

## Confirmation of the identification and establishment of the South American slipper limpet *Crepidula dilatata* (Lamarck 1822) (Caenogastropoda: Calyptraeidae) in Northern Spain

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### Abstract

Calyptraeid gastropods have been introduced frequently in bays and ports around the world, and have become rampantly invasive in several cases. Here we confirm the identification and establishment of a recently-detected population of *Crepidula dilatata* in northern Spain. Because their shells do not have many diagnostic features, introductions of calyptraeids are often accompanied by confusion about the identity and therefore origin of the species in question. We use DNA sequence data and developmental observations to verify the species identity of this population as the South American species *Crepidula dilatata*. The apparently rapid spread of this species, which lacks a larval stage, may be due to human action.

**Key words:** Galicia, *Crepidula*, barcode, COI, direct development, exotic species

Several species of calyptraeid gastropods (slipper limpets, cup-and-saucer limpets, and hat limpets) have been introduced and established populations outside their native ranges. Although many of these are restricted to small, local populations, two are now particularly widespread and abundant. *Crepidula fornicata* (Linnaeus, 1758), native to eastern North America, has invaded much of the northern coast of Europe and the English Channel, since its introduction to England at the turn of the century (Blanchard 1997; Minchin et al. 1995). In many areas of introduction, *C. fornicata* is a serious pest of commercial shellfisheries (Blanchard 1997), and is the dominant benthic species in many areas of the Solent UK, reaching densities of 1229 m<sup>-2</sup> (Farrell, unpublished). This species has also been reported in the Mediterranean (Gofas and Zenetos 2003). *Crepidula onyx* Sowerby, 1824, is a species similar to *C. fornicata* native to southern California. *Crepidula onyx* was first reported in Japan in 1968 (Woodruff et al. 1986),

and is now widespread in Korea, Japan, and Hong Kong (Choe and Park 1992; Ekawa 1985; Morton 1987).

The initial documentation of many introduced calyptraeids has been fraught with taxonomic confusion and misidentifications. For example, Asian populations of *Crepidula onyx*, a well-known species from Southern California were originally identified as *Crepidula fornicata*. This mis-identification was corrected 20 years ago (Huang et al. 1983; Woodruff et al. 1986) but is still present in online databases of introduced species (e.g., DAISIE and BIOTIC [Rayment 2008]). Similarly, a population of *Crepidula convexa* Say 1822 in Humbolt Bay on the West coast of the United States was initially identified as *C. fornicata* (Carlton 1992). The identification was corrected using evidence from DNA sequence data 16 years later (McGlashan et al. 2008). In this case, the shells of the two species can be distinguished and the mode of development also differs. However, in many

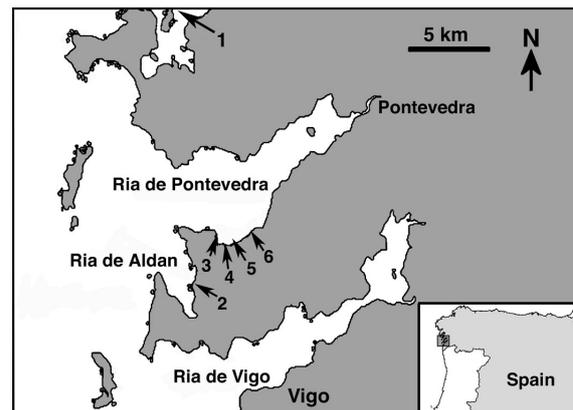
cases closely related calyptraeids cannot be distinguished without developmental or genetic data (Collin 2001, 2005; Collin et al. 2007; Veliz et al. 2003). Such propagation of taxonomic errors and the long time necessary to discover and correct them make it doubly important to carefully verify difficult species identifications when exotic species are first discovered.

Northern Europe has a single native calyptraeid, *Calyptraea chinensis* (Linnaeus, 1758), making detection of exotic calyptraeids relatively straightforward. However the correct species-level identification of exotics is not always so easy. The recent reported introduction of *Crepidatella dilatata* in Baia de Aldan (Rolán and Horro 2005) is noted by the authors to be a potentially ambiguous identification due to the existence of a morphologically cryptic sister species. The original report was based primarily on the description of shell characters and body color (Rolán and Horro 2005). An additional site was subsequently also reported incidentally for 2005 in a review of introduced gastropods in Galicia (O Grove Bay; Bañón et al. 2008).

