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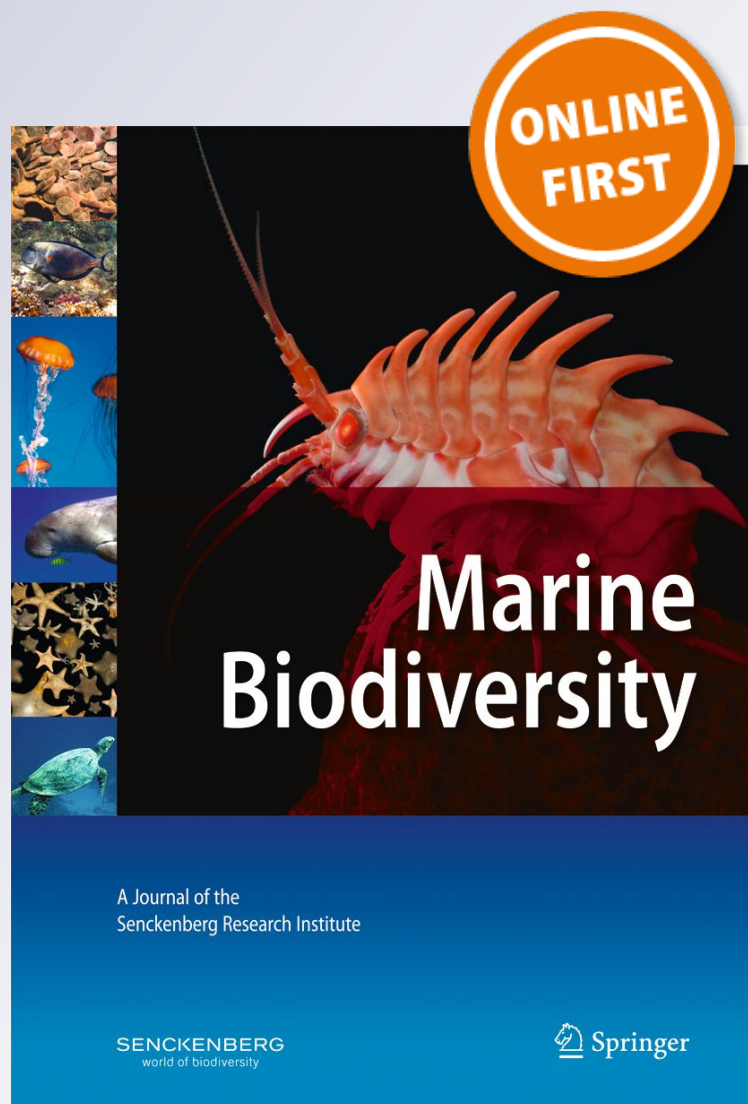
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# A survival pack for escaping predation in the open ocean: amphipod – pteropod associations in the Southern Ocean

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## Abstract

Hyperiid amphipods are a major prey for fish and seabirds. In the Southern Ocean, they are particularly abundant, with distributions ranging from the Polar Frontal Zone to Antarctic shelf waters. The species *Hyperiella dilatata* has previously been reported to show a peculiar anti-predatory behaviour: It captures chemically protected, gymnosome pteropods in the water column and carries them on its dorsum, like a backpack. We report this association at four oceanic sampling sites between latitudes 45° and 71° S. Molecular barcodes of both hosts and pteropods are provided and compared with those of other hyperiid and pteropod specimens. Morphological identifications as well as molecular analyses show a so far undocumented association of *Hyperiella antarctica* with the pteropod *Spongiobranchaea australis* in the Polar Frontal Zone (Lazarev Sea). *H. dilatata* carried *Clione limacina antarctica* specimens in the Weddell Sea, as recorded previously for the Ross Sea. Lengths of the abducted pteropods varied between 1 and 5 mm, with the biggest pteropod measuring more than half the host's size. One of the abducting amphipods was a female carrying eggs. The formation of such tandem is known to be very efficient as protection from visually hunting icefish in the crystal-clear coastal waters around the Antarctic continent; however, in the open ocean, this behaviour was so far undocumented. Here, we develop hypotheses on its origin and function.

**Keywords** Hyperiidea · *Hyperiella* · *Clione* · *Spongiobranchaea* · Gymnosomata · COI barcodes

## Introduction

Almost three decades ago, in the Antarctic shelf waters of McMurdo Sound in the Ross Sea, a peculiar association between the hyperiid amphipod *Hyperiella dilatata* Stebbing, 1888 and the shell-less pelagic pteropod *Clione limacina antarctica* E.A. Smith, 1902 was reported for the first time (McClintock and Janssen 1990). Amphipods were observed

(in situ) carrying a pteropod on their dorsal side, firmly holding it with their sixth and seventh pereopods. In the under-ice environment around the Antarctic continent, *H. dilatata* is a regular component of zooplankton communities and highly preyed upon by fish and seabirds (Foster 1987; Vacchi and La Mesa 1995). In under-ice waters nearshore and offshore, most of the notothenioid fish had *H. dilatata* in their stomachs (Foster et al. 1987; Foster and Montgomery 1993). McClintock and Janssen (1990) reported that at shallow depths (< 10 m) in McMurdo Sound, up to 74% of the *Hyperiella* individuals observed were carrying abducted *Clione* on their dorsum, apparently serving as an efficient protection from the predatory notothenioids. The authors noted that notothenioids feed selectively: They avoid *Clione* but feed avidly on hyperiid amphipods; however, they were observed to spit out the pairs consisting of amphipods with abducted pteropods. This can be explained by the fact that *C. limacina antarctica* is capable of producing an efficient predator-deterrent chemical named pteroenone, a  $\beta$ -hydroxy ketone, that has been isolated, characterized (Bryan et al. 1995) and recently also synthesized (Asao et al. 2010). A similar tandem formation was observed around Elephant Island, where *H. dilatata* carried the pteropod *Spongiobranchaea*

