equilibrium (Table 1), probably due to Wahlund effect or existence of genetic structure since the Honduras population was sampled three times over 6 months and across ~400-km range. Null alleles were evident in all these loci and in TnM541 and TnN21. Three linkage groups were detected: TnM36–TnM70–TnM53; TnM79–TnM85 and TnM41–Tnm71. The loci TnM58, TnN6 and TnN13 gave inconsistent amplification while TnM50 [$(GGTAGAA)_7$] produced uncorrectable stutter peaks. Poor amplification, also observed in TnM541 and TnN12, was probably due to null alleles, and need further optimization before use. Cross-species amplification was tested on *T. latebricola* (= *americanus*), *Abraeus* sp. *Chatabraeus* sp. and *Acritus nigricornis*) (Abraeinae), *Carcinops pumilio* (Dendrophilinae); *Saprinus bicoloroides* (Saprininae) and *Hister zulu* (Histerinae) (Table 2). All multiplex sets except A and B (Table 1), comprising differently labelled primers of nearly similar annealing temperatures, produced correct genotypes with normal and hot-start polymerases. To our knowledge, this is the first microsatellite primer set for Histeridae, many of which are important predators of necrophagous insects, and hence useful in forensic entomology. They will be useful for the population studies on *T. nigrescens* in the control of the LGB.

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A panel of microsatellite loci from two species of octopus, *Pareledone turqueti* (Joubin, 1905) and *Pareledone charcoti* (Joubin, 1905)

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Abstract

Eighteen dinucleotide microsatellite loci were isolated from two octopus species, *Pareledone turqueti* and *Pareledone charcoti*, which are endemic to the Southern Ocean. Genetic diversity was assessed in samples of *P. charcoti* and *P. turqueti* from Elephant Island and Shag Rocks

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respectively. All except one locus (which has proved to be polymorphic in other species) were variable in the focal species and amplified between six and 30 and between four and 28 alleles for *P. charcoti* and *P. turqueti* respectively; mean expected heterozygosities varied between 0.38 and 0.95 (*P. charcoti*) and between 0.34 and 0.97 (*P. turqueti*), with significant ($P < 0.05$) departures from Hardy–Weinberg equilibrium at seven loci; three of these loci provided significant ($P < 0.05$) evidence for null alleles. Two pairs of loci isolated from *P. turqueti* demonstrated significant ($P < 0.05$) linkage disequilibrium. We are presently using these genetic markers to quantify spatial genetic structure in the genus *Pareledone*.

Keywords: Antarctica, microsatellites, *Pareledone charcoti*, *Pareledone turqueti*, population structure, Southern Ocean

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The octopus genus *Pareledone* is endemic to Antarctic waters and contains 15 species (Allcock 2005; Allcock *et al*. 2007). *Pareledone charcoti* (Joubin, 1905) is restricted to the South Shetland Islands, South Orkney Islands and waters nearby to Graham Land (Allcock 2005) and inhabits shallow waters to a depth of about 400 m (Strugnell *et al*. 2008). *Pareledone turqueti* (Joubin, 1905) has been reported mainly from western Antarctic waters where it inhabits depths ranging from less than 100 m to over 1000 m (Strugnell *et al*. 2008). Both *P. turqueti* and *P. charcoti* produce large eggs that likely hatch as benthic, crawl-away young. Given that the known distributions of both these species include areas separated by great depths, it is likely that the population structure of these species is extremely heterogeneous. For example, a study using allozyme electrophoresis showed extremely limited gene flow between populations of *P. turqueti* from South Georgia and Shag Rocks that are separated by deep water (Allcock *et al*. 1997). This panel of microsatellite loci was developed to further quantify population boundaries within the genus *Pareledone* distributed throughout the Southern Ocean but separated by deep water.

Genomic DNA was extracted using a high salt method (Sambrook *et al*. 1989) from mantle tissue taken from four individuals of each species. Partial genomic libraries enriched for microsatellite repeats were constructed using the protocol described by Bloor *et al*. (2001). Briefly, ~8 μg DNA was digested with 40 U of *Sau*3A1 (Boehringer-Mannheim) and the 400–1200-bp size fraction was excised from an agarose gel and purified using QIAquick gel extraction kit (QIAGEN). Fragments were then ligated to 50 pmol phosphorylated linkers (*Sau*LA: 5′-GGCCAGAGACCCCAAGCTTCG-3′; *Sau*LB: 5′ GATCCGAAGCTTGGCGTCTCTGGCC-3′) (Refseth *et al*. 1997) with 40 U of T4 DNA ligase (Promega).

