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## Finding of the macrophagous deep-sea ascidian *Dicopia antirrhinum* Monniot, 1972 (Chordata: Tunicata) in the Tyrrhenian Sea and updating of its distribution

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### Abstract

This study reports the in situ observations of a deep-sea ascidian, *Dicopia antirrhinum* (family Octacnemidae), on the deep seabed off the Aeolian Islands (Tyrrhenian Sea), between 569 and 813 m depth. These observations represent the first record of the species in Italian waters and the second observation in vivo to date. Peculiar macroscopic features are described here and the main differences from other species of the family Octacnemidae are highlighted, in order to allow the future identification of *D. antirrhinum* through visual technologies such as remotely operated vehicles. A total of 29 specimens were observed, with a maximum density of 0.012 specimens/m<sup>2</sup>. The global geographic and bathymetric distribution of *D. antirrhinum* known so far is also updated and discussed.

**Keywords:** *Dicopia*, *Tunicata*, deep-sea, ROV, Mediterranean Sea

### Introduction

The current knowledge about Mediterranean deep-sea fauna is still scarce compared with the fauna in shallower waters, especially concerning the class Ascidiacea. Unlike their shallow-water counterparts, deep-sea ascidians are rather uncommonly found in the Mediterranean Sea (Mecho et al. 2014). Visual technologies, such as remotely operated vehicles (ROVs), provide the opportunity to find and observe deep-sea habitats and species. This is particularly true for fragile species that are destroyed by trawling or that live in irregular areas where trawls cannot be deployed, to update the knowledge about their distribution (e.g. Mastrototaro et al. 2016, 2017; Chimienti et al. 2018c). Moreover, visual techniques can allow the study of population structure and dynamics in non-invasive ways (e.g. Ambroso et al. 2014; Chimienti et al. 2018b), as well as the observation of interesting or unknown behaviours of benthic invertebrates (e.g. Chimienti et al. 2018a).

However, the overall lack of sampling, which cannot always be carried out using an ROV, represents a bias for the taxonomic identification of some marine invertebrates, such as the ascidians (Sanamyan et al. 2018), as well as for their molecular analysis.

Within the family Octacnemidae, the peculiar features of the body such as the shape of the siphons with the hypertrophy of the oral lobes, as well as the presence of a peduncle (or stalk), can be used as macroscopic diagnostic characters to allow morphological identification even without the collection of samples. The family Octacnemidae includes 10 genera and 30 species of deep-sea ascidians. Among them, the genera *Dicopia*, *Megalodicopia* and *Situla* are characterized by an oral siphon expanded into muscular lobes, forming a lip-like aperture used to catch small motile prey such as crustaceans (Monniot & Monniot 1975a). This unique morphological feature, coupled with descriptions provided in the literature (e.g. Monniot & Monniot 1974,

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1977a; Mecho et al. 2014), can help with the specific identification of some Octacnemidae species, such as *Dicopia antirrhinum* Monniot, 1972. Within this family, only *D. antirrhinum* and *Situla lanosa* Monniot and Monniot, 1973 have been found so far in the Mediterranean Sea, the former supported by scientific literature (e.g. Mecho et al. 2014 and reference therein), the latter reported only in Monniot and Monniot (1990) without any reference. The present study reports the observation of various specimens of *D. antirrhinum* in the Aeolian archipelago (Tyrrhenian Sea), with a detailed description of the external morphological appearance of the species. Moreover, an update about the currently known distribution of *D. antirrhinum* is given and discussed. The behaviour of the specimens observed in the Aeolian archipelago is described and a preliminary estimate of the density of this species is reported.

