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## Mesophotic rocks dominated by *Diazona violacea*: a Mediterranean codified habitat

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### Abstract

The football ascidian *Diazona violacea* was observed in three different sites in the Aeolian Archipelago (Southern Tyrrhenian Sea), from 53 to 116 m depth. A large population of this ascidian was found on rocky habitat, enhancing its three-dimensionality and constituting a peculiar *facies*. Colonies of *D. violacea* showed a mean density of  $0.87 \pm 0.09$  colonies  $m^{-2}$  and a maximum of 4 colonies  $m^{-2}$ . The number of zooids was used as a proxy of colony size and age (assuming that the higher the number of zooids, the larger and older is the colony), and the population showed a leptokurtic distribution with the dominant presence of colonies with fewer than 100 zooids. Large aggregations of this species proved to play an important role in terms of seabed complexity, influencing the underwater landscape and worth of being recognized as a relevant type of biogenic habitat (although ephemeral) that can characterize mesophotic rocks in the Mediterranean Sea.

**Keywords:** *Diazona*, *Tunicata*, mesophotic, Aeolian Islands, Mediterranean Sea

### Introduction

*Diazona violacea* Savigny, 1816 is a colonial ascidian, namely a species able to reproduce many zooids by budding, all of them remaining connected in the common tunic (or test) and forming a colony. The filtering zooids are long (up to 50 mm) and not arranged in systems, with the abdomen embedded in a common tunic which consists of a massive firm gelatinous matrix, while the thorax extends freely from the colony surface (Berrill 1948). This species is commonly known as “football ascidian” due to its characteristic large, massive colonies of a globular shape with the zooids only partially included in the common tunic, and is therefore easily recognizable from images. The filtering zooids are characterized by a white marking similar to that of *Clavelina lepadiformis* (Müller, 1776), consisting of white lines along the endostyle, the dorsal lamina and around the siphons but, unlike *Clavelina* species, the zooids

of *D. violacea* are embedded in a thick basal common tunic up to the abdomen and not only at their bases (Brunetti & Mastrototaro 2017; Bay-Nouailhat & Bay-Nouailhat 2020). This feature suggested the generic name *Diazona*, from the ancient Greek words *διά* (through) and *ζώνη* (belt), probably referring to the common tunic that keeps the zooids together only in the basal portion, which gives the appearance that they are connected through a basal belt. The specific name *violacea* was given by Savigny (1816), who described the species based on a colony preserved in ethanol. In fact, as established afterwards by Forbes and Goodsir (1853), the appearance of a live colony is greenish (fresh material outside of the water), while once preserved in alcohol the zooid turns violet and the preserving solution (ethanol) acquires the green colour of the ascidian. More recent *in vivo* observations showed that the colour of *D. violacea* underwater is not

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greenish, as it is outside the water, but simply translucent, whitish or yellowish. In a more in-depth study of the colour, Holt (1914) identified oil-like vesicles lying in the external portion of the tunic that are responsible for both the green and violet colours. Although the green colour seemed to be due to chlorophyll-like pigments, and the colour shift was somehow influenced by light, this author was not able to give a final explanation for this phenomenon.

Colonies of *D. violacea* can colonize both rocky and soft/detritic bottoms, settling on hard substrates such as rocks, stones, pebbles or biogenic detritus. It can be observed from euphotic to mesophotic depths from 10 to 200 m (Bay-Nouailhat & Bay-Nouailhat 2020). The species is distributed in the north-eastern Atlantic Ocean, from Scotland to Gibraltar, and in the Mediterranean Sea (Berrill 1928; Castric-Fey 1996; Boulcott et al. 2014; Chevaldonné et al. 2015; Ramos et al. 2016; Brunetti & Mastrototaro 2017; Bay-Nouailhat & Bay-Nouailhat 2020). Recently, it has also been found in the Red Sea (Shenkar 2012). In the Mediterranean Sea, *D. violacea* has been found across the basin, on both hard and soft bottoms, and frequently found as bycatch in trawling operations (Lubet & Azouz 1969; El Lakhrech et al. 2012; Petović et al. 2016; DeLaHoz et al. 2018; Demestre et al. 2018; Moya-Urbano et al. 2019). However, this species is considered characteristic of the coastal terrigenous mud biocoenosis, where it can form large aggregations of colonies (also known as *facies*) on viscous mud together with *Alcyonium palmatum* Pallas, 1766, sea pens and holothuroids (Pérès & Picard 1964).

