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Baseline

First report of the carnivorous sponge *Lycopodina hypogea* (Cladorhizidae) associated with marine debris, and its possible implications on deep-sea connectivity

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ABSTRACT

Nowadays, there are an increasing number of reports of deep-sea accumulation of marine debris, often associated with a wide array of pernicious effects on benthic fauna. Nevertheless, there is still a huge knowledge gap regarding the interaction of benthic organisms and marine debris. In this paper, we report for the first time the colonization of plastic debris by the protected sponges *Lycopodina hypogea*. The sponges were discovered growing on plastic debris tangled with nylon ropes on the Blanes canyon (northwestern Mediterranean Sea). Over 30 individuals of *L. hypogea* were identified attached on ca. 10 cm^2 plastic debris, an unusual feature for a species mostly known for low-density populations and a patchy distribution. The implications of this discovery are discussed, and it is suggested that marine debris might provide substrate for benthic species on otherwise unsuitable habitats, with its possible role as stepping-stones for deep-sea benthic connectivity needing further study.

Marine debris poses one of the main anthropogenic impacts with surging interest within both governmental and scientific communities (Ryan, 2015) due to its negative impacts on marine environments (e.g. Miyake et al., 2011; Ryan, 2015; Oliveira et al., 2015; Mecho et al., 2020). In this sense, while the real impact of marine litter is still difficult to quantify, it is, at best, underestimated (Ryan, 2015). Marine debris represents a major concern, not just for marine ecosystems, but for hundreds of marine species which are known to be directly or indirectly affected by it (Kühn et al., 2015). While the most visual example of such impacts might be that of the entanglement and/or consumption of marine litter by mammals, turtles and marine birds (Kühn et al., 2015), which ultimately leads to their death, marine litter generates a myriad of pernicious indirect effects on marine fauna (Sheavly and Register, 2007). Beneath the sea surface, marine litter accumulates on the sea floor, even making its way down to bathyal and abyssal environments (Miyake et al., 2011; Cau et al., 2018), where the accumulation of debris can smother marine organisms and facilitate hypoxic conditions on its habitats (Gregory, 2009). This phenomenon is particularly prevalent in submarine canyons as, due to their geomorphology, they transport and act as deposits for marine litter (Schlining et al., 2013; Pham et al., 2014; Oliveira et al., 2015; Pierdomenico et al., 2019).

Additionally, marine litter is known to act as substrata for several sessile benthic taxa (Carter and Gregory, 2005; Gregory, 2009) and, in the case of buoyant marine debris, it is known to act as vectors for the dispersal of organisms, including the spread of potential invasive species (Aliani and Molcard, 2003; Gregory, 2009; Suaria and Aliani, 2014; Kiessling et al., 2015). This phenomenon has been widely reported in surface-floating debris, yet it is still seldom reported for deep-sea habitats (Carter and Gregory, 2005), and mostly associated with large artificial structures (Glover and Smith, 2003) and, to a lesser extent, derelict fishing gear (Battaglia et al., 2019).

In the present paper, we report, for the first time, evidence of colonization of marine debris by the protected sponge *Lycopodina hypogea* (Vacelet and Boury-Esnault, 1996). This species is perhaps one of the most well-known sponges, as its first findings from a cave in Mediterranean French waters led to the discovery of the so-called “carnivorous sponges” (Vacelet and Boury-Esnault, 1995, 1996). As such, the species is considered emblematic of the Atlantic-Mediterranean waters (Chevaldonné et al., 2015), and it is protected under the Barcelona Convention (UNEP/MAP-SPA/RAC, 2018).

