Supplementary Materials

Gastropod assemblages associated with *Himantothallus grandifolius, Sarcopeltis antarctica,* and other subtidal macroalgae

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Contents:

Table S1. Number of individual gastropods by species per 100 g wet weight of alga found on five species of macroalgae. Means for combined depths ± S.E.

Gastropod Species	***Himantothallus grandifolius***	***Sarcopeltis antarctica***	***Desmarestia anceps***	***Desmarestia antarctica***	***Plocamium* sp.**
Cerithiella austrina	0.0005 ± 0.0005	0.01 ± 0.01			
Eatoniella caliginosa	7.42 ± 3.98	6.20 ± 1.43	2.02 ± 0.50	0.74 ± 0.62	129.8 ± 87.4
Eatoniella cana	0.88 ± 0.36	1.95 ± 0.79	0.57 ± 0.37		39.6 ± 32.7
Eatoniella kerguelensis regularis	1.41 ± 0.54	6.48 ± 3.05	0.44 ± 0.18		277.2 ± 98.5
Eatoniella sp.	0.01 ± 0.01	0.16 ± 0.15			5.00 ± 3.50
Laevilacunaria antarctica	0.57 ± 0.11	2.43 ± 0.53	4.08 ± 0.85	2.83 ± 0.62	1.21 ± 0.61
Laevilacunaria bennetti	0.01 ± 0.01	0.22 ± 0.07	0.45 ± 0.41	0.77 ± 0.41	0.98 ± 0.60
Laevilitorina calignosa	0.15 ± 0.05	0.14 ± 0.08		0.06 ± 0.06	
Laevilitorina wandelensis	0.10 ± 0.05	0.10 ± 0.05			4.33 ± 2.03
Laevilitorina umbilicata	0.02 ± 0.01	0.10 ± 0.04	0.17 ± 0.11		0.57 ± 0.46
Liotella endeavourensis		0.02 ± 0.02	0.08 ± 0.04		
Margarites sp.			0.01 ± 0.01		61.4 ± 35.8
Margarella antarctica	1.87 ± 0.35	4.08 ± 0.88	1.04 ± 0.36		12.6 ± 5.6
Microdiscula sp.		0.04 ± 0.04			
Munditia meridionalis	2.32 ± 1.12	4.88 ± 0.92	1.61 ± 0.42	0.22 ± 0.14	109.3 ± 43.6
Nacella concinna	0.17 ± 0.03	0.17 ± 0.06			0.23 ± 0.23
Omalogyra antarctica	0.17 ± 0.11	0.11 ± 0.04	0.02 ± 0.01		18.2 ± 5.5
Onoba grisea	0.28 ± 0.19	1.32 ± 0.38	0.09 ± 0.06	0.06 ± 0.06	30.0 ± 10.0
Onoba kergueleni	0.01 ± 0.01	0.55 ± 0.24	0.28 ± 0.21		3.13 ± 3.13
Pellilitorina pellita	0.03 ± 0.01	0.06 ± 0.03	0.15 ± 0.10		2.16 ± 1.45
Pellilitorina setosa	0.0003 ± 0.0003	0.63 ± 0.22	0.03 ± 0.03		0.57 ± 0.46
Prosipho sp. 1	0.01 ± 0.004	0.19 ± 0.11			13.2 ± 6.08
Prosipho sp. 2			0.01 ± 0.01		0.88 ± 0.56
Prosipho sp. 3					0.23 ± 0.23
Protonuptea sp.		0.02 ± 0.02	0.01 ± 0.01		
Rissoella powelli	0.0005 ± 0.0005	0.09 ± 0.04	0.03 ± 0.02		11.8 ± 6.01
Scissurella petermannensis	0.03 ± 0.01	0.71 ± 0.37	0.03 ± 0.02	0.06 ± 0.06	1.14 ± 0.62
Skenella paludinoides	1.36 ± 1.02	0.44 ± 0.19	0.07 ± 0.04		28.8 ± 19.8
Skenella umbilicata	12.3 ± 5.34	7.39 ± 1.76	5.57 ± 2.36	1.33 ± 0.99	208.6 ± 31.6
Subonoba turqueti	0.95 ± 0.71	0.92 ± 0.39	0.02 ± 0.02	0.14 ± 0.12	5.09 ± 3.84
Toledonia palmeri	0.11 ± 0.04	3.28 ± 1.06	0.20 ± 0.08	0.20 ± 0.13	26.9 ± 11.7
Torellia planispira	0.01 ± 0.01	0.01 ± 0.01			
Trophon minutus	0.0005 ± 0.0005	0.02 ± 0.01			0.45 ± 0.45
unknown Littorinidae			6.65 ± 3.02		2.12 ± 1.01
unknown sp. 1					0.53 ± 0.53

Table S2. Pairwise ANOSIM tests for differences between gastropod assemblages for shallow vs. deep collections at individual common sites for *Himantothallus grandifolius* and *Sarcopeltis antarctica*. Brown text highlights the only instance where the two depths at a site were not significantly different from each other.

	R	p
Himantothallus grandifolius		
Christine Is. shallow vs. deep	0.421	0.006
Southeast Bonaparte Pt. shallow vs. deep	-0.04	0.516
East Litchfield Is. shallow vs. deep	0.888	0.008
Stepping Stones Is. shallow vs. deep	0.832	0.008
Sarcopeltis antarctica		
Southeast Bonaparte Pt. shallow vs. deep	0.696	0.008
Hermit Is. cove shallow vs. deep	0.944	0.008
Stepping Stones Is. shallow vs. deep	0.676	0.008

Table S3. One-way SIMPER analysis of species making greatest contributions to similarity and differences in depth distributions gastropod assemblages on *Himantothallus grandifolius*. **Contrib%** = percent contribution to the similarity or dissimilarity. **Cum.%** = cumulative percentage of species' contributions. The analyses were terminated when the cumulative percentage exceeded 70%.

