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## Ecology of the non-native snail *Sinotaia cf quadrata* (Caenogastropoda: Viviparidae). A study in a lowland stream of South America with different water qualities

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### ABSTRACT

*Sinotaia quadrata* is a snail native from Asia recorded for the first time in South America in 2009 in central Argentina. In 2015, this species was also found in a lowland stream with different water qualities. Our aims were to contribute to the knowledge of its population ecology and to compare the individuals from the two locations anatomically. Snails were searched at 6 sites, where physicochemical and hydraulic parameters were measured. Biological samples were also taken at two sites (S3 and S4) to study the population traits of *S. cf quadrata* (density, size structure, fecundity and sex ratio) and to assess the water quality through macroinvertebrates' biological indices (richness, diversity and IBPamp). Physicochemical and biological parameters allowed us classifying sites as “moderately polluted” (S3) and “heavily polluted” (S4). At S4, the population showed a lower density, larger individuals, higher fecundity and a scarce representation of young snails. The differences observed in the radula and mantle border of snails from the two geographical regions might be attributed to environmental differences. We conclude that this species is tolerant to a wide range of environmental variables which, along with its high fecundity and morphological plasticity, could allow this species to colonize neighbor streams.

**Key words:** alien species, life history traits, lowland streams, tolerance, water quality.

### INTRODUCTION

As a consequence of a globalized world, several species have been transferred between regions through human activities, increasing the rate of species introduction between continents (Strayer 2010). Before those species can be considered as

“invasive”, they must go through different stages: in the first stage (stage of introduction), they have to overcome natural barriers, what is frequently favored by a human-aided dispersal (deliberate or accidental); in the second stage (stage of establishment), the population has to reproduce and complete all its natural life cycle successfully, overcoming all the biotic interactions that may cause

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local extinction (competition, allelopathy, diseases, etc.), and in the final stage (stage of assimilation) it must adapt to the new environmental conditions and spread out to neighbor sites (Penchaszadeh 2005, Feiner et al. 2012, Mainali et al. 2015). Although not all introduced species successfully establish or become noxious, the establishment of an alien species threatens the natural structure and function of the colonized ecosystems with potential negative consequences on the global biodiversity (Sala et al. 2000). According to the kind of recipient habitat (river, lake, stream, etc.) and the trophic role of the introduced species (primary producer, predator, grazer, etc.), different direct and indirect effects will be observed in the invaded ecosystem. Direct effects can include modifications in the biotic interactions (for example, competition for food and space), whereas the most important indirect effects include the changes in the habitat characteristics (e.g. nutrient enrichment and increased turbidity and organic matter concentrations) (Riley et al. 2008, Morrison and Hay 2011, Gallardo et al. 2016). In this frame, the early detection of a non-native species population should count with appropriate rapid response strategies to avoid its dispersal and harmful effects on the environment (Beric and Mac Isaac 2015).

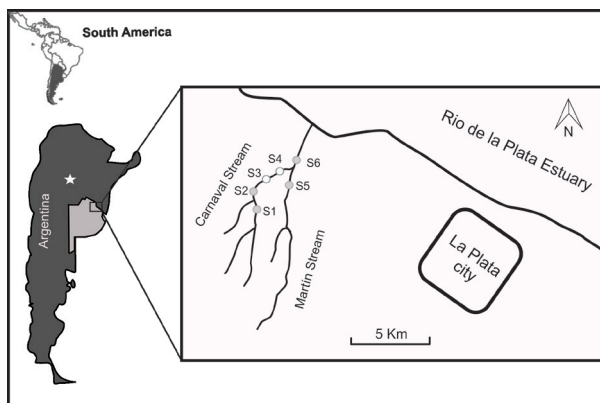
To the date, 41 alien molluscs species have been cited for Argentina, 5 of which are freshwater and estuarial snails: *Melanoides tuberculata* (Müller, 1774) (Peso and Quintana 1999, Gutiérrez et al. 2007), *Pseudosuccinea columella* (Say, 1817), *Physa acuta* (Dreparnaud, 1805), *Physa venustula* (Gold, 1844) (Rumi et al. 2008) and *Helisoma duryi* (Wetherby, 1879) in aquaria (Rumi et al. 2002). Recently, a new species was added to this list: the snail *Sinotaia quadrata* (Benson 1842) (Caenogastropoda: Viviparidae) (Ovando and Cuezco 2012). Fossil records of viviparid species had been described between the Cretaceous and the Tertiary for different areas of South America -Argentinean Patagonia, Chile, Bolivia and Brazil-

(Parodíz 1969); however, the first living viviparid population was recorded in 2009 at the Grande de Punilla River (Punilla Valley, Córdoba Province), a region of low altitude mountains (about 900 m) located in Central Argentina (Ovando and Cuezco 2012). *Sinotaia quadrata* is native to Asia and inhabits lakes, ponds, streams and rice paddies of Taiwan, China, Korea, Philippines and Japan (Hirano et al. 2015). It is frequently found in muddy sediments, associated to the freshwater clam *Corbicula fluminea* (Chiu et al. 2002). The knowledge on the ecology of this snail is scarce and fragmented, probably due to the uncertain taxonomic identification (mainly based in the shell morphology), which has derived in a multiple synonyms (i.e., *Bellamya quadrata* (Benson, 1842), *Paludina quadrata* (Reeve, 1862) and *Vivipara quadrata* (Kobelt, 1909), among others. In this context, based on the premise of conspecificity, this research aimed to contribute to the knowledge of the ecology of this snail through the study of a population of *S. cf quadrata* found in a lowland stream located at Buenos Aires Province (about 700 km away from the sites where it was first recorded in Córdoba Province). Although the information provided here is preliminary, this contribution is essential to the knowledge of this species and helps to understand the biological hazards that this snail represents to in the local fauna and environment. Also, in order to assess the tolerance ranges and morphological variability of this species, we compared the anatomy of the individuals of *S. cf quadrata* from the lowland stream with those from Córdoba and compared the population traits recorded in two sites with different water qualities.

