

# Anti-predatory chemical defences in Antarctic benthic fauna

Juan Moles<sup>1</sup> · Laura Núñez-Pons<sup>1,2</sup> · Sergi Taboada<sup>1</sup> · Blanca Figuerola<sup>1</sup> ·  
Javier Cristobo<sup>3</sup> · Conxita Avila<sup>1</sup>

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**Abstract** Antarctic benthic communities are largely structured by predation, which leads to the development of mechanisms of repellence. Among those mechanisms, chemical defences are quite extensive, yet poorly understood. To increase knowledge about the role of chemical defences in the Southern Ocean ecosystems, we assessed the incidence of feeding repellents in sessile and vagile invertebrates from nine phyla: Porifera, Cnidaria, Nemertea, Annelida, Mollusca, Bryozoa, Echinodermata, Hemichordata, and Tunicata (Asciacea). Samples were collected at depths of 120–789 m in the eastern Weddell Sea and Bouvet Island, and at depths ranging 0–100 m in the South Shetland Islands. When possible, specimens were dissected to study anatomical allocation of repellents. The common, eurybathic sea star *Odontaster validus* was chosen to perform feeding repellence bioassays, using

diethyl ether (lipophilic) and butanol (hydrophilic) extracts from these samples. Among the 75 species tested, 52 % were studied for the first time for anti-predatory properties. Results provide further evidence of the prevalence of defensive metabolites in Antarctic organisms, with 47 % of the species exhibiting significant repellence within their lipophilic extracts. They also suggest a wider use of non-polar defensive chemicals. Sessile taxa displayed highest repellence activities, with ascidians, cnidarians, and sponges being the most chemically protected. Overall, the present study indicates that natural products by mediating trophic interactions between prey and their potential predators play an important role in structuring Antarctic benthic ecosystems.

## Introduction

Antarctic benthos comprises some of the most ancient and stable marine ecosystems worldwide. There, diverse communities of sessile suspension feeders are mainly structured by biotic interactions (Dayton et al. 1974, 1994; Arntz et al. 1994). The Southern Ocean fauna is characterized by the poor presence of fish and decapods as either competitors or predators (Clarke et al. 2004; Gili et al. 2006). Echinoderms are the dominant vagile megafaunal organisms in terms of abundance and diversity (Dayton et al. 1974; Clarke et al. 2004), with many species representing important generalist predators. Hence many organisms have developed mechanisms to deter them (see Avila et al. 2008; Taboada et al. 2013).

Marine natural products (NPs), mostly secondary metabolites, often regulate the biology, coexistence, and coevolution of species without participating directly in their primary metabolism (i.e. growth, development, and reproduction; see Torssell 1983). Although the descriptive

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✉ Juan Moles  
moles.sanchez@gmail.com

<sup>1</sup> Department of Animal Biology (Invertebrates) and Biodiversity Research Institute (IrBIO), Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

<sup>2</sup> Hawai'i Institute of Marine Biology, University of Hawaii, 46-007 Lilipuna Rd., Kaneohe, HI 96744, USA

<sup>3</sup> Spanish Institute of Oceanography (IEO), Oceanographic Centre of Gijón, Av. Príncipe de Asturias 70 bis, 33212 Gijón, Spain

chemical studies on novel NPs from marine invertebrates are constantly growing in number, the ecological functions of NPs have received less attention (see Lebar et al. 2007; Avila et al. 2008; McClintock et al. 2010; Núñez-Pons and Avila 2015). In general, NPs often play important roles in predator–prey interactions, symbioses, competition, reproduction, and larval settlement (Amsler et al. 2001a; Puglisi et al. 2014). Among the ecological functions of these compounds, anti-predatory properties have raised more interest. In particular, several studies in McMurdo Sound (Ross Sea), the western Antarctic Peninsula (see McClintock and Baker 1997; Amsler et al. 2001b; Avila et al. 2008; McClintock et al. 2010; Amsler et al. 2014), and the eastern Weddell Sea and Bouvet Island (e.g. Davies-Coleman 2006; Taboada et al. 2013; Figuerola et al. 2013a; Núñez-Pons and Avila 2014b) indicate that anti-predatory chemical defences are widespread among Antarctic species.

