

Ribosomal DNA ITS–1 sequencing of *Galba truncatula* (Gastropoda, Lymnaeidae) and its potential impact on fascioliasis transmission in Mendoza, Argentina

M. D. BARGUES, R. L. MERA Y SIERRA, H. G. GÓMEZ, P. ARTIGAS & S. MAS–COMA

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Abstract

Ribosomal DNA ITS–1 sequencing of Galba truncatula (Gastropoda, Lymnaeidae) and its potential impact on fascioliasis transmission in Mendoza, Argentina.— Sequencing of the rDNA ITS–1 proved that the lymnaeid snail species *Galba truncatula* is present in Argentina and that it belongs to the haplotype HC, the same as that responsible for the fascioliasis transmission in the human hyperendemic area with the highest human prevalences and intensities known, the Northern Bolivian Altiplano.

Key words: *Galba truncatula*, Lymnaeid vectors, Human and animal fascioliasis, Transmission, Mendoza, Argentina.

Resumen

Secuenciación del ITS–1 del ADN ribosomal de Galba truncatula (Gastropoda, Lymnaeidae) y su impacto potencial en la transmisión de la fascioliasis en Mendoza, Argentina.— La secuenciación del ITS–1 del ADN demostró que la especie de gasterópodo lymnaeido *Galba truncatula* se encuentra en Argentina y que pertenece al haplotipo HC, el mismo responsable de la transmisión de la fascioliasis en el área de hiperendemia humana con las mayores prevalencias e intensidades de fascioliasis conocidas, el Altiplano Norte Boliviano.

Palabras clave: *Galba truncatula*, Vectores Lymnaeidae, Fascioliasis humana y animal, Transmisión, Mendoza, Argentina.

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M. D. BARGUES, R. L. MERA Y SIERRA, H. G. GÓMEZ, P. ARTIGAS & S. MAS–COMA, Depto. de Parasitología, Fac. de Farmacia, Univ. de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot (Valencia), España (Spain).— R. L. MERA Y SIERRA, Fac. de Ciencias Médicas, Univ. Nacional de Cuyo, Av. Libertador 80 Centro Universitario, P. O. Box 33, 5500 Mendoza, Argentina (Argentina).

Corresponding author: M. D. BARGUES. E–mail: M.D.BARGUES@uv.es

Introduction

Fascioliasis is a well-known parasitic disease transmitted by freshwater snail species of the family Lymnaeidae (Gastropoda) (Mas-Coma et al., 2005). *Fasciola hepatica* is believed to be of European origin, with the lymnaeid *Galba truncatula* as the original vector species. *G. truncatula* is a species which reproduces by selfing and spread to other continents, most probably together with the commercial export of livestock (Mas-Coma et al., 2001).

When carrying out animal fascioliasis studies in the province of Mendoza, Argentina, Mera y Sierra (2001) collected lymnaeids which he classified as belonging to the species *G. truncatula*, by comparing with morphological descriptions (Oviedo et al., 1995; Samadi et al., 2000).

This was the first time that *G. truncatula* was cited in Argentina. Studies performed during subsequent years showed that morphology is not sufficient to differentiate lymnaeid species belonging to the so-called Galba-Fossaria group (Bargues et al., 2001; Durand et al., 2002). Problems in morphological differentiation of lymnaeid species have already been detected in Mendoza (Mera y Sierra et al., 2005, 2006; Artigas et al., 2005).

The present paper aimed to verify the species classification of the lymnaeids collected by Mera y Sierra (2001) in Mendoza, by analysis of the complete sequence of the first internal transcribed spacer (ITS-1) of the nuclear ribosomal DNA (rDNA). This molecular marker has recently proved its usefulness in Lymnaeidae (Bargues & Mas-Coma, 2005; Bargues et al., 2006).

Materials and methods

The rDNA ITS-1 sequence was obtained from each of 5 lymnaeid specimens collected in the locality of El Salto, province of Mendoza, Argentina. DNA extraction procedure steps were performed according to methods outlined previously (Bargues et al., 2001). Total DNA was isolated according to the phenol-chloroform extraction and ethanol precipitation method, the ITS-1 fragment was amplified by the Polymerase Chain Reaction (PCR), sequencing performed by the dideoxy chain-termination method, and sequences were aligned using CLUSTAL-W version 1.8 (Bargues et al., 2001; Mas-Coma et al., 2001). Homologies were performed using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). The following ITS-1 sequences present in GenBank were used for comparisons: *Galba truncatula* haplotype A (AJ243018), haplotype B (AJ296270), and haplotype C (AJ272052) (Mas-Coma et al., 2001).

