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Notas / *Notes*

New location for *Bulinus truncatus* (Audouin, 1827) (Gastropoda: Planorbidae) intermediate host of *Schistosoma haematobium* and its distribution in the Iberian Peninsula

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ABSTRACT

This paper reports a new population of *Bulinus truncatus* (Audouin, 1827) (Gastropoda, Planorbidae) found in the province of Almería (Southeast Spain). *B. truncatus* is an intermediate host of *Schistosoma haematobium*, the trematode which causes urinary schistosomiasis in humans. Individuals were identified to species level by double-nested PCR, resulting in 100% homology. This population is located under the driest climate conditions of the Iberian Peninsula. Data on the distribution of this species in the Iberian Peninsula was gathered and is provided in this paper. Improved knowledge of the distribution of *Bulinus truncatus* is key to assess the risk of new outbreaks of schistosomiasis in the Iberian Peninsula.

Keywords: Planorbidae; distribution; urinary schistosomiasis; bilharziosis; urogenital; Iberian Peninsula; Portugal; Spain; Almería.

RESUMEN

Nueva localidad de *Bulinus truncatus* (Audouin, 1827) (Gastropoda: Planorbidae), hospedador intermediario de *Schistosoma haematobium*, y su distribución en la península Ibérica

En este trabajo se reporta una nueva población de *Bulinus truncatus* (Audouin, 1827) (Gastropoda, Planorbidae) en la provincia de Almería (Sureste de España). Se trata de una especie que presenta considerable interés tanto zoológico como epidemiológico por su papel como hospedador intermediario de *Schistosoma haematobium*, responsable de la esquistosomiasis urogenital humana. Los ejemplares fueron determinados a nivel específico mediante PCR doble anidada, con un 100% de homología. Se trata de la población localizada más al Sureste y en condiciones de mayor aridez en la península Ibérica. Se recopilaron los datos de presencia publicados para conocer su distribución en la península. El conocimiento de la distribución de *Bulinus truncatus* es una pieza clave para evaluar el riesgo de nuevos focos de esquistosomiasis en la península Ibérica.

Palabras clave: Planorbidae; distribución; esquistosomiasis; bilharziosis; urogenital; península Ibérica; Portugal; España; Almería.

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The genus *Bulinus* O.F. Müller, 1781 (Mollusca, Gastropoda, Planorbidae) comprises a group of species mainly distributed throughout warm regions. At least 35 species of *Bulinus* have been described subdivided between *Physopsis* and *Bulinus* s.s. -the latter including the old *Phyrgophysa* genus (Mandahl-Barth, 1965). Within the genus *Bulinus* Brown (1994) recognizes four species groups; a group of taxa related to *B. africanus*, another group related to *B. forskalii*, a third group with taxa closely related to *B. reticulatus* and finally a complex of forms which includes *B. truncatus* and *B. tropicus*. As in other hololimnic species, this group shows wide genetic variation between populations in great contrast with the greatly reduced variation at the morphological level (Jones *et al*., 2001; Stothard *et al*., 1997). This fact causes phylogenetic relationship problems, and thus the apparition of lineages of reduced geographical distribution and frequent synonyms (Hubendick, 1954, 1962; Brown, 1994; Nalugwa *et al*., 2010).

Bulinus truncatus (Audouin, 1827) [Syn.: *Physa truncata* (Auduoin, 1827), *Bulinus contortus* (Michaud, 1829)] can be found dispersedly and irregularly throughout Africa, islands and coastal areas of the Mediterranean basin and the Irano-Turanian region (Grácio, 1981; Rolán *et al*., 1987; Brown, 1994). It is the principal host of *Schistosoma haematobium*, which causes urinary schistosomiasis or bilharzia, thus its worldwide distribution has aroused great interest generating most of the international scientific literature about the species ecology and distribution (Mandahl-Barth, 1965). Bilharzia is the most important human disease caused by parasitic worms, with around 200 million infected (accumulating all variations of the illness) and up to 250.000 deaths a year. It is the second cause of morbidity and mortality worldwide, after malaria, and according to the World Health Organization, around 12% of the human population is infected (Santos *et al*., 2004).

