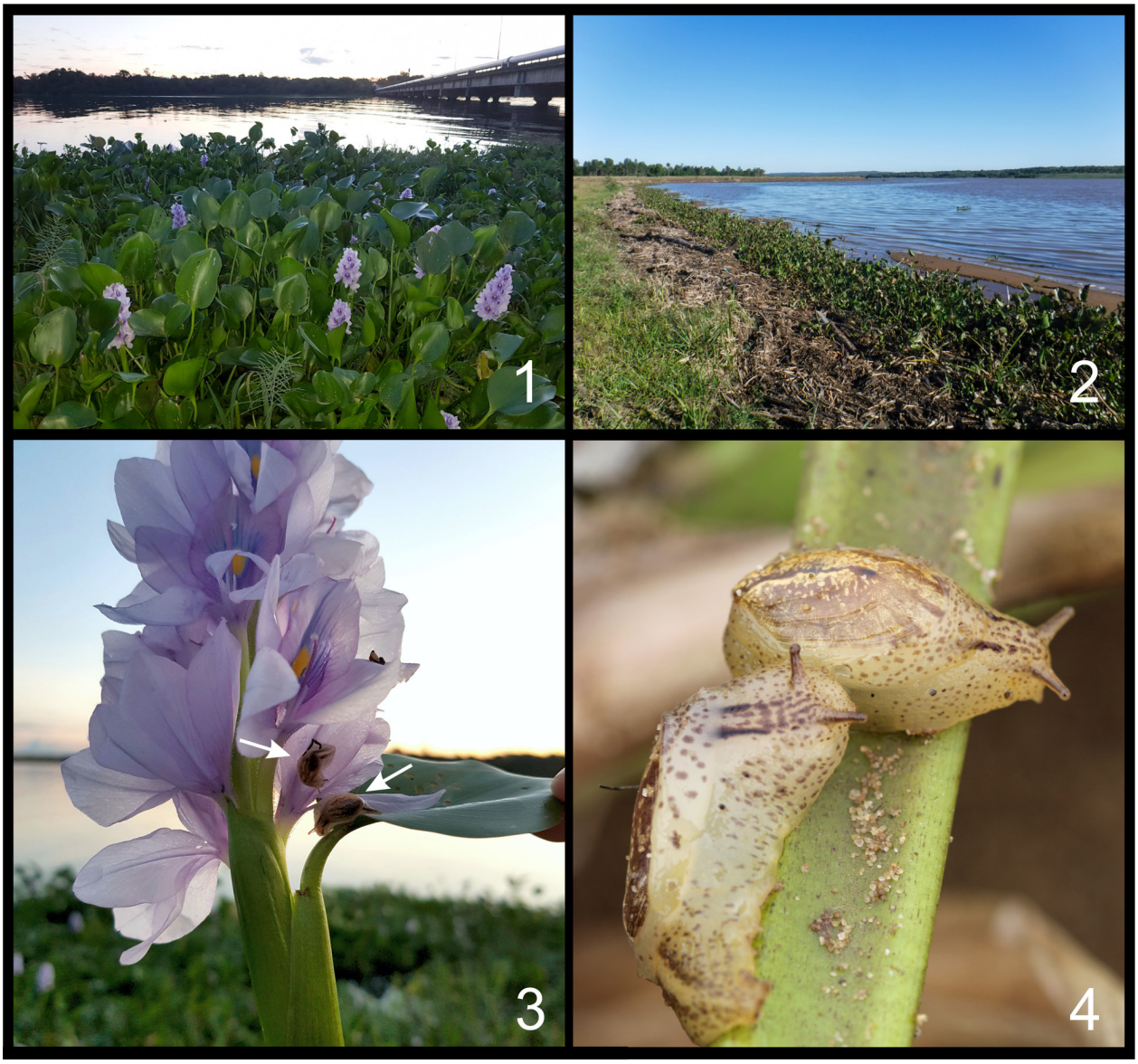
Methods

Living specimens were manually collected in the margins of the High Paraná River and 4 tributary streams between 2017 and early 2018. Individuals were captured during the sunset while they were involved in full activity on the leaves and flowers of *Eichornia crassipes* (Pontederiaceae) and on riparian vegetation (Figs 1–4). The animals were photographed using a Sony DSC Hx400v camera. Geographic coordinates were recorded with GPS Garmin eTrex Legend®. Living specimens were relaxed in water with menthol crystals for 4–10 h, subsequently immersed in hot water (80 °C), and finally fixed in 96% ethanol. Shells were photographed in dorsal, ventral, lateral and protoconch views following Arruda et al. (2016). Voucher material was deposited in the malacological collection at the Instituto de Biología Subtropical (IBS-Ma), UNaM-CONICET, Misiones Province, Argentina.