The DNA fragments were then hybridized to a 3′-biotin-labelled $(CA)_{12}$ repeat probe and captured on M2–80 streptavidin-coated magnetic beads (Dynal). After a series of stringency washes in 2× SSC and 1× SSC, the enriched DNA was recovered and used as a template in a 50 μL polymerase chain reaction (PCR) primed using 30 pmol *Sau*LA. It was then purified using a QIAquick PCR purification kit (QIAGEN), ligated into pGEM-T plasmid vector (Promega) then transformed into JM109 competent cells (Promega). Recombinant plasmids were identified by black/white screening on S-Gal (Sigma) agar/ampicillin plates; plasmids containing a microsatellite insert were identified by a double band when screened with PCR that contained *Sau*LA and $(CA)_{12}$ oligonucleotide. Positive clones were sequenced on an ABI 3130xl (Applied Biosystems). Primers flanking microsatellite regions were designed using Primer 3 version 0.4.0 (Rozen & Skaletsky 2000).

For *P. charcoti*, we sequenced 51 positive clones, of which 49 (~96%) contained a microsatellite motif greater than six repeat units. Primers were developed around 29 loci; however, 22 primer pairs were discarded subsequently because they either failed to amplify or produced multiple/spurious bands during PCR leaving seven useful loci (Table 1). For *P. turqueti*, 53 putative positive clones were sequenced, of which 51 (~96%) contained a microsatellite. Primers were developed for 28 of these loci, 17 primer pairs were discarded, which left 11 robust loci (Table 1). Each of the primer pairs for *P. turqueti* was also tested on *P. charcoti* individuals and vice versa. In many cases, polymorphic loci were amplified (Table 2).

Genetic diversity was assessed in samples of *P. charcoti* and *P. turqueti* from Elephant Island and Shag Rocks ($n = 42$ and 34 respectively); we also determined the level of cross-species amplification of each panel of loci in a small sample (eight individuals) of the nonsource species. Loci were amplified in a 10-μL PCR using a three-primer labelling system (Schuelke 2000). The PCR consisted of 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20, 0.2 mM each dNTP, 3 mM $MgCl_2$, 5–50 ng template DNA, 3 pmol of M13 tailed, reverse and M13 labelled 6-FAM, NED, PET or VIC; Applied Biosystems) primers, 10 μg BSA, 1.25 U *Taq* DNA polymerase (ABgene). PCR conditions for all loci were 95 °C 3 min, 6× (95 °C 30 s, T_a °C 45 s, 72 °C 45 s), 25× (92 °C 30 s, T_a °C 45 s, 72 °C 55 s), where T_a is the annealing temperature (Table 1). PCR products were pooled with GeneScan 500 LIZ size standard (Applied Biosystems), separated by electrophoresis on an ABI 3130xl and sized using GeneMapper software (Applied Biosystems). The online version (3.4, http://wbiomed.curtin.edu.au/genepop/) of GenePop (Raymond

Table 1 Levels of variability at microsatellite loci isolated from the Southern Ocean octopuses *Pareledone turqueti* (PT) from Shag Rocks (n = 34) and *Pareledone charcoti* (PC) from Elephant Island (n = 42). T_a, annealing temperature of primers; S_R indicates the size range per locus in base pairs; N_a indicates number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *indicates a significant ($P < 0.05$) deviation from expected Hardy–Weinberg equilibrium (after sequential Bonferroni correction for 11 and 6 tests in PT and PC respectively); bold text highlights loci that likely suffer from null alleles ($P < 0.05$)

Locus GenBank	Primer Sequence (5′–3′)	Core motif	T_a	S_R	N_a	H_O	H_E
PT_B02_07 GF091788	F: CCCACACATACACACCCATAC R: TGAACATGGCACAATAGTAAATG	$(CA)_{13}$	51	103–173	22	0.960	0.884
PT_E04_09 GF091789	F: ATGCACCCTTGCACACAG R: TTTCGTCTGTACGTGCTTGG	$(AC)_{13}$	55	94–204	26	0.647	**0.967***
PT_F04_09 GF091790	F: GAAGTCTGCATCTCATTAGC R: CAGTTGGATTGTCGTCTTGG	$(AC)_{14}(n)_{18}(AC)_8$	55	133–208	18	0.733	0.923*
PT_H02_07 GF091791	F: GGGCGGGAAGAAGATATAAGG R: ATGTGTGTGTCTCGGTGAGG	$(CA)_7$	60	171–221	13	0.735	0.782
PT_D04_09 GF091793	F: CGTTTCATTCATTGTCG R: CGTGGTTTGTGTCTGTCTATG	$(AC)_8$	55	195–203	4	0.323	0.369
PT_F01_06 GF091794	F: GTTGTGTAAGGTCTACTCTCTGG R: AGCATCAACAATGTGCA	$(TG)_6(n)_2(TG)_6$	55	194–321	7	0.516	0.667
PT_G03_08 GF091795	F: TGACCAAACTGGAAGACTCG R: CTGACACTTGTACTCTTAATGTAATC	$(TG)_7$	55	148–168	5	0.576	0.656
PT_A03_08 GF091796	F: GCACGTTGTCCCATGATA R: GACTCTACGCGCTACTTCTGC	$(TA)_7(n)_9(GC)_7(GT)_{42}$	57	169–222	25	0.719	0.963*
PT_G03_082 GF091797	F: CAGCAACAACAGAAGTG R: CGAACCAGAAACAACG	$(TG)_7(n)_{18}(TG)6(n)_2(TG)_{10}$	52	236–254	13	0.824	0.865
PT_F03_08 GF091798	F: GAGTTAATGAGTGAAGAGAAAGAGC R: CTGTGTCTGGTCGTTTAGAGG	$(TG)_9(n)_{12}(AG)_9$	58	337–344	6	0.387	0.343
PT_F02_07 GF091799	F: AGAGCTAGACAAAGAAGGAAGC R: CGAACACATACACACTTTCAGG	$(GT)_7(n)_{26}(TG)_6(n)_{102}(GT)_{11}$	58	352–434	28	0.867	0.963*
PC_B01_06 GF091781	F: TTAACCTAAACTTAGACACATTCACG R: GGCTTAAATCCAGCGGAAAC	$(AC)_5(n)(CA)_6$	58	158–184	6	0.262	0.382
PC_B03_08 GF091783	F: CCTTGCTGGCTCATAGTTGG R: AAAACAGCTGGAGTTTCAAAGC	$(CA)_{12}$	58	218–295	13	0.714	0.823
PC_C05_10 GF091784	F: TGAGACGCAAAGAGAATTTCG R: TCGCCGAAACATTATACAGG	$(TG)_{12}(n)_{24}(GT)_6$	55	178–206	14	0.548	**0.869***
PC_D02_07 GF091785	F: TGTATCCGAGTTTGAAGTTTTGG R: GAACCTCACAAAGGAAATGACC	$(AC)_9$	60	176–261	30	0.810	0.952*
PC_H02_07 GF091786	F: GCATGAACGAACTGTCATTAGACC R: CTCCACTCATCCACCGAACC	$(CA)_8$	60	167–285	15	0.429	**0.789***
PC_G03_08 GF091787	F: CATCTCAATCAGGTCCAACC R: ACCCAGCCCAACTGATGC	$(TG)_9(n)_{11}(GT)_6$	58	275–319	20	0.833	0.901