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*australis* d'Orbigny, 1836 (Loeb et al. 1998). Hence, this pteropod species likely also possesses pteroenone or a similar chemical defence compound (Phleger et al. 1999). For the first time, we report the observation of this behaviour in open oceanic waters of the Southern Ocean. We investigate whether this defensive behaviour is unique to *H. dilatata* and whether there are regional differences in the formation of such amphipod-pteropod pairs.

## Material and methods

*Hyperliella* specimens carrying pteropods were collected, along a latitudinal transect, at four different stations of a total of 30 stations sampled during the expedition PS103 (ANT-XXXII/2) of R/V *Polarstern*. A permit for sampling south of 60° S latitude (under the Antarctic Treaty law for environmental protection) was obtained from the German Environment Agency (UBA) with the number II 2.8–94,003-3/382. Amphipods and pteropods were collected using bongo nets (mesh size 500 µm, ring diameter 60 cm each), using a Hydro-Bios V-Fin depressor to create negative buoyancy. The nets were towed obliquely from the aft of the ship at a max. speed of 1.5 knots and wire length of max. 300 m. Details on the sampling sites and specimens used for analyses are listed in Tables 1 and 2; sampling sites are shown in Fig. 1. Additional hyperiids were collected by means of a Rectangular Midwater Trawl (RMT; see Table 2) during the R/V *Polarstern* expeditions PS79 (ANT-XXVIII/3) and PS82 (ANT-XXIX/9) to the Southern Ocean in 2012 and 2014. Amphipods and pteropods were preserved in 99% ethanol, except for the hyperiidean–pteropod pair HypC97 and PteC98, which was preserved in the stabilizing agent RNAlater.

Amphipod and pteropod specimens were morphologically identified to species level and their total length measured; in the case of amphipods, this was done along a straightened body from the front of the head to the tip of uropod 3. Pteropods were measured from the base of the anterior tentacles to the posterior end of the trunk after relaxation for a few minutes in a petri dish with cold seawater, and length determined comparing the scaled pictures taken when still alive. Hyperiid amphipods from the R/V *Polarstern* expeditions

PS82 (ANT-XXIX/9), PS79 (ANT-XXVIII/3) and PS103 (ANT-XXXII/2) were selected for molecular analyses, including the abducting specimens. They were identified prior to extraction based on descriptions and keys from Bowman (1973), Weigmann-Haass (1989), Vinogradov (1999) and Zeidler (2015). Genomic DNA was isolated using the Nucleospin Tissue kit (Macherey-Nagel) according to the manufacturer's protocol. For the amphipod specimens, a pleopod was used, and for the pteropods, a small piece of the tissue or the entire animal in the case of small *Clione* specimens. A fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene was amplified by PCR using the LCO1490 and HCO2198 primers (Folmer et al. 1994). PCR reactions consisted of 0.02 U/µl Hotmaster Taq (5Prime GmbH), 0.2 mM dNTPs, 0.5 µM of forward and reverse primers, 1× PCR-buffer and 1 µl (about 30 ng) of template DNA filled with molecular water to 25 µl. PCR conditions were initial denaturation at 94 °C for 2 min, followed by 36 cycles of 94 °C for 20 s, annealing at 42 °C for 20 s, extension at 65 °C for 1 min and a final extension at 65 °C for 15 min. For the PCR products, both forward and reverse strands were sequenced using the sequencing services of EUROFINs (Germany). Sequences were checked for ambiguities and aligned using the software CodonCode Aligner v.3.7.1.1. (CodonCode Corporation, Deham, MA, USA). In order to prevent inclusion of pseudogenes in the analyses, electropherograms were checked for ambiguous base calls and sequences were translated into amino acids and checked for stop codons. GenBank sequences were added for species identification and comparison in the analyses. For the amphipods, we included GenBank sequences of *Hyperliella antarctica* Bovallius, 1887 (KC74382, KC74381), *H. dilatata* (KC754384), *Hyperia macrocephala* (Dana, 1853) (KC754383), *Hyperia galba* (Montagu, 1815) (DQ889153), *Hyperoche capucinus* Barnard, 1930 (EF989665), and *Hyperoche martinezi* (Müller, 1864) (EF989668) (Browne et al. 2007; Costa et al. 2007; Cheng et al. 2013). For the pteropod analyses, sequences of *Spongiobranchea australis* and *Clione limacina antarctica* collected during the same expedition (Table 1) as well as from other localities in the Southern Ocean (retrieved from GenBank) were also added (*S. australis*:

