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# First in situ observations of the deep-sea carnivorous ascidian *Dicopia antirrhinum* Monniot C., 1972 in the Western Mediterranean Sea

Mecho A.[1*], Aguzzi J.[1], Company J.B.[1], Canals M.[2], Lastras G.[2], Turon X.[3]

[1]Instituto de Ciencias del Mar (ICM-CSIC). Paseo Marítimo de la Barceloneta, 37-49, 08003 Barcelona, Spain.

[2]GRC Geociències Marines, Departament d'Estratigrafia, Paleontologia i Geociències Marines, Facultat de Geologia, Universitat de Barcelona (UB). Martí i Franquès s/n, 08028 Barcelona, Spain.

[3]Centre d'Estudis Avançats de Blanes (CEAB-CSIC), Accés a la Cala Sant Francesc 14, 17300 Blanes (Girona), Spain.

Corresponding author:

Mecho A.: mecho@icm.csic.es; TEL: +34 932309500; FAX: +34 932309555

## Abstract

*Dicopia antirrhinum* C. Monniot, 1972 is a rare species of deep-sea ascidian belonging to the Family Octacnemidae, reported at depths of 1000-2500 m in European Atlantic waters. Adult individuals have never been reported before in the Mediterranean Sea, where only seven juvenile specimens were found in 1975 at 500 m water depth in the Central basin (Malta). The affinities of these specimens with *D. antirrhinum* were noted, but lack of some typical characters of the species in juveniles prevented a definite taxonomical identification. No other member of the Octacnemidae has ever been found in the Mediterranean. In this study we describe the sampling of an adult specimen of *Dicopia antirrhinum* at around 1100 m water depth on the flank of the La Fonera (Palamós) canyon, Northwestern Mediterranean, confirming their presence in the Mediterranean Sea. We also observed 5 individuals of this species on their natural habitat with a Remotely Operated Vehicle (ROV). Our results highlight the potential occurrence of Octacnemidae, the presence of which has been largely overlooked, in several deep-sea canyon areas within the western Mediterranean basin. These observations are important because they indicate the need for increased sampling effort with new technologies, such as ROVs, in ecologically relevant habitats such as canyons, in order to obtain a more accurate picture of deep-sea biodiversity in the Mediterranean Sea.



Key words: *Dicopia antirrhinum;* Octacnemidae; Western Mediterranean; La Fonera canyon; Remotely Operated Vehicle (ROV).

## 1. Introduction

Deep-sea ascidians are markedly different from their more abundant shallow-water counterparts. These differences relate to the dominance of solitary forms inhabiting soft bottoms as opposed to the commonly rock-dwelling and often colonial species in littoral waters (Havenhand et al., 2006; Monniot and Monniot, 1978). Another common trend in deep-sea ascidians is the adaptation to the scarcity of particulate matter, which led to partly or totally macrophagous feeding in some members (Tatián et al, 2011) of this otherwise filter-feeding group (Monniot and Monniot, 1978, 1991). Although far from reaching the abundance and diversity of shallow-water ascidian faunas, the diversity of deep-sea ascidians is surprisingly high (Monniot, 1998; Monniot and Monniot, 1973, 1991), reaching a maximum at great depth (4000-5000 m) (Monniot and Monniot, 1978). Individuals of some species have been found even down to 8000 m depth (Sanamyan and Sanamyan, 2002).

Many ascidian families have deep-sea representatives, but some groups (i.e., Octacnemidae) are exclusively found at great depths, and they feature some of the most notable morphological adaptations (Monniot and Monniot, 1991). The Family Octacnemidae, in particular, comprises deep-sea species that can reach high abundances in the continental slope and abyssal plains over a depth range from 500 to over 8000 m depth. This family has adopted a partly macrophagous diet using the oral siphon as a trap to capture prey. This siphon is often expanded into muscular lobes or lips capable of capturing motile prey (Monniot, 1998). The branchial sac is reduced to a variable degree, indicating a different mix of micro/macrophagous feeding, as confirmed by fatty acid analysis (Ogawa et al., 2005). At present, this family comprises 26 species

belonging to 10 genera (according to the World Register of Marine Species and Ascidian World Database).

