

1 **Extremely rapid withdrawal behaviour of the sea pen *Protoptilum cf. carpenteri*** 2 **in the deep Mediterranean**

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11 **Abstract**

12 Sea pens (Octocorallia, Pennatulacea) are a specialized and morphologically distinct group of
13 octocorals. The majority of them have adapted to survive on soft sediments with the help of an
14 anchoring muscular peduncle. The whip-like sea pen *Protoptilum carpenteri* is considered a deep-
15 sea North Atlantic species, which recently has been documented also in the Mediterranean Sea,
16 where its actual distribution and abundance are still unknown. Even less is known about its ecology
17 and behaviour, its reactions after disturbance, and its possible escape strategies. Several species of
18 pennatulaceans can withdraw partially or completely into the sediment, following an apparent
19 rhythmic but unsynchronized procedure that is usually preceded by the closure of the autozooids
20 and the expulsion of the water contained within the colony. The present study reports and discusses
21 for the first time the extremely fast withdrawal behaviour of *P. cf. carpenteri* after *in-situ*
22 disturbance.

23 **Key words:** Burrowing behaviour, Mediterranean Sea, soft bottoms, ROV, mega-epibenthic
24 communities.

25 **Introduction**

26 Sessile marine organisms respond to environmental disturbance and predation by means of different
27 adaptive strategies (Williams 2011). Sea pens (Octocorallia, Pennatulacea) are colonial cnidarians

28 and the majority of them are adapted to inhabit muddy and sandy bottoms of the continental shelf
29 and slope. *Protoptilum carpenteri* Kölliker, 1872 is a deep-sea species that can live in a wide
30 bathymetric range, between 400 and 4270 m depth, with a predominant North Atlantic distribution
31 (Gilkinson and Edinger 2009; Mastrototaro et al. 2015). However, since its description at the end of
32 the 18th century, only five sightings in deep-sea Mediterranean environments have been
33 documented, which were between 400 and 800 m depth (Mastrototaro et al. 2015; Prampolini et al.,
34 2020).

35 Although in the last decades a substantial increase in the exploration of the Mediterranean
36 deep-sea environment has occurred, soft sediment environments have remained widely disregarded
37 here (Grinyó et al. 2020). Consequently, there is a wide knowledge gap on the ecology and
38 biogeography of deep-sea Mediterranean megabenthic species living in soft substrates. This is
39 especially true for species showing a burrowing or withdrawing behaviour (Durden et al. 2015;
40 Chimienti et al. 2018). Most sea pen species have the capacity to withdraw into the sediment
41 following an apparent rhythmic but unsynchronized procedure (Langton et al. 1990). By means of
42 few contractions of the peduncle, sea pens can expel part of the water contained within the colony
43 withdrawing themselves partially or completely into the sediment. Although it has been suggested
44 that food availability and light may influence pennatulacean withdrawal behaviour, most existing
45 information based on laboratory studies is ambiguous and withdrawal triggering factors are not well
46 understood (Buisson, 1964; Magnus, 1966). Hoare and Wilson (1977) described a possible tidal-
47 based rhythm for *Virgularia mirabilis* while Wilson (1975), using time-lapse photography, found a
48 22- to 27-h rhythm for this species that was independent of lighting and tidal regime. For *Veretillum*
49 *cynomorium*, Buisson (1964) documented that light was controlling the rhythm of extension and
50 withdrawal. Magnus (1966) found a strong diurnal activity in *Scytaliopsis djiboutiensis*, which
51 extended only at night. On the other hand, *Ptilosarcus gurneyi* expands only for feeding (Birkeland
52 1974) and *Echinoptilum* sp. can avoid dangerous situations such as predation via migration while
53 being retracted inside the sediment (Kushida et al. 2020).