**Table 1** Information on the observed pairs of hyperiidean amphipods with abducted pteropods

Station	Depth range	Latitude	Longitude	Amphipod species	ID Nr	Length	Pteropod species	ID Nr	Length
001–4	0–40 m	45°57' S	06°17' E	<i>H. antarctica</i>	HypC34	7.5 mm	<i>S. australis</i>	PteC35	5.0 mm
003–4	0–100 m	51°58' S	02°06' E	<i>H. antarctica</i>	HypC97	7.0 mm	<i>S. australis</i>	PteC98	1.7 mm
022–7	0–250 m	69°01' S	06°57' W	<i>H. dilatata</i>	HypC36	6.5 mm	<i>C. antarctica</i>	PteC37	1.1 mm
030–1	0–40 m	71°17' S	14°06' W	<i>H. dilatata</i>	HypC99	5.5 mm	<i>C. antarctica</i>	PteC100	1.0 mm

Details for each station where hyperiidean amphipods with abducted pteropods were found during the R/V *Polarstern* voyage PS103 (ANT-XXXII/2). Depth, geographic coordinates, species name, identification number and body length are given, both for the hyperiidean and pteropod specimens



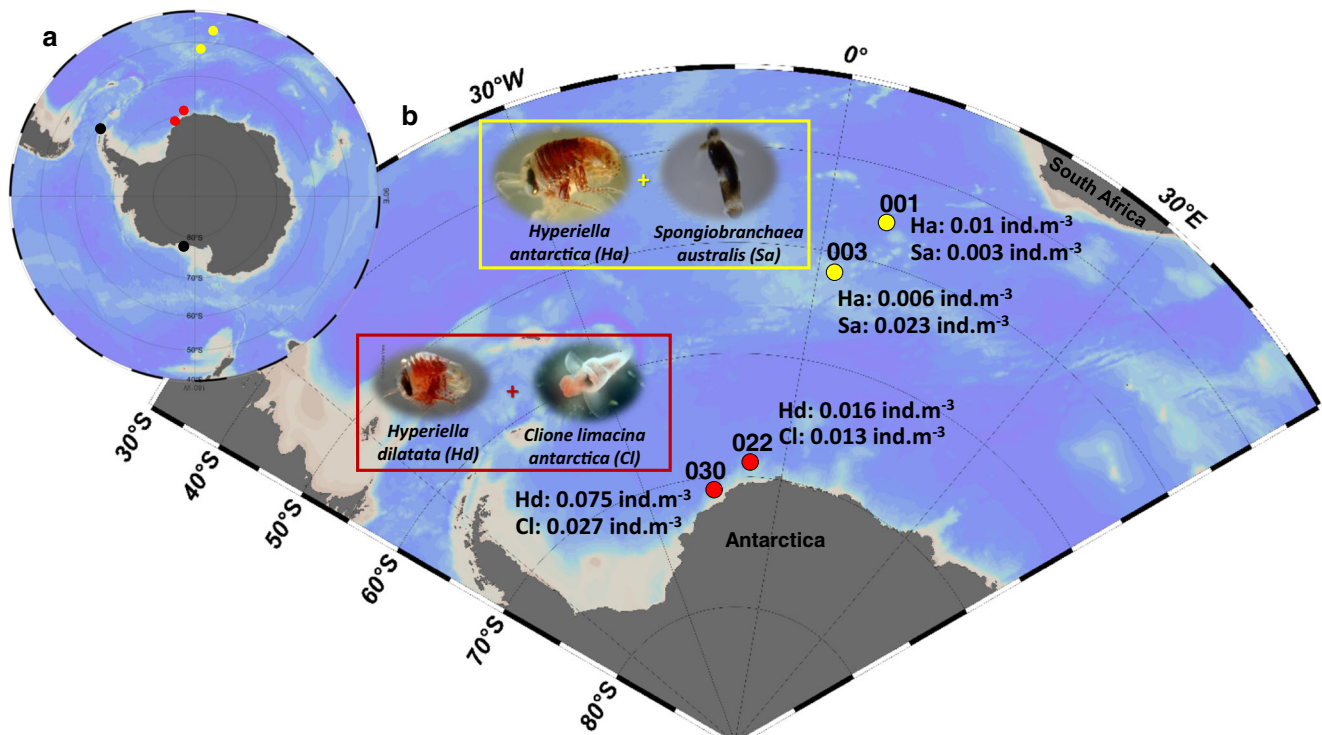
**Table 2** Details of the amphipods and pteropods used for molecular analyses

