




## SYMPOSIUM

# Every Rule Has an Exception: a Cheater in the Community-Wide Mutualism in Antarctic Seaweed Forests

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**Synopsis** Dense macroalgal forests on the Western Antarctic Peninsula serve important ecological roles both in terms of considerable biomass for primary production as well as in being ecosystem engineers. Their function within the Antarctic ecosystem has been described as a crucial member of a community-wide mutualism which benefits macroalgal species and dense assemblages of associated amphipod grazers. However, there is a cheater within the system that can feed on one of the most highly chemically defended macroalgal hosts. The amphipod *Paradexamine fissicauda* has been found to readily consume the finely branched red macroalga *Plocamium cartilagineum*. This amphipod grazer not only feeds on its host, but also appears to sequester its host's chemical defenses for its own utilization. This review summarizes what we know about both of these exceptions to the community-wide mutualism.

## Introduction

Macroalgal forests cover along the western Antarctic Peninsula (WAP) can be remarkably high, covering up to 80% or more of the hard substratum (Amsler et al. 1995; Brouwer et al. 1995). Reported biomass is comparable to that of temperate macroalgal forests (Amsler et al. 1995), underlining the fundamental importance that seaweeds play within the Antarctic coastal ecosystem. A unique feature of the Southern Ocean compared to the rest of the world is that nutrient levels naturally are consistently high on a broad geographic scale and, therefore, usually are not a limiting factor for growth. This is reflected in high nitrogen levels and low carbon to nitrogen ratios within Antarctic macroalgae (Peters et al. 2005). The majority of the macroalgal carbon and nitrogen are not readily available to grazers because most of the macroalgae are chemically defended (reviewed by Amsler et al. 2008, 2009b, 2020; Avila et al. 2008). Experiments demonstrate, however, that

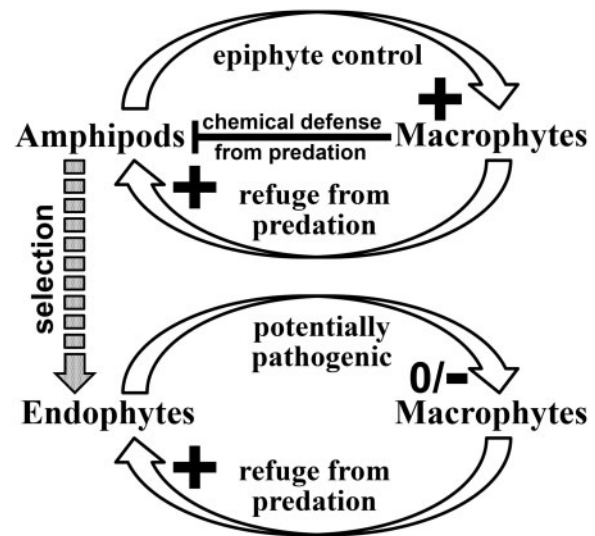
once thalli of chemically defended brown macroalgae die and begin to degrade, they become palatable to mesograzers and their stored carbon becomes available (Reichardt and Dieckmann 1985; Amsler et al. 2012a).

Chemical defenses are commonly mediated through secondary metabolites which are defined as compounds which usually have no role in the primary metabolism of the organism producing them (Williams et al. 1989). These compounds normally have relatively low molecular masses of <3000 Da (Williams et al. 1989). The majority of secondary metabolites known are produced by plants or other sessile organisms largely as a defense against predators due to their inability to escape by moving away (Harborne 1993). Similarly, secondary metabolites produced by macroalgae are not only involved in defense against grazers, but also competition for space or in anti-fouling activity (Young et al. 2015). Additionally, the presence of secondary

metabolites may indirectly affect the distribution of macroalgae depending on the presence and feeding preferences of grazers, thereby profoundly structuring ecosystems (Marques et al. 2006). Secondary metabolites, particularly phlorotannins, are also involved in ultraviolet protection and antioxidant activity, significantly impacting the depth distribution of macroalgae such as endemic brown species in Antarctica (reviewed in Gómez and Huovinen 2020).