54 Observations of withdrawal behaviour *in situ* are still quite scarce, particularly in deep-sea
55 environments (Kushida et al. 2020). The only *in situ* observations were reported by Birkeland (1974)
56 for *P. gurneyi*, by Langton et al. (1990) for *Pennatula aculeata*, by Ambroso et al. (2013) for
57 *Virgularia mirabilis*, and by Chimienti et al. (2018) for *Pennatula rubra*.

58 Pennatulacean withdrawal behaviour occurs at different velocities. For *P. rubra*, colony
59 withdrawal is preceded by polyp retraction and is proved to be a slow process requiring more than 3
60 min to be completed (Chimienti et al. 2018). On the other hand, *V. mirabilis* has been reported to
61 withdraw itself into the seabed in a few seconds when disturbed (Ambroso et al. 2013). The present

62 study reports for the first time the fastest withdrawal of *P. cf. carpenteri*, representing a behaviour
63 never reported before for a sea pen.

64

65 **Material and methods**

66 Video transects were made during the research cruise ABRIC 1, from 13 to 29 February 2020, on
67 board the *R/V Sarmiento de Gamboa*, using the ROV (Remotely Operated Vehicle) *Liropus 2000*.
68 The main goal of this cruise was to explore and characterize deep-sea benthic habitats between 600
69 and 1200 m depth in the Blanes submarine canyon. Study sites were chosen based on the seafloor
70 morphology, targeting canyon wall areas that could host habitat-forming anthozoans, such as frame-
71 work building scleractinians, gorgonians and antipatharians as well soft bottom areas hosting
72 pennatulaceans and soft corals. The ROV was equipped with a low-definition video camera for
73 navigation and a high-definition video camera for the detailed observation during the video
74 transects. The ROV also hosted a depth sensor, a compass, a grabber arm and two laser beams
75 providing a 10-cm scale as reference for dimensional measurements.

76

77 **Results and discussion**

78

79 The withdrawal behaviour of *P. cf. carpenteri* was observed just one time for a single
80 colony at 617 m depth in the east flank of Blanes Canyon (41°30'16.98"N, 2°57'2.71"W; Fig. 1),
81 showing that this species retracts extremely fast and with its polyps fully expanded (Fig. 2; Online
82 Resource 1). Mobility was also observed in free-living mushroom corals allowing them to escape
83 from interactions with other organisms (Chadwick 1988; Hoeksema and de Voogd, 2012) and in
84 solitary zoantharians by means of horizontal locomotion on sandy substratum via peristalsis
85 (Nagabhushanam and Jothinayagam 1982). Mushroom corals can also turn themselves over and
86 using peristalsis to shed sediment from their bodies (Bongaerts et al. 2012; Hoeksema and
87 Bongaerts 2016). Withdrawal behaviour, peristalsis and active movements may be a somewhat
88 common feature for anthozoans in sandy bottom environments but the reasons why some species
89 burrows and moves are still unresolved (Kushida et al. 2020). The withdrawal behaviour of *P. cf.*
90 *carpenteri* was observed after a disturbance caused approaching the arm of the ROV in an attempt
91 to sample it and it was calculated by counting the number of frames per second (30 of 50 frames
92 corresponding to 0.6 sec) it took the animal to hide (Online Resource 1).

93

94 The observed morphology of our specimen agrees well with the expanded colonies of the
95 genus *Protoptilum* photographed and videotaped in the Mediterranean Sea, although a darker
colouration is observed in the pharynx of autozooids. *Protoptilum carpenteri* is the only species of

96 this genus reported so far for the Mediterranean Sea (Mastrotorato et al. 2015; Altuna and Polisenò
97 2019). Unfortunately, the observed specimen was not available for examination.

98 The species delimitation in this genus is still an open question since part of the variability of
99 characters currently in use can be found in a single colony (López-González. com. pers.). Molecular
100 information for this genus is scarce, but suggests that species diversity is higher and that the wide
101 geographic distributions are more restricted than those assumed today (López-González, com. pers.).
102 Taking all this into account, and awaiting a revision of the genus, including Mediterranean forms,
103 we prefer to maintain the species identification based on the present observations as *Protoptilum* cf.
104 *carpenteri*.