## MATERIALS AND METHODS

### STUDY AREA

The first individuals of *S. cf quadrata* were found in benthic samples taken in November 2015 in zone with dense riparian vegetation of the



**Figure 1** - Study area. S1-6: Sites where *Sinotaia cf quadrata* was searched (white spots indicate where the species was present). The star in the map of Argentina indicates the area where *S. quadrata* was first recorded in Córdoba, Argentina, according to Ovando and Cuezco (2012).

Carnaval stream (S3), a semi-urban stream located in the proximities of La Plata city (Fig. 1). Then, the species was also searched at two sites located upstream (S1 and S2, at 3.8 km and 2.7 km from S3, respectively), and one site 500 m downstream (S4, at a portion that has recently been channeled and receives untreated domestic wastes). Also, we looked for *S. cf quadrata* individuals at Martín stream (S5), which runs near Carnaval stream, and at the Villa Elisa channel (S6), where both streams –Carnaval and Martín– converge to flow into the Río de la Plata Estuary. The most relevant characteristics of the habitat and stream morphology were recorded *in situ*: vegetation cover and richness, depth, width, flow, discharge, physical alterations derived from recent hydraulic works (3-5 years old) and other anthropogenic impacts such as presence of garbage and domestic discharges. The granulometric and organic matter analysis of the sediments were done by gravimetric and ignition methods, respectively, only for the sites where the species occurred. The following water physicochemical parameters were also measured at each site with a multiparametric sensor (Horiba U40) by triplicate in water free of vegetation as well as among vegetation stands:

pH, temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{DO mg l}^{-1}$ ), conductivity ( $\mu\text{S cm}^{-2}$ ), Redox potential (mV) and turbidity (NTU). Additionally, water samples were taken in plastic bottles for the analysis at the laboratory of chemistry of nutrients concentrations (Nitrates, Nitrites, Ammonia and Soluble Reactive Phosphorus,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$  and SRP, respectively) and oxygen demands (biochemical and chemical,  $\text{BOD}_5$  and COD, respectively) under standardized protocols (APHA 1998). The statistical differences between sites in these physicochemical parameters were assessed through One-way ANOVA; *a posteriori* Student-Newman-Keuls (SNK) tests were performed to compare sites when significant differences were detected. S6 was excluded of some analysis (nutrients and oxygen demands) because of the lack of data.

The ecological status of the two sites where the *S. cf quadrata* was present was assessed through the Shannon index (macroinvertebrate diversity), species richness (number of taxa) and through a local biotic index IBPamp (Indice Biótico Pampeano, Rodrigues Capítulo et al. 2001). Statistical differences were assessed through a t-test. We also described the molluscs fauna associated to *S. cf quadrata* in order to compare it with the community described for the mountain streams of Córdoba described by Ovando and Cuezco (2012). A two-way ANOVA with the fixed factors “Site” (with two levels, S3 and S4) and “Habitat” (with two levels, vegetated and non-vegetated areas) was performed to compare the physicochemical variables at those sites with an *a posteriori* Student-Newman-Keuls test when statistically significant differences were found between treatments.

#### POPULATION TRAITS

At the sites where *S. cf quadrata* was found (Fig. 1), five biological random samples were taken among macrophytes stands and five in non-

vegetated substrates with a bottomless 60 x 60 square (30 cm height) in February, April, May and June 2016. All the invertebrates comprised in that square -including *S.cf quadrata* individuals- were collected with a sieve (mesh of 500 µm open) and fixed in formaldehyde (5%). These samples were used for the calculation of the biotic indices named above and for the estimation of *S. cf quadrata*'s population traits: density, size distribution, sex ratio and fecundity. The sex of mature individuals was determined after Ovando and Cuezco (2012): males showing the right tentacle modified as a copulatory organ and females without that feature and/or bearing embryos inside the uterus. The fecundity was determined as the number of embryos (partially or totally developed) in the brood pouch. The shell size (shell length in mm) of *S. cf quadrata* individuals from the two sites were compared through t-test, whereas the spatial and temporal differences in density values were assessed through a two-way ANOVA with a posteriori SNK tests.