Macroalgal forests along the WAP support an extremely high abundance of mesograzers, especially amphipods. The large brown alga *Desmarestia menziesii* can support over 300,000 individual amphipods  $m^{-2}$  of the benthos, while its congener *Desmarestia anceps* and the finely-branched red seaweed *Plocamium cartilagineum* can both support approximately 30,000 individuals  $m^{-2}$  (Amsler et al. 2008). Despite the high abundance of mesograzers on many macroalgal species, most common species of amphipods, including *Gondogeneia antarctica*, *Prostebbingia gracilis*, and *Metaleptamphopus pectinatus*, do not consume their hosts as they are deterred by their chemical defenses (Amsler et al. 2005; Huang et al. 2006; Aumack et al. 2010). The only non-filamentous macroalgal species readily consumed during experiments has been *Palmaria decipiens* (Huang et al. 2006; Amsler et al. 2009b; Aumack et al. 2010). Interestingly, *P. decipiens*, when compared to 36 other macroalgal species near Anvers Island, had the lowest carbon to nitrogen ratio (7.37) indicating a very high nitrogen content (Peters et al. 2005). This species also had one of the highest protein contents varying between 9.3% and 17.3% of dry weight depending on the season of the year (Peters et al. 2005), making it overall a desirable food source for grazers. Of the unpalatable seaweeds, not all of the chemical extracts deterred feeding by amphipod grazers, suggesting that a mixture of chemical as well as structural defenses are involved (reviewed in Amsler et al. 2020). Similarly, when testing the palatability of common macroalgal species from near Anvers Island (64° S latitude), 22 of 35 tested were unpalatable to the omnivorous sea star *Odontaster validus*, and 24 of 28 tested to the Antarctic rockfish *Notothenia coriiceps* (reviewed in Amsler et al. 2020). Similar to the aforementioned amphipod grazers, a mixture of chemical and structural defenses lead to the unpalatability of macroalgae to *O. validus* and *N. coriiceps* (reviewed in Amsler et al. 2020).

Free-living, filamentous algae are mostly absent from the Antarctic subtidal due to intense amphipod grazing (Aumack et al. 2011a, 2012b). Grazers are therefore benefitting chemically defended macroalgae



**Fig. 1** Schematic representation of species interactions between algae and amphipods on the western Antarctic Peninsula. See text for details. + indicates a positive effect, 0 indicates no effect, – indicates a negative effect. From (Amsler et al. 2014). Used with permission.

by removing epiphytes while they themselves gain refuge from predators (Aumack et al. 2011a). The latter has been shown by Zamzow et al. (2010) who saw increased association of amphipods with chemically defended and structurally more complex macroalgae in the presence of predator cues. Additionally, amphipods associating with structurally complex, chemically-defended macroalgae had a higher survival rate in the presence of the predatory fish *N. coriiceps* (Zamzow et al. 2010). This relationship applies to a wide variety of macroalgal and amphipod species, benefitting both parties as outlined in the upper section of Fig. 1 and has been described as a community-wide mutualism (Amsler et al. 2014).

The intense grazing of the amphipods does not only lead to the absence of epiphytes, it also leads to the high prevalence of endophytes as their association with chemically defended macroalgae protects them from grazing (Peters 2003). Brown as well as green endophytes are common in some, but not all, macroalgae (Amsler et al. 2009a). When endophytes were isolated and grown in culture, the thalli were consumed significantly faster by amphipods than were their hosts, several fold faster even than the palatable *P. decipiens*, indicating that they are very palatable (Amsler et al. 2009a). However, endophytes are often known to have detrimental effects on their hosts. Schoenrock et al. (2013) determined that pathogenesis of endophytes varied between four different macroalgal species when measuring growth

rates of infected compared to noninfected thalli. Palatability, however, was not impacted by endophyte presence in all of nine macroalgae tested (Schoenrock et al. 2015a). Only the red macroalga *Pachymenia* sp. exhibited a weakened thallus when examining the force required to puncture it (Schoenrock et al. 2015a). The red seaweed *Iridaea cordata* had the highest reduction in growth rate when endophytes were present at high densities and experienced stress to the photosystem in tissue directly adjacent to infected areas (Schoenrock et al. 2013, 2015a). Further investigation revealed that the fertility of *I. cordata* was not effected by endophyte presence and that female gametophytes bearing carposporophytes had a lower incidence of endophytes (Schoenrock et al. 2015b). The lower half of Fig. 1 summarizes the relationship of endophytes and macroalgae. Whilst the endophytes gain refugia from the high grazing pressure of amphipods within the described ecosystem near Anvers Island (64° S latitude), their pathogenic effect on the host is variable with most being benign.

