Molluscan Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tmos20

New record for Galba neotropica (d'Orbigny, 1835) in Argentina, with a detailed analysis of its morphology and molecular characteristics

Julieta M. Pujadas[ae], Marisa Farber[be], Jean-Pierre Pointier[c], Claudio Giudici[d], Cristina Wisnivesky[ae] & Lucila Prepelitchi[ae]

[a] Unidad de Ecología de Reservorios y Vectores de Parásitos, Departamento de Ecología, Genética y Evolución, Instituto IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 4° piso, Laboratorio 55, Ciudad Autónoma de Buenos Aires C1428EGA, Argentina

[b] Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (CICVyA-INTA), Los Reseros y Las Cabañas, B1712WAA Hurlingham, Buenos Aires, Argentina

[c] USR 3278, CNRS-EPHE-Université, Perpignan, France

[d] Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Spangenberg y Bv. Colón (2170), Casilda, Santa Fe, Argentina

[e] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Ciudad Autónoma de Buenos Aires 1033, Argentina

Published online: 16 Jun 2015.

Molluscan Research, 2015
http://dx.doi.org/10.1080/13235818.2015.1044925

New record for *Galba neotropica* (d'Orbigny, 1835) in Argentina, with a detailed analysis of its morphology and molecular characteristics

Julieta M. Pujadas[a,e*], Marisa Farber[b,e], Jean-Pierre Pointier[c], Claudio Giudici[d], Cristina Wisnivesky[a,e] and Lucila Prepelitchi[a,e]

[a]*Unidad de Ecología de Reservorios y Vectores de Parásitos, Departamento de Ecología, Genética y Evolución, Instituto IEGEBA (CONICET–UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 4° piso, Laboratorio 55, Ciudad Autónoma de Buenos Aires C1428EGA, Argentina;* [b]*Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (CICVyA-INTA), Los Reseros y Las Cabañas, B1712WAA Hurlingham, Buenos Aires, Argentina;* [c]*USR 3278, CNRS-EPHE-Université, Perpignan, France;* [d]*Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional de Rosario, Spangenberg y Bv. Colón (2170), Casilda, Santa Fe, Argentina;* [e]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Ciudad Autónoma de Buenos Aires 1033, Argentina*

(*Received 25 October 2014; final version received 31 March 2015*)

We report for the first time the presence of *Galba neotropica* in Santa Fe province, Pampean Argentina. Until the present work, the identity of the intermediate host of *Fasciola hepatica* in this region, the most important livestock production area of Argentina, was unknown. This report extends the geographic distribution of *G. neotropica* and is the first to provide molecular and morphological information on this species in Argentina. During summer 2013, snails were collected on private livestock farms with high prevalence of fasciolosis in cattle. Specimens were identified by using the nuclear sequences of the internal transcribed spacers ITS-1 and ITS-2, the shell and the shape and size of the male reproductive organs. Molecularly, ITS-1 and ITS-2 nuclear sequences exhibit 100% nucleotide identity with *G. neotropica* from Lima, Peru. Morphologically, *G. neotropica* from Santa Fe province was indistinguishable from *Galba viatrix* and *Galba cubensis*, the other two cryptic species within this South American group of lymnaeids. The combination of molecular and morphological analyses is strongly recommended to identify lymnaeids at species level. The identity of the intermediate host and the subsequent knowledge of its susceptibility, behaviour, distribution, ecology and biology are important components in developing effective measures to control fasciolosis.

Keywords: cryptic species; fasciolosis; intermediate host; distribution; identity

Introduction

Snails of the family Lymnaeidae act as intermediate host of several trematodes, among which is *Fasciola hepatica* (Linnaeus, 1758), the aetiological agent of fasciolosis (Mas-Coma 2004; Correa *et al*. 2011). This zoonotic disease has a worldwide distribution and represents an important veterinary and health problem all over the world (Torgerson and Claxton 1999; Samadi *et al*. 2000). Eradication of this disease is not a feasible goal and control measures must be applied to reduce transmission (Downey 1991). The epidemiology of the parasite, which depends mainly on environmental factors that affect the intermediate host populations, provides the information needed to design strategic control programs (Stromberg and Averbeck 1999). In this context, the identity of the snails involved in the transmission becomes essential for designing effective control measures (Torgerson and Claxton 1999).

Until recently, the specific identification of lymnaeid snails has been based exclusively on morphological characteristics of the shell, radula, and renal and reproductive organs (Hubendick 1951; Paraense 1976; Pointier *et al*. 2004). However, reliance on these features failed to distinguish species with similar morphology (cryptic species) or variants within a single species (Pointier *et al*. 2006; Standley *et al*. 2013).

