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The zooxanthellate scleractinian coral *Oulastrea crispata* (Lamarck, 1816), an overlooked newcomer in the Mediterranean Sea?

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Abstract

The zooxanthellate scleractinian coral *Oulastrea crispata*, a widely distributed species across central Indo-Pacific nearshore marine habitats, has been first reported from the Mediterranean Sea (Corsica) in 2014. Here we report on two new sites for this species in the NW Mediterranean Sea and provide a general description of external morphological characters of the colonies and a detailed account of the cnidom to help future identifications. Living specimens may appear virtually identical to small colonies (~5 cm) of the Mediterranean zooxanthellate scleractinian *Cladocora caespitosa*. While this species shows long, ramified, independent corallites, with cylindrical calices, *O. crispata* has enlarged, cup-like calices, which can be joined by the coenosteum. It also shows clear differences among several groups of nematocysts, principally the presence in the filaments of large penicilli (p - mastigophore) of one type that is absent in *C. caespitosa*. Identifications based on underwater observations or even the analysis of photographs may easily lead to misleading identifications. We hypothesize that *O. crispata* may have gone unnoticed because of misidentifications as *C. caespitosa*. More detailed research is needed to get reliable maps of the actual distribution of this apparently non-indigenous species in the Mediterranean Sea.

Keywords: Zebra coral; non-indigenous species; *Cladocora caespitosa*; *Oculina patagonica*; marine habitats.

Introduction

Colonial zooxanthellate scleractinian corals are virtually absent in the Mediterranean Sea with the exception of the endemic *Cladocora caespitosa* (Linnaeus, 1767) (Fig. 1a), which is capable of reef-building (Kruzic & Pozar-Domac, 2003; Kruzic & Benkovic, 2008; Kersting & Linares, 2012), and *Oculina patagonica* de Angelis, 1908 (Fig. 1b), a species that was thought to be non-indigenous until recently (Leydet & Hellberg, 2015). *Oulastrea crispata* (Lamarck, 1816) is a colonial hermatypic scleractinian coral recently identified in the Mediterranean Sea, concretely in the shallow waters of the northwest coast of Corsica (Hoeksema & Ocaña Vicente, 2014).

Oulastrea crispata has a wide distribution across cen-

tral Indo-Pacific nearshore marine ecosystems (<http://maps.iucnredlist.org/map.html?id=132859>). Populations of this species have been found in Japan in areas where the seawater temperature can drop down to 7°C (Yajima *et al.*, 1986), and in sites like the Houtman Abrolhos Islands in Western Australia (see Veron, 2000) where seawater temperatures are between 18 and 24°C. The northernmost location for any population of the species outside the Mediterranean Sea is 38° 4' N, 138° 14' E (Honma & Kitami, 1978) and the southernmost is 28° 43' S 113° 47' E (Veron, 2000). Populations of *O. crispata* from different areas show diverse life-history traits and phenotypic characters (Yamashiro, 2000; Chen *et al.*, 2011). This plasticity may allow colonies of *O. crispata* to inhabit a wide variety of habitats along moderately shallow rocky shores (Hoeksema & Ocaña Vicente, 2014). As Hoekse-



Fig. 1: Colonies of *Cladocora caespitosa* (a) and *Oculina patagonica* (b) from the study area. Scale bars are approximately 2 and 0.5 cm, respectively.

ma & Ocaña Vicente (2014) and other authors have put forward (Lam, 2000a), *O. crispata* has the necessary traits to behave as an opportunistic coloniser and to eventually spread and become invasive in the Mediterranean and other tropical or subtropical seas worldwide. Here we report the presence of colonies assigned to *O. crispata* in two new localities in the Mediterranean Sea and add new and supporting information about the species depth range and morphological features. The latter are vital to distinguish *O. crispata* from other scleractinian species that share the same habitats in the Mediterranean Sea and particularly *C. caespitosa*, which may look very similar from underwater observations of living specimens.

Materials and Methods

Colonies assigned to *Oulastrea crispata* were ob-

served at two different locations along the coast of Catalonia (Fig. 2). A first colony was recognised as putatively belonging to this species from an underwater photograph taken at a depth of ~3 m on a rocky boulder of the outward breakwater of the Port Olímpic of Barcelona (41.386443° N, 2.203961° E, WGS84) in May 2017. At that time, the specimen was not collected, so SCUBA divers conducted a new extensive monitoring mission at the same site at a depth of between 3 and 13 m September 2017. While the putative colony observed in May was not found, the divers photographed and took a sample of a different colony of *O. crispata*, stored it in a sealed pot with seawater, and took it to the laboratory for further processing (see below for details).