Species	Contrib%	Cum.%
Shallow similarity		
Skenella umbilicata	34.41	34.41
Eatoniella caliginosa	18.71	53.12
Laevilacunaria antarctica	17.65	70.77
Deep similarity		
Skenella umbilicata	16.83	16.83
Margarella antarctica	16.77	33.6
Eatoniella caliginosa	12.28	45.88
Laevilacunaria antarctica	9.39	55.27
Eatoniella kerguelensis regularis	9.29	64.56
Munditia meridionalis	8.87	73.43
Shallow-deep dissimilarity		
Eatoniella caliginosa	10.19	10.19
Margarella antarctica	9.78	19.97
Eatoniella kerguelensis regularis	8.76	28.73
Eatoniella cana	7.68	36.41
Skenella umbilicata	7.56	43.97
Munditia meridionalis	6.86	50.83
Nacella concinna	5.71	56.54
Skenella paludinoides	4.79	61.33
Laevilacunaria antarctica	4.74	66.07
Subonoba turqueti	4.36	70.43

Table S4. One-way SIMPER analysis of species making greatest contributions to similarity and differences in depth distributions gastropod assemblages on *Sarcopeltis antarctica*. **Contrib%** = percent contribution to the similarity or dissimilarity. **Cum.%** = cumulative percentage of species' contributions. The analyses were terminated when the cumulative percentage exceeded 70%.

Species	Contrib%	Cum.%
Shallow similarity		
Skenella umbilicata	22.24	22.24
Munditia meridionalis	18.87	41.11
Eatoniella caliginosa	17.88	58.99
Laevilacunaria antarctica	16.49	75.48
Deep similarity		
Munditia meridionalis	11.41	11.41
Eatoniella caliginosa	11.07	22.48
Margarella antarctica	9.98	32.46
Eatoniella kerguelensis regularis	9.55	42.01
Skenella umbilicata	9.33	51.35
Toledonia palmeri	9.29	60.64
Laevilacunaria antarctica	5.91	66.55
Eatoniella cana	5.19	71.74
Shallow-deep dissimilarity		
Eatoniella kerguelensis regularis	9.44	9.44
Toledonia palmeri	6.9	16.34
Eatoniella cana	6.12	22.46
Margarella antarctica	5.44	27.9
Eatoniella caliginosa	5.33	33.22
Pellilitorina setosa	5.24	38.47
Onoba grisea	5.21	43.68
Subonoba turqueti	5.06	48.74
Scissurella petermannensis	5.02	53.76
Munditia meridionalis	4.35	58.11
Skenella umbilicata	4.3	62.41
Skenella paludinoides	4.06	66.47
Laevilacunaria antarctica	3.97	70.43

Table S5. Pairwise ANOSIM tests for differences between unordered species-depth groupings. Brown text highlights the only two pairs that were not significantly different from each other.

Groups	**R**	**p**
H. grandifolius-Shallow, *H. grandifolius*-Deep	0.445	0.0001
H. grandifolius-Shallow, *D. anceps*-Shallow	0.374	0.0007
H. grandifolius-Shallow, *D. anceps*-Deep	0.427	0.0003
H. grandifolius-Shallow, *D. antarctica*-Shallow	0.768	0.0001
H. grandifolius-Shallow, *S. antarctica*-Shallow	0.288	0.0001
H. grandifolius-Shallow, *S. antarctica*-Deep	0.793	0.0001
H. grandifolius-Shallow, *Plocamium* sp.-Deep	0.987	0.0001
H. grandifolius-Shallow, *Plocamium* sp.-Shallow	0.905	0.0001
H. grandifolius-Deep, *D. anceps*-Shallow	0.595	0.0001
H. grandifolius-Deep, *D. anceps*-Deep	0.546	0.0001
H. grandifolius-Deep, *D. antarctica*-Shallow	0.907	0.0001
H. grandifolius-Deep, *S. antarctica*-Shallow	0.351	0.0001
H. grandifolius-Deep, *S. antarctica*-Deep	0.317	0.0002
H. grandifolius-Deep, *Plocamium* sp.-Deep	0.858	0.0001
H. grandifolius-Deep, *Plocamium* sp.-Shallow	0.674	0.0002
D. anceps-Shallow, *D. anceps*-Deep	-0.003	0.465
D. anceps-Shallow, *D. antarctica*-Shallow	0.458	0.0001
D. anceps-Shallow, *S. antarctica*-Shallow	0.123	0.101
D. anceps-Shallow, *S. antarctica*-Deep	0.743	0.0001
D. anceps-Shallow, *Plocamium* sp.-Deep	0.928	0.0002
D. anceps-Shallow, *Plocamium* sp.-Shallow	0.689	0.0005
D. anceps-Deep, *D. antarctica*-Shallow	0.648	0.0002
D. anceps-Deep, *S. antarctica*-Shallow	0.183	0.047
D. anceps-Deep, *S. antarctica*-Deep	0.769	0.0001
D. anceps-Deep, *Plocamium* sp.-Deep	0.984	0.0003
D. anceps-Deep, *Plocamium* sp.-Shallow	0.717	0.0008
D. antarctica-Shallow, *S. antarctica*-Shallow	0.725	0.0001
D. antarctica-Shallow, *S. antarctica*-Deep	0.905	0.0001
D. antarctica-Shallow, *Plocamium* sp.-Deep	0.900	0.0001
D. antarctica-Shallow, *Plocamium* sp.-Shallow	0.864	0.0003
S. antarctica-Shallow, *S. antarctica*-Deep	0.523	0.0001
S. antarctica-Shallow, *Plocamium* sp.-Deep	0.939	0.0001
S. antarctica-Shallow, *Plocamium* sp.-Shallow	0.679	0.0001
S. antarctica-Deep, *Plocamium* sp.-Deep	0.821	0.0001
S. antarctica-Deep, *Plocamium* sp.-Shallow	0.777	0.0002
Plocamium sp.-Deep, *Plocamium* sp.-Shallow	0.851	0.004