According to the optimal defence theory (ODT), NPs should be allocated effectively in most vulnerable or valuable structures, thus compensating the energetic requirements for growth, reproduction, and defence (Rhoades 1979). The concentration and body allocation of NPs among and within individuals may vary with life history, season, and ecological interactions (López-Legentil et al. 2005; Loh and Pawlik 2014). Asteroids, which generally consume the surface of prey with their eversible cardiac stomach (Hyman 1955), may have driven the evolution of differential allocation of defences to the most exposed tissues in potential prey organisms (e.g. Furrow et al. 2003; Fairhead et al. 2005; Peters et al. 2009). Among Antarctic sea stars, *Odontaster validus* Koehler, 1906, is considered a model predator for repellence assays because of its generalist and opportunistic feeding habits, and for being commonly found throughout Antarctic seafloors (McClintock et al. 1990; Avila et al. 2000; Taboada et al. 2013). *Odontaster validus* is present in the Antarctic and sub-Antarctic at depths of 0–2900 m (Dearborn 1977; Moles et al. 2015). Furthermore, *O. validus* is the most abundant sea star on the shallow Antarctic shelf, where it exerts considerable predatory pressure on benthic assemblages (Dayton et al. 1994). As a mobile, generalist, and opportunistic predator, *O. validus* consumes a wide array of organisms, including bryozoans, cnidarians, crustaceans, diatoms, echinoderms, molluscs, and sponges (Dayton et al. 1974; Dearborn 1977; McClintock 1994).

In the present study, we evaluated the incidence of chemical defences in sessile and vagile benthic invertebrates from nine phyla: Porifera, Cnidaria, Nemertea, Annelida, Mollusca, Bryozoa, Echinodermata, Hemichordata, and Tunicata (Ascidiacea). Organisms were collected across a wide bathymetric range in three regions: (1) the eastern Weddell Sea, (2) Bouvet Island, and (3) the South Shetland Islands. Extracts of lipophilic and hydrophilic nature were

used in no-choice feeding repellence experiments against the sea star *O. validus*, following previous methodologies (e.g. Avila et al. 2000; Núñez-Pons et al. 2012a; Taboada et al. 2013; Figuerola et al. 2013a). More than half of the organisms were tested in repellence bioassays for the first time. We discuss differences in chemical defences among species tested in previous studies, contrasting different assay methodologies and sample localities. When species came from different geographic and bathymetric regions, they were compared for possible intraspecific variability in chemical protection. Several samples were also dissected anatomically in order to examine strategic allocation of defensive NPs. The use of extracts of different polarity allowed us to test the hypothesis that nonpolar (lipophilic) compounds dominate as anti-predatory defences.

## Materials and methods

### Sample collection and identification

Invertebrates were collected from Neumayer to Drescher Inlet in the eastern Weddell Sea (Antarctica) and the vicinities of Bouvet Island (sub-Antarctica) during the ANT XV/3 (February 1998) and ANT XXI/2 (November 2003–January 2004) cruises on board the R/V *Polarstern* (AWI, Bremerhaven, Germany). A total of 29 stations were surveyed at depths of 120–789 m with Agassiz trawl, bottom trawl, epibenthic sledge, and Rauschert dredge (Supplementary Table S1). During the ACTIQUIM-1 campaign (2007–2008), invertebrates from Deception, Livingston, and Snow Islands (South Shetland Islands, Antarctica) were sampled with Agassiz trawl, rock dredge, and scuba diving at 11 stations at depths of 0–100 m (Supplementary Table S1). Samples were sorted on deck, and sessile and sluggish organisms were selected, photographed, and frozen to  $-20^{\circ}\text{C}$ . A voucher specimen or a portion of each sample was fixed in 10 % formalin or in 70 % EtOH for taxonomical determination. Vouchers are deposited at the Department of Animal Biology (Invertebrates), University of Barcelona, Spain.

### Chemical extraction

When necessary, individuals of the same species and station were pooled and extracted together as a single sample to represent the average population in that location, and to obtain enough extract for the experiments. When possible, organisms were dissected for allocation of defensive chemicals to specific body parts. The resulting dissected structures were then processed separately. According to the taxonomic group, organisms were divided into: mantle, foot, and viscera, in molluscs; oral and aboral in asteroids;

pharyngeal and visceral tissues, and external tissues or tunic, in ascidians; and external (cortex)/internal (endosome), and apical/basal regions, in sponges (for a complete list of body parts, see Supplementary Table S2 and S3). Chemical extractions were carried out separately with acetone, which was subsequently evaporated *in vacuo*. The remaining aqueous residue was sequentially partitioned into diethyl ether (EE) and *n*-butanol (BE) extracts. EE yields the most nonpolar (lipophilic) molecules, while BE retains more polar (hydrophilic) compounds. All steps were repeated thrice, except for the butanol extraction, which was done once. Organic solvents were evaporated at low pressure, yielding dry EE and BE, and aqueous residues (see Supplementary Table S2 and S3). Thin-layer chromatography (TLC) screenings were carried out to provide a general profile of the chemical composition of the extracts obtained and to check for the prevalence of the extracts in the assay food items during the assays (see below). Aliquots of lipophilic and hydrophilic extracts were used at their natural concentrations in the feeding repellence tests (see below).