We examined the genital anatomy of 8 ethanol-preserved adult specimens (IBS-Ma 042, 073/1, 074/1, 280, 281/1, 282/3, 283, 284/1) following Tillier (1981), Arruda et al. (2006), Arruda (2011), and Coscarelli and Vidigal (2011). Specimens were dissected using a Labomed Luxeo 4D stereomicroscope. A 0.1% (w/v) neutral-red solution was used to increase the contrast of tissues as in Vogler et al. (2014). DNA was isolated from muscle tissue of the same 8 anatomically dissected specimens using a CTAB protocol (Beltramino et al. 2018). Partial sequences of the mitochondrial COI marker were amplified by polymerase chain reaction (PCR) through the use of the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR reaction was performed in a total volume of 50 µl containing 30–50 ng of template DNA, each primer at 0.1 µM, 1× reaction buffer, 2.5 mM $MgCl_2$, 50 µM dNTPs and 1.2 U Taq Pegasus DNA polymerase (Productos Bio-Lógicos, Argentina). Amplification was performed on a T18 thermocycler (Ivema Desarrollos) as follows: an initial denaturing for 3 min at 94 °C; 5 cycles of 30 sec at 94 °C, 40 sec at 45 °C, 1 min at 72 °C; 35 cycles of 30 sec at 94 °C, 40 sec at 51 °C, 1 min at 72 °C; followed by a final extension at 72 °C for 10 min. Success of PCR reactions was verified by agarose gel electrophoresis. The PCR products were purified by means of an AccuPrep PCR Purification Kit (Bioneer, Korea). In some cases, owing to the co-amplification of nonspecific fragments, PCR products were purified from 1.5% (w/v) agarose gel with an ADN PuriPrep-GP Kit (Inbio Highway, Argentina). Both DNA strands were directly cycle sequenced (Macrogen Inc., Seoul, Korea), and the resulting sequences were trimmed to remove the primers. The consensus sequences of the individuals between the forward and reverse sequencings were assembled using the BIOEDIT 7.0.5 software (Hall 1999). In order to confirm the species-specific identification, the consensus sequences were compared with reference sequences in GenBank using the BLASTN algorithm (Altschul et al. 1990).

Results

New records. Argentina: Misiones: Mártires stream (27.3719 °S, 055.9542 °W), collectors: R.E. Vogler, A.A. Beltramino and L.B. Guzmán, March 2018 (11 specimens, IBS-Ma 282). Argentina: Misiones: Antonica stream (27.3633 °S, 055.9420 °W), collectors: R.E. Vogler, A.A. Beltramino and L.B. Guzmán, March 2018 (1 specimen, IBS-Ma 280). Argentina: Misiones: Itá stream mouth at confluence with High Paraná River (27.3592 °S, 055.9100 °W), collectors: R.E. Vogler, A.A. Beltramino and L.B. Guzmán, March 2018 (20 specimens, IBS-Ma 281). Argentina: Misiones: High Paraná River (27.3606 °S, 055.8878 °W), collectors: R.E. Vogler, A.A. Beltramino and L.B. Guzmán, March 2018 (1 specimen, IBS-Ma 283). Argentina: Misiones: Zaimán stream mouth at confluence with High Paraná River (27.4061 °S, 055.8939 °W), collectors: V. Núñez, R.E. Vogler and L.B. Guzmán, November 2017 (2 specimens, IBS-Ma 074). Argentina: Misiones: Zaimán stream (27.4206 °S, 055.8961 °W), collectors: R.E. Vogler and L.B. Guzmán, April 2018 (5 specimens, IBS-Ma 284). Argentina: Misiones: Garupá stream beach (27.4786 °S, 055.7933 °W),



Figures 1–4. Habitat and specimens of *Omalonyx unguis* from Misiones Province. **1, 2.** *Eichornia crassipes* (water hyacinth) mats in the Mártires and Garupá streams, respectively, where *O. unguis* individuals were found. **3.** Specimens of *O. unguis* (white arrows) involved in full activity on flowers of *Eichornia crassipes* at Mártires stream (IBS-Ma 282). **4.** *Omalonyx unguis* (IBS-Ma 042) from Garupá stream floodplain.

collectors: J.G. Peso, R.E. Vogler and A.A. Beltramino, May 2017 (5 specimens, IBS-Ma 042), and collectors: V. Núñez, R.E. Vogler and L.B. Guzmán, November 2017 (5 specimens, IBS-Ma 073) (Table 1, Fig. 5).