#### ANATOMICAL ANALYSIS

Twelve individuals of *S. cf quadrata* were collected, kept alive and brought to the laboratory of Malacology of the Museo de La Plata (La Plata Museum of Natural Ciencias, MLP) in order to confirm the identity of the species based on the anatomical description provided by Ovando and Cuezco (2012). The morphology of the shell, the radula and other anatomical features (reproductive system and mantle) were analyzed. Soft parts were separated from the shell for subsequent processing, after relaxation with menthol solution for about 24h, and fixed in Railleit-Henry or 70% alcohol. Shell measurements (total length and maximum diameter) were done using a manual caliper and the dissection was done under a stereoscopic binocular microscope (LEICA MZ6). Following Holznagel proposal (1998), two radula were separated from the tissue mass and placed in 0.5 ml of NET buffer

(76 % water, 20 % of SDS 10%, 2% 0.5M EDTA, 1 % Tris pH 8.0 and 1 % NaCl 5M) and 0.02 ml of proteinase K (20 mg/ml). They were then incubated at 57°C, with renewed NET buffer and proteinase K until verify the absence of tissue. After two washes with distilled water, samples were preserved in 25% ethanol. The radula were reviewed under an electron microscope Scanning (JEOL 6360) belonging to the Museo de La Plata. The specimens examined were deposited in the Molluscs collection of the Museo de La Plata (MLP-Ma No. 14190), establishing the southernmost record of *S. cf quadrata*.

## RESULTS

#### HABITAT CHARACTERISTICS

The description of the sites surveyed for the occurrence of *S. cf quadrata* is shown in Table I. Most data were collected between late spring (November) and early autumn (April). In general, the sites analyzed were shallow slow-flow streams. The width was strongly affected by the presence of hydraulic works. The most urbanized sites presented greater amounts of garbage. Untreated domestic discharges were detected only at sites S4 (Carnaval stream) and S5 (Martín stream). Aquatic vegetation was present at all sites, although the richness and coverage was variable between sites. Among the most abundant macrophytes were *Gymnocoronis spilantoides* (D. Don ex Hook and Arn.), *Hydrocleys nymphoides* (Willd) Buch, *Hydrocotyle ranunculoides* L.F, *Ludwigia peploides* (H.B.K.) Raven, *Egeria densa* Planch, *Potamogeton sp.*, *Schoenoplectus californicus* (C.A. Mey) Sojak and *Sagittaria montevidiensis* Cham. Et Schlech. Stands of the algae *Chara sp.* were found only at S3.

No statistical differences were found between sites for temperature, turbidity, nutrients and oxygen demands, whereas the physicochemical variables pH, ORP, conductivity and OD exhibited differences between sites (Table II). No habitat differences were found for the variables measured

**TABLE I**  
General characterization of the collecting sites. Mean  $\pm$  Standard Deviation of the morphometric and physicochemical parameters. Crosses (x) indicate presence whereas the asterisks (\*) indicate abundance (from one, for the lowest abundance to three, for the highest). Nm = not measured.

	S1	S2	S3	S4	S5	S6
Latitude	34°53'7.84"S	34°52'37.37"S	34°51'36.55"S	34°51'28.22"S	34°51'59.62"S	34°51'23.12"S
Longitude	58° 5'23.62"W	58° 5'24.34"W	58° 4'32.77"W	58° 4'5.17"W	58° 4'7.15"W	58° 3'39.84"W
<i>Morphometric data</i>						
Depth (m)	0.12 $\pm$ 0.02	0.17 $\pm$ 0.02	0.23 $\pm$ 0.11	0.12 $\pm$ 0.04	0.12 $\pm$ 0.06	0.16 $\pm$ 0.11
Width (m)	4 $\pm$ 0.5	7 $\pm$ 0.5	6 $\pm$ 1.1	3 $\pm$ 2.3	6 $\pm$ 1.1	10 $\pm$ 2.4
Flow (m seg-1)	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002	0.01 $\pm$ 0.007	0.08 $\pm$ 0.05	0.04 $\pm$ 0.06	0.04 $\pm$ 0.04
Discharge (m3 seg-1)	0.01 $\pm$ 0.002	0.03 $\pm$ 0.005	0.03 $\pm$ 0.01	0.02 $\pm$ 0.008	0.01 $\pm$ 0.02	0.06 $\pm$ 0.01
Plant cover (%)	50	40	70	30	70	60
Plant richness	3	7	7	6	10	6
<i>Physical alterations</i>						
Channelization				x		x
Dredging	x				x	
Straightening					x	x
Other impacts						
Presence of garbage	*	**	*	**	***	*
Domestic discharges				**	*	
<i>Physico-chemical data</i>						
Temperature (°C)	26 $\pm$ 1	24 $\pm$ 1	21 $\pm$ 3	24 $\pm$ 3	18 $\pm$ 8	20 $\pm$ 1
pH	9.6 $\pm$ 0.2	9.2 $\pm$ 0.1	7.3 $\pm$ 0.1	8 $\pm$ 0.2	6.3 $\pm$ 2.8	7.4 $\pm$ 0.1
OD (mg/l)	10.7 $\pm$ 1.4	13.1 $\pm$ 1.8	4.4 $\pm$ 2.7	8.3 $\pm$ 0.9	2.4 $\pm$ 1.0	7.1 $\pm$ 0.2
ORP (mV)	149 $\pm$ 9	168 $\pm$ 5	208 $\pm$ 15	211 $\pm$ 22	166 $\pm$ 69	242 $\pm$ 9
Conductivity ( $\mu$ S cm <sup>-2</sup> )	366 $\pm$ 2	574 $\pm$ 7	716 $\pm$ 60	991 $\pm$ 186	720 $\pm$ 357	794 $\pm$ 2
Turbidity (NTU)	14 $\pm$ 9	24 $\pm$ 16	34 $\pm$ 14	34 $\pm$ 19	22 $\pm$ 12	47 $\pm$ 1
NO <sub>3</sub> <sup>-</sup> (mg N l <sup>-1</sup> )	0.06 $\pm$ 0.05	0.02 $\pm$ 0.03	0.25 $\pm$ 0.14	0.68 $\pm$ 0.37	0.16 $\pm$ 0.07	nm
NH <sub>4</sub> <sup>+</sup> (mg N l <sup>-1</sup> )	0.03 $\pm$ 0.04	0.004 $\pm$ 0.003	0.34 $\pm$ 0.31	0.56 $\pm$ 0.65	0.35 $\pm$ 0.08	nm
SRP (mg P l <sup>-1</sup> )	0.35 $\pm$ 0.11	0.25 $\pm$ 0.15	0.79 $\pm$ 0.11	0.53 $\pm$ 0.25	0.5 $\pm$ 0.17	nm
COD (mg O <sub>2</sub> l <sup>-1</sup> )	23.5 $\pm$ 7.7	19.5 $\pm$ 10.6	11.6 $\pm$ 9.3	6.6 $\pm$ 0.6	16.5 $\pm$ 6.6	nm
BOD <sub>5</sub> (mg O <sub>2</sub> l <sup>-1</sup> )	4 $\pm$ 1	2 $\pm$ 0	4 $\pm$ 3	3 $\pm$ 2	4 $\pm$ 2	nm
<i>Sinotaia cf. quadrata</i>			x	x		



