

## Short communication

## Identification of the South Atlantic spiny slipper limpet *Bostrycapulus odites* Collin, 2005 (Caenogastropoda: Calyptraeidae) on the Spanish Mediterranean coast

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### Abstract

Calyptraeid gastropods are common fouling organisms in some areas, and have been introduced frequently into bays and ports in the northern hemisphere. In some cases, introduced calyptraeids have spread extensively, while several other calyptraeid introductions have persisted but failed to expand geographically. Calyptraeid species are often difficult to identify to species making it difficult to identify the origin of many introductions. Here we use DNA sequence data to confirm that a Mediterranean population of the spiny slipper snail *Bostrycapulus*, previously referred to as *B. aculeatus* and *B. calyptraeformis*, is in fact *B. odites* from the South Atlantic, and that this population probably originated in South America.

**Key words:** Western Mediterranean, *Crepidula*, barcode, COI, *Bostrycapulus aculeatus*, exotic species

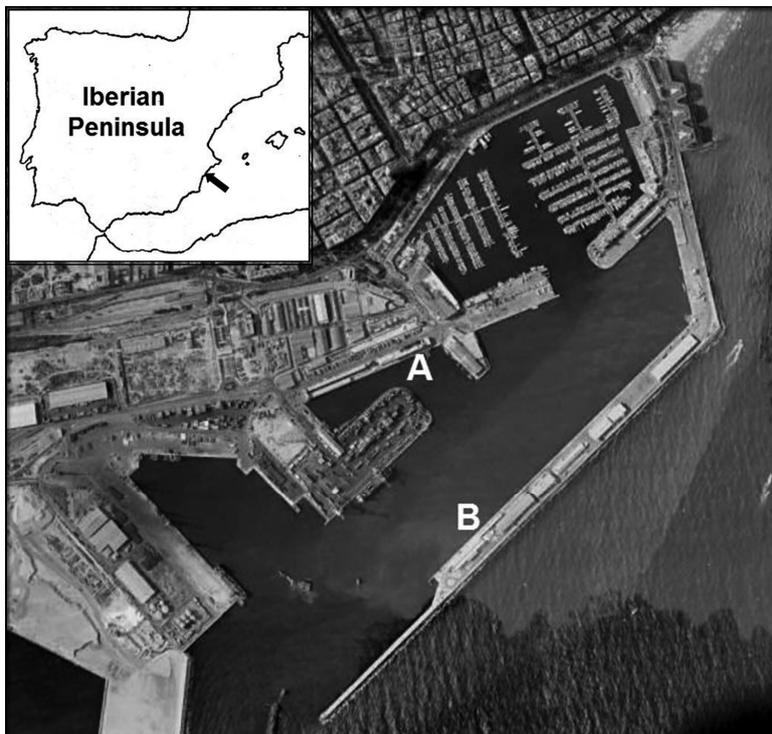
Several species of calyptraeid gastropods (slipper snails, cup-and-saucer snails, and hat snails) have been introduced and have established populations outside their native ranges. Two well known introductions, *Crepidula fornicata* (Linnaeus, 1758) introduced to northern Europe, and *Crepidula onyx* Sowerby, 1824 introduced to Asia, have become widespread and very abundant (Blanchard 1997; Minchin et al. 1995; Choe and Park 1992; Ekawa 1985; Morton 1987; Woodruff et al. 1986). However, there are several other introduced calyptraeids that occur in localized populations and have failed to spread outside protected bays and harbours. These can persist for years as small, restricted populations, presumably near the site of introduction. Examples include the documented introductions of *C. plana* Say, 1822 in San Francisco Bay (Carlton 1992), and *C. convexa* Say, 1822 in Humboldt Bay on the Pacific coast of California (McGlashan et al. 2008) and Puget Sound and the Georgia Strait in the Pacific Northwest (Collin et al. 2006). There is also currently a single restricted population of

*Crepidatella dilatata* (Lamarck, 1822) near Vigo on the northern coast of Spain (Rolán and Horro 2005; Collin et al. 2009).