Identification. The semi-slugs were firstly identified as belonging to *Omalonyx* by their typically reduced, flat, fingernail-shaped shells (Fig. 6), as well as by the animal bodies twice than shell lengths (Arruda and Thomé 2008a). Species-specific identification was achieved based on morphological features of the reproductive system, and on partial COI sequence data using the BLASTN algorithm. Anatomically, the specimens matched the morphological characters defined for *O. unguis* as described by Arruda (2011), and in particular exhibited the presence of a serpent-like fold on the outer surface of the epiphallus, a diagnostic character for the species (Figs 7–9). In addition, the following distinctive characters were observed: ovotestis spherical with hermaphroditic duct contorted in distal portion. Penial retractor muscle attached to distal region of vas deferens. Epiphallus shorter than penis, with epiphallic sphincter in proximal region. Penis uniform in diameter with inner papillae similar to those described by Tillier (1981) and Arruda (2011); diameter of proximal penis similar to epiphallus. Spermatheca spherical with duct of spermatheca thin, inserted at junction of free oviduct and vagina. Vagina cylindrical with elliptic papillae on inner surface. The complete reproductive system is shown in Fig. 9. The total lengths of the mitochondrial sequences obtained were 655 bp for all individuals. The BLASTN search results with the COI sequences here obtained confirmed their specific identities as *O. unguis*, as they showed 100% coverage and top-ranking scores between 100–96% similarity with the COI sequences available for the species in the GenBank database.

Table 1. Reports of *Omalonyx unguis* obtained here and literature records for which COI sequences are available. IBS-Ma: Malacological collection at Instituto de Biología Subtropical, Universidad Nacional de Misiones-CONICET. LMSM: Malacological collection of the Laboratório de Malacologia e Sistemática Molecular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

No.	Location	Voucher #	Latitude	Longitude	GenBank #	References
1	Mártires stream, Misiones, Argentina	IBS-Ma 282	27.3719 °S	055.9542 °W	MH396680	Present study
2	Antonica stream, Misiones, Argentina	IBS-Ma 280	27.3633 °S	055.9420 °W	MH396681	Present study
3	Itá stream mouth, Misiones, Argentina	IBS-Ma 281	27.3592 °S	055.9100 °W	MH396682	Present study
4	High Paraná River, Misiones, Argentina	IBS-Ma 283	27.3606 °S	055.8878 °W	MH396683	Present study
5	Zaimán stream mouth, Misiones, Argentina	IBS-Ma 074	27.4061 °S	055.8939 °W	MH396684	Present study
6	Zaimán stream, Misiones, Argentina	IBS-Ma 284	27.4206 °S	055.8961 °W	MH396685	Present study
7	Garupá stream beach, Misiones, Argentina	IBS-Ma 042	27.4786 °S	055.7933 °W	MG459424	Present study
8	Garupá stream beach, Misiones, Argentina	IBS-Ma 073	27.4786 °S	055.7933 °W	MH396686	Present study
9	Trevo do Lagarto, Várzea Grande, Mato Grosso, Brazil	LMSM 3349	15.6483 °S	056.1825 °W	KX261557	Vidigal et al. 2018
10	Trevo do Lagarto, Várzea Grande, Mato Grosso, Brazil	LMSM 3432	15.6483 °S	056.1825 °W	KX261560	Vidigal et al. 2018
11	Paraguay River, Cárceres, Mato Grosso, Brazil	LMSM 3391	16.0761 °S	057.7025 °W	KX261559	Vidigal et al. 2018
12	Paraguay River, Cárceres, Mato Grosso, Brazil	LMSM 3334	16.0761 °S	057.7025 °W	KX261555	Vidigal et al. 2018
13	Transpantaneira Road, Poconé, Mato Grosso, Brazil	LMSM 3333	16.3750 °S	056.6700 °W	KX261554	Vidigal et al. 2018
14	Transpantaneira Road, Poconé, Mato Grosso, Brazil	LMSM 3344	16.3750 °S	056.6700 °W	KX261611	Vidigal et al. 2018
15	Fazenda San Francisco, Miranda, Mato Grosso do Sul, Brazil	LMSM 2708	20.0989 °S	056.7094 °W	KX261550	Vidigal et al. 2018
16	Fazenda San Francisco, Miranda, Mato Grosso do Sul, Brazil	LMSM 2796	20.0989 °S	056.7094 °W	KX261551	Vidigal et al. 2018
17	Paraguay River, Asunción, Central, Paraguay	LMSM 3436	25.2758 °S	057.6378 °W	KX261563	Vidigal et al. 2018
18	Paraná River, Foz do Iguaçu, Paraná, Brazil	LMSM 3260	25.4469 °S	054.5494 °W	KX261552	Vidigal et al. 2018
19	Paraná River, Foz do Iguaçu, Paraná, Brazil	LMSM 3307	25.4469 °S	054.5494 °W	KX261553	Vidigal et al. 2018
20	Paraguay River, Pilar, Ñeembucú, Paraguay	LMSM 3433	26.8533 °S	058.2958 °W	KX261561	Vidigal et al. 2018
21	Paraguay River, Pilar, Ñeembucú, Paraguay	LMSM 3341	26.8533 °S	058.2958 °W	KX261556	Vidigal et al. 2018
22	Paraná River, Paso de Patria, Ñeembucú, Paraguay	LMSM 3350	27.2478 °S	058.5389 °W	KX261558	Vidigal et al. 2018
23	Paraná River, Paso de Patria, Ñeembucú, Paraguay	LMSM 3434	27.2478 °S	058.5389 °W	KX261562	Vidigal et al. 2018