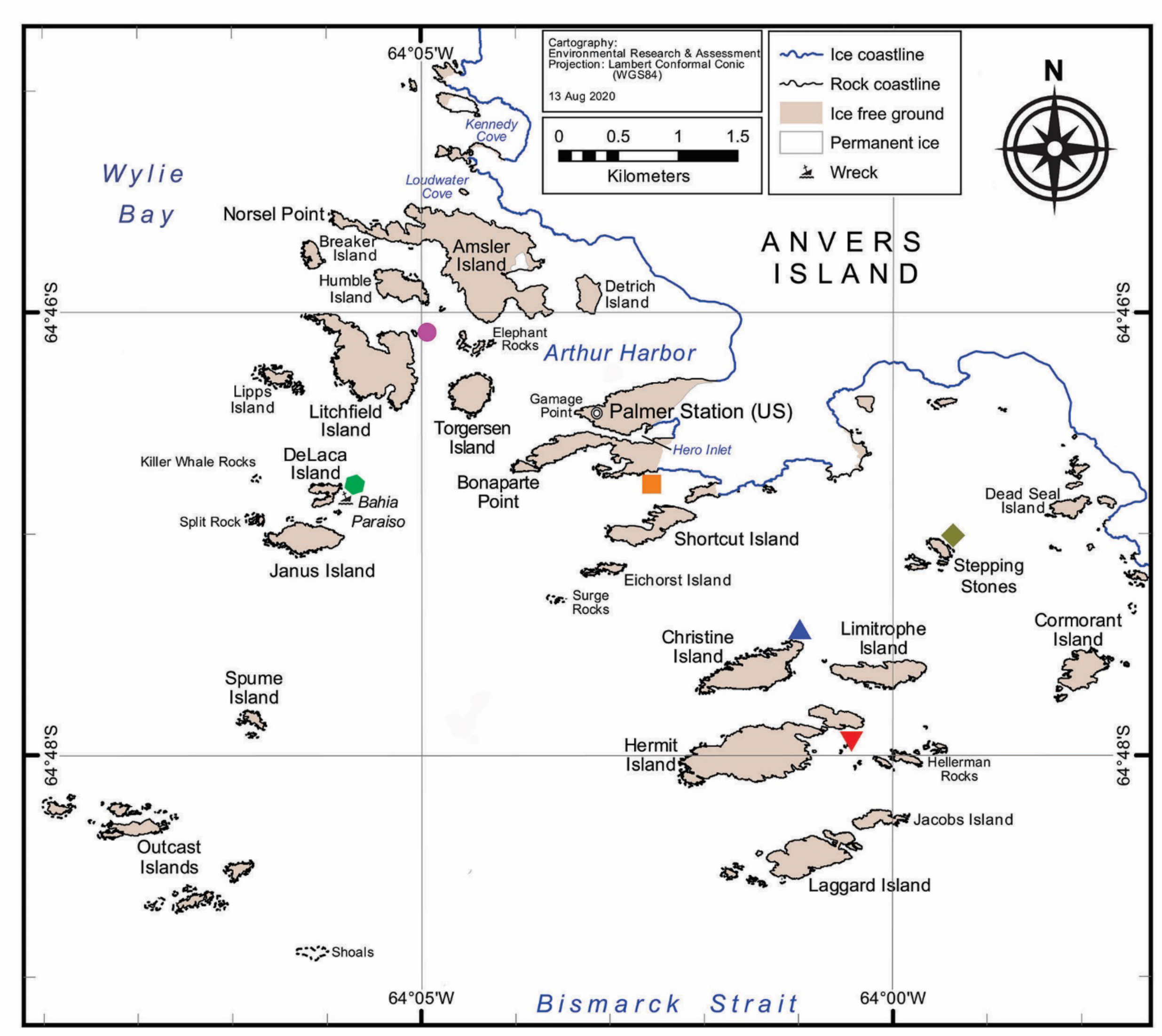

Fig. S1. Map of collection sites near Palmer Station, Antarctica: **purple circle**, "East Litchfield;" **green hexagon**, wreck of the ARA *Bahía Paraíso* and adjacent southeast corner of DeLaca Island; **orange square**, "Southeast Bonaparte;" **brownish-green diamond**, Stepping Stones Islands; **blue triangle**, Christine Island; **inverted red triangle**, "Hermit Cove."