In the last decades, molecular identification of lymnaeids appeared to have solved this problem, since several mitochondrial and nuclear DNA markers have proved to be useful in the identification of these snails at a genus, species or even population level (Bargues and Mas-Coma 2005; Correa *et al*. 2010, 2011). In particular, the two internal transcribed spacers of the nuclear ribosomal DNA (rDNA ITS-2 and ITS-1) have been shown to be the most useful for species differentiation because of their relatively fast evolution (Bargues *et al*. 2007), and

*Corresponding author. Email: jpujadas@ege.fcen.uba.ar

therefore, have been the most used in lymnaeid studies (Bargues and Mas-Coma 2005). Today, there is a general consensus that both molecular and morphological analyses are needed to correctly identify the members of the family Lymnaeidae (Samadi *et al*. 2000; Pointier *et al*. 2006; Correa *et al*. 2011).

Cryptic South American lymnaeids, namely *Galba (= Lymnaea) truncatula* (Müller 1774), *Galba (= Lymnaea) viatrix* (d'Orbigny, 1835), *Galba (= Lymnaea) neotropica* (d'Orbigny, 1835) and *Galba (= Lymnaea) cubensis* (Pfeiffer, 1839), are a good example of the way in which molecular analysis helps in their differentiation (Correa *et al*. 2011). They are considered cryptic species because they are apparently morphologically indistinguishable and some of them have been traditionally treated as a single taxon (Paraense 1976).

The four species are similar in size and shell morphology (Samadi *et al*. 2000; Correa *et al*. 2011), and only *G. truncatula* is distinguishable from the rest by the anatomy of the prostate and penial complex (Samadi *et al*. 2000; Pointier *et al*. 2006). *Galba viatrix*, *G. cubensis* and *G. neotropica* differ only by the DNA sequences of different molecular markers, such as ITS-1, ITS-2 and cytochrome oxidase subunit I (COI) (Pointier *et al*. 2006; Bargues *et al*. 2007; Standley *et al*. 2013).

Galba viatrix was described with two variants, *A ventricosa* from Rio Negro, Argentina, and *B elongata* from Lima, Peru (d'Orbigny 1835). Both variants are anatomically identical and for a long time were considered as a single species (Paraense 1976). However, the sequences of ITS-1, ITS-2 and COI markers of each variant showed significant differences, which allowed two species, *Galba neotropica* (= *Lymnaea viatrix var. B elongata* sensu Paraense 1976) and *Galba viatrix* (= *Lymnaea viatrix var. A ventricosa* sensu Paraense 1976), to be distinguished (Bargues *et al*. 2007).

The present geographic distribution of *G. neotropica* is restricted to Peru, Venezuela and Argentina (Fig. 1). In Peru it was found in Lima (type locality) and Cajamarca (Bargues *et al*. 2007, 2012); in Venezuela it was found in Carabobo and Falcón (Bargues *et al*. 2011) and in Argentina it was found in Mendoza, northern Patagonia (Mera y Sierra *et al*. 2009; Standley *et al*. 2013) and Buenos Aires, Central region (Sanabria *et al*. 2012). Identities of all these records were achieved by molecular markers.

In Argentina, *G. neotropica* was found to be naturally infected with *F. hepatica* in Mendoza (Mera y Sierra *et al*. 2009), and in experimental infections the specimens from Buenos Aires proved to be susceptible to the parasite (Sanabria *et al*. 2012). These results confirmed the role of *G. neotropica* in the transmission of fasciolosis and highlighted the need for more detailed studies on its distribution, ecology and biology.

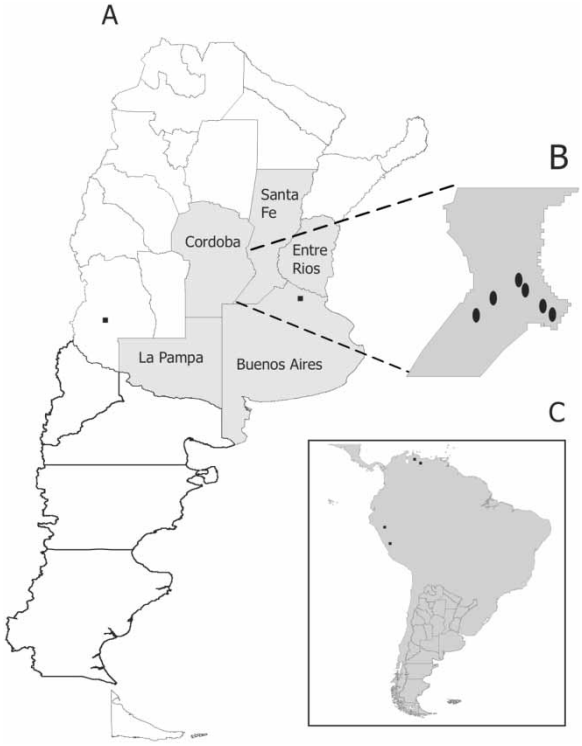

Figure 1. **A,** Map of Argentina showing the provinces of the Pampean region (grey) and previous records of *Galba neotropica* (black squares). **B,** Southern Santa Fe province showing *Galba* sp. collection sites (black ellipses). **C,** Map of South America showing *G. neotropica* distribution.