Gut content analysis of 15 amphipod species found in abundance on Antarctic macroalgae identified a mixture of food sources for these mesograzers: diatoms, filamentous, and multiseriate macroalgae, other unicellular algae, crustacean parts, and sponge spicules (Aumack et al. 2017). The food sources varied in their relative importance between amphipod species as well as depending on the habitat they were collected from, but diatoms and endo/epiphytic filamentous algae almost always comprised a major part of their diet (Aumack et al. 2017). Amphipod densities were significantly higher on the chemically defended *D. menziesii* during daytime than they were during the nighttime, whereas the reverse was the case for the more palatable *I. cordata* (Aumack et al. 2011b). This supports the hypothesis that during daylight hours, when visual predators such as fish are present, amphipods seek refuge from predation by associating with chemically defended macroalgae, while during darker hours they safely move to palatable seaweeds and other places where diatoms or other food may be available (Aumack et al. 2011b).

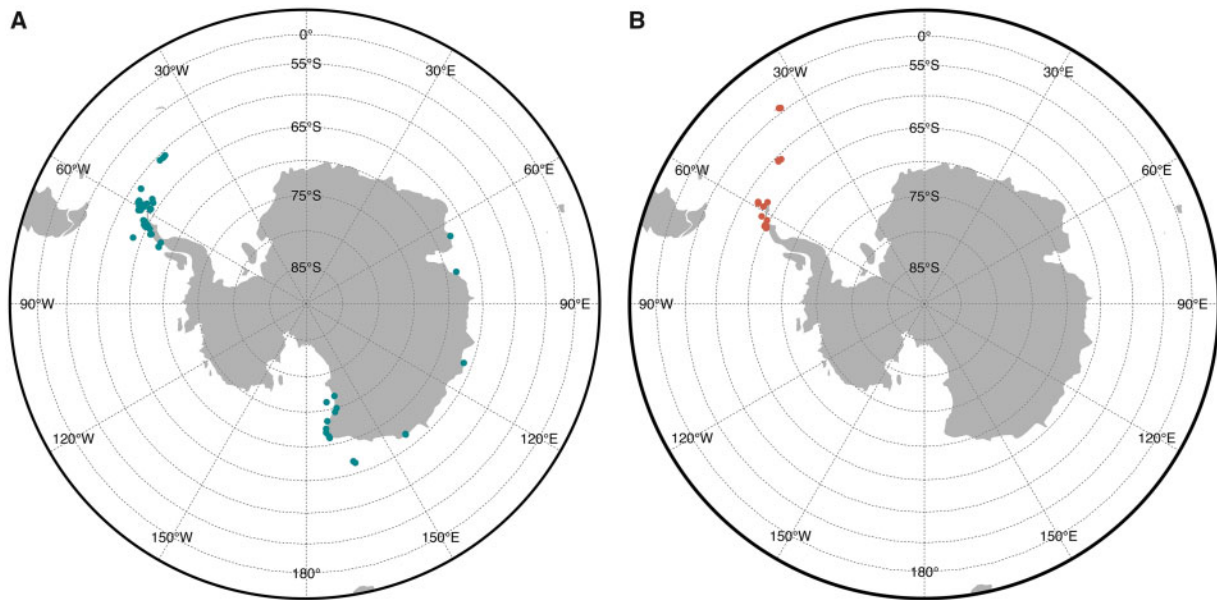
This pattern of macroalgae-amphipod mutualism appears to be a general characteristic of the community (Amsler et al. 2014). However, there is an exception: when Aumack et al. (2017) analyzed the gut content, fatty acid, and stable isotope composition of common amphipod species, they found evidence for a cheater that was consuming its host. The stable carbon isotope signature of the amphipod grazer *Paradexamine fissicauda* could only be explained if

it were consuming one of several chemically defended red macroalgae, including *P. cartilagineum* (Aumack et al. 2017, unpublished data). In addition, Huang et al. (2007) observed that *P. fissicauda* was unique compared to all other amphipod species sampled in being one to two orders of magnitude more abundant on a particular macroalgal host, *P. cartilagineum*, than on any of seven other common macroalgal species surveyed.