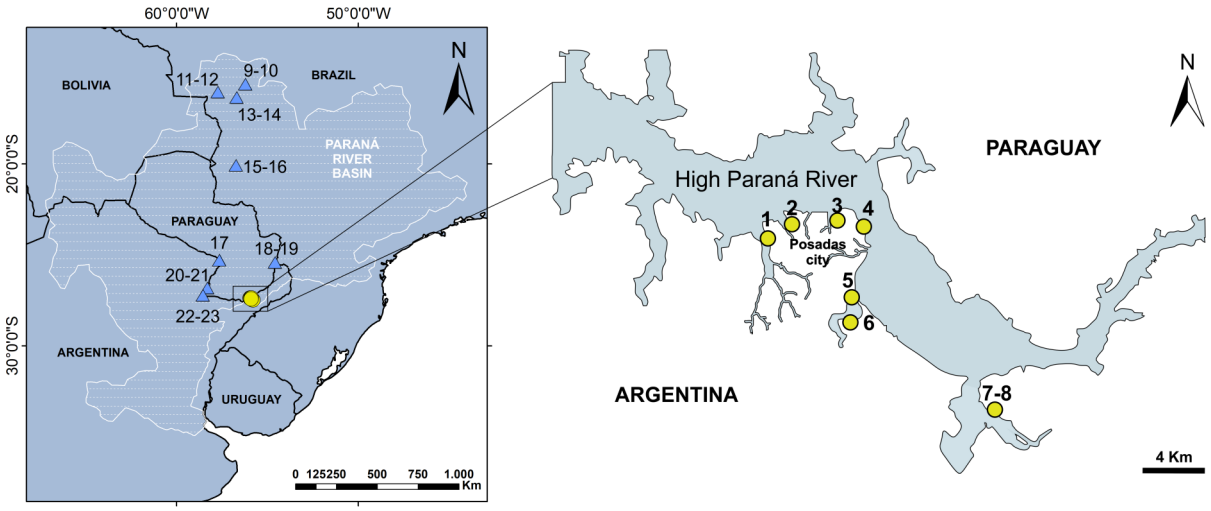


Figure 5. Distribution map of *Omalonyx unguis* in the Paraná river basin. Triangles indicate molecularly-confirmed occurrences according to Vidigal et al. (2018). Circles indicate the new localities where the specimens of *O. unguis* were found in Misiones Province, Argentina. The localities numbers correspond to those presented in Table 1.

Discussion

This work documents for the first time the occurrence of the semi-slug *O. unguis* in the Misiones Province, Argentina from 7 localities near Posadas city. *Omalonyx unguis* is the type species of the genus and its type locality was emended from Corrientes, Argentina to Asunción, Paraguay (Arruda and Thomé 2008a, b). In Argentina, the known distribution of this species—based on literature and museum data—includes the provinces of Buenos Aires, Catamarca, Chaco, Corrientes, Entre Ríos, Formosa, Santa Fe, and Tucumán (Fernández 1973, Cazzaniga 1985, Coscarelli et al. 2018 and references therein). However, due to misconceptions and contradictions in *Omalonyx* systematics, those previous records should be interpreted with caution as they may include what today is recognized as *O. convexus* (Arruda and Thomé 2008a). Despite this, the records reported herein constitute the first anatomically and molecularly confirmed occurrences of *O. unguis* in Misiones and increase