### Material and methods

ROV surveys were carried out on board the R/V *Oceana Ranger* from May to June 2018. Observations were made using a SAAB Seaeye Falcon DR ROV equipped with an HDV (high-definition video) camera of 480 TVL (television lines), with minimum scene illumination of 2.0 lux (F1.4), a 1/2" CCD (charge-coupled device) pick-up device, image sensor, spherical 1/2 of 3.8 mm and wide-angle lenses. The ROV also included a depth sensor, sonar, a compass for underwater navigation, and two laser beams for the measurement of the area surveyed and of the observed specimens. ROV transects were carried out with an average speed of 0.2–0.3 knots and a constant frame of 1.80 m of width. The position was continuously recorded using a LinkQuest Tracklink USBL Transponder with up to 0.25° accuracy.

Five different sites around the Aeolian Islands were investigated, namely Eolo Seamount, Filicudi Bank, Capo Shoal, and areas off Lipari and off Stromboli (Figure 1). A total of 51 ROV transects were carried

out in the study area, covering a linear distance of 52,316 m and a total area of 94,169 m<sup>2</sup>, from 30 to 990 m depth. A total of 76 h 11 min video of the seabed was recorded. In seven of the ROV transects, *D. antirrhinum* was observed (Table I; Figure 1), and its density was estimated considering the number of specimens and the area surveyed within each ROV transect.

Detailed footage was taken of each specimen observed to attain a complete and precise external view of their morphology. The size of each specimen (i.e. length without the stalk, considering the specimens with the oral lips open) was estimated from the video frames using metric references based on the laser beams.

### Results

A total of 29 specimens of *D. antirrhinum* were observed on the seabed off the Aeolian Islands: two at Eolo Seamount, two at Filicudi Bank, 18 off Capo Shoal, two off Lipari and five off Stromboli (Table I; Figure 1). Sampling by means of the ROV manipulator arm was not possible due to the soft consistency of the specimens.

### Systematics

Phylum Chordata  
Subphylum Tunicata  
Class Ascidiacea  
Order Phlebobranchia  
Family Octacnemidae  
Genus *Dicopia*  
*Dicopia antirrhinum* Monniot, 1972

### New record

2018, Aeolian archipelago, Tyrrhenian Sea, Central Mediterranean Sea, 569–813 m depth.

Table I. Geographic information and depth range (m) of the remotely operated vehicle (ROV) transects where *Dicopia antirrhinum* was observed, with indication of the number of specimens (N).

Site	ROV dive	Start	End	Depth (m)	N
Off Lipari	Banco del Bagno 3	38°30.450'N, 014°49.660'E	38°30.275'N, 014°49.307'E	748–834	2
Eolo Seamount	Eolo Seamount 4	38°35.233'N, 014°05.876'E	38°35.264'N, 014°05.818'E	751–771	1
	Eolo Seamount 5	38°35.227'N, 014°05.815'E	38°35.320'N, 014°05.619'E	758–902	1
Filicudi Bank	Filicudi 2	38°35.483'N, 014°28.184'E	38°35.548'N, 014°27.705'E	755–937	2
Capo Shoal	Secca del Capo 2	38°38.274'N, 014°52.695'E	38°38.305'N, 014°52.508'E	556–698	16
	Secca del Capo 6	38°38.238'N, 014°55.253'E	38°38.642'N, 014°55.502'E	649–901	2
Off Stromboli	Stromboli 8	38°47.953'N, 015°16.218'E	38°47.493'N, 015°16.529'E	136–162	5