### The cheater *P. fissicauda*

*Paradexamine fissicauda* Chevreux is a member of the family Dexaminidae (Thurston 1972). As shown in Fig. 2B, this amphipod species has been reported from the Scotia Arc (between 60° and 61° S latitude) and the Antarctic Peninsula, mainly associates with macroalgae between 5 and 49 m depth, and appears to be endemic to Antarctica (Thurston 1972; De Broyer and Jazdzewska 2014). However, the potential for cryptic speciation cannot be ignored as no study has been conducted with modern molecular genetic techniques. Such analyses would be especially relevant between the Scotia Arc and the WAP. Interestingly, the distribution of *P. fissicauda* closely matches the distribution of *P. cartilagineum* (Fig. 2A) and, as mentioned above, *P. fissicauda* closely associates with *P. cartilagineum* (Huang et al. 2007). The only major distributional difference is that *P. cartilagineum* has been recorded further south on the WAP and in East Antarctica compared to *P. fissicauda*. The amphipod visually blends in particularly well with *P. cartilagineum* due to a pigmentation that mimics that of its host and a morphology which exhibits highly spinate pleomeres that resemble the branches of *P. cartilagineum* (Huang et al. 2007).

Individuals of *P. fissicauda* in the vicinity of Signy Island (60° S latitude) were observed to commonly lay their eggs in autumn, brood over the winter, and release hatchlings during the spring (Thurston 1972). Out of 17 species examined, *P. fissicauda* fell among 8 to 10 other amphipod species that displayed a single yearly incubation period (Thurston 1972). As with most of the species examined by Thurston (1972), females were more numerous than males. Gravid *P. fissicauda* female adults varied little in size, measuring ~20 mm from the tip of the rostrum to the tip of the telson, and bearing between 53 and 56 eggs (Thurston 1972). In general, Antarctic amphipods appear to carry larger eggs than temperate amphipod species (Thurston 1972). Comparing 12 amphipod species, the length of adult individuals was positively correlated with both the volume of an individual egg and the volume of the total number



**Fig. 2** Distribution patterns of (A) the Antarctic red seaweed *Plocamium* “*cartilagineum*” and (B) the amphipod *Paradexamine fissicauda*. Information was taken from the following sources (and references within) as well as a 2019 cruise along the WAP (authors personal observations): (Thurston 1972; Wiencke et al. 2014; Guillemain et al. 2018; Barrera-Oro et al. 2019; GBIF.org 2020a, 2020b; Van de Putte et al. 2020). The map was created using R (R Core Team 2018) with the ggplot2 and rworldmap packages (South 2011; Wickham 2016).

of eggs (Thurston 1972). Whether this pattern also applies to *P. fissicauda* remains to be determined.

*Paradexamine fissicauda* was the only one of five amphipod species examined to readily consume *P. cartilagineum* (Amsler et al. 2013). In a four-way choice experiment using four chemically defended red seaweed species, *P. fissicauda* preferred to eat *P. cartilagineum* and *Picconiella plumosa* over *Pantoneura plocamioides* and *Cystoclonium obtusangulum* (Amsler et al. 2013). *Paradexamine fissicauda* maintained on a diet of *P. cartilagineum* was consumed significantly less frequently by the predatory fish *N. coriiceps* than were individuals fed non-chemically defended *P. decipiens* (Amsler et al. 2013). In the field, *P. fissicauda* is being avoided by the fish predators *N. coriiceps* as well as *Notothenia rossii* which was determined by gut content analysis and subsequent calculation of the Ivlev Index to estimate selectivity of food items (Barrera-Oro et al. 2019). In addition, halogenated compounds were identified in *P. fissicauda* that had been fed *P. cartilagineum*, but not in *P. fissicauda* fed on the non-chemically defended alga *P. decipiens* over the same period (Amsler et al. 2013). These findings indicate that *P. fissicauda* is sequestering defensive secondary metabolites produced by *P. cartilagineum*. Remarkably, to our knowledge, this pattern of sequestration of the prey’s chemical defense is the first documented case for any marine animal other than

opisthobranch molluscs (Amsler et al. 2013). This close relationship between these two species could help explain the geographic distribution of *P. fissicauda* discussed above. Dependence on *P. cartilagineum* may have restricted its capacity to spread to East Antarctica. However, *P. cartilagineum* is not the sole food source for *P. fissicauda*. In terms of relative importance (adjusted to relative fullness of the guts), *P. fissicauda* depends heavily on diatoms (45%), as well as on crustacean parts (25%), filamentous macroalgae (7%), multiserial macroalgae (13%), and other unicellular algae (12%) (Aumack et al. 2017). Fatty acid signatures did not only match *P. cartilagineum* but also *Gigartina skottsbergii* (Aumack et al. 2017) which is not consumed by the amphipods *G. antarctica* and *P. gracilis* (Huang et al. 2006). The latter, however, was likely due to the toughness of the thallus of *G. skottsbergii* which was found to be the toughest out 30 macroalgal species tested (Amsler et al. 2005; Huang et al. 2006). No feeding assay trials have been conducted with *P. fissicauda* and *G. skottsbergii*.