Figure 6. Shell of *Omalonyx unguis* from Garupá stream beach (IBS-Ma 073/1) in dorsal, ventral, lateral and protoconch views. Scale bar = 5 mm.

the known distribution of the species to northeastern corner of Argentina, bordering on Brazil and Paraguay (Gutiérrez Gregoric et al. 2013). The new records are located at about 250 km west of the nearest record in Foz do Iguaçu city, Brazil, and at about 280 km southeast of the type locality in Paraguay.

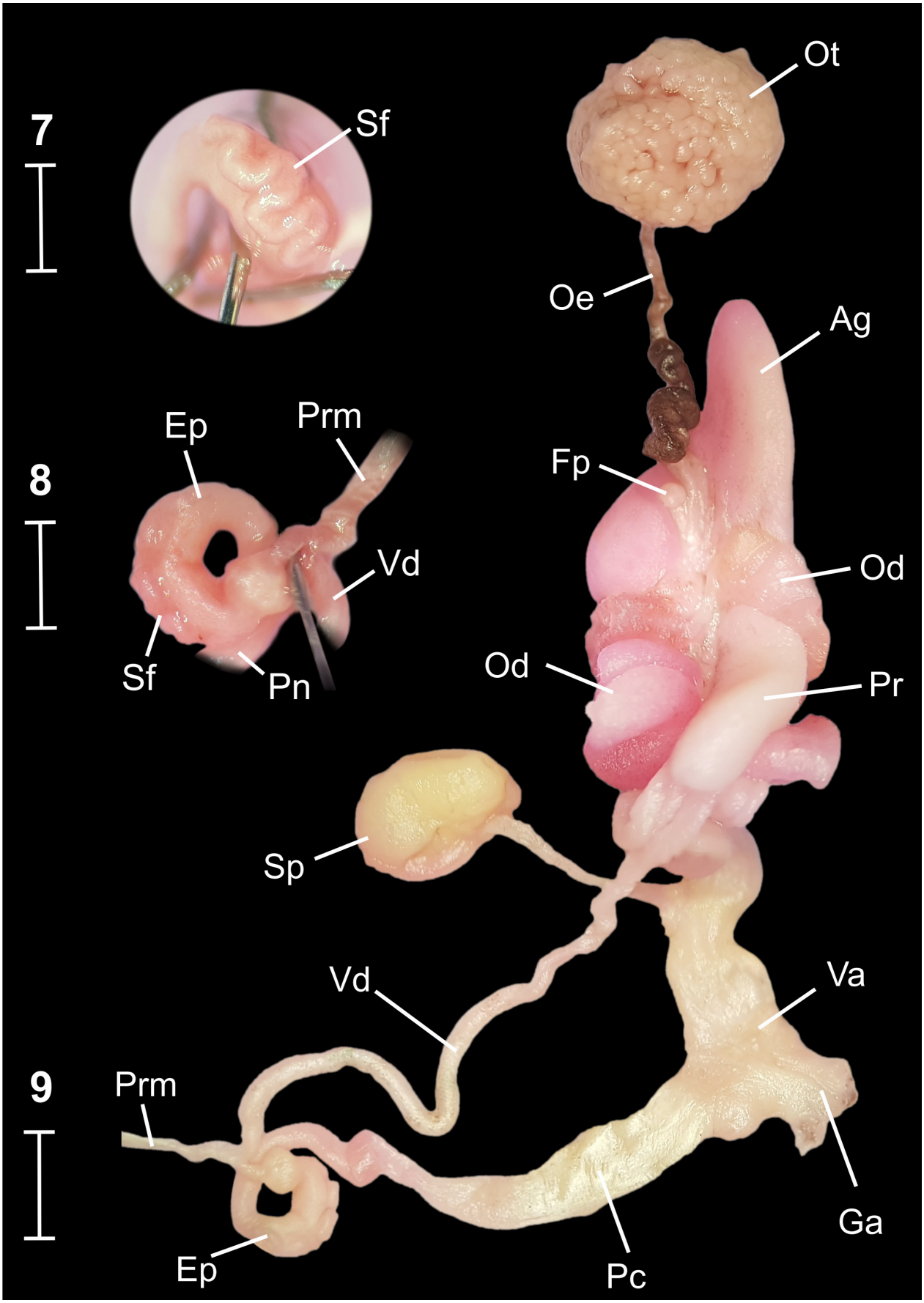
The specimens reported herein were collected in calm-water environments with abundant vegetation, at sites subject to high anthropogenic disturbance, as the Itá stream. This is an urban stream flowing through Posadas city, which is affected by the disposal of anthropogenic wastes (Secretaría de Planificación Estratégica y Territorial 2012). In addition, all the streams in the area were also affected by the filling stages of the Yacyretá Reservoir (Argentina–Paraguay) resulting in a slower surface-water flow velocity near their confluence with the High Paraná River, conditions that favor the growth of *Eichornia crassipes* (Flores et al. 2009, Fulco 2012). In agreement with observations by Poi de Neiff et al. (1977), the specimens collected in March 2018 during the flowering period of *Eichornia crassipes* showed a preference for feeding on the flowers of this plant.