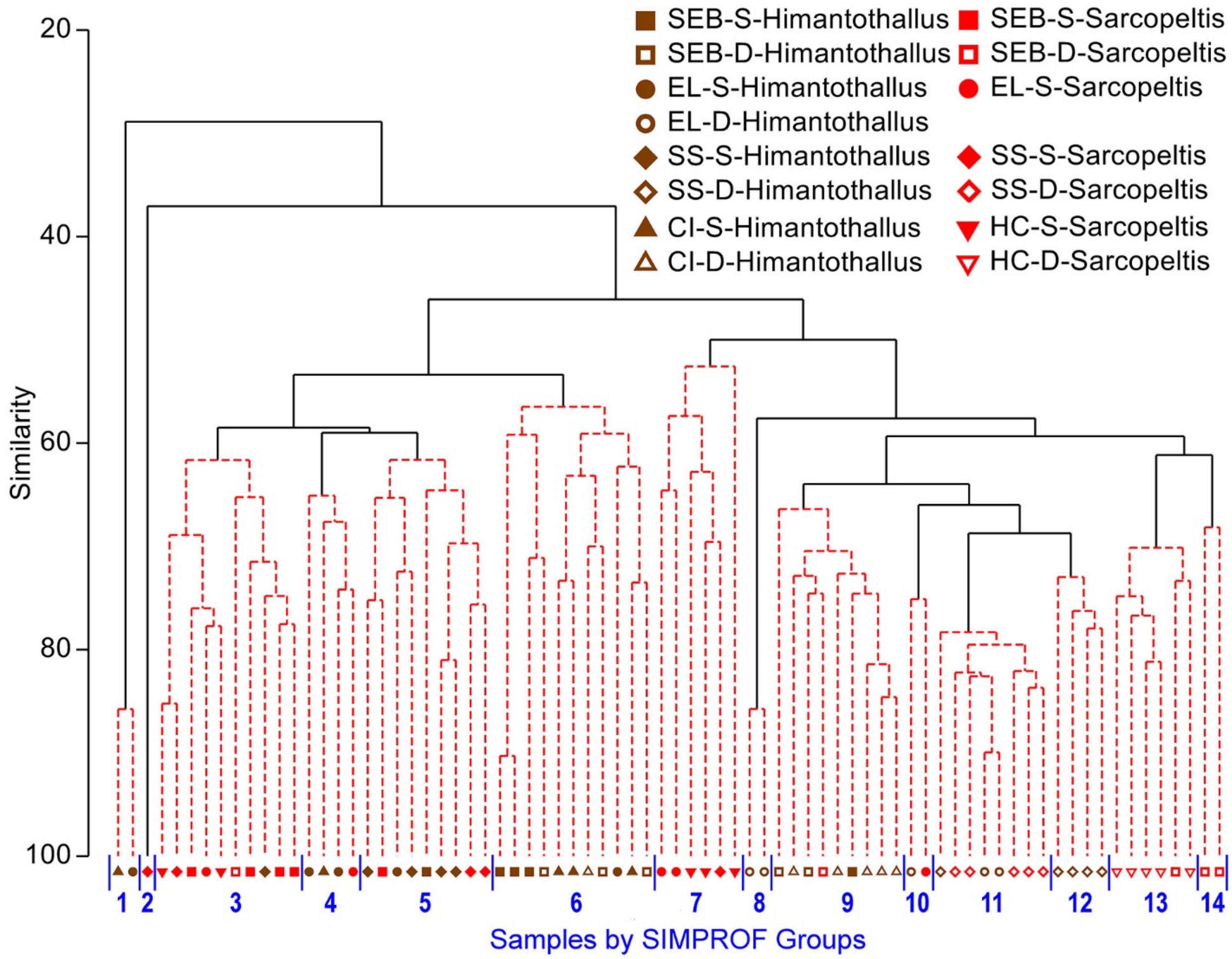

Fig. S2. CLUSTER analysis with SIMPROF test of gastropod assemblages on individual *Himantothallus grandifolius* (brown symbols) and *Sarcopeltis antarctica* (red symbols). Bray-Curtis similarities calculated from fourth-root transformed data. SIMPROF groups are significantly different from each other ($p = 0.05$). Site code: **SEB** = "Southeast Bonaparte Point", **EL** = "East Litchfield Island", **SS** = Stepping Stones Islands, **CI** = Christine Island, **HC** = "Hermit Cove". Middle code: **S** = shallow (9 m), **D** = deep (18 m).

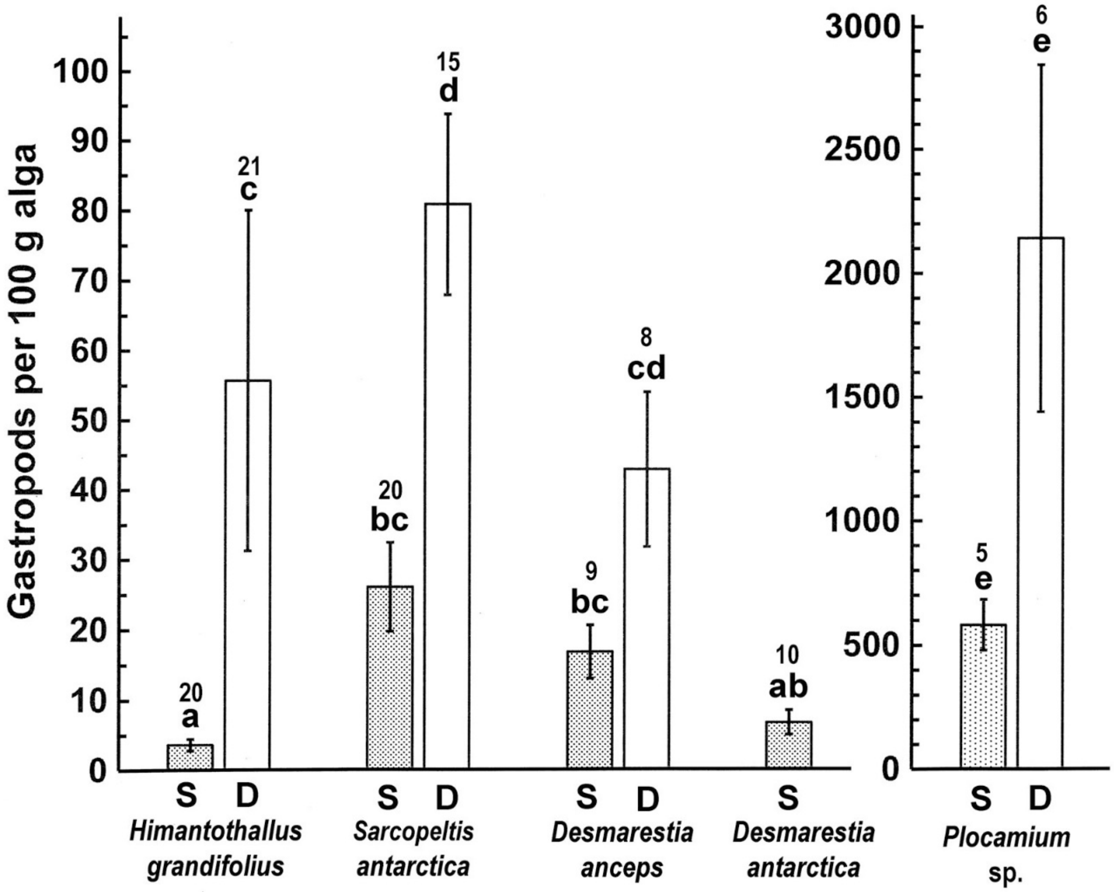

Fig. S3. Numbers of individual gastropods on macroalgal species per 100 g wet weight of alga. Means ± S.E. **S** = shallow samples (see main and supplementary text). **D** = deep samples (see main and supplementary text). Bars with same letters above mean are not significantly different from each other (Dunn-Sidak corrected p = 0.1; see supplementary text). Numbers above bars indicate sample size (n) for that depth.