### Antarctic *P. “cartilagineum”*

Antarctic *P. “cartilagineum”* (Linnaeus) P.S. Dixon 1967, originally known as *Fucus cartilagineus* Linnaeus 1753, has its closest relatives in either Chile or New Zealand (Hommersand et al. 2009).

Reports of closely related Antarctic species, such as *Plocamium aff. hookeri* or *Plocamium aff. secundatum*, have been misidentified as different species due to their similar, yet varying, morphological characteristics (Hommersand et al. 2009; Dubrasquet et al. 2018). Dubrasquet et al. (2018) utilized molecular tools to shed light on the species diversity of the Plocamiales (and other red macroalgae) along the upper half of the WAP (between 63° and 67° S latitude) and the South Shetland Islands (62° S latitude). The inaccurate evaluation of species diversity within the Plocamiales is a common problem within the *P. cartilagineum* species complex, a taxonomic group formerly described as cosmopolitan (Saunders and Lehmkuhl 2005). Although it is known that *P. "cartilagineum"* from Antarctica (hereafter 'Antarctic *P. "cartilagineum"*') is different from species with the same name elsewhere, this remains the currently accepted name for the species in Antarctica. On the one hand, cryptic species diversity has prevented us from completely describing species diversity and distribution, but on the other hand, morphological characters sometimes attributed to local adaptations represent specificity of populations but not the emergence of a new species. In the Southern Ocean, *P. "cartilagineum"* has been described as having a circum-Antarctic distribution (Wiencke et al. 2014). However, molecular studies are needed to confirm whether records from East Antarctica are indeed the same species as found on the WAP or whether those populations originated from South Africa, New Zealand, Australia, or a mixture thereof. In East Antarctica, *P. "cartilagineum"* has been recorded as far as 74°S and on the WAP as far as 68°S (Fig. 2A). It grows well at 0°C but not at all at 5°C which likely reflects a long evolutionary isolation from temperate relatives, and warranting a separate ecotype or even species status (Bischoff-Bäsmann and Wiencke 1996; Wiencke et al. 2014).

The number of cryptic species being discovered in the Southern Ocean has increased with the advancement of molecular tools and subsequent phylogeographic studies (Held 2014). In northern Europe, Saunders and Lehmkuhl (2005) used the large subunit ribosomal DNA to identify four cryptic species originally described as *P. "cartilagineum"* and more are to be expected in other regions of the world. The Antarctic continent is geographically separated from other temperate continents. This separation is further strengthened by the Antarctic Circumpolar Current which has become fully established since at least the middle Miocene ~15 Ma (Lawver et al. 2014). This has given Antarctic fauna and flora a

long time period to evolve under local conditions. Moreover, distributions and speciation events have been greatly impacted by recurring glaciation events (Crame 2014). Using the cytochrome c oxidase I (COI or *cox1*) gene, Guillemain et al. (2018) analyzed individuals of the Antarctic *P. "cartilagineum"* and five other red macroalgae species from three locations along the WAP (between 63° and 67° S latitude) and two locations in the South Shetland Islands (62° S latitude) for genetic diversity. Generally, mitochondrial DNA (mtDNA) diversity was low within all species, with the Antarctic *P. "cartilagineum"* exhibiting four haplotypes of which one dominated at all locations covering an area of 450 km (Guillemain et al. 2018). Recent recolonization following the last glaciation event has been hypothesized to have led to the low genetic diversity observed (Guillemain et al. 2018).