Samples were collected during the “ABRIC-1” cruise, from 13 to 29 February 2020, on board the R/V *Sarmiento de Gamboa*, using the ROV (Remotely Operated Vehicle) *Liropus 2000*. The main goal of this cruise

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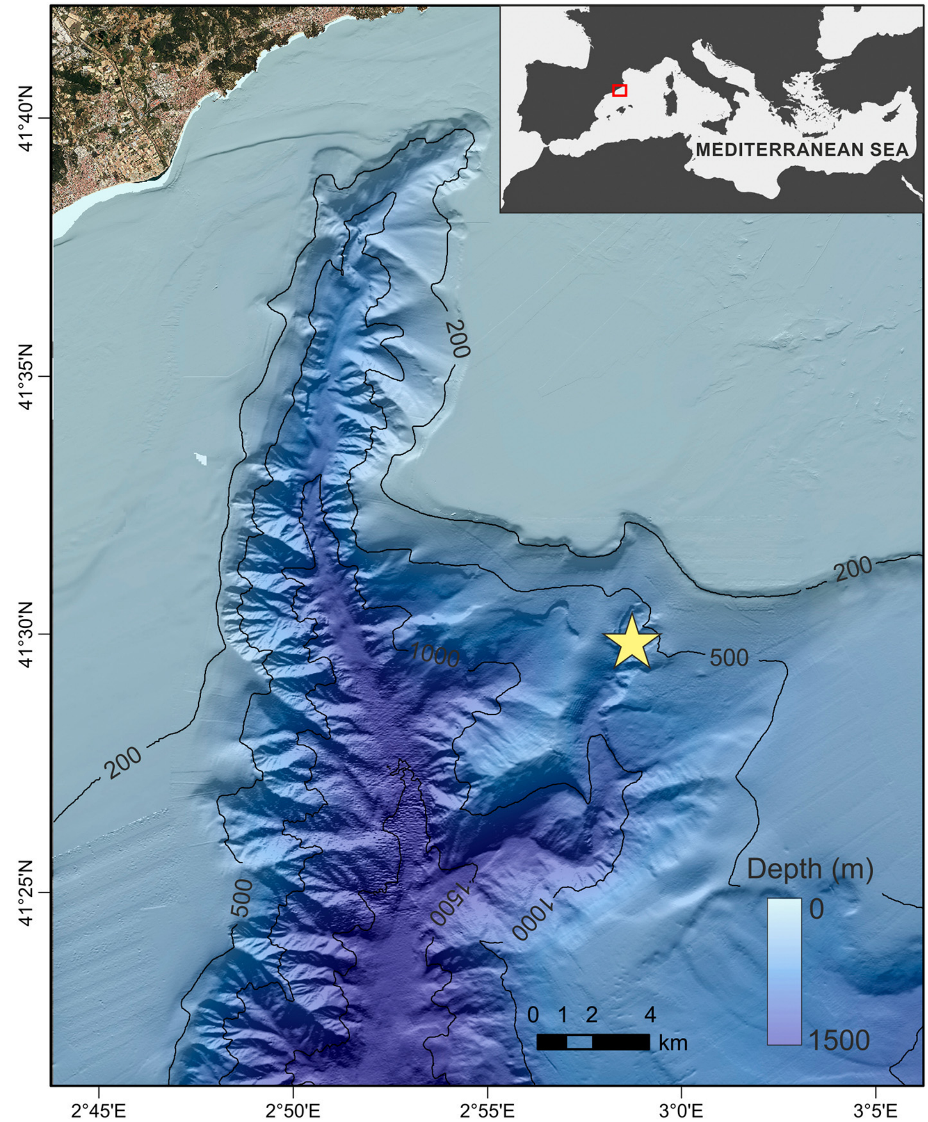

Fig. 1. Map showing the location where the nylon and PSB plastic rope was encountered (Eastern Blanes canyon, north-western Mediterranean Sea). Projected view (UTM Zone 31N (WGS84)) with geographic (WGS84) coordinates indicated for reference.

