1	Transcriptome of the Antarctic brooding gastropod mollusc Margarella antarctica
2	
3	Melody S Clark ¹ , Michael A.S. Thorne ¹
4	
5	¹ British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley
6	Road, Cambridge, CB3 0ET, UK.
7	
8	*Corresponding author : Melody S Clark, British Antarctic Survey, Natural Environment
9	Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK. Email:
10	mscl@bas.ac.uk
11	
12	
13	Abstract
14	454 RNA-Seq transcriptome data were generated from foot tissue of the Antarctic brooding
15	gastropod mollusc Margarella antarctica. A total of 6,195 contigs were assembled de novo,
16	providing a useful resource for researchers with an interest in Antarctic marine species,
17	phylogenetics and mollusc biology, especially shell production.
18	
19	Keywords
20	
21	
22	1. Introduction
23	Margarella antarctica is a small omnivorous topshell (up to 10mm) that is abundant and
24	widely distributed throughout the Antarctic. Poor genetic connectivity between populations
25	is expected in this species because it is a direct developer and the adults have low motility
26	with a limited ability to disperse over soft sediments compared with hard rock substrata

27 such as bed rock and loose rubble (Picken, 1979). It has already been subject to some 28 preliminary population analyses, using AFLPs, as part of a comparative study to investigate 29 the link between life history and genetic structure in the Antarctic ecosystem (Hoffman et al. 30 2011; 2012; 2014). The data revealed strong population structure in *M. antarctica* sampled 31 from sites along the Antarctic Peninsula separated by distances of at least 6km. At smaller 32 spatial scales *M. antarctica* populations are still significantly structured, although weakly so 33 on AFLP criteria and the isolation-by-distance pattern is non-linear (Hoffman et al. 2014) and 34 cannot be explained by simple dispersal limitation. Thus this species is now the subject of 35 further population analyses to determine the factors affecting micro-population structuring 36 and the data described here will provide a resource for those studies.

37

38 *M. antarctica* were collected by SCUBA divers from depths of 10-15 m from South Cove, 39 near Rothera research station, Adelaide Island, Antarctic Peninsula (67° 34'07" S, 68° 07 '30" 40 W) in the austral summer of 2012. 20 animals were dissected and the foot tissue flash frozen 41 in liquid nitrogen and stored at -80°C until further analysis. Total RNA was extracted from all 42 20 individuals TRIsure Reagent (Bioline) according to manufacturer's instructions. RNA 43 quality and concentration were determined using a NanoDrop ND-1000 Spectrophotometer 44 (NanoDrop Technologies, Wilmington, DE, USA), respectively. The RNAs from all the 45 individuals were pooled for RNA-Seq. The production of the 454 library and the 46 transcriptome sequencing was performed at the University of Cambridge Sequencing Service 47 in the Biochemistry Department (Cambridge, UK). The RNA library yielded 487326 million 48 reads. The contigs were trimmed and assembled using Newbler (www.454.com). De novo 49 assembly led to a total number of 6,195 contigs, of which 3,313 contained at least 10 reads 50 each. These were annotated using an in-house GenBank nr database (Benson et al., 2007) using a threshold value for annotation of below 1e⁻¹⁰. Putative annotation based on 51 52 sequence similarity searching could be assigned to 1,830 contigs (30%) of the contigs (Table

- 1). With regard to population work, 10,054 high confidence SNPs were identified in 1,974
- 54 contigs.
- 55

56 Table 1 : Statistics for the transcriptome generation from foot tissue of Margarella

57 *antarctica*

Total reads	87,326
Total Contigs	6,195
Average Contig length (bp)	597
Median length (bp)	509
Max length (bp)	4597
Min length (bp)	100
% Annotated Contigs	30

58

59

60 RNA-Seq transcriptome data for Antarctic marine invertebrates is still limited, but

61 encompasses a wide range of phyla including the Antarctic clam Laternula elliptica (Clark et

62 al., 2010; Husmann et al., 2013; Sleight et al., 2015); the krill Euphasia superba (Clark et al.,

63 2011; Toullec *et al.*, 2013; Meyer *et al.*, 2015), the brittlestar *Ophionotus victoriae* (Burns *et*

64 *al.*, 2013) and the limpet *Nacella concinna* (Fuenzalida *et al.*, 2014). Thus the data from *M*.

65 *antarctica* will form a valuable resource to understanding how life evolved and is maintained

66 in the freezing waters of the Southern Ocean. These data will also provide a useful resource

67 for researchers with an interest in mollusc biology, phylogeny, population genetics and

68 mollusk shell production.