In the Mediterranean Sea, the diversity of deep-sea ascidians seems to be lower than that of nearby Atlantic waters and they are found at relatively shallower depths. Out of 50 strictly bathyal and abyssal species in European Atlantic waters, only 11 have been reported in the Mediterranean (Monniot and Monniot, 1990). The only previous record of octacnemids in the Mediterranean was of seven juvenile individuals reported in Maltese waters at 500 m depth (Monniot and Monniot, 1975) and deposited in the Muséum National d'Histoire Naturelle. They were identified as *Dicopia* sp. because some diagnostic characters were lacking in juvenile forms, although their affinities with *D. antirrhinum* were noted (Monniot and Monniot, 1975, 1990). *D. anthirrhinum* is an Atlantic species (Monniot, 1972; Monniot and Monniot, 1974, 1985) that can be locally abundant and has been reported at depths between 1000 and 2500 m (Monniot and Monniot, 1990) . No adult octacnemid has been reported in the Mediterranean Sea until now (Coll et al., 2010), in spite of intense sampling effort in some areas, such as the Catalan margin, where scientific surveys with trawl nets or Agassiz sledges have been conducted over two decades in order to study the fauna inhabiting canyons and adjacent muddy plains areas (Ramírez-Llodra et al., 2008, 2010; Sardà et al., 1994, 2004).

New video-imaging techniques are providing a valuable tool to explore the behavior and the ecology of deep-sea fauna (reviewed by Aguzzi et al. 2010, 2012). Video surveys with Remotely Operated Vehicles (ROVs) have been increasingly performed in the past few years in the Northwestern Mediterranean, in a wide range of different deep-water geomorphological environments such as canyons, open mud plains, and submarine landslides (reviewed by Mechó et al., *in prep*.). On the Catalan margin, previous ROV video surveys did not reveal the presence of octacnemids, probably due

to the shallow nature of the dives (lower shelf and upper slope), where deep-sea coral communities occur (Orejas et al., 2009). In this study, we report outcome of the PROMARES ROV survey on the Catalan margin (Northwestern Mediterranean), where individuals of the genus *Dicopia* were observed *in situ* for several minutes prior to the collection of a specimen in La Fonera (Palamós) canyon.

## 2. Materials and Methods

Video observations were carried out with the Remotely Operated Vehicle (ROV) *Liropus* 2000 from the Spanish Oceanographic Institute (IEO) during the PROMARES survey, from 29$^{th}$ June to 15$^{th}$ July 2012. Seabed exploration was conducted onboard R/V *Sarmiento de Gamboa* on the Catalan margin and its deeply incised canyons (Cap de Creus, La Fonera and Blanes) (Fig. 1A). Footage of *D. antirrhinum* was obtained with the front-pointing video camera (HD Kongsberg OE14-502) and two small cameras (OE14376) on the extensible arm.

Navigation data (latitude, longitude and depth) were noted during each video records, making it possible to precisely locate all animals within previously-acquired swath bathymetry maps of the region (Lastras et al., 2011) (Fig. 1B).

When an animal was seen, the ROV survey was interrupted in order to obtain a detailed photographic record of it. The ROV is equipped with an extensible arm (HLK-47000), allowing the collection of specimens. This was used to recover a single individual of *Dicopia* which was placed in a lightproof box on the ROV. Immediately following the ROV dive, the specimen was preserved in 10% saltwater formalin, prior to examination in the home laboratory.