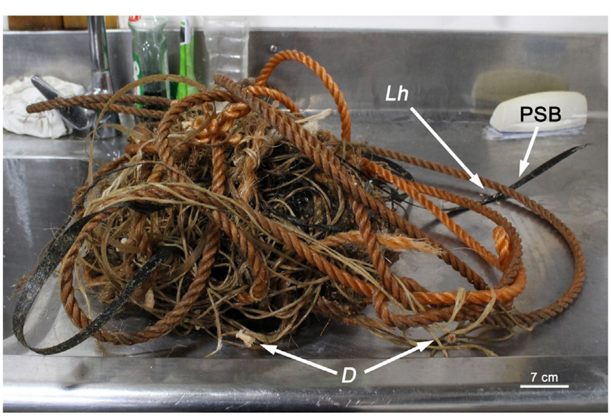

Fig. 2. On board general picture of the nylon ropes captured by the ROV upon collection, with several black PSB plastic debirs (PSB) entangled to it. The white arrow points *Lycopodina hypogea* individuals (*Lh*) observed growing on the plastic debris. Additionally, individuals of *Desmophyllum dianthus* (Esper, 1794) (*D*) can also be observed growing onto the nylon ropes.
Picture by ABRIC-1 cruise team.

was to explore and characterize deep-sea benthic habitats, between 600 and 1200 m depth, in the Blanes submarine canyon. Study sites were chosen based on the seafloor morphology, targeting canyon wall areas that could host habitat-forming anthozoans such as frame-work building scleractinians, gorgonians and antipatharians. During the exploration of a *Leiopathes glaberrima* (Esper, 1788) assemblage in the eastern canyon flank (41° 29,846′ N; 2° 58,710′ E; Fig. 1), at approximately 700 m depth, the ROV got entangled in a rope, which could have been part of derelict fishing gear. After performing several releasing maneuvers, the rope snapped and several meters remained attached to the ROV. On board, the rope was recovered and carefully examined, and was noted to be mostly composed of fragments of nylon rope and polyethylene terephthalate strapping band (PSB) (Fig. 2). On a fragment of PSB of ca. 10 cm^2 (10 cm length and 1 cm width) 31 stipitated sponges were observed attached to it, which were identified in situ as *L. hypogea* (see pointing arrow on Fig. 2). While no other *L. hypogea* individuals could be observed on the initial onboard examination of the rope, the possibility of isolated individuals occurring on the parts of the PSB plastic band or the rope cannot be ruled out. After their detection, the PSB with the attached sponges was separated from the rest and placed inside an aquarium with closed circulation at 12 °C and, posteriorly, transferred to the experimental aquarium zone at the Institut