In detail, *D. violacea* has been observed in the Gibraltar Strait (Ramos Esplá et al. 1992), the Alboran Sea (De la Torriente et al. 2018; Moya-Urbano et al. 2019), the Balearic Sea (from the Ebro area to the Gulf of Lion, including the Balearic Islands) (Turón 1985; Massutí & Reñones 2005; Ordines et al. 2011; Barberá et al. 2012; DeLaHoz et al. 2018; Demestre et al. 2018), the Tyrrhenian Sea (Bo et al. 2011; Esposito et al. 2018; Ingrassia et al. 2019), the Sicily Channel (Lubet & Azouz 1969), the Lybian Sea (El Lakhrech et al. 2012), the Adriatic Sea (Petović et al. 2016), the Levantine Sea (Çinar 2014) and the Aegean Sea (Çinar 2014; Antoniadou et al. 2016). Most of these records concern the occasional finding of one or few colonies, while quantitative records from specific areas are scarce, and the few known *facies* are reported only qualitatively as abundant or conspicuous occurrences (e.g. Pérès & Picard 1964; Lubet & Azouz 1969; Templado et al. 2012). Trawl surveys have highlighted the presence of

*D. violacea* on detritic soft bottoms with relatively low densities, such as 15–23 colonies  $\text{km}^{-2}$  off Montenegro (Petović et al. 2016), 75 colonies  $\text{km}^{-2}$  in the Gulf of Gabes, Tunisia (El Lakhrech et al. 2012), and up to 1037 colonies  $\text{km}^{-2}$  along the Catalan coast (DeLaHoz et al. 2018). Higher abundances have been observed on hard bottoms using a Remotely Operated Vehicle (ROV) at the Vercelli Seamount (Central Tyrrhenian Sea), where *D. violacea* can reach a maximum local density of three colonies  $\text{m}^{-2}$  (Bo et al. 2011).

This study reports the abundant occurrence of *D. violacea* on mesophotic rocky bottoms in the Aeolian Archipelago (Southern Tyrrhenian Sea), with a description of the main macroscopic features of the species observed *in vivo*. Furthermore, we provide a first assessment of the habitat formed by this ascidian.

## Material and methods

### Study area

The study area is the volcanic archipelago of the Aeolian Islands, in the Tyrrhenian Sea (Figure 1). This archipelago comprises seven main islands, all steep-sided volcanoes that are either active or dormant, with six offshore islets. The seafloor is characterized by volcanic outcrops, seamounts, gullies, submarine channels and canyons down to more than 2000 m depth, with the presence of hydrothermal activity from shallow to deep waters (Gugliandolo et al. 1999; Lupton et al. 2011; Romagnoli et al. 2012). Despite the occurrence of hydrothermal chimneys, the benthic community of the archipelago is composed by non-vent taxa including red algae (*Peyssonnelia* spp.), the green alga *Flabellia petiolata*, as well as sponges, bryozoans and tubeworms that can

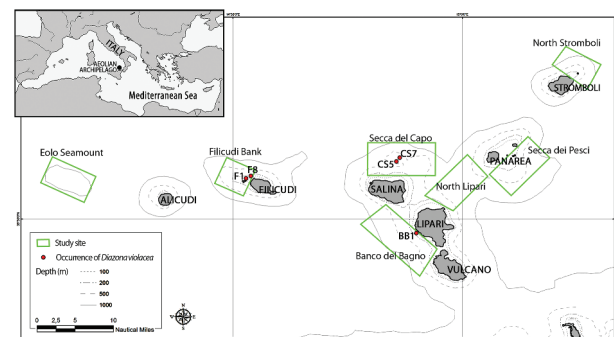


Figure 1. Study Area. The Aeolian Archipelago, with the seven study sites (in green), and occurrences of *Diazona violacea* (red points). Transects are reported in Table I

surprisingly live in the proximity of chimneys with active fluid emissions (Esposito et al. 2018). The particular mesophotic community reported by Esposito et al. (2018) in the vicinity of active chimneys included one colony of *D. violacea*. The bathyal zone of the archipelago is characterized by muddy seabed where some rare or uncommon species were recently found, including the carnivorous ascidian *Dicopia antirrhinum* Monniot, 1972 (Mastrototaro et al. 2019) and the swimming holothurian *Penilpidia ludwigi* (von Marenzeller, 1893) (Chimienti et al. 2019a).