105 Habitat-forming species have been recognized as important components of deep-sea
106 ecosystems, whilst being very vulnerable to anthropogenic impacts (Auster et al. 2011). Sea pen
107 aggregations can form important soft-bottom communities providing a three-dimensional
108 complexity from which several associated species can benefit (Chimienti et al. 2018). They are
109 relatively understudied and basic information on their ecology is largely lacking. Thus, there is a
110 need to improve the data collection of sea pen species, in particular for offshore and deep areas, in
111 order to improve the identification of Mediterranean VMEs.

112 The present study confirms that *P. cf. carpenteri* can completely withdraw into sediments
113 within 0.6 sec. This process is similar to other species, such as *V. mirabilis*, which is able to
114 withdraw in a few seconds (between 15 and 40 sec) (Ambroso et al. 2013). On the contrary, *P. rubra*
115 showed a slow process requiring between 3 and 5 min for the complete withdrawal (Chimienti et al.
116 2018). The ability of some sea pen species (e.g. *Pennatula rubra*, *Pennatula aculeata*, *Virgularia*
117 *mirabilis*, *Echinoptilum* sp. and *Protoptilum carpenteri*) to withdraw into the sediment should make
118 them less sensitive to physical disturbance, such as the one caused by bottom trawling activities
119 (Langton et al. 1990; Kenchington et al. 2011; Ambroso et al. 2013; Chimienti et al. 2018; Kushida
120 et al. 2020).

121 Dominant species found in the Mediterranean bathyal mud are the sea pen *Funiculina*
122 *quadrangularis* or the gorgonian *Isidella elongata* which are often found together with crustacean
123 species, such as *Aristeus antennatus*, *Aristeomorpha foliacea*, *Parapenaeus longirostris* and
124 *Nephrops norvegicus* (Pérès and Picard 1964). Both species *I. elongata* and *F. quadrangularis* have
125 almost completely disappeared from the trawlable bottoms of the most Mediterranean areas
126 (D'Onghia et al. 2011; Sardà et al. 2004). *F. quadrangularis* is one of the sea pen species that are
127 unable to withdraw into the sediment (Mac Donald et al. 1996). This shows that *F. quadrangularis*
128 may act as an indicator of the state of health of deep-sea mud habitats and that it can be considered
129 a Vulnerable Marine Ecosystem (VME) indicator species (Rogers and Gianni 2010). This is still

130 unknown for sea pens such as *P. carpenteri* due to the lack of knowledge about its distribution and
131 abundance in the Mediterranean Sea. The low number of *P. carpenteri* colonies found until now in
132 the Mediterranean Sea could be related to the highly patchy distribution of the species, which
133 usually characterizes sea pens and other sessile organisms in deep-sea habitats (Carpine and
134 Grasshoff 1975; Marshall 1988; Morris 2011).

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148 **Ethical approval:** No animal testing was performed during this study.

149 **Sampling and field studies:** The study does not contain sampling material or data from field
150 studies.

151 **Data availability:** All data generated or analysed during this study are included in this published
152 article and its supplementary information files.

153 **Author Contribution Statement:** SA and JG conceived and designed research. SA wrote the
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235

236 Figure captions

237 **Fig. 1** Map showing the location where the withdrawing behaviour of *P. cf. carpenteri* was
238 observed (Eastern Blanes canyon, north-western Mediterranean Sea). Projected view (UTM Zone
239 31N (WGS84)) with geographic (WGS84) coordinates indicated for reference.

240 **Fig. 2a** *Protoptilum cf. carpenteri* on the east flank of Blanes Canyon (41°30'16.98"N,
241 2°57'2.71"W). **b** Close-up showing expanded polyps.

242 **Online Resource 1** Video footage of the withdrawal behaviour of the sea pen *Protoptilum cf.*
243 *carpenteri*.