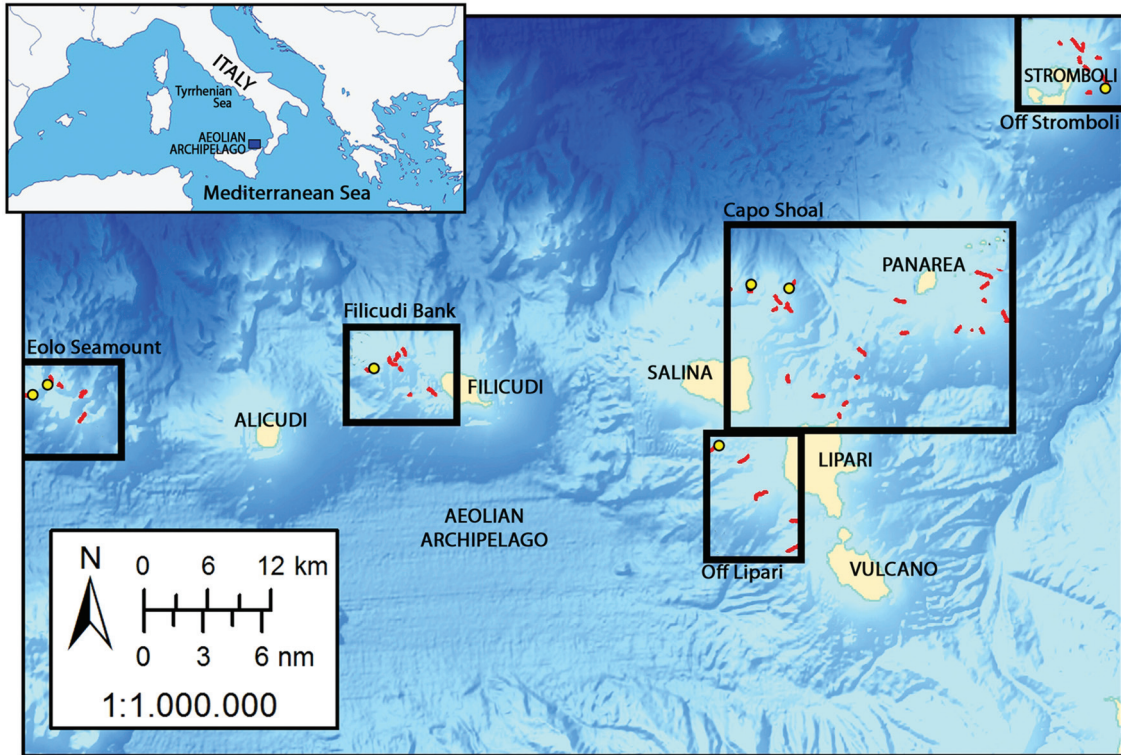


Figure 1. Study area, in the Aeolian archipelago, with five sites surveyed by remotely operated vehicle (black rectangles): Eolo Seamount, Filicudi Bank, Capo Shoal, off Lipari and off Stromboli. The locations of the ROV transects are shown in red (lines), with occurrences of *Dicopia antirrhinum* (yellow dots).

#### Previous records in the Mediterranean Sea

1975, off Malta, Sicily Channel, Central Mediterranean Sea, 500 m depth (Monniot & Monniot 1975b).

2014, La Fonera Canyon, Balearic Sea, Western Mediterranean Sea, 1000–1100 m depth (Mecho et al. 2014).

#### Description

The body was supported by a thick peduncle inserted in the substrate (Figure 2a, e–f). The oral aperture consisted of two enlarged and lengthened lips that formed a sort of horseshoe. The two lips were almost the same length, with the lower one slightly longer than the upper one (Figure 2). The distal ends of both lips appeared smooth (Figure 2a–d). The tunic was also quite smooth, except for some small digitations visible on its surface (Figure 2), likely to be papillae, that give it a velvet appearance. The narrow atrial siphon was placed dorsally (Figure 2f). The size of some observed specimens was estimated at between 3.3 and 3.7 cm height.

Among the 29 specimens observed, 18 had their lips open suggesting a feeding behaviour, and six of them were observed closing their lips and performing

a rapid contraction of their bodies to considerably reduce their size in case of stress (e.g. the presence of the ROV) (Figure 2g–j; Supplemental material). This contraction took from 2 to 15 seconds, with a mean of  $6.17 \pm 5.38$  seconds. In particular, the first step of the contraction was often slow, until contact was made between the two lips; then it was followed by a rapid contraction of the body, which seemed to collapse on the peduncle. In contrast, 10 other specimens already had their lips closed once approached by the ROV, while one of them opened its lips when illuminated by the lights of the ROV.