In this paper, the geographical distribution of the species in the Iberian Peninsula is shown and a new location is reported.

In march 2014 several hundred individuals of the genus *Bulinus* were found in an artificial pond –formed by excavation– in El Ejido, Almería (UTM ED50 30 S, X=518335 E, Y=4068085, 90×50 m^2, Fig. 1). Original material was deposited at the Museo Nacional de Ciencias Naturales (MNCN 15.05/72320). Ten specimens (n=10) were frozen to be analysed by molecular techniques (PCR). The use of these techniques is of maximum interest in the genus *Bulinus*, as identification based on morphological characters can induce confusion with other species both native and introduced (in the study site, a few specimens of *Physa* sp. were visually detected). This is especially relevant in this group as different species are specifically related to the life cycle of human or cattle parasites (López-Soriano, 2004; Andree & López, 2013).

Fifty 50 mg of tissue from each specimen were homogenized using a sterilized crystal homogenizer. DNA was extracted following the automatic method provided in the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Ibérica) and the solution was adjusted to 50 ng/µl. The amplification reaction was carried out over a total reaction volume of 50 µl containing 2 µg of DNA, 10 mM Tris HCL, 50 mM KCL, 0.1% Triton X-1'', 2 mM $MgCl_2$, 0.5 mM for each dNTPs (Promega, Madison), 4 U Taq Polymerase (GenScript Taq, Bionova, Pisgataway, USA) and 0.5 µM for each primer (in both directions). The primer sequence was used to amplify the Mitochondrial cytochrome oxydase subunit I (Nalugwa *et al*., 2010). The amplification was performed as follows; initial phase 4 min at 95 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C, 30 s at 72 °C and final extension step with 7 min at 72 °C, using a Sensoquest Labcycler termocycler. Amplicons were detected running an agarose 3% gel electrophoresis. PCR products were purified using the Purelink column (Invitrogen, Life Technologies, Löhne), following the instructions provided by the manufacturer. Amplified DNA sequences were obtained using the direct Big Dye sequencing method (Applied Biosystems, Life Technologies, Austin) on a genetic analyzer ABI PRISM 310 DNA sequencer (Applied Biosystems, Life Technologies) following the manufacturer's recommendations and normal procedures. All samples were sequenced in both directions. Sequence similarity was compared with other gastropod sequences registered in the GenBank of the National Centre for Biotechnology Information and was determined using BLAST (Basic Local Aligned Search Tool) nucleotide. Material used for analyses and DNA extracted are kept frozen at −80 °C and preserved at the Instituto Valenciano de Microbiología.

The analyses confirm the identification of the specimens as *B. truncatus*, with a 100% homology (sequence cover=100%, E. value=0.0, accession numbers of 100% ident: KJ135307.1, AM921807.1, AM286314.2). Hence, this is the southeastern most population detected in Spain, under the most arid climate conditions recorded in Europe: 260 mm mean

Fig. 1.— *Bulinus truncatus* (Audouin, 1827) specimens found in Almería province (Spain). Autor: F. Rodríguez-Luque.

Fig. 1.— Ejemplares de *Bulinus truncatus* (Audouin, 1827) recolectados en Almería (España). Autor: F. Rodríguez-Luque.

annual precipitations and 17.8 °C as mean temperature (Bayo, 2005, Fig. 2). These conditions are very similar to those populations studied by Yacoubi *et al*. (2007). Known distribution (Fig. 2) suggest a broader range of the species in the Iberian Peninsula. Nevertheless, it seems to be considered a rare species in the Iberian Peninsula as compared to the occurrence of other freshwater *Planorbidae*. A study of six species undertaken by Grácio (1983) in 62 different localities of the Portuguese Algarve only collected one site with the presence of *Bulinus truncatus*. There is a possibility that the species is in regression in some areas due to the alteration and destruction of its habitats and to the competitive displacement by species such as *Physa acuta* (Nobre, 1941; Grácio, 1983).