Two colonies resembling *O. crispata* were found in Tercer Ullastre, an underwater granitic reef located on the northern coast of Catalonia (41.884593° N, 3.203490° E,



Fig. 2: Map of the locations where *Oulastrea crispata* has been found to date in the Mediterranean Sea.

Fig. 2) in autumn 2017. The colonies were photographed and collected for further analysis at the laboratory, and the reports were uploaded to the marine citizen science platform Observadores del Mar (www.observadoresdel-mar.com).

Fresh samples of the colonies of *O. crispata* and two colonies of *C. caespitosa* collected from Barcelona and Tercer Ullastre for comparison purposes were either dried or fixed with 4-10% formaldehyde and then stored in 70% ethanol.

General morphology and anatomical features of the skeleton of *O. crispata* and *C. caespitosa* were described under the dissecting microscope. Different numbers of capsules (cnidae) of both species were used to examine and compare the nematocyst size and type under a light microscope equipped with a Nomarski differential interference contrast optic system. For the classification and terminology of nematocysts, we used Schmidt's (1972) criteria as adapted by Den Hartog (1980) and Den Hartog *et al.* (1993).

Voucher specimens were stored in the Marine Museum of Ceuta (MMC, Spain).

The SCUBA divers recorded the main plant and invertebrate species found in the vicinity of the *O. crispata* colonies at both sites.

Results

Specimens putatively identified as *Oulastrea crispata* were initially recognised underwater because of some unusual features i.e., apparently more imbricated, wider and thicker corallites than in *Cladocora caespitosa* and overall much larger corallites than *Oculina patagonica* (Fig. 2). Figure 3 shows that the polyp calices of *O. crispata* are wider than those of *O. patagonica* and that the typical coenosteum of the latter species is not nearly as conspicuous in *O. crispata*.

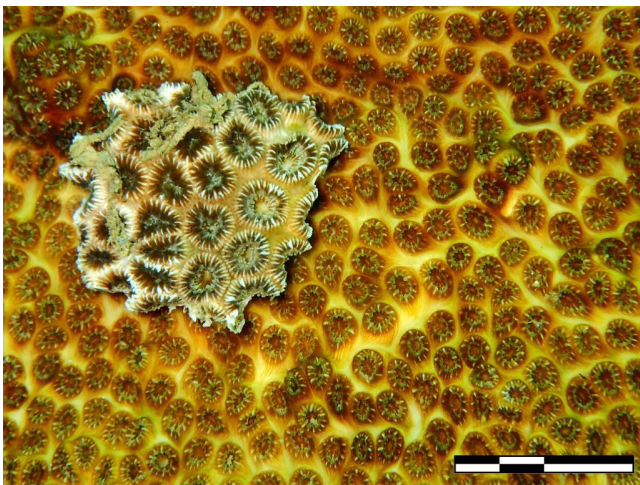


Fig. 3: A portion of the colony of *Oulastrea crispata* positioned and photographed on a colony of *Oculina patagonica* from the Port Olímpic in Barcelona for comparison purposes. Scale bar is approximately 1 cm.

Differences in colony and main nematocyst features for the three species are summarised in Table 1. The size of the colony collected in Barcelona in 2017 was ~5 cm in diameter. Among the colonies found in Tercer Ullastre, one measured ~5-6 cm and showed the characters of *O. crispata* (Fig. 4). Another actually was *C. caespitosa* and was used for the comparisons. In Barcelona, both the putative colony observed in May and the one assigned to *O. crispata* from the September samplings were found in habitats of photophilic algae with *Halopteris scoparia* (Linneus) Sauvageau, *Dictyota* spp., and *Lithophyllum incrustans* Philippi and the abundant sea urchin *Arbacia lixula* (Linneus, 1758). Mean minimum and maximum surface seawater temperatures (2012-2017) in Barcelona were 12.3 °C (± 0.5 SD) in January and 24.8 °C (± 0.8 SD) in July, respectively (data from NASA). The colony from Tercer Ullastre was found at 12 m depth in photophilic and hemisciaphilic habitats with *Corallina elongata* J. Ellis and Solander, *Dictyota* spp., *Taonia atomaria* (Woodward) J. Agarth, *Halopteris scoparia*, *H. filicina* (Grateloup) Kützing, and *Padina pavonica* (Linnaeus) Thivy. The sea urchins *Arbacia lixula* and *Paracentrotus lividus* (Lamarck, 1816) were scarcer there than in Barcelona. In Tercer Ullastre, minimum and maximum mean surface seawater temperatures (2012-2017) were 12.3 °C (0.5 SD) in January and 22.2 °C (± 1 SD) in July, respectively (data from NASA).