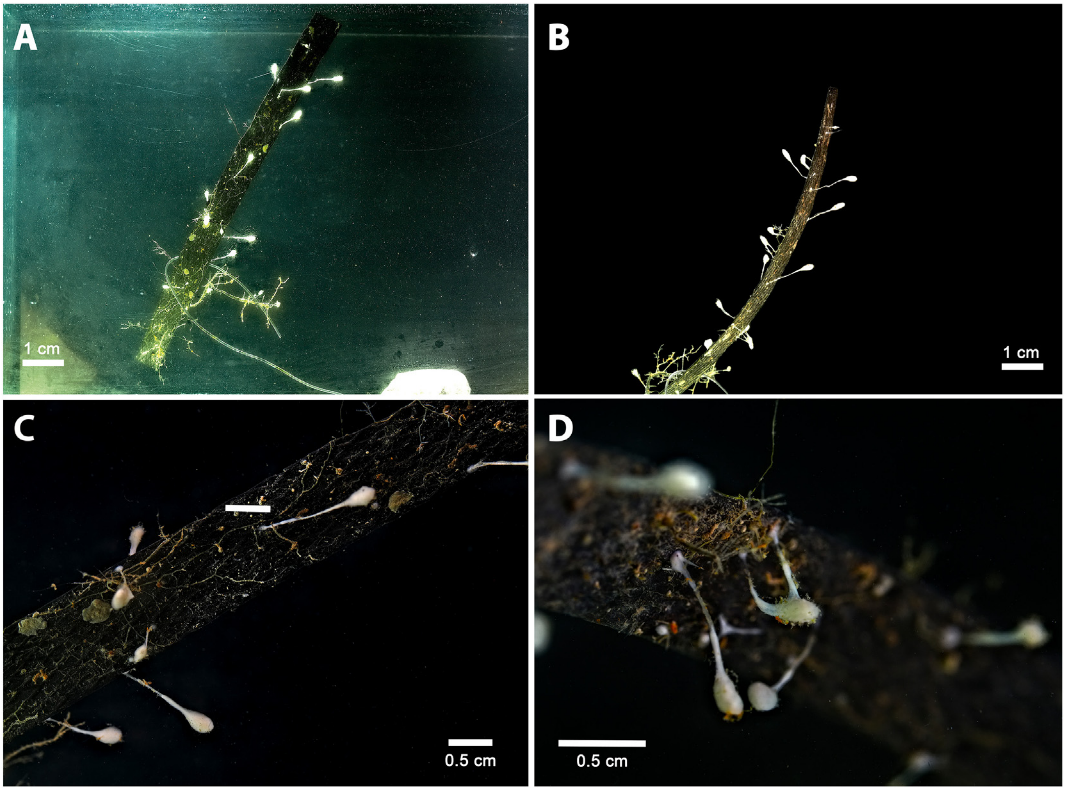

Fig. 3. A) General picture of the PSB plastic debris with the attached *L. hypogea* in the aquaria; the plastic was to be held to counter its buoyancy. B and C) Close up of the plastic debris; *L. hypogea* individuals of different sizes can be seen growing on both sides of the plastic, alongside bryozoans and hydroids D) Two individuals of *L. hypogea* fused at their heads.
Pictures by Ruben Duró (ruben@scienceintoimages.com).

de Ciències del Mar (ICM-CSIC) in Barcelona (Fig. 3). Additionally, the size of all individuals was recorded, and the demography of the PSB plastic debris' population was estimated by means of size-frequency distribution histograms and skewness and kurtosis tests as in Santín et al. (2019). Two of the sponge individuals were used for spicule preparations to certify the identification, following the standard procedures (Cristobo et al., 1993; all spicule measures are given as MIN. – MEAN ± SD – MAX.) and are described below:

Order Poecilosclerida Topsent, 1928

Family Cladorhizidae Dendy, 1922

Lycopodina hypogea (Vacelet and Boury-Esnault, 1996)

Description: Small, white, stipitate sponge, with a basal attachment area, followed by a long, thin stem made of styles, ending in a hirsute, drop-like body (Figs. 3 & 4). Most individuals did not show clear hunting filaments.

Skeleton: The stem is a dense, parallel-arranged, axis of styles, which progressively branches into tracts once inside the main body. Around these tracts there are also styles, arranged in a confused or unorganized pattern. Anisochelae are very abundant, located on the hunting filaments.

Spicules: Megascleres: styles to subtylostyles with a subtle tyle ('mycalostyles' like; Fig. 5B) or fully-formed tylostyles, mostly straight or slight flexuous (Fig. 5A). While there were no clearly distinguishable categories, the biggest ones were found in the stalk. Size 410–567.8 ± 75–930 × 3–4.7 ± 0.8–6 µm. Microscleres: characteristic palmate anisochelae (Fig. 5C), small and strongly bent, with prominent alae on its biggest ends, and greatly reduced, teeth-like alae on the other. Size 11.5–*13.7* ± 2.1–16.5 µm. Forceps were not found.

A few decades ago, the chance encounter of an unknown cave sponge without a proper filtration system resulted in paradigm-changing phenomena, crystalizing in the discovery of carnivorous sponges (Vacelet and Boury-Esnault, 1995). While sponges from the Cladorhizidae family had been known for centuries (Dendy, 1922), *L. hypogea* (mostly reported as *Asbestopluma hypogea*) was the first species where such a unique feeding habitus could be observed, becoming a model organism for the study of carnivorous sponges (e.g. Vacelet and Duport, 2004; Martinand-Mari et al., 2012; Dupont et al., 2014; Rastorgueff et al., 2015), and attracting the attention of both the scientific community and the general public towards this unique group, ultimately leading to its declaration as a protected species by the Barcelona Convention (UNEP/MAP-SPA/RAC, 2018).