### Feeding repellence tests

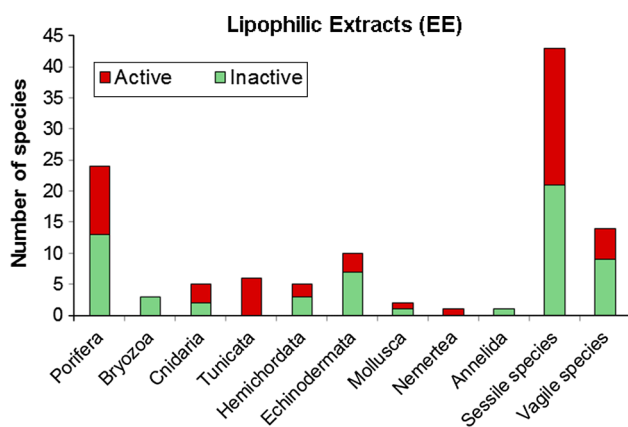
Specimens of *O. validus* (approx.  $n = 1800$ ) with a diameter of 4.5–10.5 cm were collected by scuba diving at depths from 3 to 15 m in Deception Island (Whalers Bay; 62°59.37'S, 60°33.42'W) during the ACTIQUIM-1–4 campaigns (January and February of 2008–2009, 2009–2010, 2011–2012, and 2012–2013). *Odontaster validus* exhibits large population connectivity over considerable geographic and bathymetric distances (Janosik et al. 2011), which suits its usage for testing samples from distant locations throughout circumpolar regions. After collection, sea stars were maintained in large (~1000 L) tanks with running sea water pumped directly from Deception Island's bay, in the laboratory at the "Gabriel de Castilla" Spanish Antarctic Base. Sea stars were acclimated and starved for 5 days before being used in the feeding assays.

The methodology used for the assays is described in previous studies (e.g. Avila et al. 2000). Briefly, extracts of invertebrate tissues previously diluted in diethyl ether (EE) or methanol (BE) were incorporated into wet shrimp-based cubes, hereafter termed "food cubes", at the corresponding natural concentration. The solvent was subsequently left to evaporate under a fume hood. Food cubes had an approximate volume of 0.5 cm<sup>3</sup>,  $68.92 \pm 11.01$  mg (mean  $\pm$  SD) wet weight, and  $13.09 \pm 3.43$  mg dry weight. EE or BE was added uniformly to the feeding cubes during preparation. Natural concentrations of extracts were calculated referring EE or BE weight to the total dry weight (DW<sub>T</sub>) of each sample (DW<sub>T</sub> = EE weight + BE weight + DW of the

solid residue). The choice for using dry weight is based on the fact that it eliminates the water contents, which in aquatic samples can fluctuate considerably producing significant bias in the reference values for the calculations. To discard solvent effects on food preference, daily controls (using feeding items only treated with solvent) were run simultaneously to each set of experimental treatment assays (i.e. those performed with food cubes containing extracts). Randomly selected sea stars were placed individually in the centre of 2.5-L buckets, and food cubes were deposited under the oral opening of each individual. Treatment and control assays were replicated ten times, each with a single sea star and a food cube placed in separate containers filled with sea water. After 24 h, repellent activities of the extracts were evaluated by counting the number of ingested and leftover (not consumed) feeding cubes. A food cube was considered eaten when more than 75 % of it was consumed. Sea stars that did not ingest any food cube were reused for tests after 5 days of starvation. Sea stars that ingested the food cubes were maintained in large tanks with running sea water and brought back into Whalers Bay after the assays were finished. Non-ingested food cubes treated with extracts were frozen (−20 °C) after the experiments, later extracted, and subjected to TLC screenings to confirm that the chemical profile from the extracts remained unchanged after the assays. The low temperature (1–2 °C) at which the assays were performed minimized extract degradation and diffusion into the water column. Statistics were calculated by contrasting the difference in ingestion rates between each set of treatment tests referred to the controls run simultaneously by applying the Fisher's exact test (Sokal and Rohlf 1995).