## 3. Results

### 3.1 Underwater observation

A total of five adult individuals was observed in two different locations in the La Fonera canyon (see Fig. 1B, C). First, two individuals were reported on the northeastern canyon flank at 1106 m depth. Later, between 1072 and 1085 m depth on the same flank of the canyon, three other specimens were observed; two of them only a few centimeters apart and the third one separated by approximately 35 m. Figures 2A-C show selected images of two individuals. The individual collected (Fig. 2D) corresponds to the last specimen observed by the ROV (Fig. 2E). When examined after recovery, fragments of consolidated mud were found to be attached to the base of the peduncle, suggesting that the captured specimen was originally anchored to this harder material beneath a thin layer of soft mud.

Our video filming showed the behavioral activity of *Dicopia* over short temporal scales (i.e. minutes). The animals seem to close the oral siphon when disturbed, while at the same time they presented a slow opening and closing of the atrial siphon (Fig. 2E).

### 3.2 Morphology

The specimen captured measured 5.8 cm in height, and has a thin and translucent tunic covered with muddy material (Fig. 3A, B). The body is not stalked, but tapers posteriorly and ends in a flat area with tunic rhizoids anchoring the animal to hardened mud. The oral siphon is enlarged forming a big horizontal slit surrounded by two lips. The atrial siphon is small and placed dorsally. Once the tunic is removed, the body wall is thin and has discrete muscular fascicles, consisting mainly of dense circular muscles around the rims of the lips, longitudinal muscles ventrally and dorsally (ending in the base of the body), and oblique muscles from the corners of the lips to the digestive system (Fig. 3C, D, E). The atrial siphon has some circular and radial muscles (Fig 3F).

Internally the oral siphon (i.e., the lips) is covered everywhere by small, flattened papillae.

The branchial sac is cone-shaped, limited anteriorly by a membranous velum topped with numerous filamentous tentacles. The velum is higher posteriorly. A triangular neural ganglion is anterior to the velum. The aperture of the neural gland is oblique. The pharyngeal groove is adjacent to the base of the velum and makes a slight indentation to surround the neural gland opening. The branchial wall is a three-dimensional network of sinuses, overlain by a perforated membrane with no ciliation (Fig. 3G). There are no imperforated areas in the branchial cone. The dorsal lamina is very low, while the endostyle is well developed.

The digestive system is compact and postero-dorsal. The esophagus opens at the bottom of the branchial cone and is followed by a slightly enlarged stomach and an ascending intestine. An ovarian mass lies in the gut loop, while a testicle is spread over the stomach and has a duct ending in a papilla close to the anus.

## 4. Discussion and Conclusion

Three octacnemid genera have an oral siphon in the shape of two lips: *Situla*, with a flat branchial lamina, and *Dicopia* and *Megalodicopia* with cone-shaped branchial sacs. The last two are distinguished by the presence of papillae in the inner side of the oral siphon and the absence of a muscular peduncle in *Dicopia* (Millar, 1988; Monniot, 1972; Sanamyan and Sanamyan, 2002), to which our specimens can be assigned. The morphological features of the present material agree well with the Atlantic species *Dicopia antirrhinum*, as described in Monniot (1972), and Monniot & Monniot (1974). In particular, in the latter work specimens are described as attached by

166 a flat surface with rhizoids as in the case of the individuals described here. The
167 musculature of our specimens seems to be within the variability reported for this
168 species. The other two *Dicopia* species described were Pacific forms; *D. fimbriata*
169 Sluiter, 1905 has a very different shape and musculature and has papillae in the inner
170 part of the tentacular velum (Monniot and Monniot, 1991; Sanamyan and Sanamyan,
171 1999), while *D. japonica* Oka, 1913 has a different shape and branchial wall (Millar,
172 1988).