Sample ID	Species	Expedition	Station	Coordinates	Region	Accession number
Amphipoda						
PS103HypC01	<i>Hyperiella dilatata</i>	PS103	053–3	63°47' S 50°06' W	Western WDL	MH482519
PS103HypC30	<i>Hyperiella dilatata</i>	PS103	005–7	59°04' S 00°05' E	APF zone	MH482523
PS103HypC31	<i>Hyperiella dilatata</i>	PS103	006–5	60°59' S 00°01' E	Lazarev Sea	MH482522
<b>PS103HypC36</b>	<i>Hyperiella dilatata</i> <sup>+</sup>	PS103	022–7	69°01' S 06°57' W	Kapp Norvegia	MH482521
<b>PS103HypC99</b>	<i>Hyperiella dilatata</i> <sup>o</sup>	PS103	030–1	71°17' S 14°06' W	Kapp Norvegia	MH482520
PS103HypC04	<i>Hyperiella antarctica</i>	PS103	001–5	45°57' S 06°17' E	SAF zone	MH482517
<b>PS103HypC34</b>	<i>Hyperiella antarctica</i> *	PS103	001–4	45°57' S 06°17' E	SAF zone	MH482518
<b>PS103HypC97</b>	<i>Hyperiella antarctica</i>	PS103	003–4	51°59' S 02°06' E	APF zone	MH482516
PS103HypC02	<i>Hyperoche capucinus</i>	PS103	023–4	65°59' S 12°15' W	Lazarev Sea	MH482540
PS103HypC05	<i>Hyperoche capucinus</i>	PS103	023–4	65°59' S 12°15' W	Lazarev Sea	MH482542
PS103HypC10	<i>Hyperoche capucinus</i>	PS103	040–7	65°43' S 36°41' W	Central WDL	MH482541
PS82C107	<i>Hyperoche capucinus</i>	PS82	227–1	74°19' S 37°41' W	NW Filchner area	MH482539
PS79Ant98	<i>Themisto gaudichaudii</i>	PS79	152–3	49°37' S 37°37' W	Scotia Sea	MH482546
PS103HypC08	Hyperiidea sp.	PS103	061–3	63°24' S 52°19' W	Western WDL	MH482538
PS82TgaC01	<i>Hyperiella macronyx</i>	PS82	003–1	73°45' S 25°46' W	NE Filchner area	MH482535
PS82TgaC10	<i>Hyperiella macronyx</i>	PS82	042–1	76°04' S 30°16' W	E Filchner area	MH482534
PS82TgaC18	<i>Hyperiella macronyx</i>	PS82	083–1	77°01' S 33°41' W	SE Filchner area	MH482533
PS82TgaC59	<i>Hyperiella macronyx</i>	PS82	107–1	77°55' S 38°03' W	Filchner Ice Shelf	MH482532
PS82TgaC60	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482531
PS82TgaC61	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482530
PS82TgaC63	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482529
PS82TgaC64	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482528
PS82TgaC65	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482527
PS82TgaC66	<i>Hyperiella macronyx</i>	PS82	107–1	77°55' S 38°03' W	Filchner Ice Shelf	MH482526
PS82TgaC67	<i>Hyperiella macronyx</i>	PS82	107–1	77°55' S 38°03' W	Filchner Ice Shelf	MH482525
PS82TgaC68	<i>Hyperiella macronyx</i>	PS82	117–1	77°36' S 38°55' W	Filchner Trough	MH482524
PS82TgaC84	<i>Hyperiella macronyx</i>	PS82	199–1	74°36' S 36°21' W	NW Filchner area	MH482537
PS103HypC13	<i>Hyperiella macronyx</i>	PS103	070–3	63°08' S 54°20' W	Near AP	MH482536
PS103HypC38	<i>Phronima</i> sp.	PS103	001–5	45°57' S 06°17' E	SAF zone	MH482543
PS103HypC15	<i>Cylopus</i> sp. (juvenile)	PS103	005–6	59°03' S 00°05' E	APF zone	MH482515
Pteropoda						
<b>PS103PteC35</b>	<i>Spongiobranchea australis</i> *	PS103	001–4	45°57' S 06°17' E	SAF zone	MH482544
PteSS143	<i>Spongiobranchea australis</i>	PS103	001–5	45°57' S 06°17' E	SAF zone	MH482545
<b>PS103PteC100</b>	<i>Clione limacina antarctica</i> <sup>o</sup>	PS103	030–1	71°17' S 14°06' W	Kapp Norvegia	MH482514
<b>PS103PteC37</b>	<i>Clione limacina antarctica</i> <sup>+</sup>	PS103	022–7	69°01' S 06°57' W	Kapp Norvegia	MH482513

For each amphipod or pteropod specimen, for which a COI barcode sequence was obtained during this study, species name and identification number, details on sample localities (expedition, station, coordinates) and GenBank accession numbers are given. *WDL* Weddell Sea, *AP* Antarctic Peninsula, *SAF* sub-Antarctic Front, *APF* Antarctic Polar Front, *n.d.* no data. Sample IDs in bold indicate the specimens from the amphipod-pteropod tandems and identical symbols (<sup>+</sup>, \*,<sup>o</sup>) next to the respective species names link the associated pteropod and amphipod specimens

DQ238002, GQ861834, GQ861833; *C. limacina antarctica*: GQ861822, AB859618, GQ861821) (Klussmann-Kolb and Dinapoli 2006; Hunt et al. 2010; Sromek et al. 2015). Specimen data and sequences generated and analysed during this study are available in the Barcode of Life Data System repository [<http://boldsystems.org>] (BOLD, Ratnasingham and Hebert 2007) under the project “Hyperiid amphipod –

pteropod associations in the Southern Ocean” with the code “HYPTTE” and with the GenBank accession numbers MH482513–MH482546. With all COI sequences of the hyperiidean species, a neighbour-joining tree was constructed with MEGA 6.06 (Tamura et al. 2013) with p-distances, with 2000 bootstrap replicates and applying the pairwise deletion option.