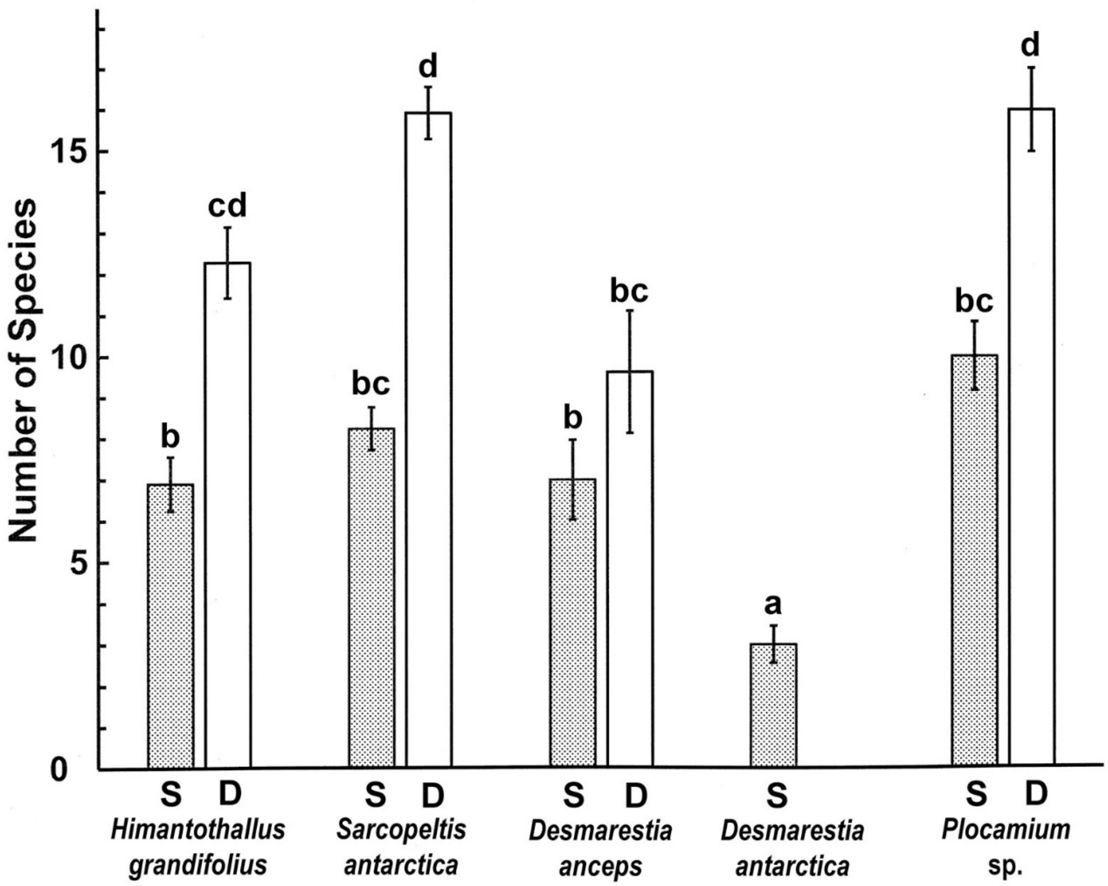

Fig. S4. Number of gastropod species on macroalgae. Means ± S.E. **S** = shallow samples (see main and supplementary text). **D** = deep samples (see main and supplementary text). Bars with same letters above mean are not significantly different from each other (p = 0.05). Sample sizes as in Fig. S3.