Most of the morphological characters of the skeleton and nematocysts were similar among the colonies of *O. crispata* and *C. caespitosa* collected. The most obvious difference was that the *O. crispata* showed enlarged, cup-like calices, which were somewhat joined by the coenosteum (Fig. 5). Differently, the long corallites forming the budding, ramified, phaceloid colonies of *C. caespitosa* are typically cylindrical, either imbricated or often with free space among them (Fig. 6). The coral calices of *O. crispata* from this study showed 45 septa.

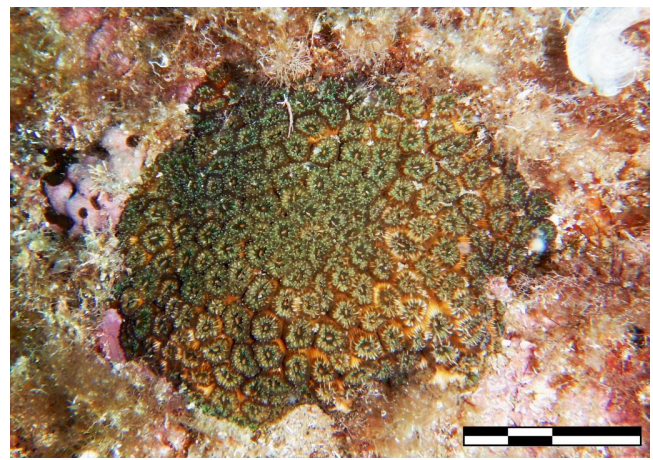


Fig. 4: A colony of *Oulastrea crispata* at Tercer Ullastre. Scale bar is approximately 2.5 cm.

Table 1. Comparison of main morphological characters of *Oulastrea crispata*, *Cladocora caespitosa* and *Oculina patagonica*.

Species	Coenosarc	Calix morphology, and size and numbers of septa	Colony growth form	Nematocyst types and features	Remarks
<i>Oulastrea crispata</i>	Arrangement of dense polyps with overall no visible or very reduced coenosarc	Cuplike, short, rounded elliptical calices Size range: 2-3 mm high and 3-8 mm wide Septa: 24-45	Placoid	Presence of large p-mastigophore in the filaments One type of nematocysts in the body wall	Colonies from Corsica (Hoeksema & Ocaña Vicente, 2014) and the Indo-Pacific do not show coenosarc. Reduced coenosarc was observed in all the colonies from Catalonia. Number of septa from the colonies in this work matched that of specimens from Asian waters (see Hoeksema & Ocaña Vicente, 2014)
<i>Cladocora caespitosa</i>	Coenosarc absent in most colonies	Cylindrical (round to elliptical in outline), enlarged calices. Size range: 2-8 mm high and 3-5 mm wide. Whole coralites can reach >100 mm height Septa: 34-40	Phaceloid	Absence of large p-mastigophore in the filaments Several categories in the body wall	No coenosarc in the colonies from the Museo del Mar of Ceuta (Spain), the Balearic Islands (MMC-573, Mallorca, 2005, one colony) and Italy (MMC-574, Lipari, 2005, one colony; MMC-575, Vulcano, 2005, two colonies). Large colonies from the Alboran Sea and the Strait of Gibraltar do not show coenosarc (see Ocaña <i>et al.</i> , 2009: 131). Septa never exceed 36 in number for all specimens observed
<i>Oculina patagonica</i>	Coenosarc always present	Round to elliptical in outline Size range: 2-3 mm high and 1-3 mm wide Septa: 24	Placoid	Unknown	New species and perhaps new genus should be erected to accommodate all the material assigned to <i>Oculina patagonica</i> from the Mediterranean Sea. Typically an encrusting coral capable of covering the substrate as thin lamina or rather conspicuous outcrops. In this case, colonies might resemble species like <i>Schizoculina</i> spp, which has recently been found in the Canary Islands (see Brito Hernández <i>et al.</i> , 2017)



Fig. 5: **a)** Skeleton of a colony portion of *Oulastrea crispata* from Barcelona (with zoomed views) and **b)** from Terçer Ullastre. Segments of the scale bar measure 1 cm.