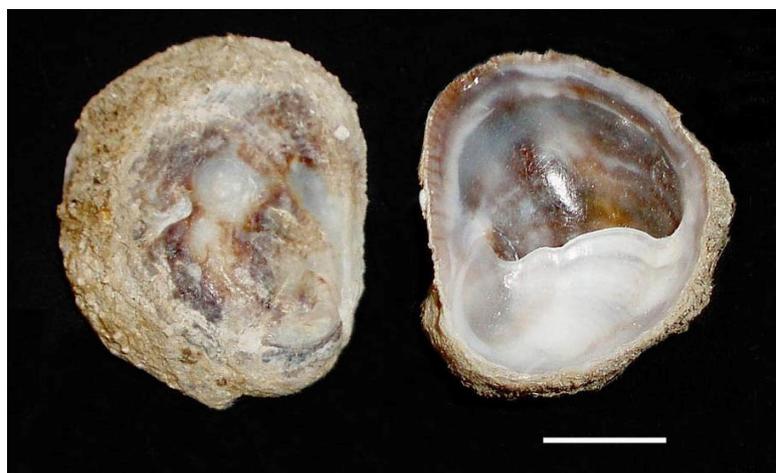
One such persistent localized population in Europe is the population of spiny slipper snails *Bostrycapulus* sp. in Alicante Harbour, Spain (Figure 1). Initially discovered in the 1970's, this population was first recognized as distinct from *C. fornicata* in 1973, but this result was not published until 1991 (Zibrowius 1991), when the animals were referred to as *Crepidula calyptraeformis* (now *Bostrycapulus calyptraeformis* [Deshayes, 1830]). Subsequently, this species was referred to as *Crepidula aculeata* (currently *Bostrycapulus aculeatus* [Gmelin, 1791]) (Gofas and Zenetos 2003; Zibrowius 2002), reflecting the taxonomy current at the time, which placed all spiny slipper limpets into a single global species (Collin 2005).

Despite its unclear taxonomy, which is due to recent revisions within the family and recognition of new distinct species, the population in Alicante is well known and has persisted since the 1970's (Gofas and Zenetos 2003). It was

**Figure 1.** Location of the Alicante Harbour (South-eastern Iberian Peninsula). **A** and **B** are sites where *Bostrycapulus odites* has been collected.

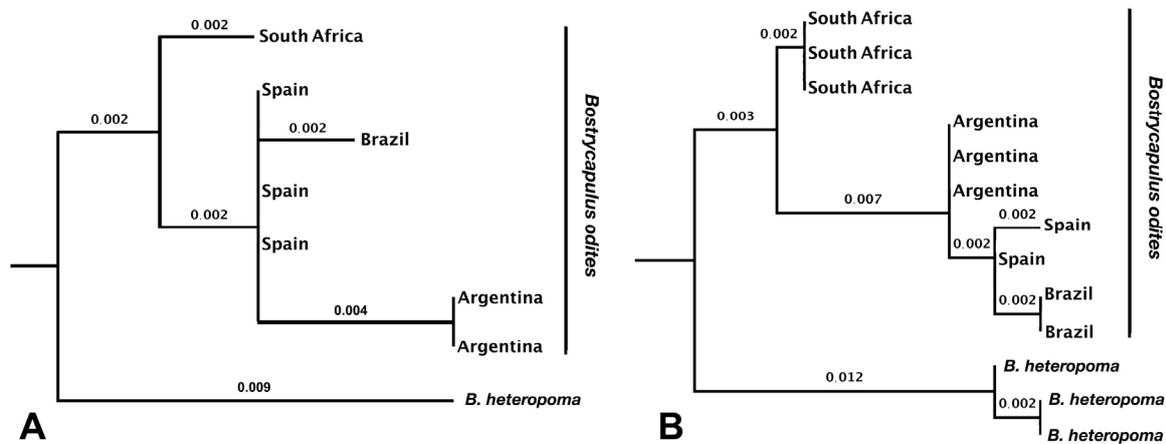


**Figure 2.** The shells of *Bostrycapulus odites* from Alicante Harbour, Spain. The shells are often eroded and fouled which usually obscures the spines and radial ridges. Most species of *Bostrycapulus* cannot be distinguished on the basis of shells alone. Scale bar = 1 cm. Photograph by A.A. Ramos-Esplá.



