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## **RESEARCH NOTE**

## OBSERVATIONS OF JUVENILE ANADARA (SCAPHARCA) BRASILIANA (BIVALVIA: ARCIDAE) ON MELLITA ISOMETRA (ECHINOIDEA: MELLITIDAE) AT ST CATHERINES ISLAND, GA, USA

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Juvenile clams were observed on Mellita isometra Harold & Telford, 1990 (Echinoidea: Mellitidae), a lunulate sand dollar, collected off of St Catherines Island, GA, USA, in June 2008. These small clams (ranging from 0.5 to 3 mm in length; Fig. 1) had closed their valves on spines along the fringe, in the lunules and on the oral and aboral surfaces of the sand dollars. The ectosymbiotic crab Dissodactylus mellitae (Rathbun, 1900) is commonly found on the oral surface of M. isometra (Ruppert & Fox, 1988; George & Boone, 2003), but we found no previous reports of juvenile clams attached to sand dollars. We addressed several questions about this clam/crab/sand dollar association: (1) Were juvenile clams commonly present on sand dollars and where on the sand dollars were they located? (2) Was there evidence for any interaction between the juvenile clams and the crab Dissodactylus mellitae? (3) What species of clam were we observing?

To answer these questions, we collected a total of 300 sand dollars at three sites on St Catherines Island: North Beach, the Ramp and South Beach (described in Prezant et al., 2002). St Catherines Island is a barrier island on the coast of Georgia and these sites are spread across about 12 km of coastline. All collecting was done at low tide in 1-2 m of water. For each sand dollar we recorded the width of the sand dollar, the number of D. mellitae crabs and the number of clams on each sand dollar. We also recorded where on the sand dollar each clam was attached (on the fringe, in a lunule, on the aboral surface or on the oral surface). All data were recorded in the field, immediately after lifting the sand dollar from the substrate. This was important as our preliminary observations revealed that the clams released their attachment if the sand dollar was placed in a container and transported any significant distance. We also collected juvenile and adult clams in 95% ethanol for microscopic examination and genetic study.

We found a total of 142 clams on 300 sand dollars. Twenty-seven per cent of sand dollars had one or more clams, with a maximum of nine clams observed on one sand dollar. The clams were most common on the sand dollar fringe (86/142 observations, 61%). The lunules were the second most common location (40/142, 28%). Clams were rarely found on the aboral surface (14/142, 10%) and almost never on the oral surface (2/142, 1%).

We found a total of 419 *D. mellitae* crabs on 300 sand dollars. Sixty-eight per cent of sand dollars had one or more crabs, with a maximum of seven crabs per sand dollar. These results are similar to observations from George & Boone (2003) at Tybee Island, GA, USA. We found no evidence for an association between the presence of one or more crabs and the presence of one or more clams on a sand dollar ( $\chi^2$  test, P = 0.11). *Dissodactylus mellitae* were found almost exclusively on the oral surface of sand dollars. As clams were rarely observed on the oral surface, the clams and crabs may not have an opportunity to interact. We found no evidence that *D. mellitae* excludes clams from the oral surface as both of the clams found on the oral surface were found on sand dollars with *D. mellitae*, and no clams were found on the oral surface of any of the 96 sand dollars without *D. mellitae*.

Based on their distinctive overlapping valves (Fig. 1), we suspected these juvenile clams were Anadara (Scapharca) brasiliana (Lamarck, 1819). Adult A. brasiliana, which are known from St Catherines Island (Prezant et al., 2002), can be identified by the overlap of the left valve over the right (Ruppert & Fox, 1988; Mikkelsen & Bieler, 2008). To confirm this hypothesis we sequenced a portion of the mitochondrial cytochrome c oxidase I (COI) gene from two adult A. brasiliana and five juvenile clams (including the largest and smallest that we collected). We first isolated DNA from one individual and amplified and sequenced a portion of the COI gene using standard methods (Snowman, Zigler & Hedin, 2010) and the HCOI and LCOI primers from Folmer et al. (1994). Based on this sequence we designed taxon-specific primers (LCO1Ana: 5'-CGGCATTAGTAGGAATTTCCTTAAG-3' and HCOI Ana: 5'-GCCAAGAAGTGACAAACATACCCC-3') and used these to amplify and sequence a portion of the COI gene from all seven individuals (GenBank accession numbers IN656612-JN656618).

Across a 458-bp alignment of these seven sequences, six nucleotide positions were variable. One adult *A. brasiliana* individual and three of the juvenile individuals had identical sequences, one individual differed from those four individuals by a single-nucleotide difference, one by two differences and one by three differences. The maximum divergence between specimens was 1.1%, and the mean divergence was 0.4%.



Figure 1. Anadara brasiliana collected on Mellita isometra in June 2008 at St Catherines Island, GA, USA. This figure appears in colour in the online version of *Journal of Molluscan Studies*.

Prior to this study there were no *A. brasiliana* sequences in GenBank, but a BLAST search identified the COI gene of eight other *Anadara* species (all from Marko, 2002) as the closest matches to our sequences. The closest match was to *A. ovalis* with a nucleotide identity of 81%. Given the morphological and genetic similarity between these juvenile clams and adult *A. brasiliana*, we are confident that these juvenile clams are *A. brasiliana*.

We answered the three questions posed above: juvenile clams were commonly found on the fringe and in the lunules of *Mellita isometra*, their presence was not obviously influenced by the presence of the ectosymbiotic crab *Dissodactylus mellitae*, and the clams were juvenile *Anadara brasiliana*. Limited sampling in June 2009 found clams on 13% (8/62) of sand dollars at St Catherines, indicating that our observations in 2008 were not unique.

In Georgia, A. brasiliana is common just offshore of coastal beaches, where they live in sandy substrates (Walker & Gates, 2001). Growth rates of juvenile A. brasiliana have not been determined, but 1-year-old A. brasiliana from Georgia can reach 25 mm in length (Walker & Gates, 2001), suggesting that the juveniles we observed (0.5-3 mm in length) are, at most, a few months post-settlement. This is consistent with juvenile growth curves for other species of Anadara (e.g. Muthiah et al., 1992).

Mellita isometra are deposit feeders that move slowly along or just below the surface of sandy substrates. They feed on particles ranging from 100 to 250  $\mu$ m in size (Telford, Mooi & Ellers, 1985), so it is unlikely that they are feeding on these juvenile clams. The attachment of juvenile clams to sand dollars may help them avoid burial under shifting sands due to waves and tides. As the sand dollars move laterally across the sand, attachment along the fringe or in a lunule might provide protection from friction with the substrate. Alternatively, *M. isometra* spines on the fringe and in the lunules are generally thicker and longer than spines elsewhere on the sand dollar (Telford, Mooi & Ellers, 1985), which might provide an easier attachment for the clam. It is not clear whether these clams settle onto sand dollars or attach to sand dollars post-settlement. George & Boone (2003) suggest that *Dissodactylus* crabs have a slight negative effect on egg production by their sand-dollar host; it is not clear if the presence of clams would have a similar effect. Further study at different times of the year could identify when this association begins and how long it persists, and close examination of sand dollars in other locations should reveal whether this association is common. *Anadara brasiliana* ranges from North Carolina to Brazil (Mikkelsen & Bieler, 2008), as do members of the genus *Mellita* (Harold & Telford, 1990), so this association could be present across a large area.

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