### Results

A total of 120 EE and 40 BE were tested against the sea star *O. validus*, comprising 75 species of invertebrates from nine phyla. A higher percentage of repellent activity was found in EE (27 out of 57 species, 47.4 %) than BE (three out of 27 species; 11 %). Within the EE tested, the highest repellent activity was found in sessile invertebrates (51 %; Fig. 1), among which ascidians recorded six repellent species (100 % of anti-predatory; Fig. 2). These were followed by cnidarians with two repellent species (60 %), sponges with 11 species (46 %) being also the best represented phylum (24 species), and hemichordates with two species (40 %); bryozoans showed no repellence (three species tested). Among the vagile species, 36 % showed repellence activity: echinoderms yielded three repellent species (30 %), followed by one species of nemertean and one mollusc out of two species tested. The only species of annelid assessed did not exhibit repellence (Fig. 2). By contrast,



**Fig. 1** Number of repellent and nonrepellent species tested for each phylum as well as for all sessile and vagile fauna. Only lipophilic extracts (EE) were considered

BE showed repellence in only one species of sponge, one mollusc, and one pterobranch hemichordate (Fig. 3).

## Discussion

Our study increases knowledge about anti-predatory defences in shallow- and deep-water marine invertebrates from a well-studied region (South Shetland Is.) and two less studied areas (Bouvet Is. and the Weddell Sea). Among the 75 species tested, 39 (52 %) were studied for the first time in repellence bioassays. Representatives of nearly all studied phyla (47 % of total species tested) showed feeding repellence against the predatory sea star *O. validus*. Our results reflect a conspicuous presence of chemical defences in many representative groups of the Antarctic benthic biota, in accordance with the few studies performed in similar areas using the same methodology (Núñez-Pons et al. 2010; Taboada et al. 2013). However, anti-predatory activity in nonrepellent species should be further assessed using different assays and predators to confirm the absence of repellence in these species (e.g. Núñez-Pons et al. 2012c). The high nutritional quality of the food cubes might have underestimated repellence activity in the species with mid to low levels of NPs (Duffy and Paul 1992; Núñez-Pons and Avila 2014a). Most species protected against the key-stone predator *O. validus* were sessile, reflecting the extensive occurrence of NPs as mediators of trophic interactions among sessile Antarctic fauna. Mobile invertebrates, although showing feeding repellence in our study, might also rely on behavioural mechanisms (e.g. escaping, hiding) and/or physical defences (e.g. plates, shells) to avoid predation. Regarding the chemical nature of the defensive metabolites, anti-predatory activity was mainly located in the lipophilic, nonpolar extracts (EE). Several studies

(recently reviewed in, e.g. Sotka et al. 2009; Puglisi et al. 2014) showed that most of the marine anti-predatory compounds are lipophilic (nonpolar).