While originally discovered in cave environments, the species was soon theorized to occur in deep-sea habitats (Vacelet, 1996) due to the shared similarities between both environments (Harmelin and Vacelet, 1997). Since then, several new sightings of the sponge have been reported in both littoral caves (Bakran-Petricioli et al., 2007) and deep-sea environments (Aguilar et al., 2011; Calcinai et al., 2013; Chevaldonné et al., 2015; Fourt et al., 2017; Sitjà et al., 2019), including the NE Atlantic (Chevaldonné et al., 2015). Nevertheless, and despite the intensive exploration of the deep-sea environments over the past decades, the species' sightings are still scarce, and it presents a rather patchy and disjunct distribution, where it very rarely occurs in great numbers (Chevaldonné et al., 2015).

While discovering the origin of organisms growing on artificial substrates might not be always possible (Goldstein et al., 2014), nearby known populations have been theorized as the most likely origin, as stated for deep-sea corals growing onto abandoned, lost or otherwise discarded fishing gear in the deep-waters of the Strait of Sicily (Battaglia et al., 2019). In this regard, the presence of *L. hypogea* is known from recently discovered Cold Water Corals (CWC) at the Blanes canyon head (Santín et al., 2018), which might be the mostly likely source population for the PSB plastic debris population presented in this study. While a lot of attention has been paid to the role of drifting litter as a potential dispersal tool for non-native species (Gregory, 2009;