#### Data collection and analysis

Seven main sites around the Aeolian Islands were investigated on board the R/V *Oceana Ranger*, from May to June 2018 (Figure 1). Surveys were carried out using a Saab Seaeye Falcon DR ROV equipped with a high-definition video (HDV) camera of 1920 × 1080 resolution, 1/2.9" Exmor R CMOS Sensor, minimum scene illumination of 3–11 Lux, and a 4.48 mm, f/1.8–3.4 zoom lens. The ROV also hosted a depth sensor, a sonar, a compass for underwater navigation, as well as two laser beams for size measurements. The ROV position was recorded continuously using a LinkQuest Tracklink USBL Transponder with up to 0.25° accuracy.

Fifty-one ROV transects were carried out in the study area, covering a total area of 94,169 m<sup>2</sup>, from 30 to 990 m depth. The sites in which *D. violacea* was present have been considered here, and one of them, where *D. violacea* was observed in large numbers (i.e. transect F8 at Filicudi Bank), was analysed in detail. In particular, videos from this ascidian *facies* were processed using Adobe Premiere Pro software by defining sampling units of 2.5 ± 0.2 m<sup>2</sup>, according to the minimal area used for visual surveys on mesophotic bottoms (Weinberg 1978; Chimienti et al. 2018, 2020; Chimienti 2020). Sequences with poor visibility or recorded outside the *D. violacea facies* were discarded, and a total of 76 sampling units were used for the analysis. The population of *D. violacea* was quantified by abundance (number of colonies per

sampling unit), and density (colonies m<sup>-2</sup>) was calculated for each sampling unit and expressed as mean ± standard error.

High-resolution still images were extracted directly from the ROV footage in order to assess the number of zooids per colony, when ROV framing and colonies position permitted it. This number was used as a proxy of the size and the age of the colonies, assuming that the higher the number of zooids, the larger and older was the colony (Berrill 1948). On the contrary, the ability of *D. violacea* to display different levels of contraction make direct size measures highly variable within the same colony and not particularly reliable. Fourteen size-classes were identified considering the number of zooids (50 zooids for each class, from 1–50 to 651–700). Population structure was analysed for each sub-population in terms of size-frequency and distribution parameters, such as skewness and kurtosis, calculated by means of the R software functions *agostino.test* (Komsta & Novomestky 2012) and *anscombe.test* (Anscombe & Gynn 1983).

## Results

### Occurrence of *Diazona violacea* in the Aeolian Archipelago

Colonies of *D. violacea* were found in five ROV transects from three study sites (Figure 1; Table I). On the Filicudi Bank (West of Filicudi Island), the species occupied rocky bottoms colonized by calcareous red algae (mostly Corallinales) and encrusting epifauna, including serpulids, bryozoans and molluscs, at 90–116 m depth. Both algae and encrusting fauna locally formed small coralligenous bioconstructions (*sensu* Ballesteros 2006; Ingresso et al. 2018) (Figure 2a).

At Secca del Capo (North of Salina Island), *D. violacea* was present on both encrusted rocks and small coralligenous formations, from 53 to 91 m depth. In particular, at transect CS7 some colonies were present within a turf of red algae (mostly Corallinales) and brown algae (mostly Dictyotales)

Table I. Geographic information and depth range (m) of occurrence of *Diazona violacea* at each study site, with indication of the number of colonies (N).

Site	Transect	Coordinates	Depth (m)	N
Filicudi Bank	F1	38°35.688'N, 014°29.483'E	115	1
	F8	38°35.971'N, 014°29.592'E - 38°35.928'N, 014°29.566'E	90–116	166
Secca del Capo	CS5	38°37.283'N, 014°54.108'E - 38°37.286'N, 014°54.166'E	82–91	7
	CS7	38°37.568'N, 014°54.510'E - 38°37.728'N, 014°54.347'E	53–66	10
Banco del Bagno	BB1	38°27.964'N, 014°52.565'E	60	1