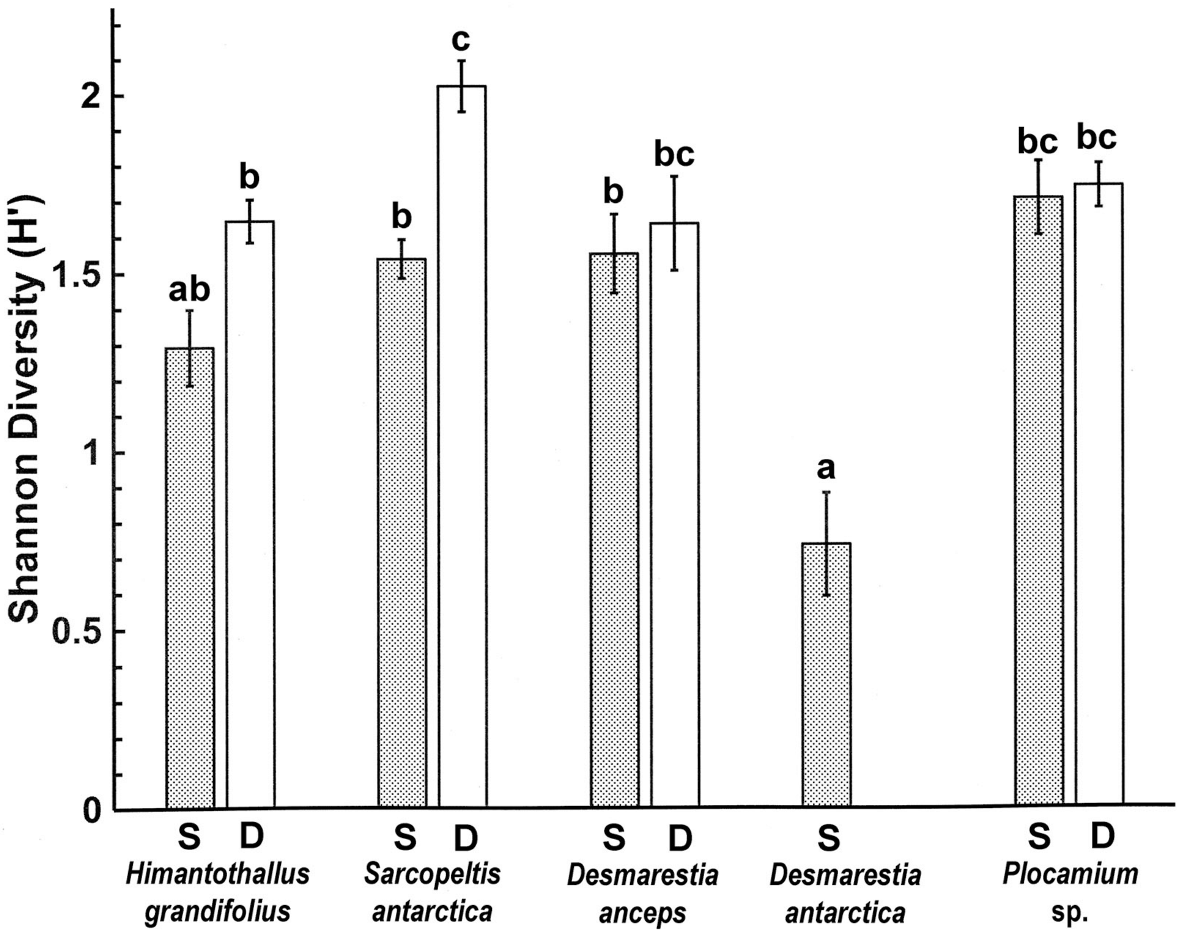

Fig. S5. Shannon Diversity index (H') for gastropod assemblages on macroalgae. Means ± S.E. **S** = shallow samples (see main and supplementary text). **D** = deep samples (see main and supplementary text). Bars with same letters above mean are not significantly different from each other (Dunn-Sidak corrected p = 0.1; see supplementary text). Sample sizes as in Fig. S3.

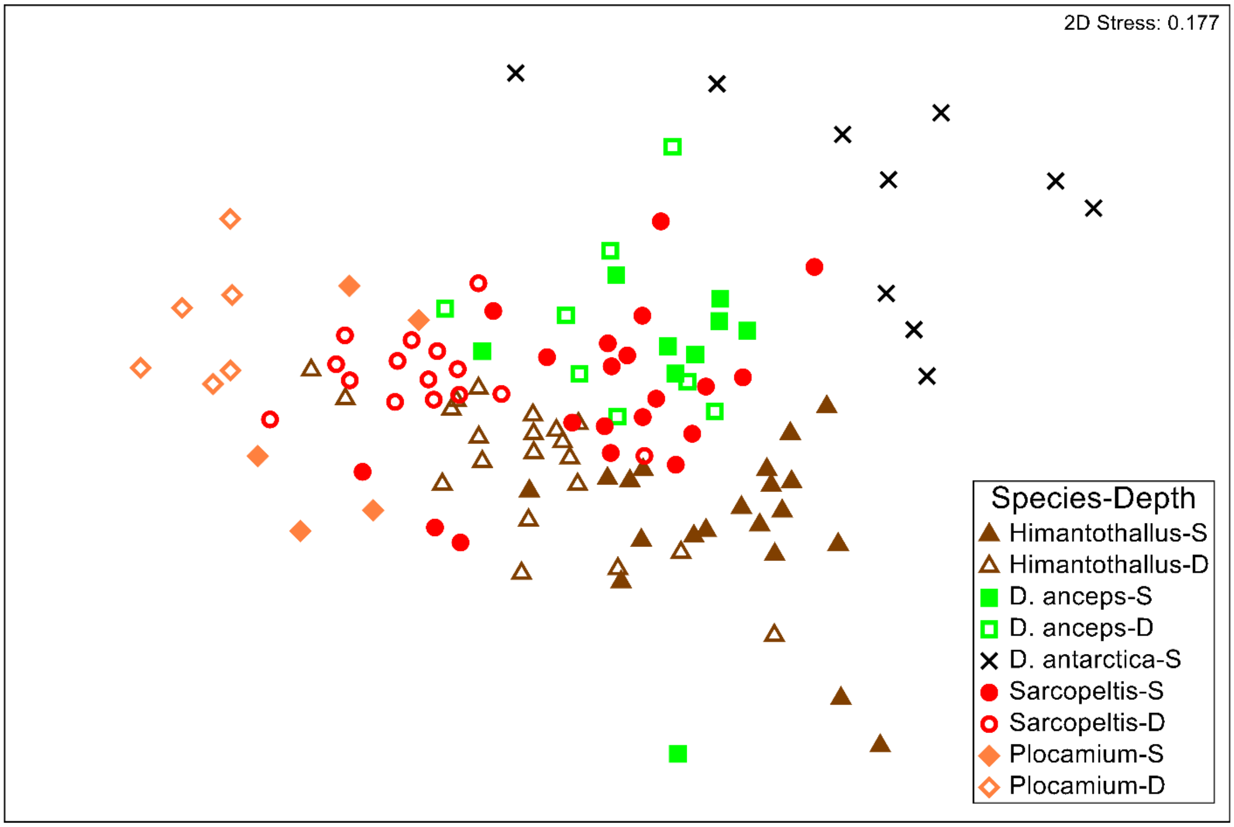

Fig. S6. Two-dimensional nMDS ordination of gastropod assemblages on all macroalgal species by depth. Bray-Curtis similarities calculated from fourth-root transformed data. S = shallow samples (see main and supplementary text). D = deep samples (see main and supplementary text).