TABLE II

Results of the Analysis of Variance (ANOVA) and *a posteriori* Student-Neuman-Keuls (SNK) test for the physicochemical data recorded in Sites 1 to 6. ORP: REDOX potential; DO: dissolved oxygen; NO<sub>3</sub><sup>-</sup>: Nitrates; NO<sub>2</sub><sup>-</sup>: Nitrites; NH<sub>4</sub><sup>+</sup>: Ammonia; SRP: Soluble Reactive Phosphorous; DBO<sub>5</sub>: Biochemical oxygen demand; COD: Chemical oxygen demand.

Variable	DF	SS	F	p	SNK
Temperature	5	15.7	2.13	0.07	NS
pH	5	20.8	4.15	<0.01	S1=S2≠S3=S6≠S5≠S4
ORP	5	12536.3	3.81	<0.01	S1≠S2≠S3≠S4=S6≠S5
Conductivity	5	2984874.9	32.22	<0.01	S1=S2≠S3=S6≠S5≠S4
Turbidity	5	6564.2	2.77	0.04	NS
DO	5	665.4	33.3	<0.01	S1≠S2≠S3≠S4=S6≠S5
NO <sub>3</sub> <sup>-</sup>	4	9.9	0.8	0.54	NS
NO <sub>2</sub> <sup>-</sup>	4	40.0	1.9	0.16	NS
NH <sub>4</sub> <sup>+</sup>	4	2.8	2.7	0.07	NS
SRP	4	0.1	0.5	0.71	NS
BOD <sub>5</sub>	4	46.5	0.3	0.82	NS
COD	4	1976.6	1.4	0.28	NS

inside sites S3 and S4 (vegetated = non-vegetated zones). At both sites, sediments were mainly composed of gravel (60 ±5% at S3 and 47±8% at S4;  $p < 0.05$ ) followed by silt (21 ±4% and 29±5% at S3 and S4, respectively), sand (16% ±2% and 25 ±7% at S3 and S4, respectively) and low percentages of clay (3 and 3.5 % at S3 and S4, respectively). Pebbles and big stones (> 10 cm) were also found at both sites –mainly at S3, where limestone outcrops were also present. The organic matter content was, on average, slightly higher at S4 (4.3 ±2 % against 3.3±1 % at S3).

The macroinvertebrate fauna was represented by 26 taxa, including species of Ephemeroptera, Odonata (Libellulidae, Coenagrionidae), Diptera (Chironomidae, Tabanidae, Ephydriidae) and Amphipoda (*Hyaella curvispina*). Molluscs, with 8 species, were the most abundant: 3 species of bivalves –*Corbicula fluminea* (Müller, 1774), *Pisidium sterkianum* (Pilsbry, 1897) and *Eupera platensis* Klapenbach, 1967–and 5 species of gastropods –*Uncancylus concentricus* (d’Orb., 1835), *Pomacea canaliculata*, *Stenophysa marmorata* (Guilding, 1828) (absent at

S4), *Heleobia parchapii* (d’Orb., 1835) and *Biomphalaria peregrina* (d’Orb., 1835) (the last absent at S3)–. The mean density of these species is shown in (Fig. 2).

The richness of taxa was higher at S3 than at S4 (15 ± 2 and 9 ± 3 taxa, respectively;  $p < 0.01$ ). Similarly, the diversity of macroinvertebrates was higher at S3, but differences were not statistically significant (H: 2.6 ± 0.6 and 2.2 ± 0.7 at sites S3 and S4, respectively;  $p = 0.24$ ). According to the IBPamp index, S3 was classified as “moderately polluted”, whereas S4 was classified as a “heavily polluted” stream (IBPamp values of 7 and 5, respectively).