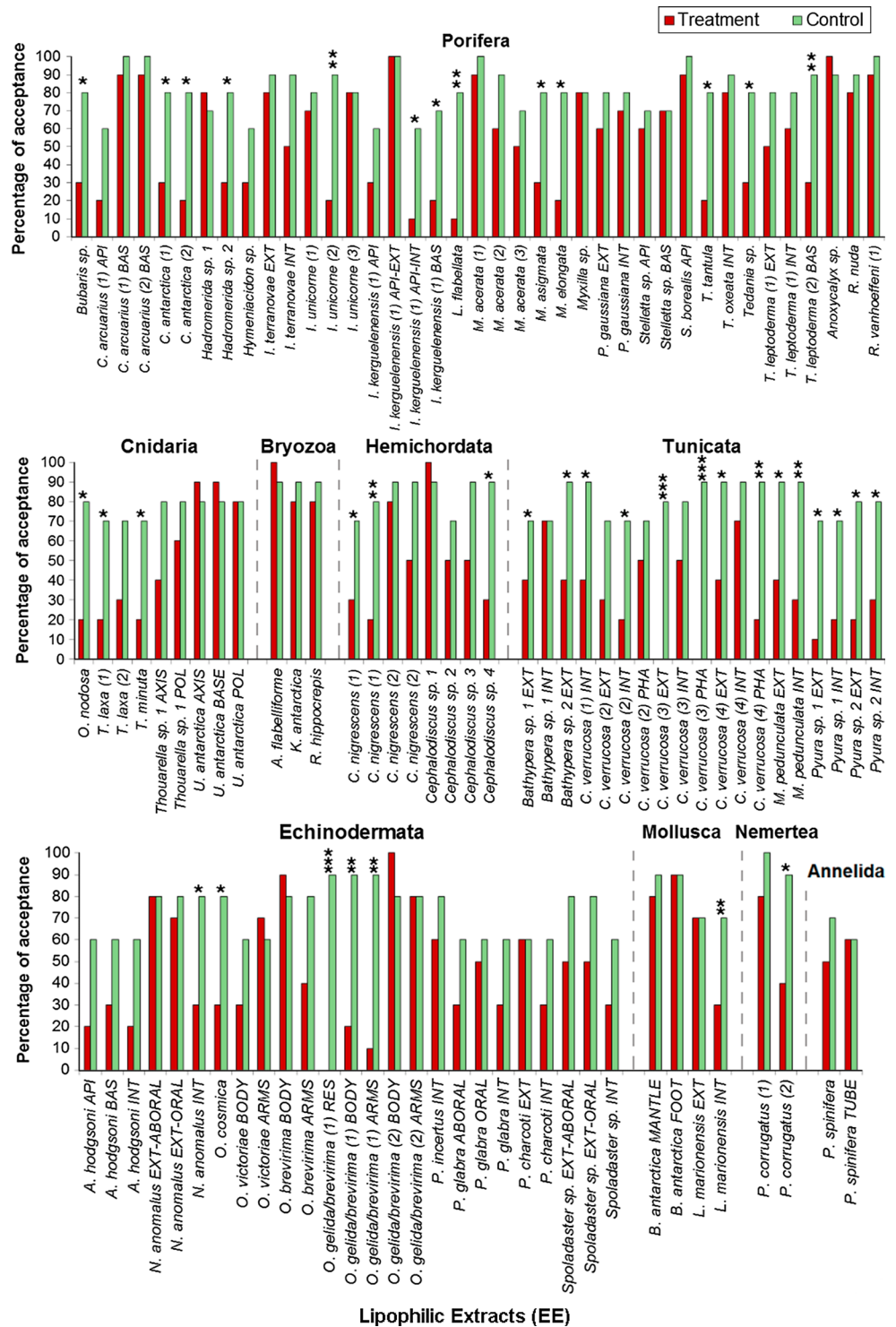
Our experiments provided evidence of variation in anti-predatory activity at two levels: among conspecifics collected from different areas and body parts in some of the species tested (see below). Different bioactivities and chemical patterns of NPs have been reported among populations of conspecific marine invertebrates worldwide (e.g. Cutignano et al. 2011; Pawlik 2012). Those variations may be attributed to differences in predation intensity (and in correlation with chemical defence-inductive processes, in which defences are produced in response to predator attacks), genetic variability among populations (providing different phenotypes/chemotypes), as well as developmental changes among individuals or shifts in microbial symbionts (when the producer organism is the endosymbiont; Bewley et al. 1996; López-Legentil et al. 2005; Loh and Pawlik 2014). Besides, anatomical dissections allowed estimating the allocation of chemical defences in some of the species suitable to such approach. Our results showed that chemical defences are often unevenly distributed within organisms. Thus, predictions of the ODT (Rhoades 1979) were supported at least in some cases, meaning that chemical defences seem to be located in those tissues that are most vulnerable and frequently more easily targeted by predators (Hines and Pawlik 2012; Figuerola et al. 2013a).

Porifera was the most represented phylum in our study, with repellence results comparable to those in previous studies testing specimens with similar geographic and bathymetric distributions (Taboada et al. 2013). Surveys using organisms from other geographic areas and shallower waters found higher chemical repellence against *O. validus* (McClintock et al. 1990; Peters et al. 2009). Those results suggest higher predatory pressure in shallower environments (McClintock et al. 1988), which may increase the production of chemical defences. However, it is important to note that different methodologies (assay types) were used to measure anti-predatory activities in such deep- and shallow-water surveys, and thus, this comparison should be contrasted with caution.