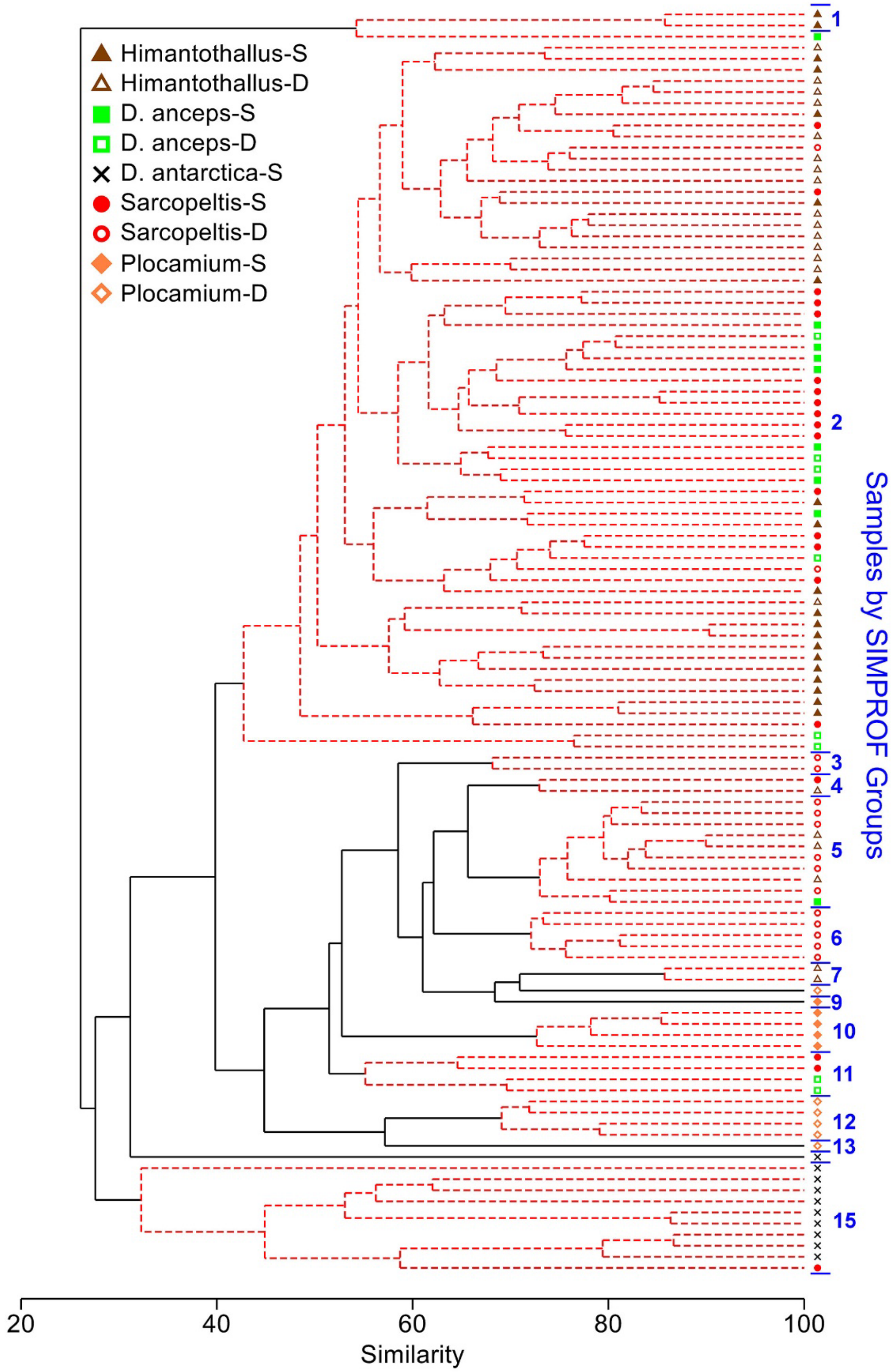

Fig. S7. CLUSTER analysis with SIMPROF test of gastropod assemblages on all macroalgal species. Bray-Curtis similarities calculated from fourth-root transformed data. SIMPROF groups are significantly different from each other ($p = 0.05$). S = shallow samples (see main and supplementary text). D = deep samples (see main and supplementary text).

Supplementary Materials and Methods:

In addition to the collecting sites referred to in the main paper, additional species were also collected from the wreck of the ARA *Bahía Paraíso* and adjacent southeast corner of DeLaca Island (S 64°46.827', W 64°05.750'; green hexagon on Fig. S1). *Desmarestia anceps* Montagne was collected from 9 ± 1 m depth and 21 ± 2 m depth at the *Bahía Paraíso* (five individuals at 9 m and four at 21 m) and "Hermit Cove" (four individuals at each depth). *Desmarestia antarctica* R.L. Moe & P.C. Silva was collected at 9 m depth only at the *Bahía Paraíso* and "Southeast Bonaparte" (five individuals at each site). *Plocamium* sp. was collected at 9 m depth and 26 m depth at "East Litchfield" (five individuals at 9 m, 2 individuals at 26 m) and 26 m depth only at "Southeast Bonaparte" (four individuals). In the past, we and others have used the name *P. cartilagineum* (L.) P.S. Dixon for *Plocamium* collected along the WAP. However, the WAP entity is genetically distinct from all other, non-Antarctic *Plocamium* spp. including *P. cartilagineum* (Hommersand *et al.* 2009, Young *et al.* 2013, Dubrasquet *et al.* 2018, Guillemin *et al.* 2018) so we now refer to the taxon as *Plocamium* sp.