The main objectives of this work are: (1) to report for the first time the presence of *G. neotropica* in Santa Fe province, Pampean region, the main livestock production area in Argentina; and (2) to provide molecular and morphological information on this species.

Materials and methods

Study area

The study was performed in southern Santa Fe province, Pampean region, Argentina (Fig. 1).

This area belongs to the Pampean ecoregion (Burkart *et al*. 1999), and is characterised by a temperate-humid climate with annual mean air temperature of 14 °C (minimum mean: 7.2 °C in July and maximum mean: 21.5 °C in January) (Bayala *et al*. 2013). Total annual precipitation ranges from 600 mm in the west to 1200 mm in the east (Magrin *et al*. 1997).

Snail collection and analyses

Snails were collected in late summer (March) 2013, from six livestock farms with local history of fasciolosis (Fig. 1, Table 1).

Table 1. *Galba* sp. collection sites in southern Santa Fe province, Pampean region, Argentina.

Collection site	Department	Coordinates (latitude; longitude)	Altitude (m a.s.l.)
R1	Rosario	33°02′17.6″S; 60°54′36.6″W	43
R2	Rosario	33°06′05.7″S; 60°51′31.5″W	36
R3	Rosario	33°05′56.1″S; 61°14′19.0″W	75
CO1	Constitucion	33°24′38.3″S; 60°38′58.6″W	0
CO2	Constitucion	33°28′45.2″S; 60°34′41.2″W	52
CA1	Caseros	33°23′24.3″S; 61°31′39.0″W	96

Note: m a.s.l.—metres above sea level.

On each farm, snails were collected in flooded zones and in permanent and temporary water bodies. At each sampling site, snails were collected with forceps for a 30-minute period by the same person. Water temperature, pH and conductivity were recorded at each site. Collected snails were transported alive to the laboratory in plastic flasks containing water, mud and vegetation from the collecting sites.

Snails were measured using a stereo microscope with a graduated eyepiece. Specimens > 4 mm were considered adults (Kleiman *et al*. 2007).

For parasitological analysis, snails were placed in plastic vials containing dechlorinated water and exposed to artificial light for at least 2 hours to stimulate the shedding of *F. hepatica* cercariae (Souza *et al*. 2002).

About 10% of the collected adults were relaxed overnight using menthol, killed by immersion in hot water (70 °C) for 40 seconds and immediately submerged for 15 seconds in cold water to avoid tissue damage (Pointier *et al*. 2006). The animal was removed from the shell. The pedal region (foot) was cut off and preserved in 70% ethanol for molecular analysis. The rest of the tissues were preserved in Railliet–Henry's fluid for morphological analyses (Paraense 1984).

Molecular analyses

DNA extraction and amplification

Six snails, one from each site, were used for molecular determination through the amplification and sequencing of ITS-1 and ITS-2 of the rDNA. Total DNA was isolated from the distal part of the foot.

Extractions were performed using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Lym1657 and ITS1-Rixo primers were used to amplify ITS-1 and News2 and ITS2-Rixo primers were used to amplify ITS-2 (Correa *et al*. 2010).

Polymerase chain reaction (PCR) amplification was performed for each pair of primers in a total volume of 50 µl containing 10 µl of PCR buffer 5 × (Promega), 200 µm of each dNTP (Promega), 0.4 pmol/µl of each primer, 1 U GoTaq DNA polymerase (Promega), and 5 µl of DNA template. Amplification was carried out in a thermocycler (Bio-Rad MyCycler Thermal Cycler).

Temperature cycling for the ITS-1 and ITS-2 was as follows: 2 minutes denaturation at 94 °C, 30 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and elongation at 72 °C for 30 seconds, followed by a final extension at 72 °C for 7 minutes.

The amplified products (10 µl) were checked on 1% agarose gels in TAE buffer.

Amplicons were purified using the Qiaquick PCR purification kit (Qiagen) according to the manufacturer's protocol, and sequencing reactions were performed using Big Dye Terminator vs. 3.1 and 3130xl Genetic analyzer from Applied Biosystem (Genomic Unit, IB-INTA; Foster City, CA).

Analysis of genetic sequence data

Sequence data were compared to all sequence information of *G. viatrix*, *G. neotropica* and *G. cubensis* in South America. The following sequences from GenBank-EMBL were used for comparison in the analyses:

rDNA ITS-1: *Galba viatrix ventricosa* (GenBank Accession No. AM412227) (Bargues *et al*. 2007); *Galba viatrix* (HQ283254) (Correa *et al*. 2010); *Galba viatrix* (JN614428) (Correa *et al*. 2011); *Galba neotropica* (AM412228) (Bargues *et al*. 2007); *Galba cubensis* (HQ283253) (Correa *et al*. 2010); *Galba cubensis* (JN614427) (Correa *et al*. 2011); *Galba cubensis* (JN614425) (Correa *et al*. 2011); *Galba cubensis* (JN614422) (Correa *et al*. 2011).

rDNA ITS-2: *Galba viatrix ventricosa* (AM412224) (Bargues *et al*. 2007); *Galba viatrix* (HQ283265) (Correa *et al*. 2010); *Galba viatrix* (JN614465) (Correa *et al*. 2011); *Galba neotropica* (AM412225) (Bargues *et al*. 2012); *Galba cubensis* (HQ283264) (Correa *et al*. 2010); *Galba cubensis* (JN614464) (Correa *et al*. 2011); *Galba cubensis* (JN614462) (Correa *et al*. 2011); *Galba cubensis* (JN614463) (Correa *et al*. 2011).