**Fig. 1** **a** Map of the Southern Ocean with the localities where hyperiid-pteropod associations have been reported previously and in this study. **b** Main map focusing on the Atlantic sector of the Southern Ocean with all four sampling localities of this study, corresponding station numbers and

the type of associations observed during R/V *Polarstern* expedition PS103. Abundance of each species is indicated per station. Maps generated with Ocean Data View (Schlitzer 2018)

## Results

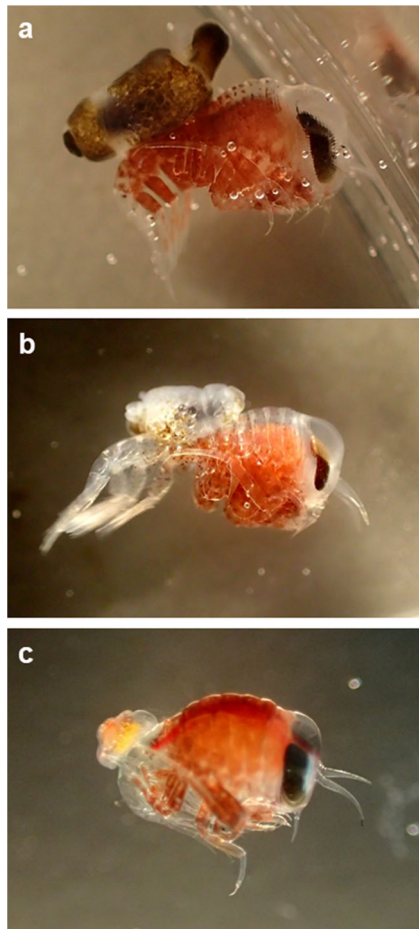
Only four hyperiid-pteropod tandem associations (Figs. 1 and 2) were observed from a total of 30 stations sampled with bongo nets during the R/V *Polarstern* expedition PS103. Each of the tandem associations occurred at a distinct station (Table 1), over a wide latitudinal range (45° to 71° S, Fig. 1) and hence, a significant gradient of sea surface temperature (6 to  $-0.2$  °C). Of the four amphipods found carrying a pteropod, the two individuals associated with *Spongiobranchea australis* were identified as *Hyperiella antarctica*, representing a new record of association (see Table 1 for details). The two specimens associated with *Clione limacina antarctica* were identified as *H. dilatata*, based on the diagnostic characters that separate the different *Hyperiella* species. These are the respective lengths of pereopods 5–7 as well as sharp vs. blunt processes on the antero-distal corners of the basis, ischium and merus of pereopods 5–7 (Weigmann-Haass 1989) and the postero-distal corners of the epimeral plates (Bowman 1973). The pteropods were identified by their morphology; COI barcodes confirmed their identification.

Hence, *Hyperiella antarctica* specimens carried the pteropod *Spongiobranchea australis* at stations ranging from the sub-Antarctic to the Antarctic Polar Front (45 and 51° S), and *H. dilatata* carried *Clione limacina antarctica* at stations in the eastern Weddell Sea (69 and 71° S) (Fig. 1). *Hyperiella*

abundances at these stations ranged from 0.006–0.010  $\text{ind m}^{-3}$ , and those of pteropods were equally low (0.003–0.027  $\text{ind m}^{-3}$ ). However, *Hyperiella* specimens were found in much higher abundances (0.14–0.75  $\text{ind m}^{-3}$ ) at stations situated near the sampling localities of the associations, whereas pteropods were equally abundant at the stations with or without associations.

Both male and female amphipods carried a pteropod and one of the four amphipods (HypC36) investigated was an ovigerous female. The size of the pteropods from the associations varied from one fifth to almost half of the amphipod's body size but never exceeded 5 mm (see Table 1 for details of all specimens). Other hyperiid species besides the ones from the association sampled during PS82 and PS103 were morphologically identified as *Hyperiella macronyx*, *Hyperoche capucinus*, *Themisto gaudichaudii* Guérin, 1825, *Phronima* sp. and an unidentified hyperiid specimen (see Table 2 for details).

In the neighbour-joining tree (Fig. 3) based on hyperiid COI sequences, species-level clusters are well-supported by high bootstrap values (100%). The two specimens associated with a *Clione* pteropod and identified as *H. dilatata* (HypC36 and HypC99) clustered together with other conspecifics. The specimens identified as *H. antarctica* (HypC34, HypC97), associated with *S. australis*, clustered together with another specimen identified as belonging to the same species



**Fig. 2** **a** *Hyperiella antarctica* carrying a large *Spongiobranchaea australis* at station 001–4 (45° S). **b** *H. antarctica* with a smaller *S. australis* at station 003–5 (51° S). **c** *H. dilatata* carrying a very small *Clione limacina antarctica* at station 022–7 (69° S)

(HypC04). Sequences retrieved from GenBank appeared to cluster within different groupings, which did not always correspond to their species identification. *H. dilatata* from East Antarctica (GenBank acc. KC754384; Cheng et al. 2013) clustered together with sequences from this study belonging to *H. macronyx* collected in the Filchner area. Two sequences identified as *H. antarctica* (GenBank acc. KC754381, KC754382) and sampled in East Antarctica (Cheng et al. 2013) were found in the same cluster as all *H. dilatata* sequences from this study. Hence, these specimens have most likely been misidentified by the authors. The node grouping *H. dilatata* and *H. antarctica*, as well as the node grouping these two species and *Hyperia macrocephala*, also received high bootstrap support. *Hyperiella macronyx* did not appear as sister species to the other two *Hyperiella* species; however, caution should be taken in interpreting distance-based analyses—most commonly used for identification purposes—as true phylogenetic relationships. Deeper nodes all received low bootstrap values (< 75%), showing that interrelationships