There are six species of *Crepidatella* (Collin et al. 2007) and the three species that co-occur in Chile (*C. dilatata*, *C. fecunda* (Gallardo 1979) and a currently un-described species) cannot be distinguished on the basis of shell characters or soft anatomy alone (Veliz et al. 2003; Collin et al. 2007). Some details of development can be used to distinguish between the three: *Crepidatella fecunda* has planktotrophic development, *Crepidatella dilatata* has non-planktotrophic development with nurse eggs that never cleave, and the undescribed species has non-planktotrophic development with nurse eggs that cleave and appear to gastrulate (Veliz et al. 2003; Collin et al. 2007). These species can also be distinguished on the basis of DNA sequences of the DNA barcoding gene cytochrome oxidase I (COI) (Collin et al. 2007). Therefore the provisional identification of Rolán and Horro requires verification with additional data.

In 2008 one of us (PF) discovered a previously unreported subtidal population of *Crepidatella*, at Playa de Beluso, in Bueu, on the Ria de Pontevedra (42°19'45.98"N, 8°47'44.83"W; Figure 1, site 4) next to a mussel-processing wharf. The snails were found almost exclusively attached to mussels (*Mytilus galloprovincialis* (Lamarck, 1819)) (Figure 2) and some empty shells of abalones (*Haliotis tuberculata* Linnaeus, 1758). Approximately 50 individuals from 5 – 36 mm in length were collected between 10-13 September 2008 and some were

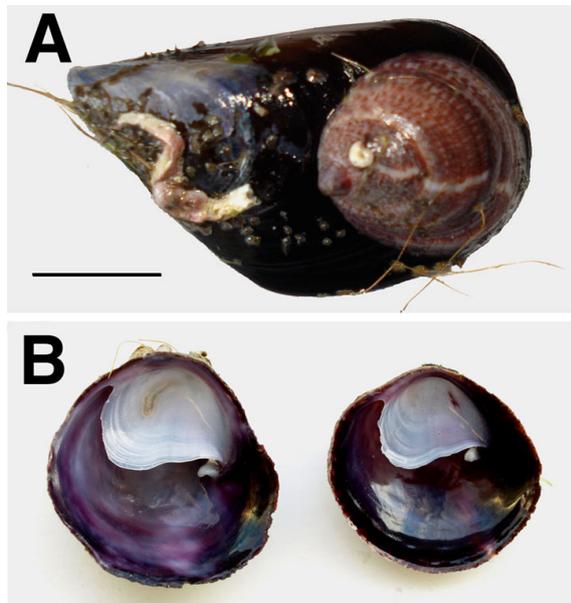
preserved in 95% ethanol for subsequent DNA sequencing. Nearby sites (Figure 1, sites 3, 5 and 6) with similar habitats, e.g. mussel-covered rocks in the subtidal area were also searched, and only one other specimen was found, at site 5. This animal was on an empty abalone shell being transported as cover by an urchin (*Paracentrotus lividus* (Lamarck, 1816)).



**Figure 1.** Survey sites for *Crepidatella dilatata* in Northern Spain. Site 1 (O Grove Bay), Site 2 (Baia de Aldan), and Site 4 (Playa de Beluso) are reported locations of *Crepidatella* populations. Site 3 (42°20'09.11"N, 8°47'55.40"W), Site 5 (42°19'39.33"N, 8°47'32.15"W) and Site 6 (42°19'57.02"N, 8°46'20.48"W) were searched but no *Crepidatella* were found

Fifteen females (30 % of total animals collected) were brooding and the embryos were examined and photographed with a dissecting microscope (Figure 3). Egg capsules contained uncleaved nurse eggs and embryos with well-developed coiled shells and velar lobes. Hatchlings crawled away from the capsules at shell lengths of about 1 mm. These features match those described for *C. dilatata* (Veliz et al. 2003; Collin et al. 2007).