most recently reported as being common and widely distributed in Alicante Harbour in 2002 (Zibrowius 2002) and 2007, but that the population had not expanded outside the harbour (Izquierdo et al. 2007). Despite the uncertainty over the taxonomic designation, these reports suggested that a likely origin of these snails is the Atlantic coast of South America, because they are commonly found in the same locations as the South American coral *Oculina patagonica* (Zibrowius and Ramos 1983; Zibrowius 1991).

The taxonomic uncertainty surrounding the Alicante *Bostrycapulus* is not unusual for calyptraeids. The genus *Bostrycapulus* is particularly difficult to identify since the 9 species have highly variable and indistinguishable shell morphologies (Figure 2; Collin 2005; Collin and Rolán 2008). They are so similar morphologically that, based on anatomical data, Simone (2002) suggested that all *Bostrycapulus* belonged to a single species, which had achieved a worldwide distribution due to anthropogenic dispersal.



**Figure 3.** Phylogenetic placement of 16S (A) and COI (B) sequences from Alicante Harbour, Spain, with respect to those from the native range in South America and South Africa, and the sister species *B. heteropoma*. Numbers above the branches are GTR distances.

Nine, well defined species can, however, be distinguished on the basis of DNA sequence data and mode of development. In some cases the protoconch morphology is distinctive (Collin 2005; Collin and Rolán 2008), but it is not always useful for distinguishing species with direct development and large eggs.

In 2004 we collected samples from Alicante Harbour at the “Fishing Dock” (38°20'11.1"N, 00°29'11.8"W, 6 m depth) and preserved them in ethanol. To identify the samples to species and determine the original geographic source of the population, DNA was extracted from 3 animals and 553 base pairs of cytochrome oxidase *c* subunit I (COI), and 460 base pairs of 16S were sequenced, following the methods of Collin (2001). DNA sequences have been deposited in GenBank (numbers GU646881-646883).

Maximum likelihood analyses of these sequences with *Bostrycapulus* sequences previously reported by Collin (2005) and Collin and Rolan (2008), using Garli (version 0.951) showed that the animals from Spain were *Bostrycapulus odites* Collin, 2005 and not *B. calyptraeformis* or *B. aculeatus*. None of the COI or 16S haplotypes were identical to available haplotypes of *B. odites*. However, for both genes the haplotypes from Spain were placed between the *B. odites* haplotypes from Brazil and those from Argentina (Figure 3), to the exclusion of those from South Africa and the closest sister species, *B. heteropoma* Collin and

Rolan 2010 (Collin and Rolán 2008). This suggests that the source of the introduction was probably in South America, but is not likely to have been either of the localities from which sequences were available (São Paulo, Brazil and San Antonio Oeste, Argentina).

The mode of development of *Bostrycapulus odites* in Alicante has not been reported, although they have been found to brood in November (Izquierdo et al. 2007). Observations of *B. odites* from South America and South Africa show that development in this species is direct, with crawling juveniles that develop from small eggs that consume nurse eggs (Collin 2005). Direct development may help this geographically limited population maintain high densities (as many as 275 animals/m<sup>2</sup>; Izquierdo et al. 2007) as well as limiting its ability to spread outside the harbour. It is worth noting that the two highly invasive calyptraeid species have planktotrophic larvae, while most of those that have established localized populations in Europe and North America have direct development and lack a free-living larval stage.

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