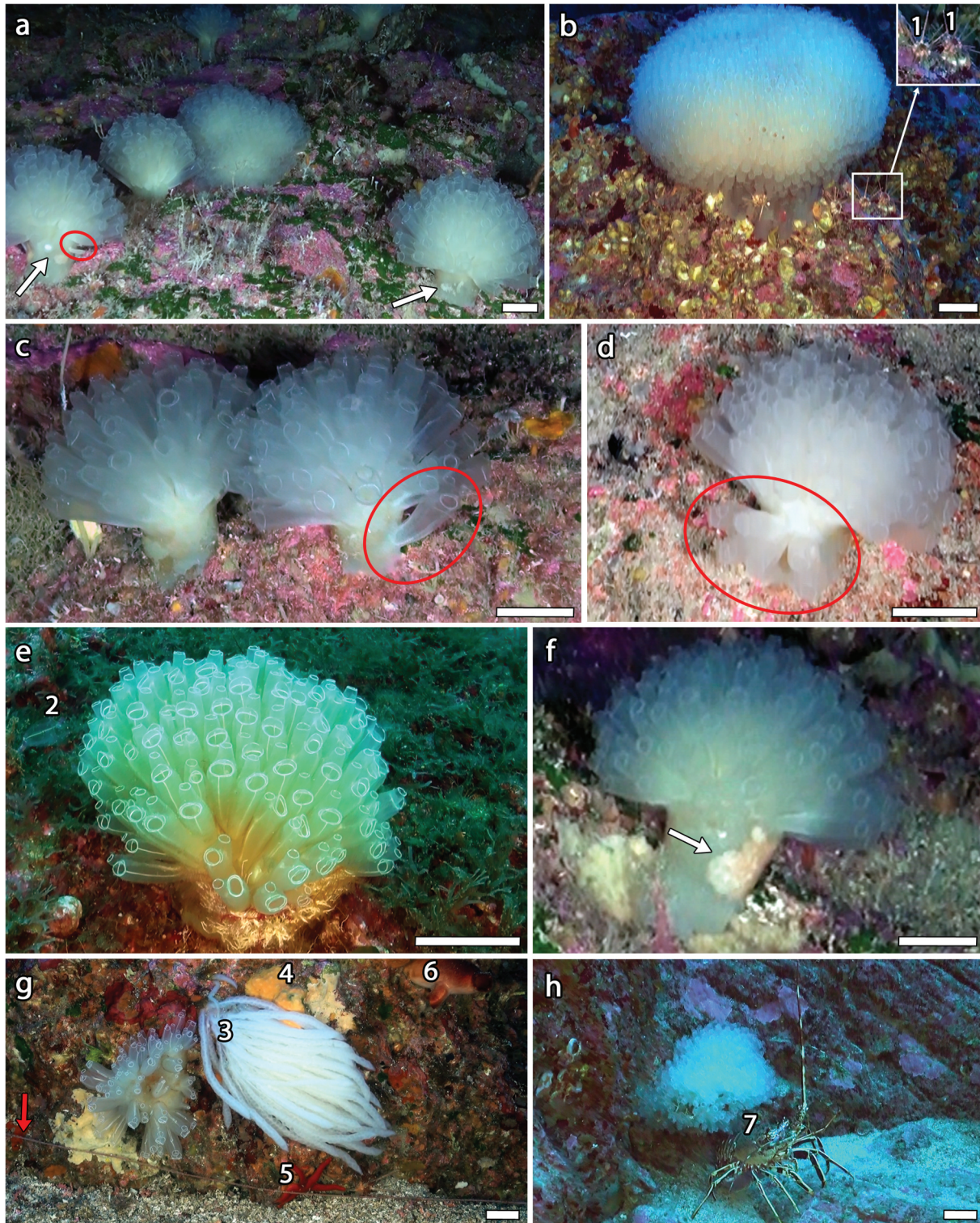


Figure 2. Mesophotic rocks with *Diazona violacea*. (a) Facies of *D. violacea* on coralligenous bioconstructions, with Plathelminthes present on the stem (white arrows) and a single zooid arising from the side of the stem in one colony (red circle). (b) Large colony on rocky bottom dominated by *Neopycnodonte cochlear*, with cidarids (1). (c) Colonies of *D. violacea* on rocks encrusted with red algae and epifauna; one zooid (red circle) arises laterally from one of the colonies. (d) Group of zooids arising laterally (red circle). (e) Yellow colony on coralligenous habitat dominated by red and brown algae, with *Clavelina dellavallei* (2). (f) Large Plathelminthes (white arrow) on the stem of a colony. (g) Squid eggs (3) laid in proximity of a small colony of *D. violacea*, on a coralligenous bioconstruction with encrusting algae and epifauna. Encrusting bryozoans (4), the sea star *Echinaster sepositus* (5) and the ascidian *Halocynthia papillosa* (6) are also present. At the base, a lost longline (red arrow) is entangled in the coralligenous bioconstruction. (h) The lobster *Palinurus elephas* (7) finding temporal refuge under a colony of *D. violacea*. Scale bars: 10 cm.



on coralligenous outcrops (Figure 2e), while at transect CS5 the hard bottom with occasional colonies of *D. violacea* was dominated by the grypheid oyster *Neopycnodonte cochlear* (Poli, 1795). This latter species entirely covered the seabed, possibly representing the initial stage of an oyster-dominated bioconstruction (Figure 2b).