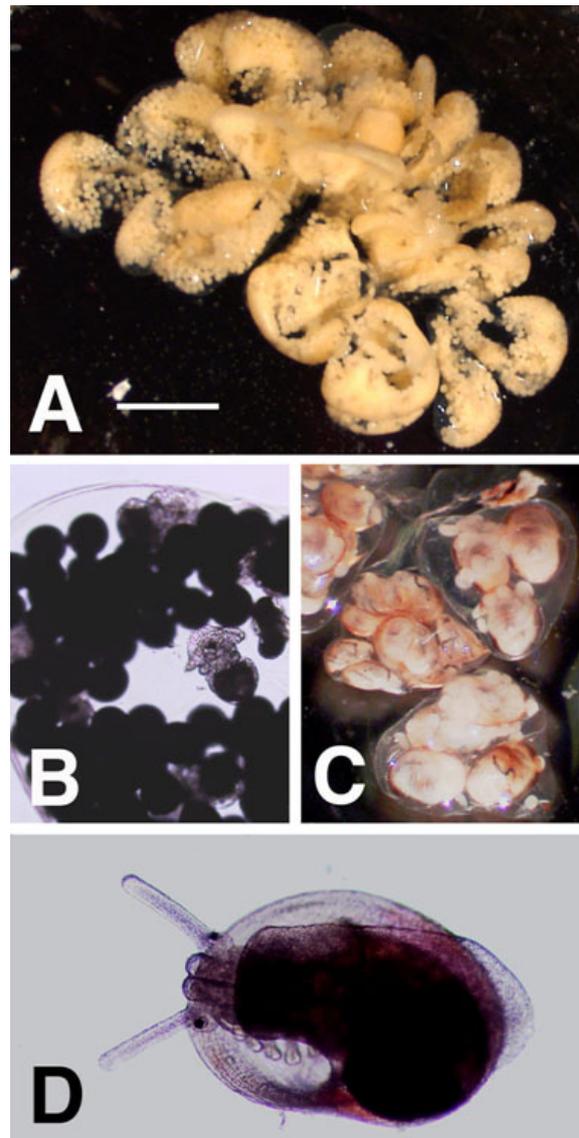
To confirm this identification genetically and to determine the source population, DNA was extracted from 5 specimens and 600 base pairs of cytochrome oxidase c subunit I (COI), and 506 base pairs of 16S were sequenced for two animals, following the methods of Collin (2001). Comparisons with sequences previously reported by Collin et al. (2007) showed that the animals from Spain were indeed *Crepidatella dilatata*. Two of the COI haplotypes were identical to haplotype Dil5 (Genbank DQ811124), two were identical to Dil3 (Genbank DQ811118) and one was identical to haplotype Dil4 (Genbank AF546052). Both 16S sequences were identical to the single sequence from *C. dilatata* in Genbank.



**Figure 2.** (A) Living specimen and (B) dorsal and ventral views of a shells of *Crepipatella dilatata* collected from the shells of *Mytilus galloprovincialis* in September 2008. Scale bar = 1 cm

Collin et al. (2007) sequenced 22 individuals of *C. dilatata* from Chile and Argentina. The haplotypes from Spain fall into both major haplotype clades reported in that paper. These haplotypes have been found along most of the central coast of Chile (Coquimbo, San Carlos, near Puerto Montt, and Chiloe). No Argentine haplotypes were detected in the Spanish sample. However the numbers of animals sequenced are not adequate to definitively exclude Argentina as the origin of the introduction. These haplotypes demonstrate that at least 3 original females founded this Spanish population.

*Crepipatella dilatata* fits the definition of an established exotic species put forward by Gofas and Zenetos (2003). They consider any species that is documented by three reliable records or for which there is information on perennial populations as being established. The previous reports of *Crepipatella* in Baia de Aldan and O Grove Bay (Rolan and Horro 2005; Bañon et al. 2008) in 2005 and the current report provide three records of populations of *C. dilatata* in a 15-km area, over three years. The 2008 samples from Playa de Beluso and the 2005 samples from Baia de Aldan both contained brooding females as well as animals as large as 25 mm (Rolan and Horro 2005) and 36 mm (reported here). *Crepipatella dilatata* is thus reproducing in Europe and should be considered established in Galicia.



**Figure 3.** Developmental stages of *C. dilatata*. (A) A cluster of egg capsules early in development. Scale bar = 4 mm. (B) Early intracapsular embryos showing the early development of the ridge that will become the velum surrounded by uncleaved nurse eggs. Scale bar = 600  $\mu$ m. (C) Fully developed embryos that have already lost the velum and are almost ready to hatch. Scale bar = 1 mm. (D) A recent hatchling. Scale bar = 400  $\mu$ m

What is the possibility of an aggressive expansion or invasion of this species in northern Spain? Rolan and Horro (2005) report that no *Crepipatella* were detected in the region during extensive dredging surveys in 1997 and that the mode of introduction is not clear. If the original introduction was in this area the expansion has been fairly rapid for a species without a free-living larval stage. It should be noted that the

two known invasive calyptraeids both have planktotrophic larval development. In general, introduced calyptraeids with direct development have not expanded outside the protected bays from which they were first reported (e.g., *Crepidula convexa* and *C. plana* Say 1822 on the West Coast of North America). The fact that none were found after thorough searches at locations either side of the site at Bueu, and that this site is adjacent to a mussel processor, suggests that they are being or could be inadvertently spread via mussel culture. Rolan and Horro (2005) also suggested that this species might have been transported by dredging and re-distribution of substrate in the area. Such human activities could increase the spread of an animal with little ability to naturally spread rapidly.

If *C. dilatata* populations expand in Spain, this species certainly has the potential to become a pest. Its preference for other marine mollusks as a substrate, makes this species a possible pest on mussel beds and aquaculture facilities. At Bueu, many mussels were colonised by *C. dilatata* (Figure 2), which could compete with them for food and be a nuisance for bivalve production, as has been the case with *C. fornicata*. *Crepidipatella dilatata* is a species that can also reach extraordinary high densities in its native range (Collin, unpublished), occurring on almost every mussel in some Chilean and Argentine mussel beds.

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