All sequences were aligned using ClustalW version 1.4 (Thompson *et al*. 1994), with default parameters.

Bayesian phylogenetic inference

Phylogenetic trees were inferred using Bayesian Inference. Model selection was based on Akaike's Information Criterion using ModelTest 2.0 (Posada 2008). Bayesian

analyses were performed by MrBayes 3.1.2 (Huelsenbeck and Ronquist 2005) using Markov chain Monte Carlo for 1 million generations, sampling every 100 generations, under GTR + I model.

Morphological analyses

Snails were dissected under a stereo microscope. The following features of the male reproductive system were measured: penis sheath length (ps), preputium length (pp) and prostate length and width (Paraense 1976). The pp/ps ratio was calculated and the shape and size of the prostate gland were analysed (Paraense 1976). In addition, shell length (SL) and maximum shell width (SW) were recorded and the SL/SW proportion was calculated.

Measurements were made using a stereo microscope with a graduated eyepiece and are expressed in mm as the mean ± standard deviation (SD).

Drawings were made from printed colour photographs captured with a digital camera attached to the stereo microscope.

Voucher specimens were deposited in the National Collection of Invertebrates at the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN-In 39.211).

Results

A total of 703 lymnaeid snails were collected in streams, ditches and marshes of southern Santa Fe province. In these habitats, in late summer, water temperature (WT), conductivity (C) and pH varied (min–max [mean ± SD]) as follows: WT = 20.6–29.0 °C (24.7 ± 3.5), C = 0.6–2.3 mS/cm (1.3 ± 0.6) and pH = 7.6–8.3 (7.9 ± 0.3). Snails were also collected in muddy marshes without a water column, and consequently, these environmental variables could not be measured.

All the specimens analysed showed the same ITS-1 and ITS-2 sequences. The ITS-1 sequence was 671 base pairs (bp) long and exhibited 100% nucleotide identity with *G. neotropica* (AM412228) from Lima, Peru (Bargues *et al.* 2007). The ITS-2 sequence was 553 bp long and exhibited 100% nucleotide identity with *G. neotropica* (AM412225) from Lima, Peru (Bargues *et al.* 2007). Phylogenetic analysis also revealed a high sequence similarity between *G. neotropica* from Peru and lymnaeids collected in this study (Fig. 2).

Based on these comparisons, our specimens from Santa Fe, Pampean region, Argentina, were identified as *G. neotropica*. ITS-1 and ITS-2 sequences were deposited in GenBank under accession numbers KJ425594 and KJ425596, respectively.

The morphological analyses were performed on 64 adult snails (> 4 mm). The shell was brown in colour and composed of five whorls that decreased slowly in diameter towards the apex (Fig. 3). The spire was long, narrow and pointed while the body whorl was poorly developed. The aperture was oval and about half as long as the total shell length (Fig. 3).

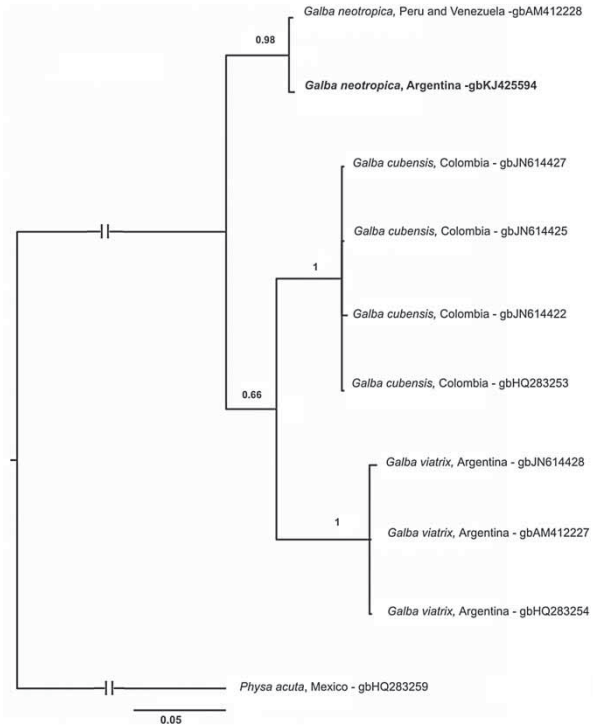

Figure 2. Bayesian inference tree of cryptic lymnaeids species (*Galba neotropica*, *Galba viatrix* and *Galba cubensis)* based on internal transcribed spacer 1 (ITS-1) (same topology was obtained with ITS-2 sequences, data not shown). Numbers above nodes denote posterior probability values. *Physa acuta* was used as outgroup.