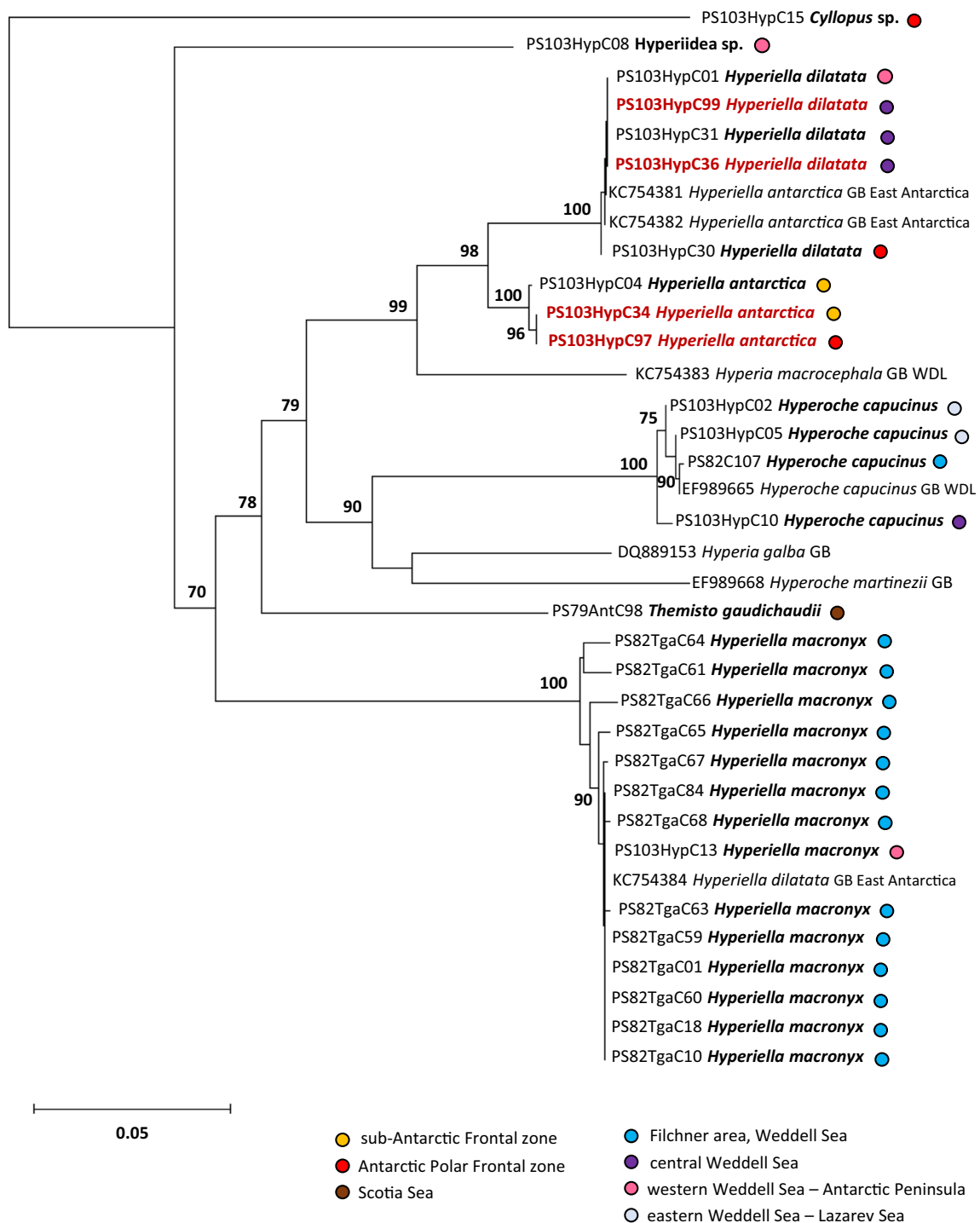
between the different hyperiidean species remain largely unresolved based on COI sequences.

## Discussion

### Distributional patterns of hyperiideans, pteropods and tandem associations

The genus *Hyperiella* currently consists of three species and is endemic to the Southern Ocean, found generally south of 55° S (Zeidler and De Broyer 2009, 2014). Of the three species, *H. antarctica* has been found furthest north with records from the Antarctic continent to the sub-Tropical Front (Zeidler and De Broyer 2009, 2014) and seems to have a lesser affinity to sea ice than its congeners (Flores et al. 2011). In this study, it was collected between the sub-Antarctic and the Antarctic Polar Front, corroborating previous studies. Weigmann-Haass (Weigmann-Haass 1989) recorded *H. dilatata* as relatively common around the tip of the Antarctic Peninsula and in the Weddell Sea, corroborated by our records (see Fig. 3). In our samples, we found *H. macronyx* throughout the entire Filchner area in the southeastern Weddell Sea, whilst no other *Hyperiella* species was encountered here, which may be explained by its affinity to colder waters and ice conditions compared to *H. antarctica* and *H. dilatata*.