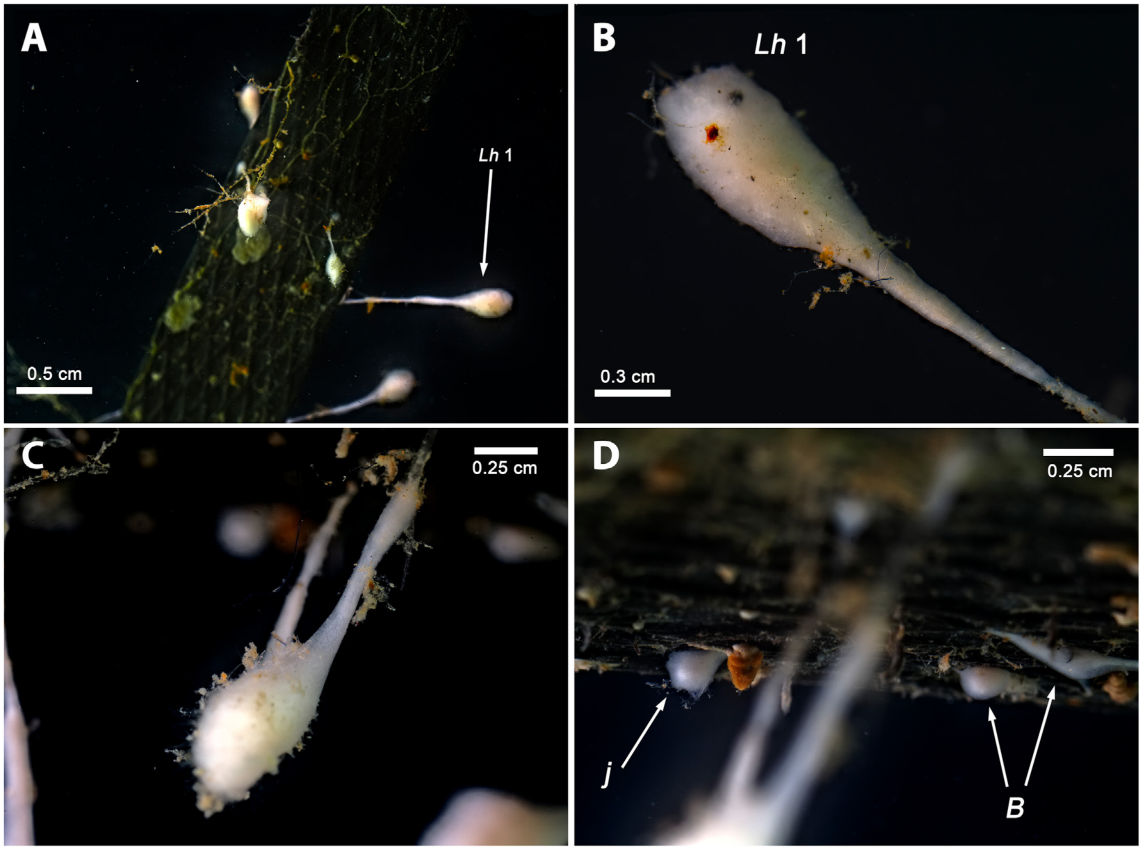

Fig. 4. A) Several *L. hypogea* individuals of different sizes, alongside bryozoans and hydroids. B) Close up of an *L. hypogea* individual (marked as *Lh* 1 in Fig. 2A). The body is fused in a single mass and hunting filaments cannot be distinguished. C) Two individuals of *L. hypogea* fused together, as previously reported in north Atlantic populations (Chevaldonné et al., 2015). D) *L. hypogea* buds (B) with a button-like appearance and still completely attached to the substrate, and a juvenile (j), which has already developed a roundish body separated from the plastic by a short stalk.
Pictures by Ruben Duró (ruben@scienceintoimages.com).

Aliani and Molcard, 2003; Suaria and Aliani, 2014; Kiessling et al., 2015), little attention has been paid to its role in the connectivity of non-invasive species (Barnes and Milner, 2005; Goldstein et al., 2014; Kiessling et al., 2015), especially in deep-sea environments (Carter and Gregory, 2005). So far, oil platforms are the most iconic anthropogenic deep-sea structures providing substrate for benthic taxa (van Elden et al., 2019; Sommer et al., 2019; Birchenough and Degraer, 2020), even in otherwise unfavorable environments (Friedlander et al., 2014), and acting as stepping stones for organisms' dispersal and connectivity between populations (Sammarco et al., 2012; Adams et al., 2014; van der Molen et al., 2018). In this sense, sponge larvae are generally seen as poor swimmers (Lanna and Riesgo, 2020) with little dispersal capabilities (Uriz et al., 2008), even at a local scale (Uriz et al., 1998). While the PSB plastic debris upon which the sponges were attached was stationary at the time, entangled ropes and fishing lines are known to break periodically due to wearing, which might set free any buoyant pieces (Battaglia et al., 2019). Marine litter is known to be persistent in time and, if buoyant, it can be transported over great distances (Kiessling et al., 2015), ultimately acting as a major dispersal vector for both invasive and non-invasive species hitchhiking on surface-floating litter (Aliani and Molcard, 2003; Barnes and Milner, 2005; Gregory, 2009; Goldstein et al., 2014). Nevertheless, it is also worth noting that non-buoyant marine litter is also subjected to transport processes, especially in canyons (Schlining et al., 2013; Oliveira et al., 2015), where sedimentary transport processes and intense bottom currents carry marine litter across the deep-sea floor (Schlining et al., 2013; Pham et al., 2014; Oliveira et al., 2015; Pierdomenico et al., 2019). As such, while the potential effect of buoyant debris on benthic organisms' connectivity might be more visible, especially in shallow areas (Gregory, 2009; Kiessling et al., 2015), in deep-sea ecosystems, and particularly canyons, both buoyant and non-buoyant debris could have a similar potential undisclosed role in the connectivity and dispersion of deep-sea benthic organisms with patchy distributions, as is the case for *L. hypogea*.