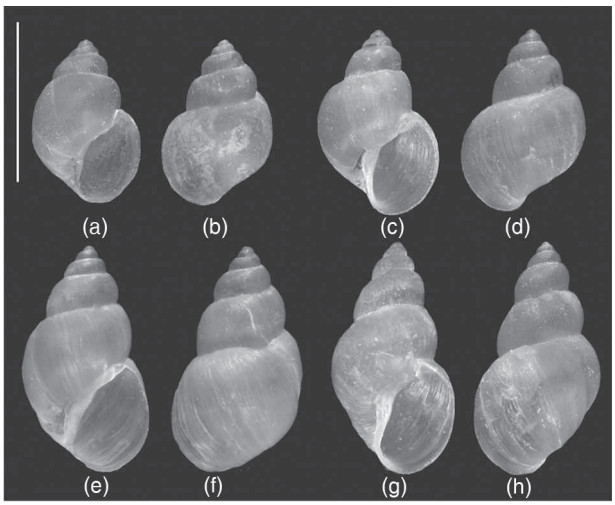

Figure 3. External aspect of the shell of *Galba neotropica* from southern Santa Fe province, Argentina, in ventral and dorsal view. **A–B,** Collection site CO1. **C–D,** Collection site CO2. **E–F,** Collection site R3. **G–H,** Collection site R1. Scale bar = 6 mm.

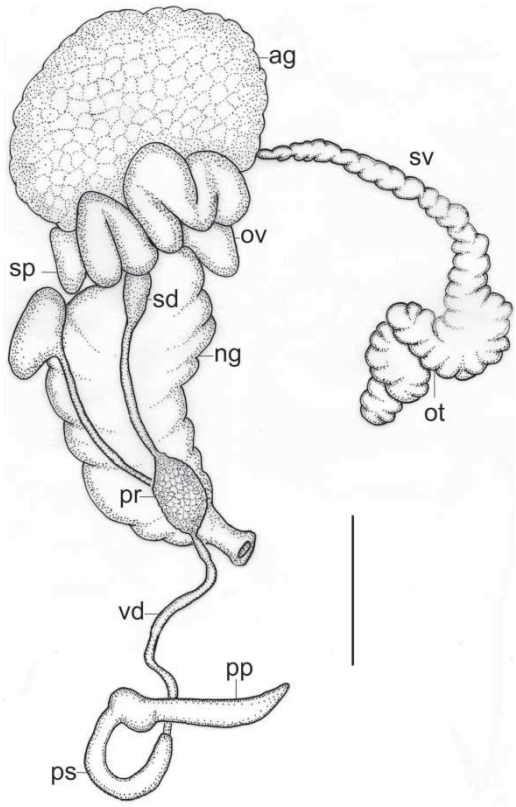

Figure 4. Anatomy of the reproductive system of *Galba neotropica* from southern Santa Fe province, Pampean region, Argentina. Abbreviations: ag—albumen gland; ng—nidamental gland; ot—ovotestis; ov—oviduct; sd—spermiduct; pr—prostate; pp—preputium; ps—penis sheath; sp—spermatheca; sv—seminal vesicle; vd—vas deferens; va—vagina. Scale bar = 1 mm.

Shell length (SL) varied between 4.1 mm and 8.5 mm (5.4 ± 0.9 mm) and shell width (SW) between 2.5 mm and 5.1 mm (3.6 ± 0.7 mm). The SL/SW ratio varied from 1.2 to 2.1 (1.5 ± 0.2). Examples of shells showing some of the intraspecific variability in *G. neotropica* are shown in Fig. 3.

The prostate gland had a granulate surface and was ovoid and pear-like in shape (Fig. 4). It was located below the nidamental gland, on the posterior half of the ventral surface. The length of the prostate varied between 0.3 mm and 0.7 mm (0.5 ± 0.1 mm) and the width between 0.2 mm and 0.4 mm (0.3 ± 0.04 mm).

The penial complex showed a preputium (pp) slightly longer than the penial sheath (ps) (Fig. 4). The pp/ps ratio varied from 0.9 to 1.6 (1.1 ± 0.1).

Morphological comparisons between the specimens analysed in this work and the other members of the South American cryptic lymnaeid species are shown in Table 2.

Comparisons with *G. cubensis*, the third member of this cryptic group, could not be made because of the lack of information on the anatomical measurements of this species. Specimens from southern Santa Fe province, Argentina, had the widest range of SL/SW ratio and the lowest range for both pp/ps ratio and prostate length (Table 2).

None of the snails were found to be infected with either *F. hepatica* or any other trematode.

Discussion

This paper reports the first finding of *G. neotropica* in Santa Fe province, Pampean Region, Argentina. This new record widens the geographical distribution of this species in Argentina, which until now was only known from the Mendoza province, northern Patagonia (Mera y Sierra *et al.* 2009; Standley *et al.* 2013) and Buenos Aires province, central region (Sanabria *et al.* 2012) (Fig. 1). This finding may suggest that the geographic distribution of *F. hepatica* could be wider, as *G. neotropica* is an intermediate host of this parasite, as was observed in Mendoza province (Mera y Sierra *et al.* 2009).