Results

The five ITS-1 sequences from the 5 lymnaeid specimens analyzed presented the same length of 504 base pairs (bp) and a scarcely biased GC content of 57.5%. The nucleotide sequence was in all cases the same and it is shown in table 1. The Argentinian lymnaeids presented an ITS-1 sequence showing a 99.6% similitude with the *G. truncatula* haplotype A (HA) from Europe. The nucleotide differences detected were only two mutations: the transition G / A in position 74 and the transversion T / G in position 75. Concerning *G. truncatula* haplotype B

Table 1. Complete nucleotide sequence of the first internal transcribed spacer ITS-1 of the nuclear ribosomal DNA of lymnaeids from the population of El Salto, Mendoza, Argentina.

Tabla 1. Secuencia nucleotídica completa del primer espaciador transcrito interno ITS-1 del ADN ribosomal nuclear de los lymnaeidos de la población de El Salto, Mendoza, Argentina.

1	ATCATTAACG	AGCAGCCAAC	CGAGCGTTGA	CTACTTTGTT	GTCTCAGTCA	GTCAGTCAGT
61	CAGGCCCGC	GCGTGCACG	CATGAAGCGC	TGTCGCGGG	CTGTGTCCG	TTCGTCTTTC
121	GGGGTACCTA	TTACTGTCCT	CGATGCGACC	CACGGTGACG	GCTTAGAGCC	CGTGTGCTCG
181	CCGGGTGCG	ACGGTTCAA	GAGTGGCCGG	CTTGGCTCAG	CTCGAGAGTC	AGCCGGCGAC
241	CGCCCGCCG	TCGCAAAAA	ACAGGAGGTT	AGTCCGGGGT	ACCTATGCCC	TGCCCGCGCT
301	CGCTCTCGCG	CCGGCAAGGC	GGTAGCTCCA	GCTCGCTATT	TGGCCGCGAG	GTTCAAAGAG
361	ACGACCGTGC	CTTAACCTGC	TCTCTCCGTG	GGCAACGGTC	GCCGCCCCGG	GCCTCCTAAA
421	ATTTCTTTTA	ATAAACGAA	ATTATTTTTT	AAAAATGTGT	GTCGGCTCGA	TCGTGGCACA
481	CGAAAAACAA	ACAAAAGTC	TTAT			

Table 2. Sequence length, nucleotide contents and differences found in the comparison of the sequences of ITS-1 of the nuclear ribosomal DNA of lymnaeid populations from Argentina and known haplotypes of *Galba truncatula*.

Tabla 2. Longitud, composición y diferencias nucleotídicas encontradas en la comparación de las secuencias del primer espaciador transcrito interno ITS-1 del ADN ribosomal nuclear de los lymnaeidos argentinos y los haplotipos conocidos de *Galba truncatula*.

<i>G. truncatula</i> haplotypes	Origin	Length	% GC	Positions			GenBank Acc. No.
				74	75	132	
HA	Spain, Portugal, Switzerland, Corsica	504 pb	57.5	A	G	T	AJ243018
HB	Morocco	504 pb	57.5	A	G	C	AJ296270
HC	Northern Bolivian Altiplano	504 pb	57.5	G	T	T	AJ272052
HC	El Salto, Mendoza, Argentina	504 pb	57.5	G	T	T	present work

(HB) from Morocco, the similitude was of 99.4% and a total of three mutations were detected: two transitions G/A and T/C in positions 74 and 132, respectively, and one transversion T/G in position 75 (table 2). When comparing the ITS-1 sequence of the Mendoza lymnaeid specimens with that of *G. truncatula* haplotype C (HC) from the Northern Bolivian Altiplano, no nucleotide differences appeared (table 2).

Discussion

The present work demonstrates that the lymnaeids of Mendoza belong to *G. truncatula* haplotype HC. The presence of *G. truncatula* HC in the Andean area of Argentina represents a great potential risk of fascioliasis. The haplotype HC of this lymnaeid is the same as that responsible for transmission of the disease in the endemic area with the highest prevalences and intensities known in humans, the Northern Bolivian Altiplano (Mas-Coma et al., 1999). Although of a somewhat lower level, prevalence and intensity situations found in other Andean areas of Peru are similar (Mas-Coma et al., 2005).

The province of Mendoza is also located in the Andean area and although the altitudes are not as high as those of hyperendemic zones in Bolivia and Peru, temperatures are similar because of the southern latitude (Fuentes et al., 1999). This suggests a high disease transmission capacity in Mendoza.