- 69
- 70

71 2. Nucleotide sequence accession numbers

72	
73	The sequence data for this transcriptome has been deposited in the GenBank SRA, accession
74	number: SRP058232. The contigs are available from http://tinyurl.com/p9nlwww
75	
76	
77	Acknowledgements
78	
79	This study was funded by National Environment Research Council (NERC) core funding to the
80	British Antarctic Survey Polar Sciences for Planet Earth programme. We would like to thank
81	the Rothera Dive Team for help in collecting animals. The NERC National Facility for Scientific
82	Diving (Oban) provided overall diving support.
83	
84	
85	References
85 86	References Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids
86	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids
86 87	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25.
86 87 88	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the
86 87 88 89	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i> . Marine Genomics 9: 9-15.
86 87 88 89 90	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i> . Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454
86 87 88 89 90 91	 Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i>. Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454 Pyrosequencing Reveals Chaperone and Stress Transcriptome. PLoS One 6: e15919.
86 87 88 89 90 91 92	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i> . Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454 Pyrosequencing Reveals Chaperone and Stress Transcriptome. PLoS One 6: e15919. Clark MS, Thorne MAS, Vieira FA, Cardoso JCR, Power DM, et al. (2010) Insights into shell
86 87 88 89 90 91 92 93	 Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i>. Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454 Pyrosequencing Reveals Chaperone and Stress Transcriptome. PLoS One 6: e15919. Clark MS, Thorne MAS, Vieira FA, Cardoso JCR, Power DM, et al. (2010) Insights into shell deposition in the Antarctic bivalve <i>Laternula elliptica</i>: gene discovery in the mantle
86 87 88 90 91 92 93 94	Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i> . Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454 Pyrosequencing Reveals Chaperone and Stress Transcriptome. PLoS One 6: e15919. Clark MS, Thorne MAS, Vieira FA, Cardoso JCR, Power DM, et al. (2010) Insights into shell deposition in the Antarctic bivalve <i>Laternula elliptica</i> : gene discovery in the mantle transcriptome using 454 pyrosequencing. BMC Genomics 11: 362.
86 87 88 90 91 92 93 94 95	 Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2007) GenBank. Nucleic Acids Research 35: D21-D25. Burns G, Thorndyke MC, Peck LS, Clark MS (2013) Transcriptome pyrosequencing of the Antarctic brittle star <i>Ophionotus victoriae</i>. Marine Genomics 9: 9-15. Clark MS, Thorne MAS, Toullec JY, Meng Y, Guan LL, et al. (2011) Antarctic Krill 454 Pyrosequencing Reveals Chaperone and Stress Transcriptome. PLoS One 6: e15919. Clark MS, Thorne MAS, Vieira FA, Cardoso JCR, Power DM, et al. (2010) Insights into shell deposition in the Antarctic bivalve <i>Laternula elliptica</i>: gene discovery in the mantle transcriptome using 454 pyrosequencing. BMC Genomics 11: 362. Fuenzalida G, Poulin E, Gonzalez-Wevar C, Molina C, Cardenas L (2014) Next-generation

- 98 Hoffman JI, Clark MS, Amos W, Peck LS (2012) Widespread amplification of amplified
- 99 fragment length polymorphisms (AFLPs) in marine Antarctic animals. Polar Biology 35: 919-
- 100 929.
- 101 Hoffman JI, Clarke A, Clark MS, Peck LS (2013) Hierarchical Population Genetic Structure in a
- 102 Direct Developing Antarctic Marine Invertebrate. PLoS One 8: e63954.
- 103 Hoffman JI, Clarke A, Linse K, Peck LS (2011) Effects of brooding and broadcasting
- 104 reproductive modes on the population genetic structure of two Antarctic gastropod
- 105 molluscs. Marine Biology 158: 287-296.
- 106 Husmann G, Abele D, Rosenstiel P, Clark MS, Kraemer L, et al. (2014) Age-dependent
- 107 expression of stress and antimicrobial genes in the hemocytes and siphon tissue of the
- 108 Antarctic bivalve, Laternula elliptica, exposed to injury and starvation. Cell Stress &
- 109 Chaperones 19: 15-32.
- 110 Meyer B, Martini P, Biscontin A, De Pittà C, Romualdi C, et al. (2015) Pyrosequencing and de
- 111 novo assembly of Antarctic krill (Euphausia superba) transcriptome to study the adaptability
- 112 of krill to climate-induced environmental changes. Molecular Ecology Resources.
- 113 DOI: 10.1111/1755-0998.12408.
- 114 Picken GB (1979) Non-pelagic reproduction of some Antarctic prosobranch gastropods from
- 115 Signy Island, South Orkney Islands. Malacologia 19: 109-128.
- 116 Sleight VA, Thorne MAS, Peck LS, Clark MS (2015) Transcriptomic response to shell damage
- 117 in the Antarctic clam, *Laternula elliptica*: Time scales and spatial localisation. Marine
- 118 Genomics 20: 45-55.
- 119 Toullec JY, Corre E, Bernay B, Thorne MAS, Cascella K, et al. (2013) Transcriptome and
- 120 Peptidome Characterisation of the Main Neuropeptides and Peptidic Hormones of a
- 121 Euphausiid: The Ice Krill, *Euphausia crystallorophias*. PLoS One 8. e71609.