#### POPULATION TRAITS

The mean density of *S. cf quadrata* was significantly higher at S3 (23 ±8 ind m<sup>-2</sup>) than in S4 (9 ±5 ind m<sup>-2</sup>) ( $p < 0.05$ ), with a maximum value of 55.7 ind m<sup>-2</sup> recorded in June in S3. No temporal differences were found inside each site ( $p > 0.05$ ).

A total of 411 individuals were measured and sexed (297 from S3 and 114 from S4). Snails from S4 were bigger than snails from S3 (mean S3= 16.4

$\pm 4.7$  mm; mean  $S4 = 21.3 \pm 5.9$ ;  $p < 0.05$ ). Since the sex of snails was possible to be determined in individuals  $\geq 17$  mm, we categorized individuals as “immature” (shell length  $< 17$  mm), and “adults” (shell length  $> 17$  mm).

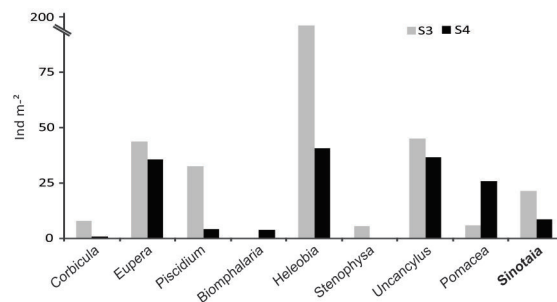
The sizes of the individuals ranged between 4.0 mm and 34.4 mm and were included in 10 size classes (Fig. 3). During the whole sampling period, the age structure of the population was dominated by immature individuals at S3, while at S4 the young individuals were scarcely represented and the structure was dominated by adults comprised between 20 and 32 mm (Fig. 3).

Gravid females were collected through February to June, although only one gravid specimen was recorded at each site in the last sampling opportunity. A maximum number of 58 embryos were recorded in May in an individual from S4 (female size: 28.08 mm). The size of recognizable embryos present at the brood pouch was very variable; from 1.9 to 5.0 mm. The mean fecundity was lower in S3 than in S4 ( $6 \pm 2$  versus  $16 \pm 2$  embryos/female, respectively  $p < 0.05$ ). Mean sex ratio was slightly biased toward females ( $0.6:0.4$  at S3 and  $0.7:0.3$  at S4).

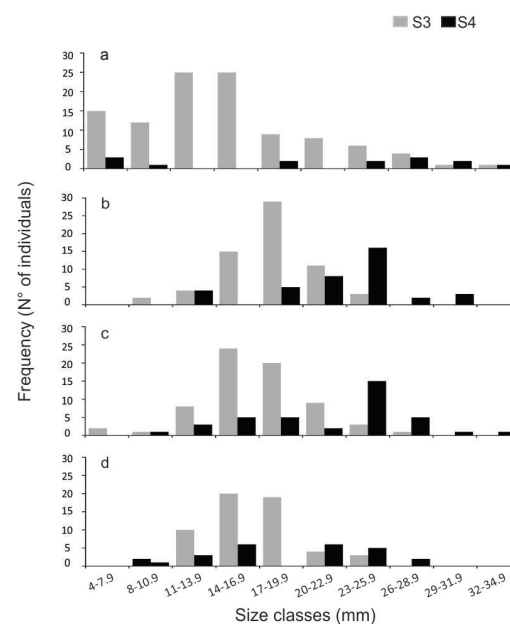
#### ANATOMICAL ANALYSIS

We examined males and females, some of the last bearing latter-stage embryos within the brood pouch. Although the specimens collected at Carnaval stream were similar to material described by Ovando and Cuezco (2012) for the population from Córdoba, we emphasize here on some morphological variations and peculiarities observed with respect to the material described by those authors (Table III).

The shells used for anatomical studies sized between 17.16 and 28.38 mm of total length; 12.42 and 19.92 mm of maximum diameter and 6 to  $6\frac{1}{8}$  whorls. Most of the thick shells showed hairs or lamellae (Fig. 4a), except for some large specimens (Fig. 4b). Their color was in general greenish-



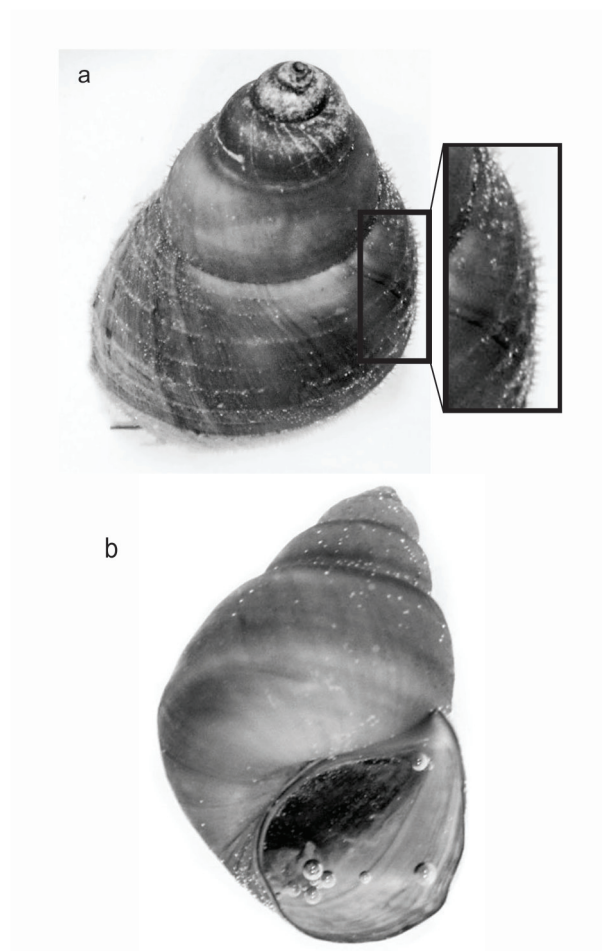
**Figure 2** - Mean density of molluscs at sites 3 (S3) and 4 (S4).