173 In this study, we reported the presence of 5 adults of the deep-sea ascidian
174 *Dicopia antirrhinum,* previously represented only by possible juvenile specimens
175 collected near Malta (Monniot and Monniot 1975), and never filmed before. Few
176 observations of living Octacnemidae have ever been made, a notable exception being
177 *Megalodicopia hians* (Oka, 1918) which is displayed in Monterey Bay Aquarium
178 (USA), Uozu Aquarium and Enoshima Aquarium (Japan) (Sanamyan and Sanamyan,
179 2012). Our specimens were observed at around 1100 m in two different ridges between
180 gullies on the northern flank of La Fonera canyon, which suggest that deep canyon areas
181 constitute the preferred habitat of this species in the Catalan margin and possibly
182 beyond in the Mediterranean Sea.

183 The discovery of *Dicopia* on the northern flank of La Fonera canyon, but not in
184 its southern flank may also indicate a preference for settings where current conditions
185 favor encounters with free-swimming prey. La Fonera canyon in particular is crossed by
186 the mesoscale Northern current flowing in a southward direction, so that the leeward
187 position of the northern canyon flank and the associated low-energy turbulence might
188 favor such encounters.

189 *Dicopia* has been suggested to have a mixed diet (Monniot, 1972; Monniot and
190 Monniot, 1978). This genus may passively capture organic detritus, as suggested for

*Megalodicopia hians*, where feeding habit were studied at all levels, from gut contents to fatty acid and isotopic analyses ( Zhang et al., 2001; Okuyama et al., 2002; Ogawa et al., 2005). Unfortunately, an analysis of the diet of *D. antirrhinum* was not possible since only one specimen was captured. Moreover, this species has a reduced branchial sac in the form of a perforated cone, and no cilia were present in the stigmatal openings, all of which suggest weak filtration ability. On the other hand, the development of the oral siphon is an adaptation to macrophagous feeding (Monniot and Monniot, 1978). Individuals seem to be passive trappers of swimming zooplankton and micronekton like other octacnemids (Havenhand et al., 2006; Lescano et al., 2010; Okuyama et al., 2002).

The presence of five individuals of *Dicopia* on a very small area of this deep-sea canyon once more highlights the role of these submarine structures as hotspots for biomass concentration and elevated biodiversity (Company et al., 2012; Danovaro et al., 2010; Ramírez-Llodra et al., 2010). The previous absence of *Dicopia* from other more accessible and much more extensively investigated slope zones could be the combined result of limited sampling in operationally complicated zones, the fragility of these kinds of species, which are usually recovered in a damaged and unrecognizable state in trawl samples, and the lack of taxonomical expertise to identify them.

Here we report for first time the presence in the Mediterranean Sea of adults of the genus *Dicopia* that can be confidently assigned to the species *D. antirrhinum*. This new record increased the lower depth limit of this genus in the Mediterranean Sea from 500 to 1100 m depth. This finding contributes to the knowledge of the poorly investigated Mediterranean deep ascidian fauna (Monniot and Monniot, 1990), in sharp contrast with the relatively well-known shallow-water Mediterranean ascidians (Coll et al., 2010)

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## Figure captions

**Figure 1**. Study area. A) Location of La Fonera canyon on the Catalan margin and in the Western Mediterranean Sea. B) Detailed bathymetric map and location of the sites where *Dicopia* individuals were observed and sampled during ROV transects. C) Detailed slope map and sampling location.

**Figure 2**. A) Two individuals detected during ROV video-surveys within the La Fonera canyon, fully closed and fully open. Laser points are 10 cm apart. B, C) Detail of the fully open specimen. D) Capture of an individual by the ROV arm. E) Complete behavioral sequence of closure of the oral siphon.

**Figure 3**. Different morphological features of the collected specimen of *Dicopia antirrhinum*. A- B) Ventral and lateral morphology of the animal with tunic intact. C-E) Morphology of the animal without tunic: ventral (C), lateral (D), and dorsal (E) views. F) Enlarged view of the atrial siphon. G) Detail of branchial structure. Scale bars: C, D, E: 1 cm; F: 1 mm; G: 0.3 mm.