#### Remarks

The detailed observation of specific macroscopic features is essential to avoid the misidentification of *D. antirrhinum* with respect to similar species. In particular, within the family Octacnemidae, described by Herdman (1888), only three genera are characterized by the presence of an oral siphon developed into two lips: *Megalodicopia* Oka, 1918, *Situla* Vinogradova, 1969 and *Dicopia* Sluiter, 1905. The first genus includes species characterized by a wide body attached to the substratum through

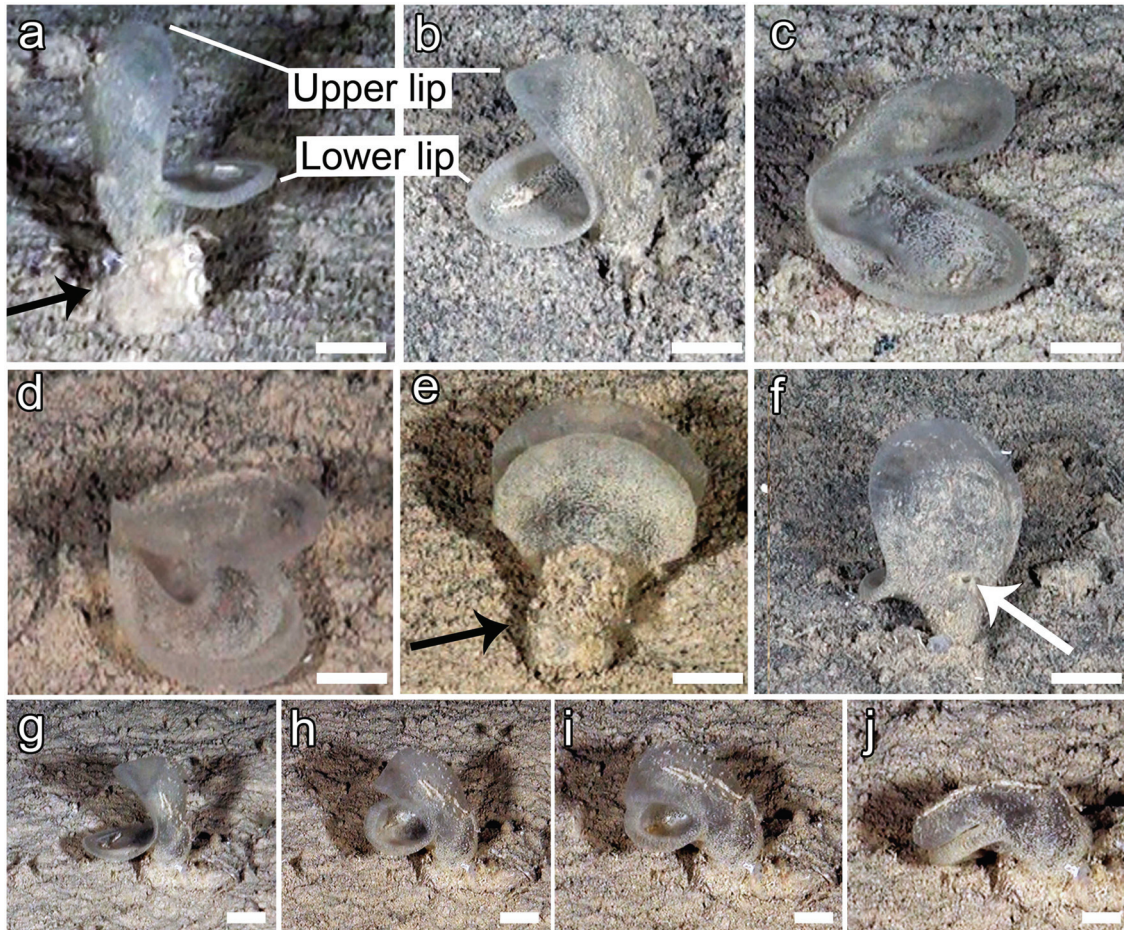


Figure 2. *Dicopia antirrhinum* observed off the Aeolian archipelago. (a–c) Lateral views of three specimens with the oral siphon open, delimited by upper and lower lips (the black arrow indicates the peduncle); (d) frontal view of a specimen with the oral siphon open; (e–f) dorsal view of two specimens where the peduncle (black arrow) and the atrial aperture (white arrow) are visible. Behaviour of *Dicopia antirrhinum*: (g) oral siphon open, (h) initial closure of the oral siphon, (i) oral siphon almost closed, and (j) oral siphon completely closed. Scale bars: 1 cm.

a very long, thick and muscular peduncle (Oka 1918; Monniot 1989; Sanamyan 1998; Sanamyan & Sanamyan 2002), unlike the genus *Dicopia*.

The two genera *Situla* and *Dicopia* are very similar, and can be distinguished by the shape of the branchial sac, which is conical in *Dicopia* and like a flat branchial lamina in *Situla* (Brunetti & Mastrototaro 2017). Unfortunately, for this type of