Around 40 species of gymnosome pteropods have been reported in the Atlantic sector of the Southern Ocean; however, only four species represent the typical Southern Ocean diversity (Roberts et al. 2014). These are *C. limacina antarctica* and *Platybrachium antarcticum* Minichev, 1976 as most common species in the Antarctic, whereas *S. australis* and *Pneumodermopsis brachialis* Minichev, 1976 are the common sub-Antarctic species (Roberts et al. 2014). Both *C. limacina antarctica* and *S. australis* reported here are distributed from the sub-Tropical convergence to the Antarctic coast; however, *S. australis* is more common north of the Polar Front, in the Polar Frontal Zone and the sub-Antarctic zone, whereas *C. limacina antarctica* is considered a typical Antarctic species (Hunt et al. 2008; Loeb et al. Loeb and Santora 2013). *Clione* can reach high abundances during the austral summer in nearshore Antarctic waters (Foster 1989) and is seasonally found more frequently under sea ice (Flores et al. 2011). This was reflected in our sampling: In the two northernmost localities (45° S and 51° S), *Spongiobranchaea* was found, whereas *Clione* was not present; conversely, in the two southernmost stations (69° S and 71° S), *Clione* was present, but no *S. australis*. Maximum abundances of gymnosome pteropods could not be linked to the occurrences of tandem associations with amphipods. At the stations, where tandems were found, abundances of *Hyperiella* were much lower compared to other stations without tandems, whereas pteropod abundances did not vary to such an extent between these



**Fig. 3** Neighbour-joining tree based on p-distances of COI sequences belonging to the sampled hyperiidean amphipods from the tandem associations (indicated in red), as well as other hyperiidean specimens sampled during PS82 and PS103 (species names indicated in bold; for sample details see Table 2) and GenBank sequences (Browne et al. 2007;

Costa et al. 2007; Cheng et al. 2013, indicated as GB). For the specimens sampled in this study, sampling regions were indicated by coloured circles, for which a legend is added. For the GenBank sequences, if known, localities are specified (WDL Weddell Sea). Bootstrap values (number of replicates = 2000) are indicated when  $\geq 75\%$

stations. Possibly, tandem associations are present in localities, where predation pressure on the hyperiid amphipods is higher, resulting in lower amphipod abundances. Since the

pteropod abduction is facultative and instantly reversible, the occurrence of this association could well be dependent on abundances of amphipod predators.



## Hypotheses on the occurrence of the anti-predatory behaviour in shelf waters versus the open ocean

Despite the fact that *Hyperiella* specimens, as well as gymnosome pteropods, were sampled at more than half of the total number of stations, observations on the abduction of pteropods remain limited to four individuals from the 342 sampled during the expedition. This contrasts with the high occurrences of tandem associations (74% of all observed amphipods) reported for the Antarctic shelf waters of McMurdo Sound (McClintock and Janssen 1990). It is possible that abducted pteropods could have been released by the amphipods during the sampling process, resulting in these few records of tandems. Also, net catches used for this study are not comparable to the in situ observations by divers for the Antarctic shelf study (McClintock and Janssen 1990). However, the sampled amphipods did not release the pteropods in stressful conditions, e.g. during observations in a Petri dish or after several days in an aquarium. Moreover, the great tenacity with which the “abductor” grasps the pteropod even persists after fixation in a preservative (RNA later, pers. obs.). This is plausible bearing in mind the anti-predatory nature of this behaviour. Hence, it is likely that the observed lower frequency of pteropod abduction is not merely a sampling artefact but reflects a truly lower frequency of this association in the Atlantic sector of the Southern Ocean than in Ross Sea shelf waters.

This may be explained by the fact that the clear, shallow waters do not offer much protection from fish predators and anti-predation behaviour may be more frequent, whereas the colonization of the open ocean can already be interpreted as a strategy to avoid demersal predators. McClintock and Janssen (1990) and McClintock and Baker (1998) demonstrated that avoidance of the tandem associations by notothenioid fish is certainly based on chemical cues, since fishes rejected the ingested pairs, but the deterrence also works visually, based on observations of fish actively swimming away from the encountered pairs. Other studies report notothenioid prey detection to be visual, particularly in the absence of chemical cues, as shown for the Antarctic fish *Trematomus newnesi* Boulenger, 1902 (Fanta et al. 1999). In situ observations around McMurdo Sound (McClintock and Janssen 1990) showed that further offshore at greater depths (50 m), the portion of hyperiideans carrying pteropods was significantly lower (6%) than in shallow nearshore waters (up to 74%). Whether this reflects a lower abundance of notothenioid and other predators, or a higher efficiency of tandem pair formation in clear waters as a visual deterrent for predators, needs to be clarified. Near the Antarctic Peninsula, where tandem associations occur as well, hyperiideans are highly preyed upon by bottom-dwelling and benthopelagic fishes relying on pelagic prey as major component of their diet (e.g. Casaux et al. 1990; Barrera-Oro Barrera-Oro 2003).