Figure_1

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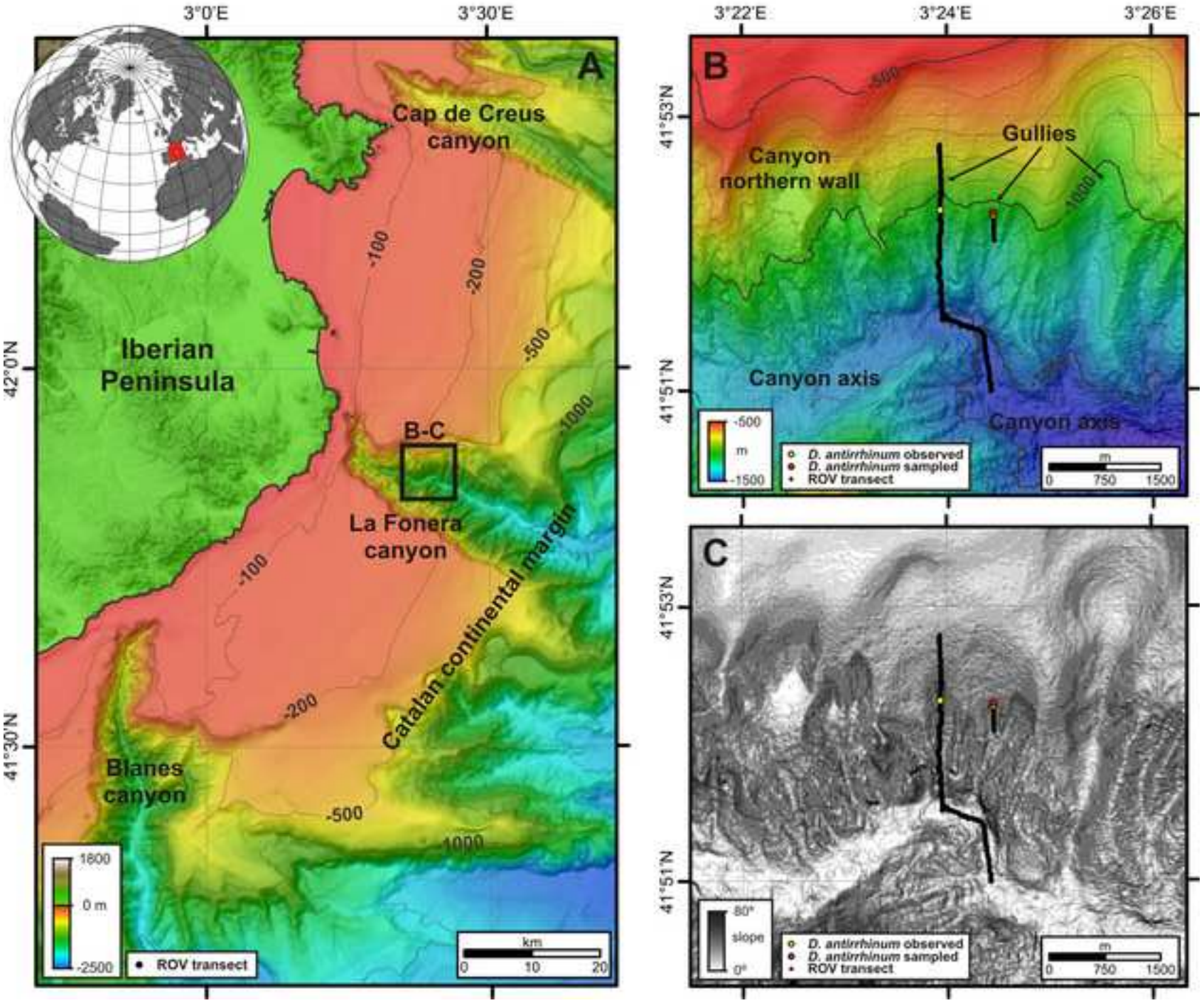