There is no information available about the current presence and life cycle of the associated parasite (*Schistosoma haematobium*) in the Iberian Peninsula, de Azevedo & Xavier (1966) considered likely that the life cycle of the parasite was being completed naturally in different sites of Portugal, where the authoctonous cases of schistosomiasis have occurred in the Iberian Peninsula. The latest case described (Grácio, 1981) occurred in the Algarve region and had *Planorbarius metidjensis* as intermediate host (possibility the Portuguese strain of *S. haematobium* had its origin in North Africa). These works conducted in Portugal have highlighted the role of *Bulinus truncatus* as potential intermediate host of *S. haematobium*. Recently, the possibility of a resurgence of the parasitic disease has emerged

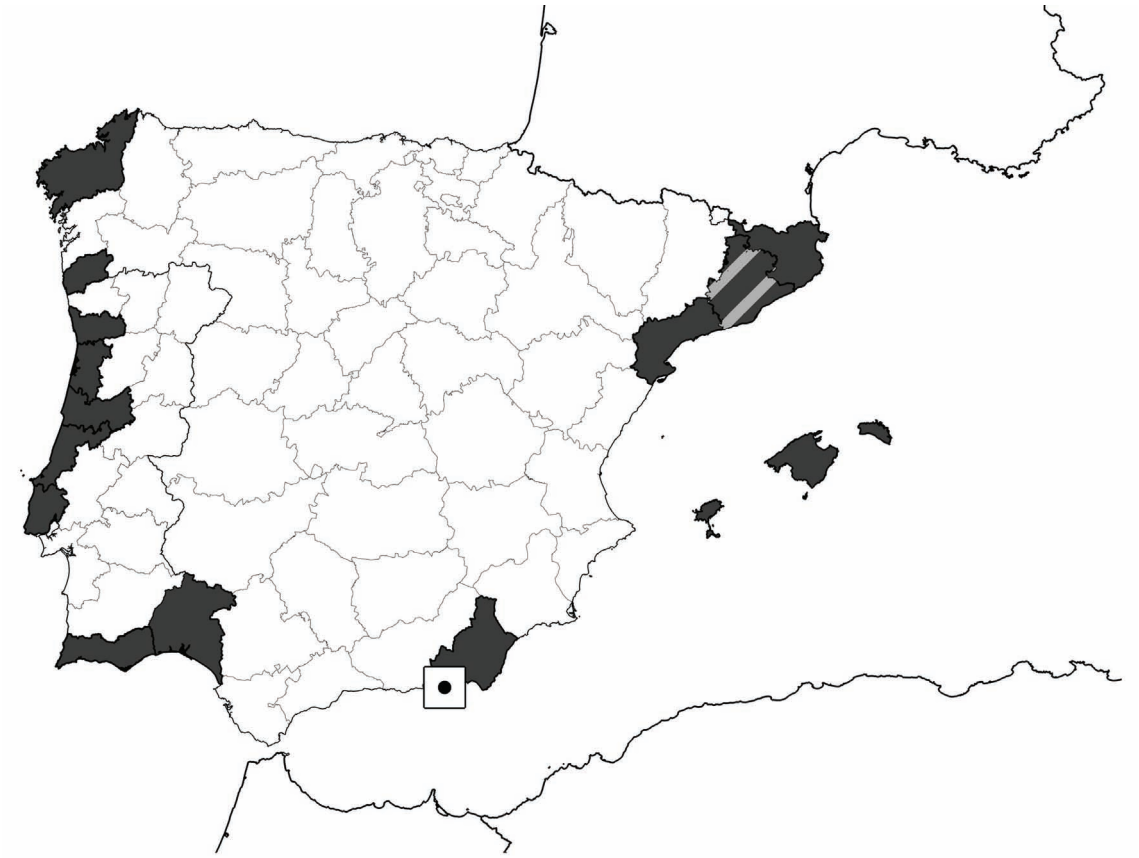

Fig. 2.— Provinces (in black) in which the presence of *Bulinus truncatus* (Audouin, 1827) has been referenced, with indication of the new record. For simplicity, provinces of occurrence are shown, since most published information does not include detailed geo-referenced locations and specific points cannot be shown. Sources: Morelet (1845)*, de Chía (1863; 1887), Martorell & Bofill (1888), Fagot (1892), Locard (1899)*, Maluquer (1917), Bofill & Haas (1920), Haas (1929), Aguilar-Amat (1933), Nobre (1941), Carvalho (1945)*, Margalef (1951), Sacchi (1957a,b), Jaeckel (1952), de Azevedo & Xavier (1965; 1966; 1969)*, Gasull (1965), Marazanof (1966), Vilella (1967), Compte (1968), Altimira (1969), Marqués-Roca (1974) –Barcelona province, in Quaternary sediments–, Colom (1978), Paul (1982), Grácio (1983)*, Rolán *et al*. (1987), Pons & Sureda (1995), Bayo (2005), Pérez-Quintero *et al*. (2004). Sources marked with (*) belong to Portugal. Strips in Barcelona province correspond to findings in Quaternary sediments.

Fig. 2.— Provincias (en negro) donde se ha documentado la presencia de *Bulinus truncatus* (Audouin, 1827) y localización de la nueva cita aportada en este trabajo. Por simplicidad se muestran las provincias, ya que la mayoría de la información publicada no contiene localizaciones georreferenciadas con detalle y los puntos específicos no pueden mostrarse. Fuentes: Morelet (1845)*, de Chía (1863; 1887), Martorell & Bofill (1888), Fagot (1892), Locard (1899)*, Maluquer (1917), Bofill & Haas (1920), Haas (1929), Aguilar-Amat (1933), Nobre (1941), Carvalho (1945)*, Margalef (1951), Sacchi (1957a,b), Jaeckel (1952), de Azevedo & Xavier (1965; 1966; 1969)*, Gasull (1965), Marazanof (1966), Vilella (1967), Compte (1968), Altimira (1969), Marqués-Roca (1974) –en sedimentos cuaternarios–, Colom (1978), Paul (1982), Grácio (1983)*, Rolán *et al*. (1987), Pons & Sureda (1995), Bayo (2005), Pérez-Quintero *et al*. (2004). Las referencias con (*) corresponden a Portugal. La trama en Barcelona indica hallazgos en estratos cuaternarios.

after the confirmation of several cases of transmission in Corsica (Berry *et al*., 2014; Holtfreter *et al*., 2014). This suggests the completion of the life cycle in the wild and the role of *Bulinus truncatus* in the transmission also for European countries. In Spain it has been considered an imported infection (mainly by immigrants, army personnel and travellers who have been in the endemic areas, Sánchez-Molina *et al*., 2010).

Due to the lack of specific studies, the presence of the parasitic worm in the natural environment cannot be ruled out, which confers a greater interest in knowing the distribution of this group of snails in areas not considered as schistosomiasis endemic regions. So far, available studies were focused on the description of patients -immigrants from African countries or travellers who had been in the areas where the parasite is endemic-, who showed the symptoms of infection (Sánchez-Molina *et al*., 2010). Some authors (Díaz & Florencio, 2001; Casimiro *et al*., 2006) suggest that in the actual scenario of global change, the rise in infected vectors increases the risk of transmission of the disease in areas where the adequate hosts exists is of greater importance than any rise in temperatures (Casimiro *et al*., 2006). This trend has been observed in the study area, where the prevalence of the disease

is growing (more than 200 cases of vesicle schistosomiasis, Parrilla-Ruiz *et al*., 2004; Salas-Coronas *et al*., 2013). According to estimations of these authors only a small fraction of affected patients is being detected, thus the presence of the parasitic diseases and the risk of completion of the life cycle seem to be considerable. This fact recommends a better knowledge of the distribution *of Bulinus truncatus* in the Iberian peninsula.

This study is an example of the utility and social impact of increasing our knowledge on water bodies and their associated biota.

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