An embedding method for the analysis of tiny statoliths: preliminary results for the Mediterranean box jellyfish Carybdea marsupialis

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Statoliths are inorganic crystals located on the rhopalia of jellyfish that serve as gravity sensors. Morphological and geochemical analysis of these rhopalia are considered useful means of understanding their taxonomy and ecology. Cubozoan statoliths are made of calcium sulfate hemihydrate (bassanite) and tend to disintegrate rapidly once removed from the rhopalium and preserved (e.g. frozen or preserved in 4% buffered formaldehyde). Moreover, the small size of the statoliths of some species, such as Carybdea marsupialis (~40μm juveniles, ~400μm adults), makes them even more difficult to work with. The aim of this study is to provide a method which enables us to slice the statolith with micrometer precision, making their study and characterization possible, focusing on C. marsupialis from Malta and Spain. Although the literature includes some references to epoxy resin embedding procedures for statoliths of other species, none of these papers indicate the component proportions in the embedding medium or other specific requirements. Steps: 1) dehydration in ethanol; 2) infiltration with several ratios of propylene oxide and Embed 812 embedding medium; 3) transfer to a mold filled with Embed 812 embedding medium 100%; 4) curation at 60ºC in an oven for 48h; 5) sectioning in a microtome (~4μm precision); 6) the embedded sample was polished using 0.25 μm diamond-dispersion powder. Different techniques can then be used to characterize the statolith section, including whole analysis, mapping, compositional profiles and punctual data. Scanning electron microscopy, X-ray photoelectron and raman spectroscopies and micro-X ray diffraction were successfully applied.