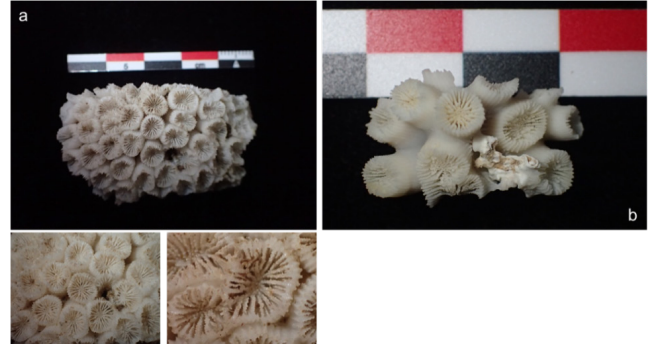


Fig. 6: **a)** Skeleton of a small colony of *Cladocora caespitosa* from Barcelona (with zoomed views) and **b)** from Terçer Ullastre. Segments of the scale bar measure 1 cm.

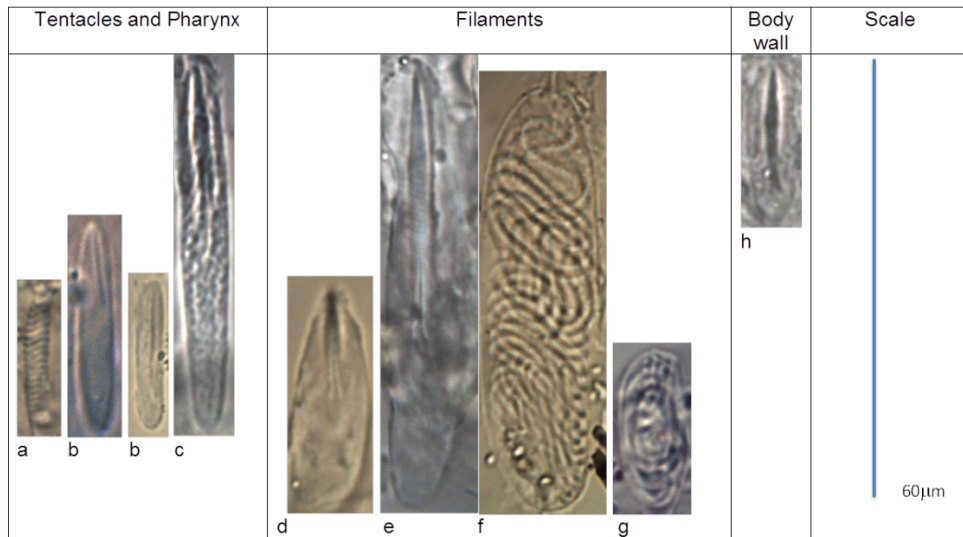


Fig. 7: The nematocyst types of different kinds of tissue in *Oulastrea crispata* (see Table 2).

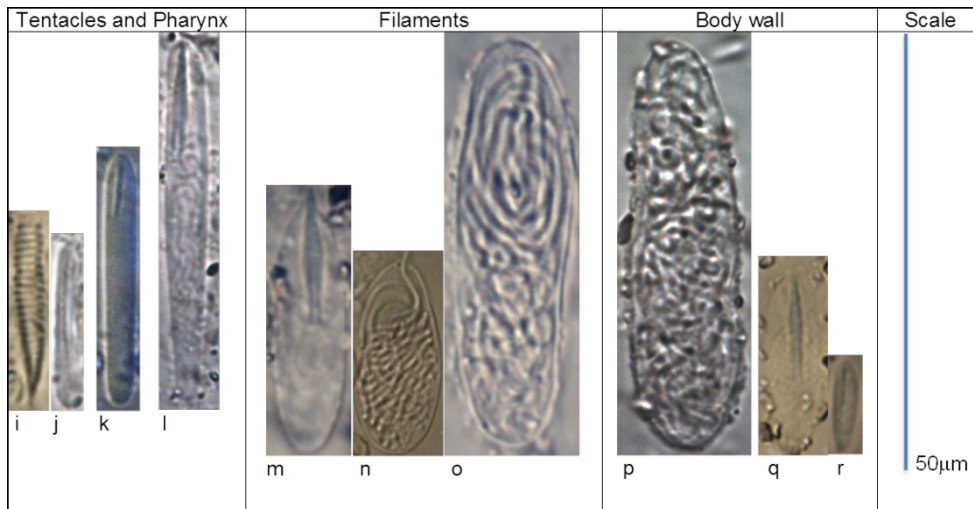


Fig. 8: The nematocyst types of different kinds of tissue in *Cladocora caespitosa* (Table 3).