diagnosis it is necessary to sample the animals and to properly anesthetize them in order to observe the branchial sac. However, specimens belonging to *Dicopia* can also be distinguished from *Situla* on the basis of macroscopic features, such as the proportions of the upper and lower lips, or the shape of the peduncle. In particular, six species have been described to date within the genus *Situla*: *S. pelliculosa* Vinogradova, 1969, *S. lanosa*, *S. rebainsi* Vinogradova, 1975, *S. macdonaldi* Monniot and Monniot, 1977b, *S. galeata* Monniot and Monniot,

1991 and *S. cuculli* Monniot and Monniot, 1991. *Situla pelliculosa*, the type species of the genus, does not have the ability to close its mouth, having a weak radial musculature (Vinogradova 1969; Sanamyan & Sanamyan 2002), resulting in an easily recognizable feature for differentiating *S. pelliculosa* from species that actively close their oral aperture, such as *D. antirrhinum* (Figure 2g–j). *Situla lanosa* appears very similar to *D. antirrhinum* (Brunetti & Mastrototaro 2017), but the two species can be distinguished due to the different proportions of the lips. In particular, the upper lip of *D. antirrhinum* is almost comparable in size to the lower one, while in *S. lanosa* the upper lip is larger than the lower one (Figure 3). Moreover, the tunic, the distal end of the lips and the peduncle are characterized by numerous filaments that give *S. lanosa* a hairy appearance (Figure 3c), hence the name *lanosa* (woolly) (Monniot & Monniot 1973).

