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Short Communication

Getting information from ethanol preserved nematocysts of the venomous cubomedusa *Chiropsalmus quadrumanus*: a simple technique to facilitate the study of nematocysts

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ABSTRACT. Preserved specimens make it difficult to study nematocysts. We tested an hydration protocol on ethanol preserved tentacles of *Chiropsalmus quadrumanus* (Cubomedusae) during a period of ten days, and quantified the success of retrieval of information. After six days of hydration, it was possible to observe discharged and undischarged capsules of the three types of nematocysts. We conclude that hydration is a possible solution to study various aspects of the cnidome, recovering information otherwise lost.

Keywords: Cubozoa, nematocysts, taxonomy, venom, hydration.

Obteniendo información de nematocistos de la cubomedusa venenosa *Chiropsalmus quadrumanus,* conservados en etanol: una técnica sencilla para facilitar el estudio de nematocistos

RESUMEN. Es difícil estudiar los nematocistos de muestras conservadas. Se ha probado un protocolo en los tentáculos de *Chiropsalmus quadrumanus* (Cubomedusae), conservados en etanol, consistente en la hidratación, durante un plazo de diez días, después de los cuales se cuantifica el éxito de la recuperación de la información del cnidoma. Después de seis días de hidratación, se observaron las cápsulas íntegras y descargadas de los tres tipos de nematocistos. Se concluye que la hidratación es una solución posible para estudiar diversos aspectos del cnidoma, recuperando información que al contrario se perdería.

Palabras clave: Cubozoa, nematocistos, taxonomía, veneno, hidratación.

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Cnidarians are venomous marine invertebrates inhabiting several different marine ecosystems. Some of them can be lethal to humans, such as the sea wasp *Chironex fleckeri* Southcott, 1956 (Baxter & Marr, 1969) or the Portuguese man-o-war *Physalia physalis* (Linnaeus, 1758) (Lane & Dodge, 1958). Non-lethal cases are abundant for several groups (*e.g.*, Marques *et al.*, 2002; Collins *et al*., 2011). Commonly known as jellyfish, anemones and corals, cnidarians are characterized by specialized cellular organelles named cnidae (phylogenetically defined by Marques & Collins, 2004; Collins *et al*., 2006; Van Iten *et al*., 2006). Some of these organelles, known as nematocysts, may inject toxins through a penetrative thread into the victim (Carrette & Seymour, 2004). In addition, nematocysts can be very useful to identify species (*e.g.*, Williamson *et al*., 1996; Marques, 2001), even cryptic ones (*e.g.*, Collins *et al*., 2011), and to infer feeding behavior and ecology (Endean & Rifkin, 1975), or even estimate the size of the individual (Carrette *et al*., 2002).

Ideally, the study of nematocysts is based on fresh and live material, for which discharging of nematocysts is accomplished by adding ethanol, distilled water or even saliva (Gershwin, 2006a). Discharged nematocysts allow the study of their microstructures, like shafts, barbs, and spines, making it easy to identify the different types of nematocysts, which are important characters to identify organisms at the species level (*cf*. Gershwin, 2006b).

However, the study of fresh material is not always possible, creating a major obstacle for many taxonomic and derived studies. The most common and

immediate source of material are specimens stored in museum collections preserved in formaldehyde or ethanol. The likelihood of finding enough discharged nematocysts in good conditions to be studied in these cases is very low. Besides, preservation in ethanol may cause opacity in the capsules of nematocysts, hampering identifications. Therefore, the quantification and description of the distribution of nematocysts in different parts of the individual is complicated, compromising further research.

The aim of this study is to present the efficiency of a simple hydration protocol in order to obtain discharged nematocysts from ethanol preserved tissue, improving the observation of types and quantity of nematocysts.

Our protocol was based on tentacular tissue as a model, because tentacles are generally regions with the highest concentration of nematocysts per area. The species model used was the box jellyfish *Chiropsalmus quadrumanus* (Müller, 1859), a venomous species from the western tropical and subtropical Atlantic. The cnidome of *C. quadrumanus* consists of three types of nematocysts, isorhizas (ovoid and ellipsoid), microbasic euryteles and microbasic mastigophores (Calder & Peters, 1975; Carrette *et al*., 2002; Gershwin, 2006a). The study of the nematocysts of this species, as well as for many other cubozoans, is also made more difficult by the thick mesoglea, which gets hardened after fixation. The experiment was carried out using part of tentacles destined for molecular research. The material had been preserved for 18 months in nearly pure ethanol. We left the tentacle tissue submerged in freshwater at room temperature (*ca.* 20-30°C) for 10 days. We defined day zero as having no hydration at all, followed by 10 days of hydration treatment. We cut off samples of *ca.* 1 mm of tentacle daily to compare the efficiency of the successive days of hydration. This tentacular sample was placed onto a glass slide, covered with a drop of freshwater and a cover slip, gently squashed and then observed under an optic microscope using the oil immersion objective (1000 x magnification). We carried out ten replicates on different glass slides every day. Under the microscope, nematocysts were examined, counted and photographed using the Zoom Browser 6.3.1.8. software and identified after Mariscal (1974), Calder & Peters (1975), Östman (2000) and in consideration of the comments by Gershwin (2006a). Statistical analysis was done using GraphPad Prism5 software. Results were expressed as means ± standard deviations (SD) of the indicated numbers of different types of nematocysts; nonparametric Tukey's significant difference test was also carried out with $P < 0.05$ for statistical significance.

The hydration protocol was found to be efficient (Fig. 1), both in making it easier to find the different types of nematocysts and in making a portion of the nematocysts discharge. Preserved material had all isorhizas undischarged in between a thick gelatinous tissue, making it difficult to study the material (Fig. 2a), and no euryteles or mastigophores were observed. Days 1-4 (represented together in Figure 1 under number "1" at X axis) already had some discharged isorhizas, but still no euryteles or mastigophores were observed. The difference between the number of nematocysts found in the non-hydrated material and hydrated material was statistically significant.