Table 2. The different sizes for nematocyst type and tissue in *Oulastrea crispata*. The numbers of capsules (cnidae) measured is shown. C = common; RC = rather common; R = rare. Letters in brackets (a to h) refer to nematocysts in Fig. 7.

Tissue	Nematocyst type	Range (in brackets) of length and width of nematocystcapsules in μm	Number of capsules measured	Frequency
Tentacles and Pharynx	Spirocysts (a)	(15-20) x (2-3)	5	C
	Spirulae (b-mastigophore) (b)	(20-29) x (3-5)	10	RC
	Penicilli (p-mastigophore) 1 (c)	(35-45) x (5-7)	25	C
Filaments	Penicilli (p-mastigophore) 2 (d)	(22-26) x 6-10)	20	C
	Penicilli (p-mastigophore) 3 (e)	(50-60) x (8-10)	9	RC
	Penicilli E (homotrichs, holotrichs) (f)	(40-70) x (9-15)	20	C
	Penicilli E (homotrichs, holotrichs) (g)	20 x 7	1	R
Body wall	Penicilli (p-mastigophore) 4 (h)	(18-20) x (6-7)	10	RC

There were also some differences in size and the presence of several groups of nematocysts among the specimens of both species (Tables 2 and 3, and Figs. 7 and 8). The main difference was the presence in the filaments of *O. crispata* of large penicilli (p-mastigophore) of a particular type (type 'e', in Table 2 and Fig. 7), a nematocyst type absent in *C. caespitosa* (Table 3 and Fig. 8).

Discussion

Our findings and those from Hoeksema & Ocaña Vicente (2014) are the only records of the presence of *Oulastrea crispata* in the Mediterranean Sea so far. Colonies of this species can be easily overlooked *in situ* because of their morphological resemblance to *Oculina patagonica* (see Hoeksema & Ocaña Vicente, 2014), and in particular to relatively small (~5 cm), young *Cladocora caespitosa*, two corals that grow abundantly in similar habitats. Nevertheless, *Oulastrea crispata* shows distinctive densely packed cup-like corallites that never enlarge or ramify from budding. Colonies of *O. patagonica* show a flatter, more expanded growth form than *O. crispata*, with polyps widely sharing common tissue and rarely with overhanging corallites. While the colony collected in Corsica had 36 septa in the calices, the colonies from

Barcelona and Tercer Ullastre had 45, in accordance with those found in *O. crispata* from Indo-Pacific areas (24 to 45, see Hoeksema & Ocaña Vicente, 2014). Unfortunately, these differences are not always clearly visible in living specimens observed underwater, particularly when the polyps are fully expanded. Additionally, Oculinidae show large penicilli of type E (holotrichs or homotrichs) in the tentacles (Pires, 1997), a character lacking in *O. crispata*. The importance of cnidae in taxonomic studies on scleractinians has been noted by Pires (1997). Later, several studies supported the relevance of observing cnidae, as well as other useful characters for identifying scleractinians (see Terrón-Sigler & López-González, 2005; Ocaña & Brito, 2013).

The specimens of *O. crispata* collected in the Mediterranean Sea are no larger than 6 cm and can be easily confused with young colonies of *C. caespitosa*, especially due to the wide plasticity in shape and morphological characteristics recorded in the latter species (Kersting *et al.*, 2017). Besides, the characteristic dark-bright pattern of the skeleton and septa observable when the polyps are retracted in *O. crispata* from some Indo-Pacific areas (see comparisons in Hoeksema & Ocaña Vicente, 2014), which apparently gives the species the English vernacular name zebra coral, is not remarkable in the Mediterranean

Table 3. The different sizes for nematocyst type and tissue in *Cladocora caespitosa*. The numbers of capsules (cnidae) measured is shown. C = common; RC = rather common; R = rare. Letters in brackets (i to r) refer to nematocysts in Fig. 8.