In this study, tandem associations were reported from two distinct open-water regions across a wide environmental gradient: the oceanic Polar Frontal zone and near-shelf sites in the eastern Weddell Sea. Potential *Hyperiella* predators at open ocean sites around 50° S include gulp-feeders such as myctophids and grappling raptorial predators such as juvenile squid, as well as the predatory amphipod *Themisto gaudichaudii*. Myctophids are known to be very abundant (Duhamel et al. 2014), and their predation impact is substantial in this area (Filin et al. 1991). Several species prey on hyperiidean amphipods (Pakhomov et al. 1999). Squid are major predators in the Polar Frontal Zone in the Scotia Sea, mostly feeding on myctophids in this region (Rodhouse and White 1995); however, hyperiidean amphipods could represent an important food source for juvenile squid. Unlike gulp-feeding fish, small squid or predatory amphipods may be able to dislodge the *Clione* specimen, before feeding on *Hyperiella*. However, in feeding experiments, we exposed *Hyperiella* individuals to the predatory amphipod *T. gaudichaudii*, but the latter did not feed upon *Hyperiella* in any of the replicates (pers. obs.). Apparently, *Hyperiella* does not seem to belong to its usual prey items.

### Benefit or trade-off?

A balanced trade-off for *Hyperiella* may exist between slower swimming speeds to feed and escape whilst carrying the pteropod versus an instant protection from predation, as hypothesized by McClintock and Janssen (1990) and McClintock and Baker (1998). Regardless of this hypothesis, the ability to swim faster for animals such as amphipods becomes irrelevant, when the dominant predators are large and faster-moving nektonic animals such as squid and fish. As mentioned by McClintock and Janssen (1990), the amphipod has the energetic cost of carrying the pteropod but gains the benefit of predator protection. One associated amphipod observed here was a female carrying eggs, and hence, the effort of carrying a pteropod may result in protection for both the female and the offspring. This may be strongly beneficial for its inclusive fitness and result in a strong selective pressure for abducting individuals.

The benefits for the pteropod remain unclear. Despite saving energy while being carried, it may be enforced starvation, as it is held tight between the amphipod's legs, which renders it incapable of feeding. This was reflected in the lipid composition of both abducted and free-living pteropods (Phleger et al. 1999): Abductees bear significantly less triacylglycerols—a rapidly mobilized lipid class for energy storage—indicating feeding inhibition. Nonetheless, the Arctic *Clione* can survive 1 year in the aquarium without being fed (Böer et al. 2006). Hence, if the Antarctic individuals—despite their high genetic divergence from the Arctic ones (Sromek et al. 2015)—have a similar physiology (Kattner et al. 1998), they could be carried

around for quite some time without having to replace it by a 'fresh' pteropod too frequently. Lacking speed or armour to escape predation, gymnosomes invested in chemical defence, but it is yet unknown how physiologically expensive the production of pteroenone is and if production continues even during starvation. Conover and Lalli (1974) found that feeding greatly increased the metabolic intensity in *Clione*, suggesting that a fed animal is 20 times more metabolically active than a starved one. However, since pteroenone is not derived from the diet (Phleger et al. 1999), starvation does not necessarily impact the production of this metabolite.

### Species-specific interactions and co-evolution?

Associations between amphipods and pteropods are rare (reviewed in Laval 1980). Known examples include the hyperiideans *Lycaea* spp., *Tetrathyrus forcipatus* Claus, 1879 and *Streetsia steenstrupi* (Bovallius, 1887) which have been found on the pseudothecosome pteropods *Corolla spectabilis* Dall, 1871 or *Gleba cordata* Forsskål in Niebuhr, 1776 and the hyperiidean *Brachyscelus rapacoides* Stephensen, 1925, which was attached to the euthecosome pteropod *Diacavolinia longirostris* (Lesueur, 1821) (Harbison et al. 1977; Gasca and Haddock 2004). However, these reports mention the use of pteropods as a host for hyperiideans rather than the amphipods carrying them as defence mechanism. No damage to the pteropod was reported in these cases, and therefore, the nature of these associations remains unclear (Harbison et al. 1977). For other associations of hyperiids with gelatinous zooplankton, cases of host specificity often are held responsible for separating niches of sympatric species (e.g. for *Primno* species, Shearer and Batten 1995). Contrary to previous observations, our results indicate that amphipod-pteropod associations may be species-specific and occur in at least two sister species, *H. dilatata* and *H. antarctica*; however, our sampling size is too small to confirm this. *Hyperiella* species are distinguished by distinctly shaped projections on the corners of pereopods 5, 6 and 7, which are the legs being used to hold the pteropod in position on the dorsum whilst swimming (see Fig. 2). Hence, these processes may serve a function in grasping the abductee and could be specific for the species of pteropod used. In case of a benefit for the pteropod, a potential co-evolution between the amphipod and pteropod species could also have resulted in a tolerance of the abducting amphipod to the nuisance of pteroenone. If these molecules differ between the different gymnosomes, species-specific interactions may have been more likely to evolve. Further analyses should reveal whether this specificity holds true, in particular in the geographic regions, where these associations are very common (Ross Sea: McClintock and Janssen 1990; Elephant Island: Phleger et al. 1999), or whether the use of a particular pteropod species as defence is determined locally by its abundance in the water column. Does the proportion of abducting amphipods increase in relation with predation

pressure? Which regional predators are being deterred by the chemical defence? These questions need to be answered in order to unravel the evolutionary pathways that determined this phenomenon, so far only reported from the Southern Ocean. With the rise of technological advances in underwater imaging, it may well be that such interactions will appear to be much more common across the World's Oceans than what we have been able to document so far.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national and/or institutional guidelines for the care and use of animals (invertebrates) were followed.

**Sampling and field studies** The necessary permit for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.

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