In this regard, while mostly acknowledged as pernicious for benthic organisms (Glover and Smith, 2003; Ryan, 2015), marine litter has also been observed to act as a substrate for benthic taxa (Pace et al., 2007; Gregory, 2009; Oliveira et al., 2015) with the potential to, paradoxically, increase biodiversity in impacted areas due to an increase in habitat heterogeneity (Katsanevakis et al., 2007; Pace et al., 2007). Moreover, some taxa larvae have been shown to preferentially settle on anthropogenic substrate rather than natural ones, which could translate into an enlargement of the distribution area for said species (Carter and Gregory, 2005; Holst and Jarms, 2007). As such, the unexpected high density of *L. hypogea* (ca. 3.1 ind./cm^2) reported here attached to PSB plastic debris could imply that marine litter might be a favorable substrate for their settlement and survival in certain conditions. Nevertheless, such a role would be highly dependent on the ability of a given taxa to sustain itself in an otherwise hostile, nutrient-less substrata (Kiessling et al., 2015). In this study, 31 individuals of *L. hypogea* could be observed attached to rather limited-size substratum (ca. 10 cm^2). This is noteworthy, as the species usually has low densities, even in stable populations (ca. 0.04 ind./cm^2; Chevaldonné et al., 2015), with only one other report of a massive occurrence of *L. hypogea* at a small scale, with 71 individuals associated with coral rubble (35 cm^2) in the Gulf of Cadiz (Sitjà et al., 2019). Additionally, the size-structure of the population was non-skewed and well-distributed, being dominated by medium-sized individuals (1–2 cm; Fig. 6), which corresponds with

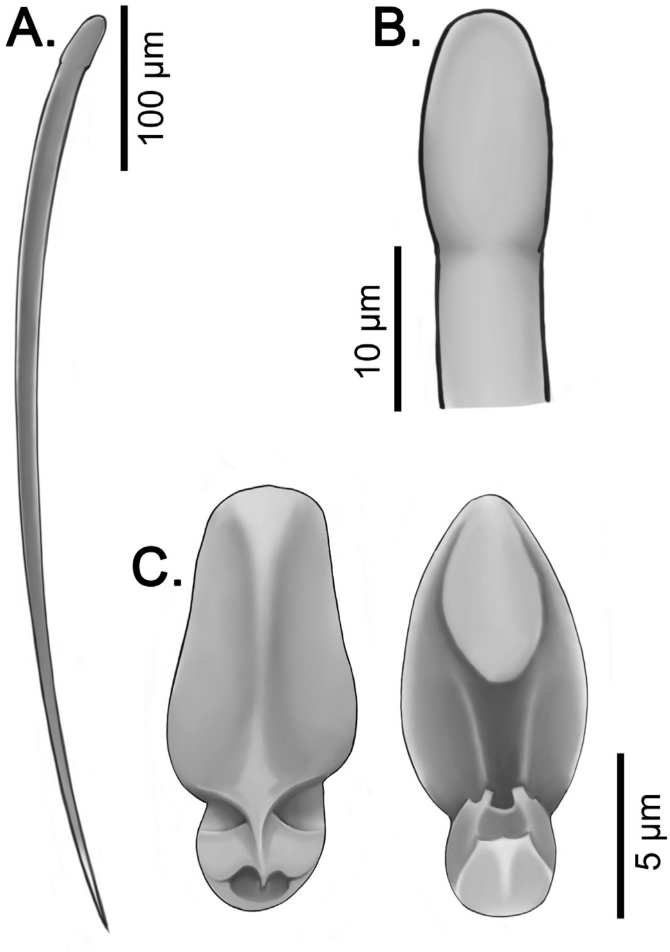

Fig. 5. Schematic representation of *L. hypogea* spicule set. A) Style B) Detail of the tyle of the subtylostyles; C) Anisochelae.
Schemes were digitally produced by C. Traboni (traboni@icm.csic.es).