The Antarctic *P. "cartilagineum"* has a life cycle with an alternation of isomorphic generations (Wiencke and Clayton 2002). Haploid tetraspores produced by meiosis in the diploid tetrasporophyte generation develop into male and female gametophytes which produce gametes when mature. Nonmotile male gametes, referred to as spermatia, fertilize female gametes, referred to as carpogonia, resulting in diploid zygotes. A small diploid stage called the carposporophyte develops from the zygote and remains attached to the females. Carposporophytes produce carpospores mitotically. They are eventually released and grow into diploid tetrasporophytes. Whilst the presence of female thalli with cystocarps containing carpospores suggests that they have isomorphic dioicous gametophytes like closely related *Plocamium* species elsewhere, to date no records of male gametophytes have been found in Antarctica. This could be due to the fact that sampling has occurred too late in the season that previously males have not specifically been targeted, or that parthenogenesis is occurring. It has been proposed that parthenogenesis is common in higher latitudes and marginal environments (reviewed in Tilquin and Kokko 2016). However, studies in the occurrence of parthenogenesis around Antarctica have not been conducted.

The Antarctic *P. "cartilagineum"* has a very low C:N ratio of 9.59 indicating a high nitrogen content compared to macroalgal species from other biogeographic regions (Peters et al. 2005). Compared to co-occurring macroalgal species from around Anvers Island (64° S latitude), however, the carbon to nitrogen ratio is not unusual for the region, while protein content is at the lower range (Peters et al. 2005). Overall, nitrogen does not appear to be growth-

limiting for Antarctic macroalgae as the majority have a C: N ratio of 10:1 or lower and a high protein content making them desirable food sources for grazers, if it was not for their aforementioned chemical defenses (Dunton 2001; Peters et al. 2005). Such defenses also protect the Antarctic *P. "cartilagineum"* from grazers other than *P. fissicauda*. Individuals belonging to closely related *Plocamium* species have been found to vary in their halogenated monoterpene production worldwide, for example, in Chile (San-Martín and Roviroso 1986; Palma et al. 2004), Spain (König et al. 1990), Portugal (Abreu and Galindro 1996), and New Zealand (Blunt et al. 1985). Monoterpenes are comprised of two isoprene units which make up the C<sub>10</sub> carbon backbone (Young et al. 2015). The Antarctic *P. "cartilagineum"* is known to produce both cyclic and acyclic halogenated monoterpenes (Young et al. 2015).

Near Anvers Island (64° S latitude), the Antarctic *P. "cartilagineum"* has been found to produce a variety of different mixtures of secondary metabolites referred to as chemogroups, including those producing acyclic polyhalogenated monoterpenes in abundance while others express primarily cyclic compounds (Young et al. 2013; Shilling et al. 2019). Out of 21 individuals collected in 2012, five unique chemogroups were initially identified (Young et al. 2013). Two haplotypes were identified using the plastid *rbcl* gene and the mitochondrial *cox1* gene, which correlated to individuals producing either of two or three chemogroups, respectively (Young et al. 2013). This indicates that there is an underlying genetic factor influencing chemogroup production. Additionally, the distribution of chemogroups and haplotypes were strongly site-specific which indicates that within haplotype variation of chemogroups could be correlated to biotic or abiotic differences between sampling sites (Young et al. 2013).

Worldwide, the genus *Plocamium* has frequently been shown to exhibit seasonal and/or geographic variation in secondary metabolite production (Capon et al. 1984; Blunt et al. 1985; König et al. 1990; Abreu and Galindro 1996; Young et al. 2013). An underlying explanation for the variation has not yet been fully established. Palma et al. (2004) found that only temperature, but not photon flux density, had a significant effect on the quantity of one of three secondary metabolites produced by a *P. cartilagineum* species from a population in Chile. This could reflect changes of chemogroup composition between seasons. Whilst the actual compounds produced might not change, the relative abundances of the main secondary metabolites could be affected driven by temperature changes. However, Palma

et al. (2004) did not extract and quantify any other than the three secondary metabolites. Therefore, it cannot be concluded that the treatments did not change the type of other secondary metabolites produced. Furthermore, Palma et al. (2004) only ran the experiment for 16 days which might not be long enough to detect any changes and they did not examine genetic markers of the collected thalli to address whether more than one cryptic species had been included. In *Plocamium brasiliense*, individuals from two different locations in Brazil were also found to exhibit different chemotypes (Pereira and Vasconcelos 2014). Unfortunately, bulk extractions were performed at each site, so intra-population variability could not be investigated. Herbivory by a common sea urchin but not a crab was significantly inhibited by individuals from both populations (Pereira and Vasconcelos 2014). One chemotype, when extracted, was more deterrent than the other (Pereira and Vasconcelos 2014). As mentioned above, Antarctic *P. "cartilagineum"* near Anvers Island (64° S latitude) has been found to produce a variety of different chemogroups which had a strong site-specific distribution, but could also be correlated to different haplotypes, indicating an underlying geographic and/or genetic explanation for the observed variation (Young et al. 2013).