At Banco del Bagno (West of Lipari Island), one single colony was observed at 60 m depth on one of the isolated rocks scattered on sandy bottom.

All the observed colonies were characterized by groups of zooids joined in a basal common tunic up to abdomen, with a free thoracic portion of 2–3 cm in length. The zooids emerged from the apex of the common basal portion of the colony, although one or few zooids could also protrude from the side of the stalk (Figure 2a,c–d). The marked white line was clearly visible along the endostyle, the dorsal lamina and around the siphons of each zooid, which were mostly translucent, whitish or, more rarely, yellowish (Figure 2a–e).

Some colonies of *D. violacea* showed the presence of one or more unidentified Plathelminthes on their basal portion, possibly representing ectoparasites (Figure 2a,f).

Visible human impacts included only few abandoned longlines entangled in the rocks (Figure 2g).

#### Mesophotic rocks dominated by *Diazona violacea*

The Filicudi Bank was characterized by a *facies* of *D. violacea* from 90 to 116 m depth, at transect F8, where 166 colonies were observed (Table I; Figure 2a). Colonies of *D. violacea* showed a mean density of  $0.87 \pm 0.09$  colonies  $m^{-2}$  (1 to 10 colonies per sampling unit; maximum of 4 colonies  $m^{-2}$ ) over an area of about 300  $m^2$ . The size of the colonies broadly ranged from 8 to 65 cm of maximum width, while the number of zooids was approximately between 5 and 696 (59 colonies considered). The size-frequency distribution was leptokurtic and highly skewed, with a right tail represented by few, large colonies (with a number of zooids  $\geq 550$ ), while colonies with fewer than 100 zooids were the most abundant (Figure 3).

The *facies* of *D. violacea* occurred on rocks encrusted by green algae, calcareous red algae (Figure 2a) and epifauna, among which were sponges, bryozoans and serpulids (Figure 2g). Further ascidian species were also present, including small colonies of *Clavelina dellavallei* (Zirpolo, 1925), as well as specimens of *Halocynthia papillosa* (Linnaeus, 1767) and *Rhopalaea neapolitana* Philippi, 1843. Sponges such as the *Calcarea Ute glabra* Schmidt, 1864 and the Demospongiae *Haliclona* sp., sea cucumbers of the

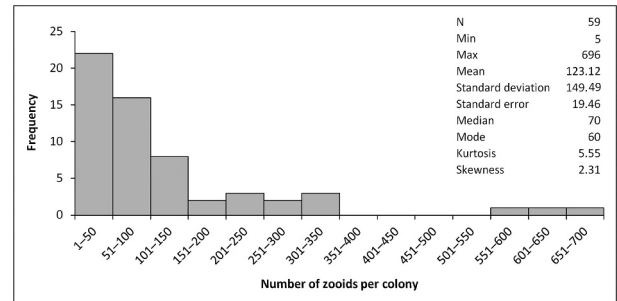


Figure 3. Frequency distribution of the number of zooids of *Diazona violacea* at Filicudi Bank.

genus *Holothuria* and sea urchins belonging to the family Cidaridae were also commonly present, although not abundant. *D. violacea* was the dominant and the largest erect organisms in the area, characterizing the underwater landscape. This was particularly evident on encrusted rocks, where *D. violacea* enhanced the habitat three-dimensionality and acted as temporary refuge for vagile organisms, due to the lack of natural niches and crevices. Among such organisms, a specimen of the spiny lobster *Palinurus elephas* (Fabricius, 1787) was observed taking refuge under a colony of *D. violacea* (Figure 2h). Moreover, squid egg masses observed in the area were mostly laid in close proximity to *D. violacea* colonies (Figure 2g).

#### Discussion

Ecosystem engineers are defined as those species that create, modify or maintain habitats by causing physical changes that enhance the three-dimensionality of the seabed and, directly or indirectly, modulate the availability of resources to other species (Jones et al. 1994). Besides bioconstructors, that build durable hard structures, ephemeral organisms can also act as ecosystem engineers, although they may be present only during specific periods of the year following seasonal patterns.