In the present study, geographic differences in sponge repellent properties were tested for five species from at least two different sampling stations. Previous studies reported that Ross Sea shallow-water specimens of *Calyx arcuarius* and *Mycale acerata* can induce tube-foot retraction in the spongivorous asteroid *Perknaster fuscus* (McClintock et al. 1993), while only water residues (the remaining molecules after the organic extractions) deterred *O. validus* (McClintock et al. 1990). In contrast, no repellence was found in *C. arcuarius* and *M. acerata* from deep bottoms of the Weddell Sea in the present study. This suggests that either different species of sea stars may have different capabilities



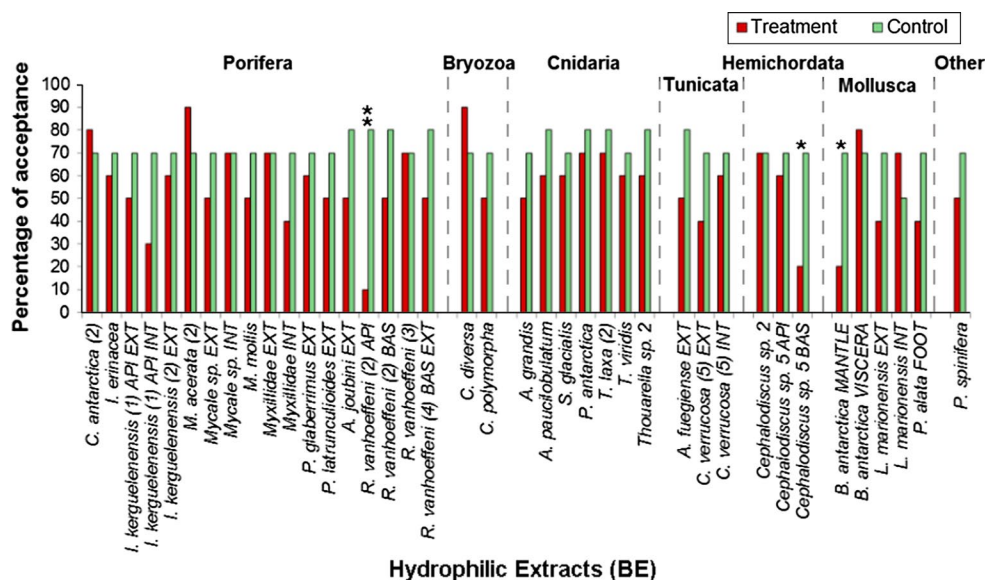
**Fig. 2** Results of the feeding deterrent tests using lipophilic extracts (EE). Percentage of acceptance for treated food cubes (*red*) versus the simultaneous control extract-free cubes (*green*). Dissected parts: *ABORAL* aboral part, *API* apical part, *ARMS* ophiuroid arms, *AXIS* central axis, *BAS* basal part, *BASE* base, *BODY* ophiuroid disc, *EXT* external part, *FOOT* foot, *INT* internal part, *MANTLE* mantle, *ORAL* oral part, *PHA* pharynx, *POL* polyparium, *TUBE* polychaete tube, and *RES* residual water. Statistical differences calculated using Fisher's exact test; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$



of perceiving and responding to sponge chemical defences, that different methodologies may produce different results, or that sponges possess different concentrations of NPs between distant locations. The repellent activities displayed by the two astrophorids *Cinachyra antarctica* and *Tetilla leptoderma* agreed with previous studies (McClintock et al. 1994, 2000), suggesting that the presence of

repellent metabolites in these species is conserved among populations. In contrast, different individuals of the epibiont sponge *Iophon unicorne* displayed different anti-predatory activity in our study. This species commonly lives encrusted on top of the ophiuroids *Ophioplathus brevirma* and *O. gelida* (Dearborn 1973), altering disc and arm plates, which makes the identification between both

**Fig. 3** Results of the feeding deterrent tests using hydrophilic extracts (BE). Percentage of acceptance for treated food cubes (*red*) versus the simultaneous control extract-free food cubes (*green*). Dissected parts: *API* apical part, *BAS* basal part, *EXT* external part, *FOOT* foot, *INT* internal part, *MANTLE* mantle, and *VISCERA* viscera. Statistical differences calculated using Fisher's exact test; \* $p \leq 0.05$ ; \*\* $p \leq 0.01$