As noted above, Guillemain et al. (2018) found low mtDNA diversity for the Antarctic *P. "cartilagineum"* with four haplotypes of which one dominated at all locations covering an area of around 450 km. Near Anvers Island (64° S latitude), two haplotypes were identified using the plastid *rbcl* and the mitochondrial *cox1* genes (Young et al. 2013). These markers are good tools for defining interspecific as well as intraspecific differences in red seaweeds (Robba et al. 2006). However, analyzing more polymorphic markers, such as microsatellites, facilitates the discrimination of individuals. As described above, the Antarctic *P. cartilagineum* exhibits an alternation of isomorphic generations which are impossible to tell apart in the absence of reproductive structures. Microsatellites enable the determination of life cycle stages, and hence ploidy levels (e.g., Engel et al. 2004; Krueger-Hadfield et al. 2013, 2016), making subsequent population genetic analysis possible (see Krueger-Hadfield and Hoban 2016). The *cox1* and *rbcl* single genes provide useful tools to detect inter- and intraspecific genetic differentiation, but microsatellite loci provide additional insights into gene flow and, consequently, the mating system of a population that can have profound eco-evolutionary consequences (e.g., Eckert et al. 2010). This would also allow for a more detailed and definitive

comparison between distribution patterns of individual genotypes and their respective assigned chemogroups.

As discussed above, the Antarctic *P. "cartilagineum"* supports one of the highest abundances of amphipods compared to seven other macroalgal species, despite being highly chemically defended, with *P. fissicauda*, its only known grazer, being the most common of those amphipod species (Huang et al. 2007). However, amphipod communities on *P. "cartilagineum"* are also significantly different between the two sites investigated (Huang et al. 2007, M.O. Amsler, personal communication). Whether this is related to chemogroup production remains to be determined. Barrera-Oro et al. (2019) found that both fish species examined, *N. rossii* and *N. coriiceps*, were selecting for macroalgae as a whole as a dietary resource and more specifically for *Desmarestia antarctica*, *P. decipiens*, and *G. skottsbergii*. Further stable isotope analysis revealed that *N. rossii* and *N. coriiceps* depend on organic matter originating from fresh as well as detrital phytoplankton and macroalgae, likely through their prey which are consuming those resources (Zenteno et al. 2019). When consuming macroalgae directly, only *N. coriiceps* also selected for *P. "cartilagineum"* amongst two other red macroalgae, with *P. "cartilagineum"* only comprising 0.42% of the stomach contents (all macroalgae together made up 7.21%) (Barrera-Oro et al. 2019). *Notothenia rossii*, on the other hand, was actively avoiding consuming *P. "cartilagineum"* according to the calculated Ivlev index value which takes stomach contents and natural abundances of prey into account (Barrera-Oro et al. 2019). The authors attributed these species-specific differences in feeding preferences to life style, with *N. rossii* being slightly more pelagic than *N. coriiceps* (Barrera-Oro et al. 2019).

Overall, secondary metabolites do not only serve as feeding deterrents. The secondary metabolites of *Plocamium hamatum*, which occurs in the tropics, have a negative allelopathic effect on *Sinularia cruciata* as well as other soft coral species and epiphytes (de Nys et al. 1991). Therefore, secondary metabolites produced by the Antarctic *P. "cartilagineum"* species could also have allelopathic effects against commonly occurring sessile invertebrates such as sponges. Antarctic *P. "cartilagineum"* does not appear to have endophytes, but it does provide surface area for epiphytes (Peters 2003). Epiphytic diatom abundance and species richness and diversity varied significantly between individuals from Admiralty Bay (South Shetland Islands, 62° S latitude) and Terra Nova Bay (Ross Sea, 74° S latitude). These

differences were hypothesized to be due to regional differences in respective dominant grazers (Majewska et al. 2015). Even at sites near Anvers Island (64° S latitude) that occurred within a few kilometer or less of each other, differences in diatom and other epiphyte cover on *P. "cartilagineum"* are apparent (authors' qualitative observations). Majewska et al. (2015) found that around 6.8% of the thalli of *P. "cartilagineum"* were covered with associated fauna which was more diverse than two other flat-bladed red seaweeds examined (*I. cordata* and *Phyllophora antarctica*). The branching morphology of the Antarctic *P. "cartilagineum"* has been hypothesized to lead to differences in the epiphytic community (Majewska et al. 2015). But, whether chemogroup production impacts epiphytic diatom assemblages or vice versa remains to be investigated.