In Argentina, fasciolosis is endemic and is the fourth most important disease for cattle (SENASA 2013). The central part of Argentina, known as the Pampean region, is the main livestock production area, producing more

Table 2. Some morphological characteristics of the shell and male reproductive system of the *Galba neotropica* described in this work ($n = 64$), and comparisons with *Galba neotropica* from Peru and *Galba viatrix* from Argentina.

	Galba neotropica	*Galba neotropica*		*Galba viatrix*
Source	This study ($n = 64$)	Paraense (1976)	Bargues *et al.* (2007)	Paraense (1976)
Collection site	Santa Fe, Argentina	Lima, Peru	Lima, Peru	Rio Negro, Argentina
Shell				
SL/SW	1.2–2.1 (1.5 ± 0.2)	1.6–1.8 (1.7)	1.7–1.9 (1.8 ± 0.1)	1.7–1.9 (1.8)
Male reproductive system				
pp/ps	0.9–1.6 (1.1 ± 0.1)	1.1–3.9 (2.7)	1.3–3.4 (2.1 ± 0.3)	1.2–2.8 (1.6)
Prostate (mm)	0.3–0.7 (0.5 ± 0.1)	no data	0.6–1.1 (0.8 ± 0.01)	no data

Abbreviations: SL—shell length; SW—shell width; pp—preputium; ps—penis sheath.
Note: Values are expressed as min–max (mean ± standard deviation).

than 90% of the total cattle stock (SENASA 2013). The prevalence of fasciolosis in cattle in this region is one of the highest in the whole country, and contributes nearly 80% of the total number of livers condemned due to fasciolosis (SENASA 2013). Despite the importance of the Pampean region for fasciolosis transmission, little is known about the identity of the intermediate hosts involved in the parasite life cycle. At present, *G. viatrix* has been found in Buenos Aires and Entre Rios provinces (Castellanos and Landoni 1981; Paraense 1982; Venturini and Fonrouge 1985), and *G. neotropica* in Buenos Aires province (Sanabria *et al*. 2012). Lymnaeids have never been reported in La Pampa province and in Santa Fe province nothing was known about the identity of the intermediate host of *F. hepatica* until the present work.

Despite the fact that no infected *G. neotropica* snail was found in the present study, we suspect that this species can act as an intermediate host of *F. hepatica* in the area, as its susceptibility to this parasite has already been confirmed (Mera y Sierra *et al*. 2009; Sanabria *et al*. 2012). For this reason, more detailed studies in Santa Fe province are needed to determine the importance of *G. neotropica* in the transmission of fasciolosis, its real geographic distribution in the area, and consequently, the identification of potentially high-risk areas of fasciolosis transmission to livestock.

The identification of *G. neotropica* in Santa Fe was only achieved by the combination of morphological and molecular information. The morphology and the ITS-1 and ITS-2 sequences of the specimens analysed in this work were identical to those reported for *G. neotropica* by Pointier *et al*. (2006) and Bargues *et al*. (2007). Supporting this, Bayesian inference clustered together *G. neotropica* from Peru and from Argentina (Fig. 2).

Morphologically, the *G. neotropica* analysed in this work exhibited some variability in its shell morphology (Fig. 3). These results are in agreement with Pointier *et al*. (2006), in that shell morphology is often not a reliable character for species identification, and therefore should be supplemented with anatomical features and molecular analyses. The anatomy of the male reproductive system does not itself provide clear species-level distinction in this group of species (Pointier *et al*. 2006; Standley *et al*. 2013). As can be seen in Table 2, this feature is characterised by high variability within populations, which makes species identification difficult and is why they are considered to be cryptic species. Although anatomical measurements of *G. cubensis* have never been reported, drawings of internal anatomy of that species are very similar to those of *G. viatrix* and *G. neotropica* (Pointier *et al*. 2009). The high variability of morphological characteristics analysed in this paper (as in the most performed in this group of lymnaeids), does not allow us to know the identity of the snails because their values are overlapping (Table 2). It would be useful to include other morphological characters of the different species within the group of cryptic species to assess whether diagnostic characters exist that have not been evaluated so far.

In agreement with Bargues *et al*. (2012), we believe that without the molecular analyses, the specimens studied in this work would be erroneously identified as *G. viatrix* or *G. cubensis*. In Mendoza province *G. neotropica* was first identified as *G. viatrix* based on the morphology of the shell and the internal organs, and then correctly identified by using COI sequences (Standley *et al*. 2013). In this sense, we suspect that the distribution of *G. neotropica* in Argentina could be wider, if we consider the specimens morphologically identified as *G. viatrix*. Most, if not all, of the studies on *G. viatrix* in Argentina have identified the specimens only by its morphology (Paraense 1982; Rubel *et al*. 2005; Kleiman *et al*. 2007) and, as mentioned above, this could lead to the misidentification of the species belonging to the cryptic South American species group.