After 5 days of hydration, we observed undischarged and discharged isorhizas, and a few euryteles were visible through the layer of tissue, but still no mastigophores were found (Fig. 2b). After 6 days of hydration, we found the first mastigophore among some well defined isorhizas and euryteles. After 7 days of hydration, we observed some bare capsules of isorhizas with few discharged ones, we also detected few euryteles and mastigophores discharged (Fig. 2c). After 8 days of hydration, the amount of isorhizas and euryteles decreased, but more mastigophores appeared (Fig. 1) at this time, the majority of isorhizas were empty and the euryteles and mastigophores observed were either undischarged, discharged, or represented by empty capsules. After 9 and 10 days of hydration, we found the three types of nematocysts of

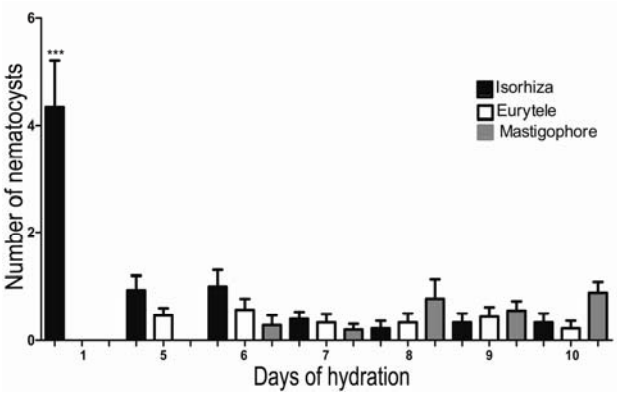

Figure 1. Nematocysts observed using hydration protocol (5-10 days) compared to the nematocysts found at the first day (1) without hydration (the second measurement was done on day 5). Each bar represents the average number of nematocysts found on ten preparations of tentacle tissue and error bars indicate SD. ***, significant difference ($P < 0.05$, determined by Tukey´s significant difference test) compared to the isorhizas found after 5 to 10 days of hydration.

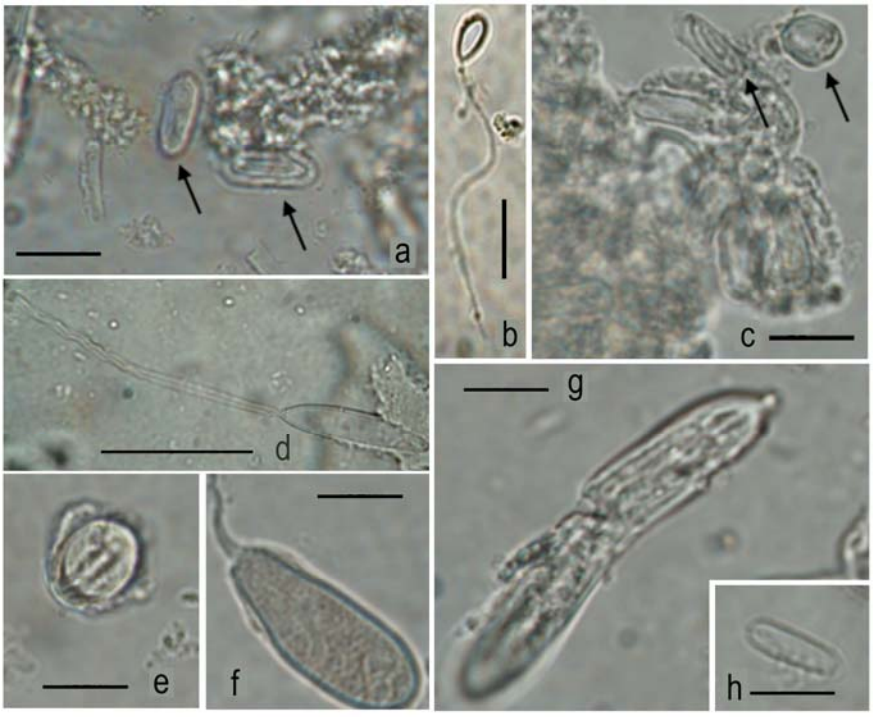

Figure 2. Tentacular nematocysts of *Chiropsalmus quadrumanus* before and after hydration. a) Two ellipsoid isorhizas (indicated by arrows) in ethanol preserved and non-hydrated tissue, b) discharged isorhiza, after 5 days of hydration, c) undischarged isorhiza (left) and eurytele (right) (indicated by arrows), after 5 days of hydration, d) discharged mastigophore, after 7 days of hydration, e) undischarged eurytele, after 7 days of hydration, f) discharged eurytele, after 7 days of hydration, g) broken capsule of a mastigophore, after 9-10 days of hydration, h) empty capsule of isorhiza, after 9-10 days of hydration. Scale bars: a, b, c, e, f, g, h = 10 micrometers, d = 50 micrometers.

Chiropsalmus quadrumanus, but broken capsules or groups of mastigophores between gelatinous tissue were difficult to examine (Fig. 2d). This deteriorated material after 10 days made us interrupt the experiment.

Time of hydration may vary depending on type and time of preservation, as well as the species, ontogenetic stage and part of the body to be studied. However, hydration may resolve situations such as that described by Gershwin (2006a: 7): "*Every effort was made to study inverted and everted nematocysts; however, with fixed material, this was not always possible*" improving the quality of nematocyst surveys. Because more material is being preserved directly in ethanol for the purposes of DNA studies, we believe that more information on cnidomes will be available in the near future.

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