## The physiology and ecology of chemical defenses

Organisms, such as predators (both carnivores and herbivores) or even parasites, evolve mechanisms to resist defenses that evolved within their prey or hosts. Some herbivores, for example, have traits which benefit them significantly by allowing them to increase their feeding rate on a specific plant or alga; this is called herbivore offense (Sotka and Whalen 2008). Herbivore offense varies from the least aggressive, which includes making a feeding choice increasing its fitness, over a change in behavior, morphology, or physiology, to the most aggressive response of manipulating the prey's production of defenses (Karban and Agrawal 2002). It does not necessarily cause damage or a reduction of fitness to the prey (Sotka and Whalen 2008). These responses can, however, be metabolically costly to the herbivore, especially if it involves detoxification or even the sequestration of secondary metabolites. In the marine realm, opisthobranch molluscs are well known to sequester dietary secondary metabolites for their own defense. The predator must not only be able to feed on and tolerate chemically defended prey, but selectively transport and store these metabolites, and in some instances, metabolize the compounds into something less harmful (reviewed in Karban and Agrawal 2002). As discussed earlier, *P. fissicauda* was rejected by an omnivorous fish predator when it had previously been fed a *P. "cartilagineum"*-only diet, indicative of the sequestration of defensive secondary metabolites (Amsler et al. 2013). It is likely that this sequestration process requires the input of limited energy that could otherwise be allocated to such key processes as

growth and/or reproductive output. The amphipod *Orchomene plebs*, for example, displays a significantly decreased rate of molting and increased rate of mortality when fed artificial food containing extracts of the Antarctic sponge *Isodictya erinacea* (Moon et al. 2000). In a related unpublished study, the impact that Antarctic *P. "cartilagineum"* harboring different chemogroups have on the fitness of *P. fissicauda*, is currently under investigation.

*Paradexamine fissicauda* appears to have evolved a physiological adaptation to deal with the bioactive secondary metabolites produced by the Antarctic *P. "cartilagineum"* (Amsler et al. 2013). Because the halogenated compounds found in *P. fissicauda* closely match those in *P. "cartilagineum"* (Amsler et al. 2013), it is unlikely that a multi-step metabolic pathway to modify and subsequently excrete the chemical defenses is involved (Karban and Agrawal 2002; Sotka and Whalen 2008). However, whether sequestration takes place for all of the secondary metabolites found in all of the chemogroups, as well as for secondary metabolites synthesized by the readily consumed red seaweed *P. plumosa*, remains to be determined (Amsler et al. 2013). In order to process chemical defenses for excretion cytochrome P450s, glutathione, S-transferases, and adenosine triphosphate-binding cassette transporters are involved in a 3-phase pathway which partially or completely deals with lipophilic compounds commonly produced as a feeding deterrent (Karban and Agrawal 2002; Sotka and Whalen 2008). Rather than sequestering the secondary metabolites, they are transformed into more hydrophilic compounds which can be excreted (Karban and Agrawal 2002; Sotka and Whalen 2008).

## Future directions

The community-wide mutualism summarized in Fig. 1 explains the co-occurrence of high numbers of amphipods within Antarctic macroalgal forests. The amphipod *P. fissicauda* and the red alga *P. "cartilagineum"* pose intriguing questions in terms of the co-evolution of chemical defenses and herbivore offense. The amphipod grazer readily feeds on a highly chemically defended and chemically diverse macroalga. It is still unknown what the evolutionary drivers are that are maintaining the high chemical diversity in populations of *P. "cartilagineum"* near Anvers Island (64° S latitude) or elsewhere. But with the advancement of molecular techniques, future investigators will be able to investigate the underlying genetic drivers for chemogroup production. Further questions of the differential costs associated

with the production of each chemogroup, as well as the costs associated with the capacity of *P. fissicauda* to feed upon and sequester compounds that serve as chemical defenses, remain open. While studies of the community-wide mutualism between most amphipods and macroalgae on the WAP are valuable to understanding how this key coastal ecosystem is structured and functions, the exception to the rule is just as intriguing.

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## Data availability

No new data were generated or analyzed in support of this research.

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