ophiuroids difficult (Mortensen 1936; JM pers. obs.). Only one sample of the sponge repelled *O. validus*, as well as did the disc and arms of its ophiuroid host. Peters et al. (2009) also showed that this sponge alone is chemically protected. Our findings, thus, suggest that the epibiotic sponge may protect the ophiuroid against predators, as proposed in a previous study of Gutt and Schickan (1998). Extracts of dissected parts of *Isodictya kerguelensis* showed an homogeneous distribution of defensive metabolites, which might indicate that this species could also be defended against other potential predators able to access inner parts of the sponge, as it has been shown in experiments using small foraging amphipods (Peters et al. 2009; Núñez-Pons et al. 2012c). The chemical activity of other repellent species—*Bubaris* sp., *Lissodendoryx flabellata*, *Myxilla asigmata*, *M. elongata*, *Tedania* sp., and *T. tantula*—was tested here for the first time. Among these species, only the NPs from *L. flabellata* have been described to date, i.e. flabellatenes and flabellone, both of lipophilic nature (Fontana et al. 1999; Cutignano et al. 2012). Their ecological function has not been reported yet, and hence, it remains to be ascertained whether these compounds caused the repellence in our assays. Finally, our tests provided evidence of hydrophilic metabolites defending against *O. validus* in the hexactinellid *Rossella vanhoefeni*, as was previously suggested by Núñez-Pons et al. (2012a).

Despite their tough protective tunic, the solitary ascidians examined in the present study possessed nonpolar molecules as means of defence against *O. validus*. In agreement with the ODT (Rhoades 1979), only the exposed tissues (i.e. the tunic) of the ascidians *Bathypera* sp. 1 and 2 yielded unpalatable extracts (EE) in our bioassays. No particular allocation of chemical defences was found in *Molgula pedunculata* and *Pyura* sp. 1 and 2, as both tunica

and internal organs were repellent. The widely studied circumpolar ascidian *Cnemidocarpa verrucosa* had repellent lipophilic extracts, as reported in previous studies from different localities applying different protocols (McClintock et al. 1994; Koplovitz et al. 2009). Similarly to *Bathypera* spp., two samples of *C. verrucosa* from nearby localities in the eastern Weddell Sea were repellent only in the most exposed tissues, pharynx and tunica. In contrast, two other *C. verrucosa* specimens from a different locality also in the eastern Weddell Sea were repellent only in the internal visceral extracts. This suggests that other trade-offs (e.g. providing protection to larval forms) could be contributing to a different allocation of chemical defences to body parts (López-Legentil et al. 2005; Núñez-Pons et al. 2010; 2012b). McClintock et al. (1991) showed that mature ovotestis (and gametes) of *C. verrucosa* were noxious to sympatric fish, suggesting a possible compensatory strategy to the considerable investment to reproduction in some species, by the production of chemically defended larvae, as shown by Lindquist et al. (1992). This may explain the presence of repellents in inner tissues (gonads; Lindquist et al. 1992; Pisut and Pawlik 2002) at the expense of external tissues. This pattern was also observed in the internal distribution of rosinone B within the colonies of the Antarctic synascidian *Aplidium fuegiense* (Núñez-Pons et al. 2012b), which was also attributed to an allocation of defensive metabolites in the gonads.

Among the sessile phyla less represented in this study, nonpolar extracts of the congeneric octocorals *Thouarella* sp., *T. minuta*, and *T. laxa* showed a strong feeding repellence against *O. validus*, being also repellent to the amphipod *Cheirimedon femoratus* (Núñez-Pons et al. 2012c; Núñez-Pons and Avila 2014b). Hydrophilic extracts of *Alcyonium grandis*, *A. paucilobulatum*, *Primnois*

*antarctica*, *Symplectoscyphus glacialis*, and *Thouarella viridis* were not repellent, although these species appeared to contain defensive compounds within their ether extracts in previous studies (Núñez-Pons et al. 2012c; Núñez-Pons and Avila 2014a). Among these octocoral representatives, soft corals of the order Alcyonacea are described to rely mostly on NPs, as their nematocysts are not potent enough to be used against predators (Hines and Pawlik 2012). Hemichordate pterobranchs have been poorly studied in chemical ecology studies, with only a few examples in the literature (Núñez-Pons et al. 2012c; Taboada et al. 2013; Núñez-Pons and Avila 2014a). Pterobranchs live protected in colonies partially buried in the substrate and hidden inside a coenocium (Dilly 1988). Recent studies have suggested that these organisms use such physical protection or polar compounds to dissuade predation (Núñez-Pons and Avila 2014a). In the present study, nonpolar and polar extracts from a graptolithoid species of the genus *Cephalodiscus* exhibited repellent activity. One of such extracts came from an unidentified *Cephalodiscus* species from the Weddell Sea, while its nonpolar extract was inactive (Taboada et al. 2013). Different specimens of *C. nigrescens* on shelf bottoms also showed differences in palatability, suggesting that physiological and ecological factors may influence distinctive production of repellents. Finally, none of the three bryozoan species showed repellence in this study. Some species of this group of sessile organisms are known to have physical mechanisms of protection such as avicularia, hard exoskeleton, or spines, which may offset the use of defensive chemicals (Hayward 1995; Figuerola et al. 2013a). This could partly explain why samples of *Reteporella hippocrepis* reported palatable extracts to *O. validus*. Although the soft and fleshy *Alcyonidium flabelliforme* showed no repellence, EE extracts of this species yielded cytotoxicity (Figuerola et al. 2013b). Therefore, other indirect chemically mediated mechanisms, not tested in the present study, could act against *O. validus* predation. The BE of *Cellaria diversa* and *Cornucopina polymorpha* were not repellent in our assays, suggesting that chemical defences may be driven by nonpolar molecules, as seen in previous studies testing the same species (Taboada et al. 2013; Figuerola et al. 2013a).