Tissue	Nematocyst type	Range (in brackets) of length and width of nematocyst capsules in μm	Number of capsules measured	Frequency
Tentacles and Pharynx	Spirocysts (i)	(15-20) x (1-3)	5	C
	Spirulae (b-mastigophore) 1 (j)	(12-23) x (2)	10	RC
	Spirulae (b-mastigophore) 2 (k)	(25-35) x (4-5)	20	C
	Penicilli (p-mastigophore) 1 (l)	(40-50) x (4-7)	20	C
Filaments	Penicilli (p-mastigophore) 2 (m)	(21-35) x (6-8)	25	C
	Penicilli E (homotrichs, holotrichs) 1 (n)	(15-25) x (10)	3	R
	Penicilli E (homotrichs, holotrichs) 2 (o)	(30-55) x (10-14)	20	C
Body wall	Penicilli E (p-mastigophore) 3 (p)	55 x 14	1	R
	Penicilli (p-mastigophore) 3 (q)	20 x 4	1	R
	Spirulae (b-mastigophore) (r)	10 x 3	1	R

living colonies, or at least is not so different from the pattern observable in *C. caespitosa* and *O. patagonica*. This could be due to a lack of some chemical components (e.g. metal and trace element) in the waters from the area studied (see Lam, 2000a) or to the genetic relatedness of the Mediterranean colonies to populations from northern Japan (Yamashiro, 2000). Molecular analyses like those performed with *O. patagonica* (Leydet & Hellberg, 2015) are needed to elucidate possible genotypic relationships between the colonies found in the Mediterranean Sea and those from native areas. For all these reasons, we strongly recommend collecting at least part of the colony of putative *O. crispata* in future research since both underwater observations and photographic analyses may easily cause misleading identifications.

The colonies of *O. crispata* collected so far are scattered across different and relatively distant areas and grow on different substrates (from man-made rocky structures to granitic and basaltic natural reefs) at depths of between 2 (Hoeksema & Ocaña Vicente, 2014) and 12 metres. Nonetheless, no other specimens of *O. crispata* have ever been observed in our annual monitoring surveys at 60 different sites along the coast of Catalonia (García *et al.*, 2015).

Although *C. caespitosa* shows the widest habitat breadth, thriving well among dense algal cover (Schiller,

1993; Kersting & Linares, 2012; Kersting *et al.*, 2017), all three scleractinian species share similar habitats in the Mediterranean Sea. In fact, they seem to thrive well in photophilic environments devoid of dense algal cover where crustose coralline algae and sea urchins dominate (Coma *et al.*, 2011, Hoeksema & Ocaña Vicente, 2014).

While considering the possibility of the previous general failure in detecting the species being on account of its resemblance to *C. caespitosa*, and to a lesser extent to *O. patagonica*, the discovery of the few colonies assigned to *O. crispata* in Catalonia and Corsica and their relatively small sizes would seem to support the conclusion of a recent introduction in the Mediterranean Sea. The species is thought to grow close to one mm per month (see Lam, 2000b), thus observed colonies might be about five years old. Nevertheless, colonies may not survive to older ages because of competition and/or predation, thus in the absence of colony monitoring the introduction timing remains merely putative. As for all analogous cases, much can also be speculated about the vehicles of arrival. Nonetheless, shipping appears as the most probable way, possibly through colonies attached to the hulls of vessels or other transported structures, rather than through the presence of short-lived planulae in ballast waters (Creed *et al.*, 2017), rafting on oceanic currents (Hoeksema *et al.*, 2012, Hoeksema *et al.*, 2018) or release from aquariums

(Wiedenmann *et al.*, 2001; Mantelatto *et al.*, 2018). While we recognise the relevance of research aimed at determining the origin of species introductions in new areas, we hope that this study, coupled with that by Hoeksema & Ocaña Vicente (2014), will help produce the essential baselines for the presence of *O. crispata* in the Mediterranean Sea. This is especially relevant to avoid generating confusing knowledge about past and present species distributions, particularly non-indigenous ones. As long as the correct quality procedures for species identification are applied, the inputs from citizen science initiatives like Observadores del Mar (www.observadoresdelmar.com) may be excellent tools to acquire new knowledge about the distribution of *O. crispata* in the Mediterranean and other seas.

If *O. crispata* becomes established in the Mediterranean Sea, it will represent a significant addition to the reduced number of zooxanthellate colonial scleractinian corals inhabiting the basin. Future studies aimed at exploring the role of *O. crispata* in Mediterranean photophilic communities should reveal its ability to adapt and, ultimately, to outcompete indigenous species.

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