Ascidians can play a marginal role in some bioconstructions, acting as a sort of “connecting tissue” with other soft-bodied taxa in the complex processes that lead to the formation and maintenance of coralligenous and other biogenic habitats (Ingrosso et al. 2018 and references therein). At the same time, conspicuous colonial ascidians such as *D. violacea* can act as principal habitat formers in areas where the benthic community is not particularly structured, creating typical *facies* on hard bottoms. Mesophotic rocks with *D. violacea* represent a peculiar Mediterranean habitat already codified by Templado et al. (2012) along the Spanish coast as

“Circalittoral rocks at seamounts summit dominated by ascidians (e.g. *D. violacea*, Ascidiidae, Pyuridae and Styelidae)” (habitat number 0302022609), but not yet quantified. This study supports the identification of such specific *facies*, providing quantitative evidence of their importance in terms of colonies density, surface area and habitat provision. This *facies* is ephemeral, since *D. violacea* alternates seasonal periods of development and regression of the colonies. Sexual maturity is reached in summer, from July to September, when eggs are released in the water and embryos develop outside the mother colony (Mukai 1977). In late autumn and early winter, after sexual reproduction, zooids undergo regression and the colony degenerates into a hard globular mass to survive the cold season (Berrill 1948). This mass has a smooth surface and the regressed zooids inside form so-called “yellow bodies” (Della Valle 1884). Then, the zooids regenerate during late winter or early spring, and each of them produces from three to eight new zooids, regenerating the whole colony (Berrill 1948). This is in accordance with the leptokurtic and skewed distribution found at Filicudi Bank (Figure 3), where most of the colonies were relatively young (broadly fewer than 100 zooids and likely no more than 3 years old), while few colonies were older. Although not precise and not always easy to assess, the number of zooids can represent a valuable proxy for population structure in *D. violacea* considering that ascidians, like many other marine invertebrates (e.g. Chimienti et al. 2018, 2019b), can show a highly variable contraction state that makes size measurements ineffective.

The asexual reproduction of *D. violacea* concerns solely the establishment and growth of the colony by increasing the number of zooids each year, but it does not generate further colonies. In fact, *D. violacea* shows a survival budding (i.e. passive budding used to survive adverse conditions) instead of a propagative budding (i.e. asexual reproduction used to increase the number of the colonies) (Nakauchi 1982). For this reason, the different colonies forming the *facies* are the product of sexual reproduction.

The persistence of colonies throughout the year can vary based on environmental conditions, particularly water temperature (Della Valle 1884; Berrill 1948). Sometimes the fully developed colonies can last for most of the year, from late winter to autumn, as observed along the Catalan coast where DeLaHoz et al. (2018) reported the presence of *D. violacea* at 100–200 m depth during both summer and winter, with 878 to 1037 colonies km<sup>-2</sup>, respectively.

However, the presence of seasonal species is a typical element of rich and diversified habitats, hosting a complex biodiversity varying not only in space, but also in time (Coma et al. 2000). A population of *D. violacea* with comparable density to that here described was documented at Vercelli seamount (North Tyrrhenian Sea), within a well-structured coralligenous assemblage also dominated by sponges and polychaetes (Bo et al. 2011). In areas like the Aeolian Archipelago, where the volcanic activity does not allow the development of climax benthic habitats over long time scales (e.g. extensive coralligenous bioconstructions in the mesophotic zone and cold-water coral frameworks in the aphotic zone), temporary habitats such as ascidian *facies* can be even more important in enhancing seabed complexity. Although ephemeral, the *facies* of *D. violacea* can represent a temporary habitat and refuge for non-exclusive species, including some of commercial importance such as lobsters and squids. The latter are likely to prefer the proximity of *D. violacea* for spawning, suggesting a sort of camouflage strategy for their egg masses in the absence of overhead coherent substrata such as slender sponges, corals or anthropogenic objects.

ROV explorations are revealing how aggregations of colonial ascidians such as *D. violacea* can enhance the underwater landscape not only on detritic bottoms, but also on rocky and biogenic hard ones. Among the different *facies* or dominances that can characterize the still scantily known mesophotic zone, those of *D. violacea* deserves a specific codified identity among the Mediterranean hard-bottom habitats.

#### Disclosure statement

No potential conflict of interest was reported by the authors.

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#### Geolocation information

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

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