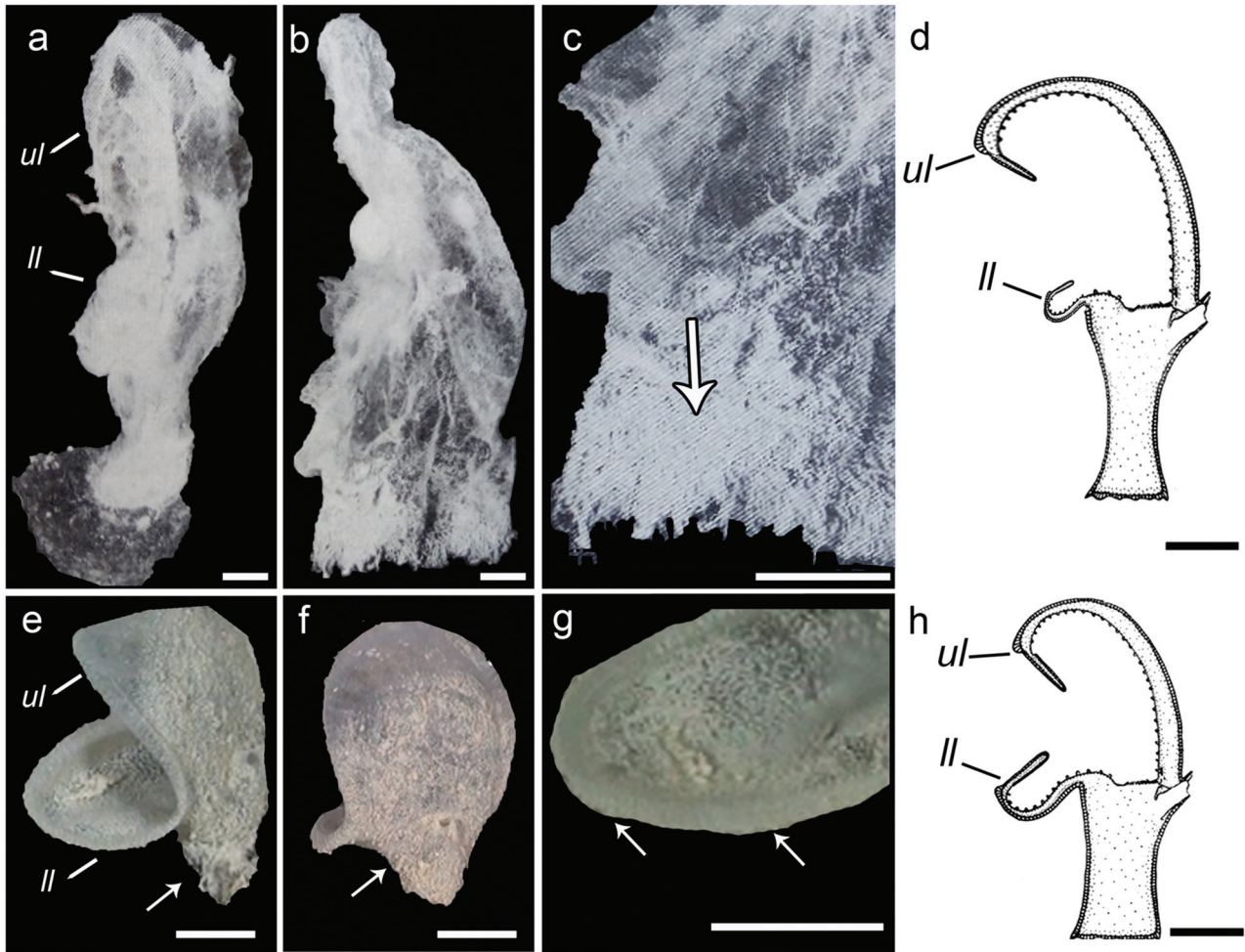


Figure 3. Comparison between *Situla lanosa* and *Dicopia antirrhinum*. *Situla lanosa* (from Monniot & Monniot 1973): (a) pedunculated specimen with a wide oral aperture and with the upper lip (*ul*) larger than the lower lip (*ll*); (b) specimen with filaments at the base of the peduncle, and (c) detail of the filaments (white arrow); (d) schematic drawing of *S. lanosa* showing the different proportions of the lips. *Dicopia antirrhinum*: (e–f) specimen with wide oral aperture, and peduncle without filaments (white arrow); (g) detail of the lower lip showing a smooth border; (h) schematic drawing of *D. antirrhinum* showing the similar proportions of the two lips. Scale bars: 1 cm.

Also, in *S. galeata* and *S. cuculli* the lower lip appears smaller than the upper one (Monniot & Monniot 1991), while *S. rebaini* is characterized by a body which resembles a flat, oval disc, attached to the substratum by a very long peduncle (Vinogradova 1975; Sanamyan & Sanamyan 2002). Lastly, *S. macdonaldi* is represented by only one specimen collected off the Kerguelen Islands (Indian Ocean) which, unlike *D. antirrhinum*, had a muscular peduncle similar to those of the genus *Megalodicopia* (Monniot & Monniot 1977b).

