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


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 ADNr 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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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...
(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.

**Acknowledgements**


**References**


**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*.

<table>
<thead>
<tr>
<th>G. truncatula haplotypes</th>
<th>Origin</th>
<th>Length</th>
<th>% GC</th>
<th>Positions</th>
<th>GenBank Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA Spain, Portugal, Switzerland, Corsica</td>
<td>504 pb</td>
<td>57.5</td>
<td>A</td>
<td>G</td>
<td>T</td>
</tr>
<tr>
<td>HB Morocco</td>
<td>504 pb</td>
<td>57.5</td>
<td>A</td>
<td>G</td>
<td>C</td>
</tr>
<tr>
<td>HC Northern Bolivian Altiplano</td>
<td>504 pb</td>
<td>57.5</td>
<td>G</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>HC El Salto, Mendoza, Argentina</td>
<td>504 pb</td>
<td>57.5</td>
<td>G</td>
<td>T</td>
<td>T</td>
</tr>
</tbody>
</table>

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*.