well-established populations. Furthermore, both buds and 'juvenile' (ca. 0.5 cm) sponges could be identified (Fig. 4D), suggesting the population could be reproductive. As such, the high density of *L. hypogea* recorded here could respond to an initial stochastic colonization, with just a few or several larvae settling on the PSB plastic strand, with a posterior sexual or asexual reproduction, which would result in the observed size-structure distribution. Nevertheless, the colonization of litter by benthic fauna seems to be subjected to a myriad of factors rather than random processes, ranging from the species' own biology to their behavior in relation to space and litter quality (Walker, 1994). Furthermore, as marine debris provide no nutrient source, the ability for food acquisition is one of the key elements in determining the successful colonization on marine debris (Kiessling et al., 2015). In this sense, the nylon rope, suspended approximately 20 m above the sea floor, might have provided carnivorous sponges with access to water masses exposed to stronger currents, similarly to the role provided by Cold Water Corals and other ecosystem engineers (Roberts et al., 2009). In this scenario, access to new food sources could have boosted *L. hypogea*'s population settlement and growth on the PSB plastic debris. Interestingly, upon collection, all individuals presented roundish bodies (Figs. 3 & 4), which has been observed as a symptom of senescence and an indicator of starvation (Chevaldonné et al., 2015). The most likely explanations for this might be possible adverse environmental conditions surrounding the nylon rope at the time or stress upon recovery, yet no conclusions can be made. Nevertheless, after two weeks in aquaria the sponges reverted back to their normal shape, with clearly visible hunting filaments.

In conclusion, the indirect effects of marine debris on benthic communities is of major concern for marine conservation policies, yet we still have a poor understanding of these effects, especially in deep-sea environments. In this regard, marine debris is reported here as a viable habitat for deep-sea fauna, particularly for carnivorous sponges, indicating that these organisms might have a higher environmental plasticity than originally thought. Finally, it is theorized that derelict fishing gear and other anthropogenic debris might have, to a certain extent, a yet undisclosed role for deep-sea organisms' dispersion and connectivity, the magnitude of which is currently unknown.

CRediT authorship contribution statement

A. Santín: Conceptualization, Formal analysis, Writing - original

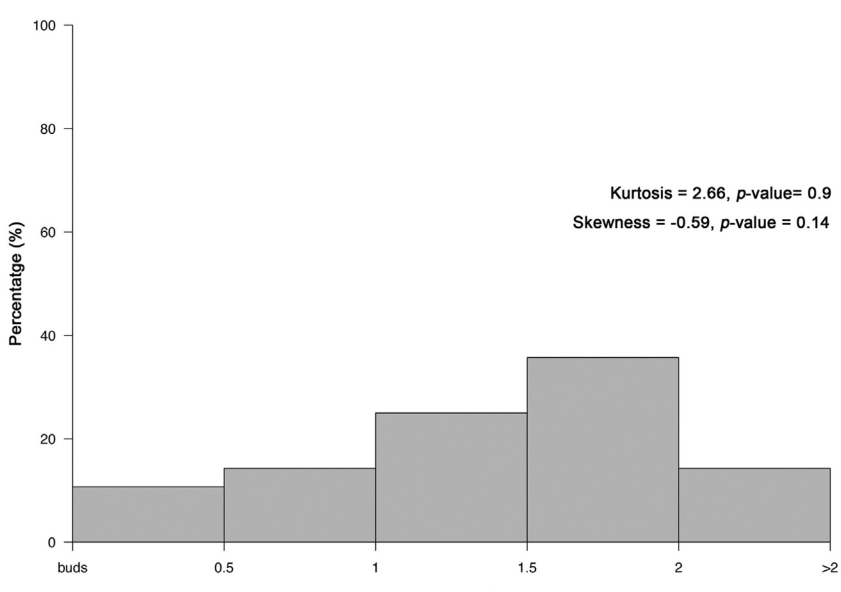

Fig. 6. Size-structure distribution for the *L. hypogea* individuals growing onto the PSB plastic debris.

draft, Writing - review & editing, Visualization.**J. Grinyó**:Resources, Writing - original draft, Writing - review & editing, Supervision.**M. Bilan**:Resources, Writing - review & editing.**S. Ambroso**:Resources, Writing - review & editing, Visualization.**P. Puig**:Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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