Within the genus *Dicopia*, three species are currently known: *D. antirrhinum*, *D. fimbriata* Sluiter, 1905 and *D. japonica* Oka, 1913. *Dicopia fimbriata* was described for the first time from Indonesia (Sluiter 1905), and four specimens were then sampled off New Caledonia (Monniot & Monniot

1991), one in the north Tasman Sea (Sanamyan & Sanamyan 1999) and, recently, one in Papua New Guinea (Monniot & López-Legentil 2017). This species is characterized by large flat lips and a small oral aperture similar to a fissure, with a smooth vitreous tunic featuring a smooth and not rolled margin of the oral lobes, as well as several papillae in the inner part of the tentacular velum (Monniot & Monniot 1991; Monniot & López-Legentil 2017). As recently highlighted by Monniot and López-Legentil (2017), *D. fimbriata* can be distinguished from *D. antirrhinum* because the latter has a tunic covered with papillae, giving it a velvet aspect, and a different organization of the musculature. Finally, *D. japonica* was described by Oka (1913), and three specimens were sampled by Millar (1988) off the coast of northern Peru.

According to Monniot (1972), this species can be distinguished from *D. antirrhinum* mainly on the basis of its highly reduced peduncle, and the oral aperture consisting of two flatter and rectangular-shaped oral lobes.

### Ecology

Specimens are usually found on the deep muddy seabed, directly attached to small rocks or pebbles covered by mud (Monniot 1972), or fixed to the hardened mud, due to the compacting ability of their anchoring rhizoids (Mecho et al. 2014). All the specimens found in the Aeolian Islands were on muddy bottoms.

### Distribution

The macrophagous ascidian *D. antirrhinum* is considered a deep-sea species, characterized by a wide bathymetric range, from 500 to 4300 m depth (Monniot & Monniot 1975b, 1977a). This species shows a North Atlantic distribution, having been recorded mainly along the Atlantic European continental slope (Monniot & Monniot 1990). It was first collected in the Gulf of Biscay between 600 and 1130 m depth, with only one specimen sampled (Monniot 1972).

Later, 22 specimens (17 juveniles and five adults) were collected in the same area at 2100–2200 m depth (Monniot & Monniot 1974), and 95 more specimens from 1845 to 4300 m depth (Monniot & Monniot 1977a). Moreover, 50 specimens of *D. antirrhinum* were sampled in 1976 along the Rockall Trough (West of Ireland), at about 2500 m depth (Monniot & Monniot 1985), and two specimens in 1988 southwest of the Gibraltar Strait, between 1200 and 2000 m depth (Monniot & Monniot 1988). In 1975, seven juveniles of *Dicopia* sp. were collected in the Mediterranean Sea, off Malta (Sicily Channel), at 500 m depth (Monniot & Monniot 1975b). More recently, in 2011, five adults were observed in situ for the first time and one of them was sampled by Mecho et al. (2014) in La Fonera Canyon (Balearic Sea), at 1072–1106 m depth, using a ROV (Figure 4).

The 29 specimens observed in this study were widespread along the five ROV transects, at depths ranging from 569 to 813 m. The maximum observed density was 0.012 specimens/m<sup>2</sup>, at Capo Shoal (Figure 1).

### Discussion

Considering the macroscopic features of the species, and based on the comparison with previous observations (Mecho et al. 2014), it was possible to assign

