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SHORT COMMUNICATION

Sponge perforating lace coral with anticancer activity

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This short note reports results from a pilot study to investigate new anticancer agents from deep-sea corals in which colonizing sponges were encountered. The pure white stylasterid coral fans of *Errina dabneyi* (Pourtalès, 1871) are a conspicuous feature on the upper bathyal slopes in Azorean waters and can be found in depths from 215 to more than 500 m (Wisshak et al. 2009; Braga-Henriques et al. 2013). From the 26 species of *Errina* known worldwide (most from deeper waters) (Cairns 1983), *E. dabneyi* seems to be endemic to the Azores Archipelago and the adjacent Mid-Atlantic Ridge region (Zibrowius & Cairns 1992; Braga-Henriques et al. 2013).

The anticancer activity of the lipid extract from the lace coral *Errina dabneyi* (Figure 1A) was being investigated as part of a broader ongoing bio prospecting research on deep-sea invertebrates from the Azores area. Deep-sea fisheries in the Azores use longline gears at depths where cold-water corals are common (Braga-Henriques et al. 2011; Pham et al. 2014). The specimens studied were collected accidently (bycatch) at Princess Alice Seamount (near the Azorean central group of islands) at 200 m in April 2012 by a local commercial fishery fleet. Observers onboard froze samples immediately after collection and kept them at -20ºC until being extracted. A subsample was taken and preserved for the biological reference collection at University of the Azores (DOP/UAc COLETA), with ID number - DOP 9044. When processing *Errina dabneyi*, we noted that part of the coral (from the base to the middle of the primary branches) had been bored by what appeared to be a sponge in the interior of the skeleton (Figure 1B). Since it was impossible to fully separate the sponge from the coral, the part of the coral that was colonized was treated as an independent sample (CS; Figure 1c). For identification purposes, a small fragment of sponge tissue was excised, digested in sodium hypochlorite, and washed in a water-ethanol series. The obtained spicules were then mounted in Canada balsam, observed and measured under a microscope. The sponge was identified as *Thoosa armata* Topsent, 1888 (Demospongiae, Astrophorida), a species previously known to occur associated with deep-sea corals in the Azores (Topsent 1904). *Thoosa* is one of several demosponge genera known for their ability to excavate calcareous substrates (see review in Cairns 1983), a genus of demosponge in the family Thoosidae. This genus is known for boring holes in corals (Schoenberg 2008) as can be seen in Figure 1B. Total lipids were extracted using a modified Bligh and Dyer method as described in Lino et al. (2013). Cytotoxicity essays were performed on HCT-116 human colon adenocarcinoma (tumorous) cell line. Briefly, cells were inoculated into plates at $2{,}5\times10^4$ cells/ml (150 µl/well), and incubated overnight 37ºC and 5% CO_2 before treatment. Series of dilutions in DMSO of each of the