To remove gastropods from the algae after transport to Palmer Station, the mesh bags were inverted into buckets and thoroughly rinsed with seawater followed by visual inspection and by-hand removal of any gastropods remaining on the bags. The thalli were further rinsed in successive buckets with fresh seawater and then examined visually to ensure that all gastropods had been separated from the thalli, removing by hand any that remained.

Gastropod abundance and Shannon diversity data could not be transformed to satisfy assumptions of parametric statistics so were analyzed by the nonparametric Kruskal-Wallis H test. Post hoc pairwise comparisons of diversity between species and depths utilized pairwise Mann-Whitney U tests corrected for type I error with the Sequential Dunn–Sidak Method (Sokal & Rohlf 1995). Because of the very large number of pairwise comparisons, alpha was set at 0.1 for the error corrections. Gastropod species number data were transformed by square-root (x+0.5) as recommended by Zar (1998) in order to meet equality of error variances as determined by Levene's tests and normality as determined by both the Kolmogorov-Smirnov and Shapiro-Wilk tests and were then compared with univariate General Linear Models tests. Post hoc analyses between individual species and depths were made with a Tukey multiple comparison test.

Supplementary Results:

A total of 35 shelled gastropod species were identified on the five macroalgal species (Table S1). To enable comparisons between bladed and finely-branched macroalgal species, gastropod numbers in the supplementary material are expressed on a per 100 g wet weight of algal thallus basis. Gastropod numbers per algal biomass differed significantly across depths and species ($H_8 = 74.722$, $p < 0.0005$; Fig. 2). Both *H. grandifolius* and *S. antarctica* supported significantly higher total numbers of gastropods at 18 m depth compared to 9 m depth (Fig. S3). (Note that the relative differences in numbers of individual gastropods between *H. grandifolius* and *S. antarctica* in Fig. S3 are somewhat different than when they are expressed on a surface area basis as in the main text; cf. main Fig. 1a.) This same trend was apparent between collection depths in *D. anceps* and *Plocamium* sp. but the variability and smaller sample sizes presumably precluded statistical significance (Fig. S3). *Plocamium* sp. supported many-fold higher densities of gastropods than the other four macroalgal species but otherwise, the only significant differences within the two depths of the other species was that *H. grandifolius* had significantly fewer gastropods than *S. antarctica* at either shallow or deep depths and that shallow *H. grandifolius* had significantly fewer gastropods than shallow *D. anceps* (Fig. S3).

There were significant differences between depths and macroalgal species in both numbers of gastropod species ($F_{8,105} = 25.115$, $p < 0.0005$; Fig. S4) and Shannon diversity ($H_8 = 48.042$, $p < 0.0005$; Fig. S5) although the differences in magnitude were much less than observed for gastropod density (Fig. S3). Of the four macroalgal species sampled at two depths, for individual species there were significantly more gastropod species in the deeper collections except for *D. anceps*, and the shallow-only collected *D. antarctica* had significantly fewer gastropod species than the shallow collections for the other macroalgal species (Fig. S4). Of the four species collected at two depths, gastropod diversity within species differed only in being slightly but significantly higher in the deep *S. antarctica* collections (Fig. S5). There were significantly fewer gastropod species on the shallow-only collections of *D. antarctica* than on any of the other species except for shallow *H. grandifolius* (Fig. S4).

Multivariate analysis of Bray-Curtis similarities also revealed differences between species and between depths between species. Although there is considerable overlap between species-depth groupings in a two-dimensional nMDS plot (Fig. S6), some differences between species and depth groupings are apparent in a CLUSTER analysis with SIMPER test (Fig. S7). The *D. antarctica* individuals (all shallow) clustered together in SIMPROF groups 14 and 15 with just a single shallow *S. antarctica* individual also in group 15 (Fig. S7). The shallow *Plocamium* sp. individuals clustered together an alone in groups 9 and 10 while the deep *Plocamium* sp. individuals clustered together and alone in groups 8, 12, and 13 (Fig. S7). ANOSIM analysis indicated strong, significant differences ($R = 0.562$, $p = 0.0001$). Of the 36 combinations of species-depth groupings, pair-wise ANOSIM analysis identified significant differences between all species-depth pairs (Table S5) except shallow vs. deep *D. anceps* ($R = -.003$, $p = 0.465$) and shallow *S. antarctica* and shallow *D. anceps* ($R = 0.123$, $p = 0.101$).

DUBRASQUET, H., REYES, J., SANCHEZ, R.P., VALDIVIA, N. & GUILLEMIN, M.-L. 2018. Molecular-assisted revision of red macroalgal diversity and distribution along the Western Antarctic Peninsula and South Shetland Islands. *Cryptogamie, Algologie,* **39**, 409-429, 10.7872/crya/v39.iss4.2018.409.

GUILLEMIN, M.-L., DUBRASQUET, H., REYES, J. & VALERO, M. 2018. Comparative phylogeography of six red algae along the Antarctic Peninsula: extreme genetic depletion linked to historical bottlenecks and recent expansion. *Polar Biology,* **41**, 827-837, 10.1007/s00300-017-2244-7.

HOMMERSAND, M.H., MOE, R.L., AMSLER, C.D. & FREDERICQ, S. 2009. Notes on the systematics and biogeographical relationships of Antarctic and Sub-Antarctic Rhodophyta with descriptions of four new genera and five new species. *Botanica Marina,* **52**, 509-534.

SOKAL, R.R. & ROHLF, F.J. 1995. *Biometry*. New York: WH Freeman and Company, 887 pp.

YOUNG, R.M., VON SALM, J.L., AMSLER, M.O., LOPEZ-BAUTISTA, J., AMSLER, C.D., MCCLINTOCK, J.B. & BAKER, B.J. 2013. Site-specific variability in the chemical diversity of the Antarctic red alga *Plocamium cartilagineum*. *Marine Drugs,* **11**, 2126-2139.

ZAR, J.H. 1998. *Biostatistical Analysis. 4th Edition*. Englewood Cliffs, NJ: Prentice-Hall, 663 pp.