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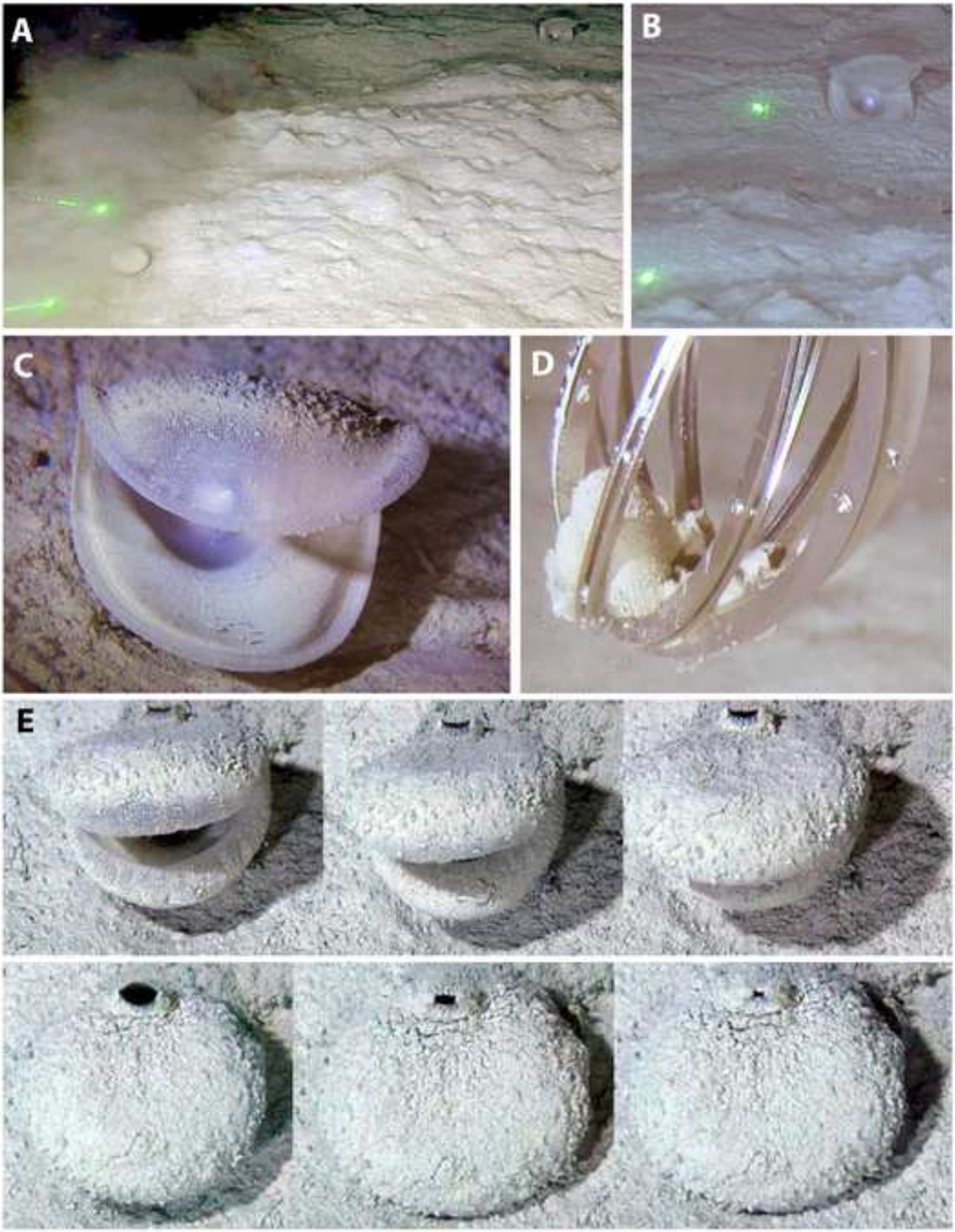

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