& Rousset 1995) was used to calculate measures of genetic diversity, the significance of any deviations from Hardy–Weinberg equilibrium and for linkage disequilibrium between all pairs of loci. Micro-Checker (van Oosterhout *et al*. 2004) was used to detect the most probable cause of Hardy–Weinberg equilibrium departures such as null alleles or large allele dropout. Sequential Bonferroni corrections for k multiple tests were applied where appropriate (Rice 1989).

All loci except PC_G01_06 (but which amplified two alleles in *P. turqueti*) were polymorphic in the focal species, amplifying between six and 30 and between four and 28 alleles per locus for *P. charcoti* and *P. turqueti*, respectively. Mean expected heterozygosities varied between 0.38 and 0.95 (*P. charcoti*) and between 0.34 and 0.97 (*P. turqueti*) (Table 1), with significant ($P < 0.05$; $k = 6$ and $k = 11$ for *P. charcoti* and *P. turqueti* respectively) departures from Hardy–Weinberg equilibrium at seven loci that may be indicative of unrecognized population substructure (as the samples comprised several trawls); three of these loci (PT_E04_09, PC_C05_10 and PC_H02_07) provided significant ($P < 0.05$) evidence for null alleles (with estimated frequencies of 0.158, 0.180 and 0.217 respectively, calculated using van Oosterhout *et al*. 2004 algorithm). Just two pairs of loci (PT_G03_08 with PT_F02_07, and PT_E04_09 with PT_G03_082) demonstrated significant ($P < 0.05$ $k = 15$ and $k = 55$ for *P. charcoti* and *P. turqueti* respectively) linkage disequilibrium. Thirteen loci were polymorphic in the nontarget species (Table 2) and this successful cross-amplification indicates that these genetic markers may be used in other members of the genus *Pareledone*. We are presently using these genetic markers to determine patterns of spatial structure exhibited by *P. turqueti* and *P. charcoti* in the Southern Ocean.

Table 2 Cross-species amplification of microsatellite markers isolated from *Pareledone turqueti* and *Pareledone charcoti* in the alternative *Pareledone* species

Taxon	Locus	N_a	S_R
P. charcoti	PT_B02_07	—	—
	PT_E04_09	6	144–151
	PT_F04_09	7	133–208
	PT_H02_07	1	211
	PT_D04_09	—	—
	PT_F01_06	5	250–450
	PT_G03_08	5	124–169
	PT_A03_08	—	—
	PT_G03_082	—	—
	PT_F03_08	5	323–344
	PT_F02_07	4	369–373
P. turqueti	PC_B01_06	3	151–155
	PC_B03_08	11	295–450
	PC_C05_10	6	146–156
	PC_D02_07	2	212–214
	PC_H02_07	6	168–212
	PC_G03_08	8	333–419
	PC_G01_06	2	166–168

N_a indicates the number of alleles observed in eight individuals of the nonsource species of *Pareledone*; S_R indicates the size range per locus in base pairs.

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Isolation and characterization of microsatellites in the bird-pollinated, autohexaploid, *Eremophila glabra* ssp. *glabra* (R.Br. (Ostenf.)) (Myoporaceae), an Australian endemic plant

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