**Figure 3** - Size classes of *Sinotaia cf quadrata* individuals recorded in February (a) April (b), May (c) and June (d) at sites 3 and 4 (S3 and S4, respectively).

brown, with a blackish outer margin of aperture. Some individuals exhibited a dark collar on the margin of the mantle, folds and papillae (Fig. 5). The taenioglossate radula was narrow and long and presented between 115 and 130 rows of teeth. The rachidium tooth presented a large central cusp and 3 smaller accessory cusps on either side (Figs. 6a and d). In the female specimen, the central cusp presented a recess towards the base of the peak and the distal portion ended laterally acuminate (Fig.





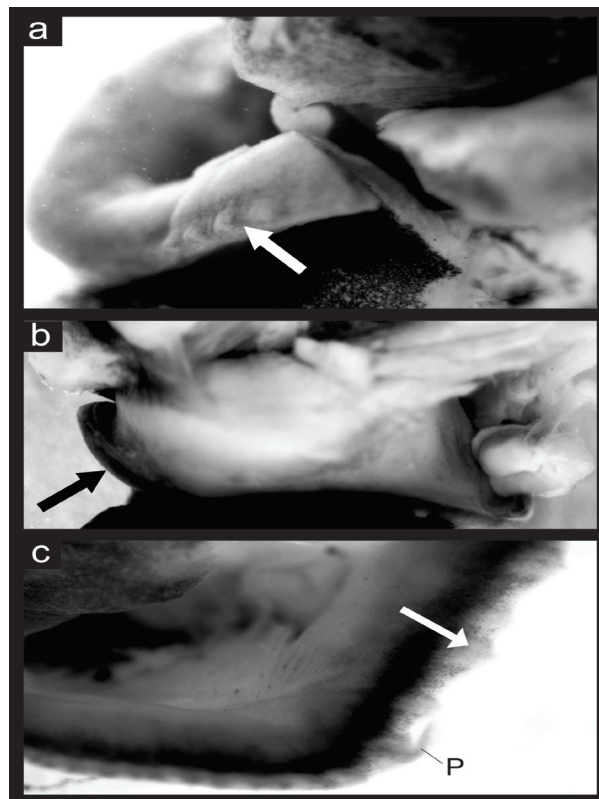
**Figure 4** - Shell morphology of *Sinotaia cf quadrata*. **a.** Histic shell (individual size: 17.16 mm). **b.** Glabrous shell (individual size: 24.3 mm)

6a). In the male specimen, in contrast, that feature was hardly seen (Fig. 6c). Two rows of lateral teeth (inner and outer) were found at each side of rachidian teeth in both sexes, with marginal teeth alternating with the laterals of the outer raw (Figs. 6b and c).

## DISCUSSION

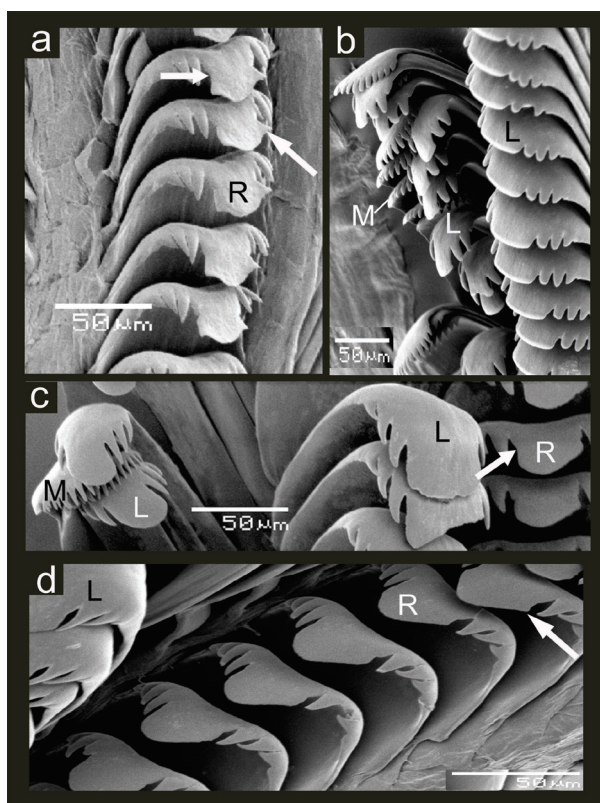
### HYPOTHESIS OF INTRODUCTION

The introduction of alien species can occur by either intentional or non-intentional ways. In the case of aquatic species, shipping (via ballast and fouling) is responsible of the dispersion of many of