Recently, a barcoding analysis carried out by Correa *et al*. (2011) on several lymnaeids raised questions about the biological reality of several species such as *G. cubensis*, *G. neotropica* and *G. viatrix*. According to these authors, the status of these taxa as separate species seems questionable on the basis of COI and ITS. Our study only included specimens of *G. neotropica*, but it would be interesting to obtain snails of the rest of the cryptic species (*G. viatrix* and *G. cubensis*) for species delimitation studies and determine if they are different species or variants within the same species.

In agreement with Standley *et al*. (2013), a conceptual taxonomic framework is required for carrying out biological and ecological studies on lymnaeids, such as population dynamics, spatial distribution, dependence on abiotic conditions and experiments to determine parasite susceptibility. Correct identification and characterisation of this group of snails is the first step towards meeting these objectives.

Acknowledgements

We thank the Secretaría de Medio Ambiente of Santa Fe province for permission to collect snails. We are deeply indebted to the owners of the private livestock farms that were visited. The help by M.V. Juan Ventura is acknowledged. We also thank Verónica Ivanov for her collaboration in drawing figures and Marcela Rodriguero for her valuable help.

We thank the referees for their critical comments on an earlier version of this article that have helped to improve our manuscript. This work received financial support from Agencia Nacional de Promoción Científica y Tecnológica of Argentina through grant PICT 2011–00031 and from Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina through grants PIP 112–200801–00821 and CONIET-Ibol 2011.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Bargues, M.D. & Mas-Coma, S. (2005) Reviewing lymnaeid vectors of fascioliasis by ribosomal DNA sequence analyses. *Journal of Helminthology* 79, 257–267.

Bargues, M.D., Artigas, P., Mera y Sierra, R.L., Pointier, J.P. & Mas-Coma, S. (2007) Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. *Annals of Tropical Medicine and Parasitology* 101, 621–641.

Bargues, M.D., González, L.C., Artigas, P. & Mas-Coma, S. (2011) A new baseline for fascioliasis in Venezuela: Lymnaeid vectors ascertained by DNA sequencing and analysis of their relationships with human and animal infection. *Parasites & Vectors* 4, 200–217.

Bargues, M.D., Artigas, P., Khoubbane, M., Ortiz, P., Náquira, C. & Mas-Coma, S. (2012) Molecular characterisation of *Galba truncatula, Lymnaea neotropica* and *L. schirazensis* from Cajamarca, Peru and their potential role in transmission of human and animal fascioliasis. *Parasites & Vectors* 5, 174–189.

Bayala, M.I., Rivas, R.E. & Scavuzzo, M. (2013) Generación de mapas de temperatura de alta resolución mediante técnicas de remuestreo. *Interciencia* 38, 502–508.

Burkart, R., Barbaro, N.O., Sanchez, R.O. & Gomez, D.A. (1999) *Eco-Regiones de la Argentina*. Reporte Secretaría de Recursos Naturales y Desarrollo Sostenible. Administracion de Parques Nacionales, Buenos Aires, Argentina.

Castellanos, Z.A. & Landoni, N.A. (1981) La familia *Lymnaeidae* Rafinesque, 1815, en la Argentina. In: Ringuelet, R.A. (Ed), *Fauna de agua dulce de la República Argentina*. Fundación para la Educación, la Ciencia y la Cultura, Buenos Aires, Argentina, pp. 55–82.

Correa, A.C., Escobar, J.S., Durand, P., Renaud, F., David, P., Jarne, P., Pointier, J.P. & Hurtrez-Boussès, S. (2010) Bridging gaps in the molecular phylogeny of the Lymnaeidae (Gastropoda: Pulmonata), vectors of fascioliasis. *BMC Evolutionary Biology* 10, 381–393.

Correa, A.C., Escobar, J.S., Noya, O., Velásquez, L.E., González-Ramírez, C., Hurtrez-Boussès, S. & Pointier, J.P. (2011) Morphological and molecular characterization of Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. *Infection, Genetics and Evolution* 11, 1978–1988.

D'Orbigny, A. (1835) Synopsis terrestrium et fluviatilum molluscorum, in suo per Americam Meridionalem itinere collectorum. *Magasin de Zoologie* 5, 1–44.

Downey, L. (1991) *Bovine TB Programme. What are the Realistic Expectations?, Eradication of Animal Disease Board*. University College Dublin Veterinary College, Dublin, Ireland.

Hubendick, B. (1951) Recent Lymnaeidae: Their variation, morphology, taxonomy, nomenclature and distribution. *Kungliga Svenska Vetenskapsakademiens Handlingar* 3, 1–223.

Huelsenbeck, J.P. & Ronquist, F. (2005) *Mr Bayes v 3.1. 2. Bayesian Analysis of Phylogeny*. University of California, San Diego and Florida State University, Tallahassee, FL.