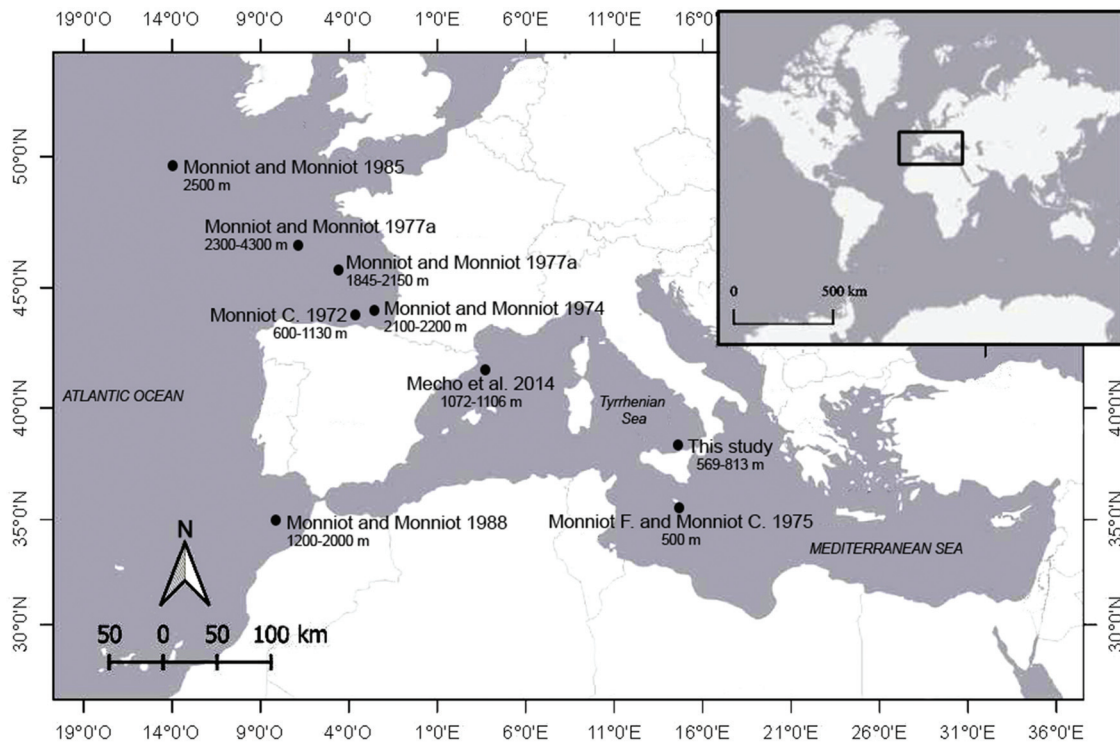


Figure 4. Known distribution and depth range of *Dicopia antirrhinum*, with reference to each record.

all the specimens observed off the Aeolian Islands to *D. antirrhinum*. Although the morphological analysis of anesthetized samples is often necessary for the correct identification of ascidians, macroscopic diagnostic characters such as body shape and the peculiar lip-like siphons permit the identification of *D. antirrhinum* from high-quality *in vivo* images, with a high level of reliability.

The size of the specimens observed (ca. 3.5 cm in height) is comparable to that measured by Mecho et al. (2014) in the Balearic Sea (about 6 cm in height), considering that those authors measured the whole specimen collected, including the part of the rhizoids below the sediment, which is not visible with ROV imaging.

The lack of observations of this species to date could be due to the difficulty in recognizing it from most deep-sea samples, which are mainly collected using sledges and dredges that damage the specimens, making them unclassifiable. For this reason, it is possible that the presence and distribution of *D. antirrhinum* have been underestimated or not detected with common sampling tools. Due to this difficulty in finding samples for molecular analysis, molecular characterization to describe the species is also missing. Our findings add one more tile to the still incomplete mosaic of distribution of *D. antirrhinum* in the Mediterranean Sea, extending its known presence to the Tyrrhenian Sea, where the species inhabits muddy bathyal bottoms between ca. 500 and 900 m depth. These new data confirm that the species is less rare than was considered in the past. Future prospects include the possibility to sample some specimens for barcoding purposes, as a complementary taxonomy tool for the morphological and visual analysis. Deep-sea visual surveys are providing continuous new findings and information about these still poorly known environments. In particular, such surveys are primarily improving knowledge about soft-bodied species (e.g. gelatinous), which are difficult to sample and identify with traditional tools as trawling, grabs and dredges.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

### Geolocation information

The study area is in the Aeolian archipelago, southern Tyrrhenian Sea: 38°30.477'N, 14°52.325'E.

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