**Figure 5** - Mantle border of *Sinotaia cf quadrata*. **a.** Folds (white arrow) in a lateral view; **b.** Black collar observed in the border of the mantle; **c.** Folds (white arrow) and papillae (P) seen in a plain view

the most noxious species, such as the zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Karatayev et al. 2015). The Carnaval stream, where *S. cf quadrata* was found, flows into the Rio de la Plata estuary, a way of entrance of several aquatic alien species (Penchaszadeh 2005); however since this snail is not a fouling species and its embryos develop inside the female's uterus (*i.e.*, no larvae state can be transported in ballast water), there is a low probability that this snail has been introduced in Argentina through this way. Moreover, if we consider that the two populations found in Argentina are the same species, this way of introduction could be discarded in views of the clear geographical and hydrological discontinuity between the lowland streams and the mountainous streams where the



**Figure 6** - Radular variability of *Sinotaia cf quadrata*. **a.** Rachidian teeth (R) of a female with basal constriction and distal expansion (arrows); **b.** Female's lateral (L) and marginal (M) teeth; **c.** Rachidian teeth of a male individual with constriction (arrow), lateral (L) and marginal teeth (M); **d.** Rachidian teeth of a male with basal constriction and small lateral expansions (white arrow).

species was first reported, suggesting different events of introduction. Aquarium trade is also an important way of introduction of non-native species in aquatic environments (Kerr et al. 2005, Strecker et al. 2011). In Asia, *S. quadrata* and the clam *C. fluminea* are used in aquaculture as a food source of carp species (NACA 1989). Considering that Ng et al. (2014) suggested that the *Sinotaia guangdongensis* (Kobelt, 1906) was accidentally introduced in Singapore from China with various carp species -fish widely used in artificial ponds in a Argentina- we consider that occasional extraordinary floods could have connected near artificial ponds with the Carnaval stream, favoring

the dispersion of the snail and the clam in that natural water body. Future genetic studies will help to elucidate whether the individuals found in Córdoba and the ones described in the present research are the same species and share their origin and ways of introduction.

#### POPULATION TRAITS

The presence of adults, immature individuals and females bearing embryos at all the sampling opportunities, indicate that the population of *S. cf quadrata* has been established at this stream, having gone through the first two stages necessary to be considered an invasive species (stages of introduction and establishment).

Some life history traits of viviparid species can be influenced by the seasonality and the characteristics of the environment (Hirai et al. 2004, Jakubik 2012). However, the population traits analyzed in this research showed some remarkable differences between the two sites (scarce representation of young snails, a lower density, higher fecundity and larger individuals in S4 than in S3) that could be explained under three hypotheses: first, the population traits recorded at S4 could indicate a more recent colonization of the species at this site (Sakai et al. 2001). In fact, our results are similar to those found by Konečná et al. (2015), who compared the population traits of a newly established population of the pumpkinseed, *Lepomis gibbosus* (Linnaeus 1758) with a long-established population (>10 years) finding that the newly established population presented lower density, higher fecundity and larger individuals. Those authors, however, found a higher representation of the youngest stages at the newly-colonized site, whereas in our study, we found mainly adult specimens at S4. Although the abundance of youngest individuals of some viviparids can decrease in summer and autumn (Jakubik 2012), the lower representation or absence

TABLE III

Anatomical similarities (merged columns) and differences (separated columns) between specimens found in Córdoba Province (after Ovando and Cuezco, 2012) and Buenos Aires Province (this research).

	Córdoba	Buenos Aires
SHELL		
Color	Yellowish green or pale brownish; blackish outer margin of aperture	
Whorls	6-7, slightly convex; conic obtuse spire; sutures well marked and deep	
Protoconch sculptures	Spiral lines with triangular thin hairs in juveniles (absent in adults)	
Maximum shell length	42 mm	34.4 mm
MANTLE (border)		
	Simple, smooth, thick	With folds, papillae and a black collar
RADULA		
Shape	Taenioglossate	
Rachidian tooth	Rectangular central cusp, 5-6 triangular lateral cusps	Central cusp laterally acuminate (♀); 3 triangular lateral cusps
Lateral teeth	2 rows: central cusp wide and rounded; multicuspid marginal teeth in the outer row	
GENITAL SYSTEM		
Male	Right cephalic tentacle modified as copulatory organ; testis large and curved on columellar surface	
Female	Oviduct functioning as brood pouch (“uterus”) with individual capsules containing each embryo	

of the smallest size classes at S4 could be related to the second hypothesis: an interspecific competition with *P. canaliculata*, a snail with similar anatomic characteristics which density was inversely proportional to *S. cf. quadrata*'s (Fig. 2). Moreover, Kwong et al. (2009) found that mature individuals of *P. canaliculata* predate on young individuals of *S. quadrata*, evidencing an interaction (predation) between these snails. This hypothesis, however, will be tested after the study of gut content in future studies, in which not only it will be elucidated if *P. canaliculata* is predating on young individuals of *S. cf. quadrata*, but also if there is a competition for other food sources. Our last hypothesis is that the differences observed between S3 and S4 in the population traits analyzed are due to the differences in the water quality of both sites. For example, the lower density recorded at S4 can be a

consequence of the human impact at that site, as it was observed in populations of other invertebrates of this area (Jergentz et al. 2004, Ambrosio et al. 2014). Similarly, studies on other viviparid species demonstrated that the fecundity of these snails can increase under unfavorable or unstable environmental conditions (Ribi and Gebhardt 1986, Jakubik 2012), as it was also observed in the most polluted site (S4). Future long-term studies (which have already begun), will help to clarify which of this hypothesis explains the differences in the population traits observed in the present study.