The wide ecological features of *G. truncatula* do allow it to come close to human settings (Mas-Coma et al., 1999). This explains why this lymnaeid is in the background of many human infections. Moreover, *G. truncatula* is markedly linked to areas where livestock is present, enabling its passive transportation by domestic animals from one place to another. The disease-spreading risk in Mendoza is evident.

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References

- Artigas, P., Mera y Sierra, R. Correa, A. P., Morales, J. R., Zuriaga, M. A., Jiménez, M., Khoubbane, M., Barges, M. D. & Mas-Coma, S., 2005. Comparison of the conchiologic morphology of lymnaeid molluscs from eastern and western Andean slopes of the endemic zones of fasciolosis in Argentina and Chile. *Acta Parasitologica Portuguesa*, 12: 243-244.
- Barges, M. D. & Mas-Coma, S., 2005. Reviewing lymnaeid vectors of fascioliasis by ribosomal DNA sequence analyses. *Journal of Helminthology*, 79: 257-267.
- Barges, M. D., Vigo, M., Horak, P., Dvorak, J., Patzner, R. A., Pointier, J. P., Jackiewicz, M., Meier-Brook, C., & Mas-Coma, S., 2001. European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiasis, based on nuclear ribosomal DNA ITS-2 sequences. *Infection, Genetics and Evolution*, 1: 85-107.
- Barges, M. D., Artigas, P., Jackiewicz, M., Pointier, J. P. & Mas-Coma, S., 2006. Ribosomal DNA ITS-1 sequence analysis of European

- stagnicoline Lymnaeidae (Gastropoda). *Heldia, München*, 6: 29–40.
- Durand, P., Pointier, J. P., Escoubeyrou, K., Arenas, J. A., Yong, M., Amarista, M., Bargues, M. D., Mas-Coma, S. & Renaud, F., 2002. Occurrence of a sibling species complex within Neotropical lymnaeids, snail intermediate hosts of fascioliasis. *Acta Tropica*, 83: 233–240.
- Fuentes, M. V., Valero, M. A., Bargues, M. D., Esteban, J. G., Angles, R. & Mas-Coma, S., 1999. Analysis of climatic data and forecast indices for human fascioliasis at very high altitude. *Annals of Tropical Medicine and Parasitology*, 93: 835–850.
- Mas-Coma, S., Angles, R., Esteban, J. G., Bargues, M. D., Buchon, P., Franken, M. & Strauss, W., 1999. The Northern Bolivian Altiplano: a region highly endemic for human fascioliasis. *Tropical Medicine and International Health*, 4: 454–467.
- Mas-Coma, S., Funatsu, I. R. & Bargues, M. D., 2001. *Fasciola hepatica* and lymnaeid snails occurring at very high altitude in South America. *Parasitology*, 123: S115–S127.
- Mas-Coma, S., Bargues, M. D. & Valero, M. A., 2005. Fascioliasis and other plant-borne trematode zoonoses. *International Journal for Parasitology*, 35: 1255–1278.
- Mera y Sierra, R. L., 2001. Distribución de Lymnaeidos en el sistema hidrográfico andino de la provincia de Mendoza, Argentina. Tesis de Maestría, Master Internacional en Enfermedades Parasitarias Tropicales (XIV Edición). Tutor: M. D. Bargues. Depto. de Parasitología, Fac. de Farmacia, Univ. de Valencia, 22 Junio 2001.
- Mera y Sierra, R. L., Artigas, P., Abraham, C., Hynes, V., González, M., Jiménez, M., Khoubbane, M., Bargues, M. D. & Mas-Coma, S., 2005. Conchiologic morphology of lymnaeid molluscs in the endemic zones of fasciolosis in Mendoza province, Argentina. *Acta Parasitologica Portuguesa*, 12: 313–314.
- Mera y Sierra, R. L., Artigas, P. & Mas-Coma, S., 2006. Lymnaeid vectors of fascioliasis in endemic areas of Mendoza, Argentina. In: *XI International Congress of Parasitology-ICOPA XI (Glasgow, UK, 6–11 August 2006)*: CD-ROM.
- Oviedo, J. A., Bargues, M. D. & Mas-Coma, S., 1995. Lymnaeid snails in the human fascioliasis high endemic zone of the Northern Bolivian Altiplano. *Research and Reviews in Parasitology*, 55: 35–43.
- Samadi, S., Roumegoux, A., Bargues, M. D., 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.
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