Although being less repellent than sessile invertebrates in our assays, the incidence of anti-predatory chemicals among Antarctic vagile fauna suggests that notoriously NPs seem to play a role in protection. Antarctic echinoderms are commonly included among the diet items of many other echinoderms, including *O. validus* (Dearborn 1977; authors' pers. obs.). Only three out of ten asteroids and ophiuroids were repellent in our study. Asteroids and ophiuroids may respond to predatory attacks with escape, although they can also use chemical repellence to gain protection from other quick predators, such as fishes

(McClintock 1989). Among the molluscs tested here, we found polar repellents (BE) against *O. validus* in the mantle of the pleurobranch *Bathyberthella antarctica*. The mantle is the most exposed tissue in pleurobranch gastropods, and it is presumably protected by low pH secretions (Thompson 1960; Taboada et al. 2013). Here we demonstrate the hydrophilic nature of anti-predatory NPs in the mantle of *B. antarctica*, which, along with acidity, may be protecting this exposed body part against predators. Bivalves have received less attention in chemoeological studies. We found lipophilic defensive compounds in the viscera of *Limopsis marionensis* that may be diet-derived and could likely be released to the water column prior to contacts with sea stars. Moreover, this internal allocation of defences may defend reproductive tissues and larval stages, as suggested for ascidians (Lindquist et al. 1992; Pisut and Pawlik 2002). As for nemerteans, *Parborlasia corrugatus* is a major predator in the Antarctic benthos. Out of the two specimens tested here, only EE of the specimen collected from Livingston Island caused repellence to *O. validus*, suggesting the occurrence of different chemicals for organisms inhabiting different geographic areas. This species secretes copious amounts of acidic mucus (pH = 3.5; Heine et al. 1991), which contains a potent toxic neuropeptide, called parbolysin. Parbolysin is known to possess cytotoxic and feeding repellent properties, which likely assists its defence against possible predators (Berne et al. 2003). Finally, the only species of annelid polychaete (*Pista spinifera*) tested showed no repellence neither in chemical extracts nor in the organic tubes they build. This terebellid lives inside a membranous tube that may be used as a shelter against predation, and hence, it may not rely on defensive metabolites.

Overall, our results provide further support against the hypothesis that Antarctic benthic marine invertebrates are poor in chemical defences due to the lack of fish predation (Amsler et al. 2000; Avila et al. 2008; Núñez-Pons et al. 2012c; Taboada et al. 2013; Figuerola et al. 2013a; Núñez-Pons and Avila 2015). Benthic invertebrates from the Southern Ocean ecosystems seem to be efficiently protected from echinoderm predation through chemical defences, sometimes in the most exposed parts. The intraspecific variation found in this study remains to be explained, but may be beneficial for organisms exposed to changing predator abundances since synthesis of NPs likely occurs at the expenses of growth and reproduction (Hines and Pawlik 2012; Loh and Pawlik 2014). Nonpolar extracts were the main cause of repellence against *O. validus* in our assays. Further studies should attempt to isolate and characterize the main NPs from the nonpolar extracts tested to identify the compounds that cause the repellence. In addition, further investigations on the less studied hydrophilic extracts as well as on water residues

will be essential to better understand the nature of the molecules responsible for repellence in the different taxa. Finally, existing data from ecological assays using different predator types will be highly useful to reconstruct how trophic interactions mediated by metabolites structure Antarctic benthic ecosystems.

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