Recently, Coscarelli et al. (2018) made predictions on the potential distribution area of 5 *Omalonyx* species, where Misiones Province is indicated as a suitable area for the occurrence of *O. unguis* and *O. convexus*. Nonetheless, although more than 150 years have passed since both species were described, these species continue to be poorly represented for Misiones Province and occurrence data are almost lacking in the literature for the area. To our knowledge, only Gutiérrez Gregoric et al. (2013) reported the presence of the genus in Misiones Province based on fieldwork conducted between 2009 and 2010; however, species-level identification was not achieved.

Specific morphological identification of *Omalonyx* species rely on characters of the genital system (Tillier 1981, Arruda et al. 2006, 2008a, b, Coscarelli and Vidigal 2011). All the individuals analyzed in this study presented the serpent-like fold on the outer surface of the epiphallus, the most robust diagnostic taxonomic character for diagnosing *O. unguis*, exhibiting different extent of undulation among specimens. *Omalonyx unguis* differs from *O. matheroni, O. pattersonae*, and *O. brasiliensis* (Simroth, 1896), in having the insertion of the penial retractor muscle on the distal region of the vas deferens, and differs from *O. matheroni*, *O. pattersonae*, *O. brasiliensis*, and *O. convexus* in having the diameter of proximal penis similar to that of epiphallus. *Omalonyx unguis* differ from *O. geayi* Tillier, 1980 and *O. pattersonae* in lacking longitudinal folds on the inner surface of the free oviduct. In addition, *O. unguis* differs from *O. geayi*, and the whole genus, in the shape and distribution of the inner penis surface papillae. Further information on the anatomical differences amongst *Omalonyx* species can be found in Arruda (2011) and Vidigal et al. (2018).

Our BLASTN searches of the COI sequences revealed that samples of *O. unguis* from Itá, Zaimán, and Garupá streams were identical (100% query coverage, 100% maximum identity) to Foz do Iguaçu, Brazil samples, whereas our sample from Mártires stream was identical (100% query coverage, 100% maximum identity) to sequences from a tributary stream of the Paraguay River, as well as with High Paraná River samples from locations situated before its confluence with the Paraguay River. On the other hand, DNA sequences from the remaining samples differed from the previously characterized by Vidigal et al. (2018), thus representing new haplotypes. Further research is required to gain insights into the genetic background of the *O. unguis* populations in Misiones Province, as the molecular data presented here represent the first DNA sequences for the species in Argentina.

By last, considering the previous records of *O. unguis* in the bordering countries of Brazil and Paraguay where similar environmental conditions exist, the lack of previous reports in Misiones Province might be attributable



Figures 7–9. Reproductive system of *Omalonyx unguis* (IBS-Ma 281/1). **7.** Detail of the serpent-like fold on the outer surface of the epiphallus. Scale bar = 1 mm. **8.** Detail of the insertion of the penial retractor muscle and epiphallus in lateral view. Scale bar = 1 mm. **9.** General view of the reproductive system. Scale bar = 2 mm. Abbreviations: **Ag**, albumen gland; **Ep**, epiphallus; **Fp**, fecundation pouch; **Ga**, genital aperture; **Od**, oviduct; **Oe**, ovuliespermiduct; **Ot**, ovotestis; **Pc**, penial complex; **Pn**, penis; **Pr**, prostate; **Prm**, penial retractor muscle; **Sf**, serpent-like fold; **Sp**, spermatheca; **Va**, vagina; **Vd**, vas deferens.

to low-sampling efforts for this molluscan fauna, and further fieldwork is required to comprehensively assess the distribution pattern of this semi-slug species in the region.

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Authors' Contributions

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

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