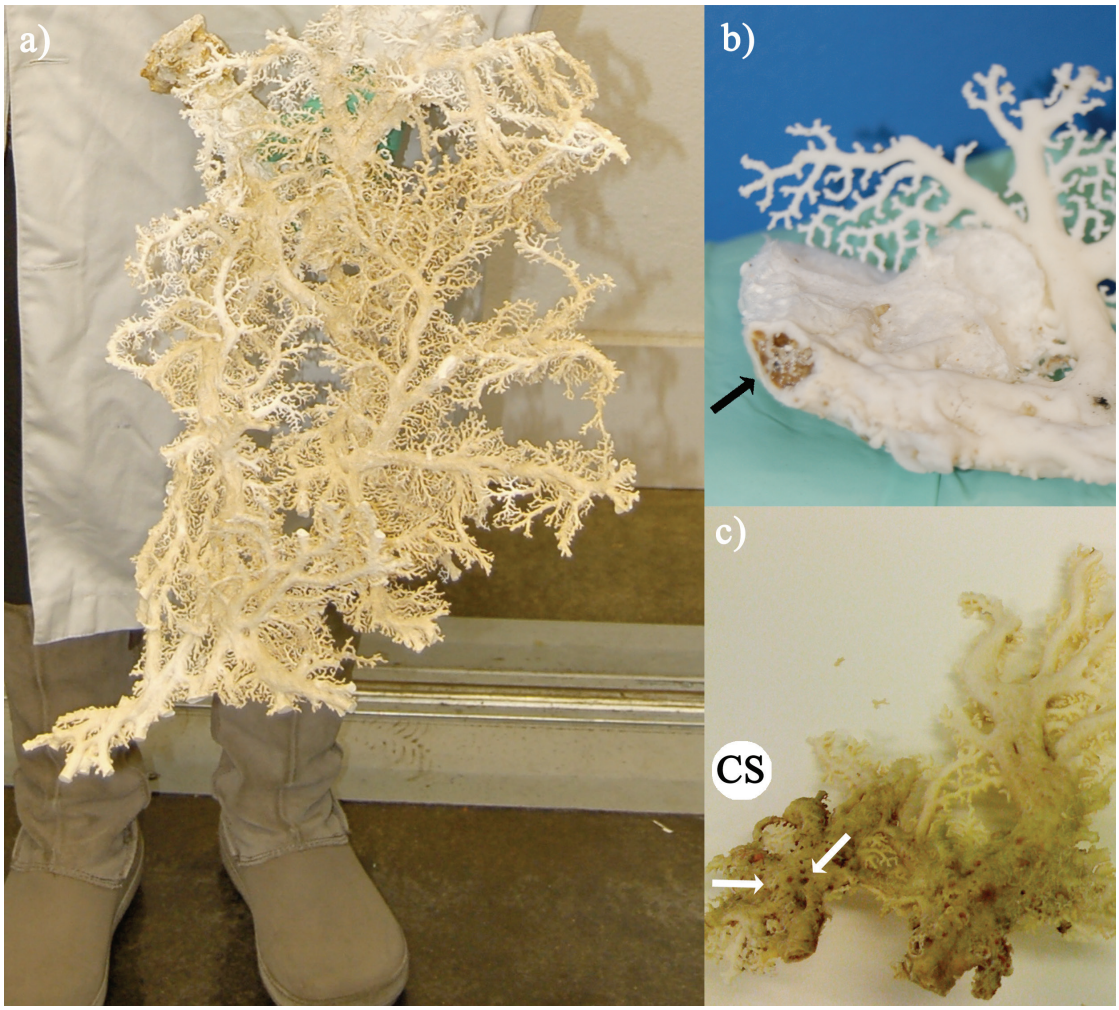

Fig. 1. a) *Errina dabneyi* specimen deposited in the reference collection at the University of the Azores DOP/UAç COLETA); b) *Errina dabneyi* specimen where it is possible to see the sponge inside (pointed by the black arrow); c) Specimen of coral *E. dabneyi* plus sponge (CS) investigated for their anticancer potential in this study - the white arrows indicate the holes done by the *Thoosa* sponge in the coral skeleton in order to connect with the exterior and which indicates the presence of the sponge within the coral.

extracts (*Errina dabneyi* (C) and *Errina* plus sponge (CS)), were added to the cells followed by further incubation at 37ºC for 72 h. Each extract was essayed in duplicate. Cell viability was determined by a colorimetric [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphen-yl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay. After the exposure time, 20 µL of MTS/ PMS (phenazine methosulfate - electron coupling agent) solution was added to each well. Plates were incubated for 3 h before absorbance was measured at 490 nm using an EMax® Endpoint ELISA Plate Reader. Cells in DMSO were used as negative control, and cells treated with Etoposide (VP-16) were used as positive control. Relative cellular survival was determined by using the measured optical density (OD) and was calculated as follows: (% Survival for sample) = [(OD of sample)/(Average of negative control)] x100. Concentration that killed 50% of the cancer cells

(IC50 values) was automatically calculated by SoftMax Pro software. Results for anticancer activity showed that the extract from *Errina dabneyi* did not show any activity but the extract from the coral sample harbouring the sponge was active presenting IC50 values of 25.29 and 32.72 µg/mL for the two essays. To our knowledge this is the first time that anticancer activity is reported in a coral perforating sponge.

Considering the interesting preliminary results on the anticancer activity for the sponge and taking in consideration the high bio eroding ratio observed in *Errina dabneyi* specimens deposited in the collection at University of the Azores, more consistent studies should be made to address this matter.

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