#### REGIONAL COMPARISON

The low-mountain streams of Córdoba province where *S. quadrata* was first recorded are, in general, pristine water bodies which main variability is related to the flood regimes (Principe et al. 2007,

Gualdoni et al. 2011), whereas the lowland streams of Buenos Aires province are generally eutrophic low-flow streams with high values of conductivity and moderate to high human impact (Feijoó and Lombardo 2007). Despite these differences, the collecting sites at the mountain region of Argentina described by Ovando and Cuezco (2012) exhibited some similarities with the lowland streams sampled at the current research: at both study areas there was aquatic vegetation present, low water flow and a substrate with rocks, sand and soft mud; the values of pH and conductivity at the mountain region, in contrast, were lower than in the lowland streams. The molluscs fauna composition was also similar at both kinds of environments, though at the lowland streams we recorded a higher richness, with two more species of bivalves (*P. sterkianum* and *E. platensis*) and the gastropods *U. concentricus* and *H. parchapii*, all at high densities. Solomon et al. (2010) found that the snail assemblage structure was not affected by the presence of the invasive viviparid species, *Bellamya chinensis* at the Wisconsin lakes (USA), but the described mollusc community found at Carnaval stream should be monitored to assess any possible impact of *S. cf quadrata* on the biodiversity of this lowland stream.

The detailed anatomical description provided by Ovando and Cuezco (2012) allowed us to compare the individuals of *S. cf quadrata* found in the lowland stream with those described for the mountain region of Córdoba, Argentina, finding slight differences in the radula and the collar mantle. The radula of females examined at the current research, presented the rachidium with 3 lateral denticles at both sides of median cusp, with a constriction to its base and lateral expansions to its distal portion (the last absent at male individuals), whereas the specimens from Córdoba presented 5 lateral denticles of median cusp, without such constriction in the base or lateral expansions (as it was also observed by Richter 2015). In a study of radular variability, Anistratenko et al. (2013) found

similarities in the radular morphology of close European species of Viviparidae, what suggests that the differences observed in this research do not exceed the average level of interpopulation variability for the viviparids radula. Moreover, such variability between the two different locations could be related to differences in the food offer, as it was observed for other snail species (Radwin and Wells 1968, Padilla 1998).

The mantle border is exposed to the environment, reason why it can present appendices that constitute special structures involved in the reception of stimuli (Simone 2011). Specimens of *S. cf quadrata* inhabiting Carnaval stream presented papillae, tentacles and other pallial structures found in several species of Caenogastropoda, but those macroscopic structures were absent in snails environments from Córdoba (smooth border mantle). This observation also suggests an adaptation of snails from lowland streams to different environmental conditions.

All the mentioned differences in the radular structure and collar of the mantle between snails from the two locations of Argentina could be explained by the morphological plasticity of this species, which is probably manifested in response to habitat differences. Nevertheless, genetic studies will confirm the possible existence of two different entities and shed light on the observed differences between the two Argentinean populations

#### APPROACH TO THE ENVIRONMENTAL TOLERANCE OF *S. CF QUADRATA*

The human disturbances frequently observed at urban and semi urban streams, increase the chances of success of the recently arrived species, threatening the local biodiversity (Dukes and Mooney 1999, Schreiber et al. 2003). The fact that *S. cf quadrata* was present not only at two different environments such as the mountain and lowland streams, but also at sites with different water qualities (S3 and S4), indicates that this species is tolerant to a wide range



of environmental parameters. Such tolerance, along with the lack of differences between the streams for most of the environmental variables analyzed, increases the chances of the population to reach the stage of assimilation in which it can spread to neighbor streams with similar environmental characteristics such as those described by Rodrigues Capítulo et al. 2001, Bauer et al. 2002, Cortelezzi et al. 2013. Moreover, the SNK test performed for those variables in which statistical differences were detected, allowed us to find similarities between S3 and S4 with S6 (Villa Elisa Channel), suggesting that this site is vulnerable to be invaded by this snail. Considering all this, preventive measures should be taken soon to avoid the spread and establishment of *S. cf quadrata* at new areas of invasion (Beric and Mac Isaac 2015).

In conclusion, the present research would constitute the southernmost record of *S. cf quadrata* and, although it is a preliminary study, it provides the first data on the population ecology of this species. We also point out the tolerance of this gastropod to a wide range of environmental variables, feature that allows the species to inhabit very different environments such as mountain and lowland streams, and even sites with high human impact. This wide environmental tolerance, in conjunction with its high fecundity and morphological variability, highlights the potential of *S. cf quadrata* as an invasive species and the risk of colonization of neighbor water bodies. Future studies will provide, not only more information about the population dynamics (what would help to find countermeasures for the eradication and for stopping the spread of this potential invader), but will also assess the impact of this non-native snail on the biodiversity of the already colonized environments, with special emphasis on a potential competitor as the native species *Pomacea canaliculata*. At the same time, genetic studies will elucidate whether the differences observed with the population from Córdoba are consequences of the differences in the

environmental characteristics or they are two (sub) species introduced independently.

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