Kleiman, F., Pietrokovsky, S., Prepelitchi, L., Carbajo, A. & Wisnivesky-Colli, C. (2007) Dynamics of *Fasciola hepatica* transmission in the Andean Patagonian valleys, Argentina. *Veterinary Parasitology* 145, 274–286.

Magrin, G.O., Traverso, M.I., Díaz, R.A. & Rodriguez, R.O. (1997) Vulnerability of the agricultural systems of Argentina to climate change. *Climate Research* 9, 31–36.

Mas-Coma, S. (2004) Human fascioliasis: Epidemiological patterns in human endemic areas of South America, Africa and Asia. *Southeast Asian Journal of Tropical Medicine and Public Health* 35, 1–11.

Mera y Sierra, R., Artigas, P., Cuervo, P., Deis, E., Sidoti, L., Mas-Coma, S. & Bargues, M.D. (2009) Fascioliasis transmission by *Lymnaea neotropica* confirmed by nuclear rDNA and mtDNA sequencing in Argentina. *Veterinary Parasitology* 166, 73–79.

Paraense, W.L. (1976) *Lymnaea viatrix*: A study of topotypic specimens (Mollusca: Lymnaeidae). *Revista Brasileira de Biologia* 36, 419–428.

Paraense, W.L. (1982) *Lymanea viatrix* and *Lymnaea columella* in the Neotropical region: A distributional outline. *Memórias do Instituto Oswaldo Cruz* 77, 181–188.

Paraense, W.L. (1984) *Lymnaea diaphana*: A study of topotypic specimens (Pulmonata: Lymnaeidae). *Memórias do Instituto Oswaldo Cruz* 79, 75–81.

Pointier, J.P., Noya, O., Amarista, M. & Théron, A. (2004) *Lymnaea cousini* Jousseaume, 1887 (Gastropoda: Lymnaeidae): First record for Venezuela. *Memórias do Instituto Oswaldo Cruz* 99, 567–569.

Pointier, J.P., Cazzaniga, N., Gonzalez-Salas, C., Gutierrez, A., Arenas, J., Bargues, M. & Mas-Coma, S. (2006) Anatomical studies of sibling species within neotropical lymnaeids, snail intermediate hosts of fascioliasis. *Memórias do Instituto Oswaldo Cruz* 101, 431–435.

Pointier, J.P., Noya, O., Alarcón de Noya, B. & Théron, A. (2009) Distribution of Lymnaeidae (Mollusca: Pulmonata), intermediate snail hosts of *Fasciola hepatica* in Venezuela. *Memórias do Instituto Oswaldo Cruz* 104, 790–796.

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25, 1253–1256.

Rubel, D., Prepelitchi, L., Kleiman, F., Carnevale, S. & Wisnivesky-Colli, C. (2005) Estudio del foco en un caso de fasciolosis humana en Neuquén. *Medicina (Buenos Aires)* 65, 207–212.

Samadi, S., Roumegoux, A., Bargues, M., Mas-Coma, S., Yong, M. & Pointier, J.P. (2000) Morphological studies of lymnaeid snails from the human fascioliasis endemic zone of Bolivia. *Journal of Molluscan Studies* 66, 31–44.

Sanabria, R., Mouzet, R., Pankrác, J., Djuikwo, F., Teukeng, F., Courtioux, B., Novobilský, A., Höglund, J., Rašný, M., Vignoles, P., Dreyfuss, G., Rondelaud, D. & Romero, J. (2012) *Lymnaea neotropica* and *Lymnaea viatrix*, potential intermediate hosts for *Fascioloides magna*. *Journal of Helminthology* 87, 494–500.

Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA). (2013) Oficina de Estadisticas. Available online at http://www.senasa.gov.ar/. [Accessed on 9 June 2015.].

Souza, C.P., Magalhaes, K.G., Passos, L.K., Pereira dos Santos, G.C., Ribeiro, F. & Katz, N. (2002) Aspects of the maintenance of the life cycle of *Fasciola hepatica* in *Lymnaea columella* in Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz* 97, 407–410.

Standley, C.J., Prepelitchi, L., Pietrokovsky, S.M., Issia, L., Stothard, J.R. & Wisnivesky-Colli, C. (2013) Molecular characterization of cryptic and sympatric lymnaeid species from the Galba/Fossaria group in Mendoza province,

northern Patagonia, Argentina. *Parasite & Vectors* 6, 304–314.

Stromberg, B.E. & Averbeck, G.A. (1999) The role of parasite epidemiology in the management of grazing cattle. *International Journal for Parasitology* 29, 33–39.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) Clustal w: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.

Torgerson, P. & Claxton, J. (1999) Epidemiology and control. In: Dalton, J.P. (Ed), *Fasciolosis*. CABI International, London, UK, pp. 113–149.

Venturini, L. & Fonrouge, R. (1985) *Lymnaea viator* (d'Orbigny 1835): Aspectos de su biología en condiciones naturales. *Revista de Medicina Veterinaria (Bs. As.)* 66, 340–345.