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PERMANENT GENETIC RESOURCES

Microsatellite development suggests evidence of polyploidy in the social sponge-dwelling snapping shrimp *Zuzalpheus brooksi*

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Abstract

We isolated and characterized 33 novel, polymorphic microsatellite loci from the social sponge-dwelling snapping shrimp *Zuzalpheus brooksi*. We screened all 33 loci in approximately 31 individuals from a population of *Z. brooksi* from the Florida Keys, USA and found an average of 16 alleles per locus. Approximately 25% of the loci showed more than two bands per individual, suggesting evidence of high gene duplication, or more likely, polyploidy, which is common in crustaceans. The 25 disomic loci had an observed heterozygosity of 0.57 (range = 0.03–1.00) and will be useful for studying the social organization in *Zuzalpheus* shrimp.

Keywords: cooperative breeding, eusocial, microsatellite, polyploid, snapping-shrimp, *Zuzalpheus*

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Eusociality, the rarest and most advanced form of social complexity, is characterized by group living, reproductive division of labour, overlap of generations, and cooperative care of young (Wilson 1971). Outside of the insects, classic eusociality has only been observed in African mole-rats (Bennett & Faulkes 2000) and *Zuzalpheus* snapping shrimps (Duffy 1996a). The genus *Zuzalpheus (*formerly part of *Synalpheus*; Ríos & Duffy 2007) is a socially diverse and species-rich group of snapping shrimp in the family Alpheidae that usually associates with sessile invertebrate hosts (Ríos & Duffy 2007). Although the phylogenetic relationships among species within this group have been studied (Duffy *et al*. 2000; Morrison *et al*. 2004), the genetic relationships among individuals within colonies and their consequences for social organization have received less attention (but see Duffy 1996a; Duffy 1996b). Here, we report the development of 33 polymorphic microsatellite loci for *Zuzalpheus brooksi* that will be useful for studying familial relations and the evolution of sociality in *Zuzalpheus* shrimp.

Microsatellites were developed from *Z. brooksi* genomic DNA pooled from seven individuals (three apparent males and four ovigerous females) using a modified enrichment protocol (Hamilton *et al*. 1999) described previously (Rubenstein 2005). The enrichment step used a different set of single-stranded biotintylated dimeric, trimeric and tetrameric repeats (dimers: GT_8, $TC_{9.5}$; trimers: TTA_{12}, GAT_7, $GTT_{6.33}$, $GTA_{8.33}$, TTC_7, GCT_8, $GTG_{4.67}$, GTC_{12}, TCC_5; tetramers: $TTTA_{8.5}$, $GAAT_{5.5}$, $GATA_7$, $GATT_{5.5}$, $GTAT_{6.25}$, $GTTA_{6.25}$, $GTTT_{8.5}$, $TTAC_{6.75}$, $TTTC_6$, $GATG_{4.25}$, $GGTT_4$, $GCTT_{3.75}$, $GTAG_{4.5}$, $GTCA_{4.25}$, $GTCT_{4.25}$).

We sequenced 190 plasmid clones and then designed primers for 66 unique microsatellite-containing sequences. Loci were screened for variability on a panel of 27–32 *Z. brooksi* individuals (mean = 31.4), which included three to five adults taken from each of eight loggerhead sponges (*Spheciospongia vesparium*) from the Florida Keys, USA.

For a subset of the loci, forward primers were labelled using the fluorescent dyes 6-FAM, NED, PET and VIC (Applied Biosystems). Each 10 L polymerase chain reaction (PCR) contained: 0.5–1 U JumpStart *Taq* DNA polymerase (Sigma), 0.8 L 1× PCR buffer (Sigma), 1–4 mM $MgCl_2$ (Sigma), 0.2 mM dNTPs (Invitrogen), 0.15 M of forward and reverse primer, and 1–50 ng of genomic DNA. PCRs consisted of 35 cycles of 94 C for 50 s, T_a for 1 min, 72 C for 1 min, and a final extension of 72 C for 4 min 30 s

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Table 1 Characterization of 33 polymorphic microsatellite loci from the sponge-dwelling snapping shrimp *Zuzalpheus brooksi*. Flanking primer sequences, recommended annealing temperatures (T_a) and $MgCl_2$ concentrations are listed for each locus. The product size range, number of alleles per individual, number of alleles for each locus, and presumed mode of inheritance are given for sample sizes of *N* individuals. Observed (H_O) and expected (H_E) heterozygosities were calculated for the disomic loci using the program CERVUS version 3.0 (Kalinowski *et al*. 2007). A BLAST analysis in GenBank confirmed that all published sequences are unique

Locus	GenBank Accession No.	Repeat motif	Primer sequence (5′–3′)	T_a (C)	$MgCl_2$ (mM)	Product size range (bp)	No. of alleles per individual	Presumptive inheritance	No. of alleles per locus	*N*	H_O	H_E
25 LOCI WITH PRESUMED DISOMIC INHERITANCE												
ZB-P1A12	EU135992	$(GA)_{27}$	F: AATAATCGGCATGCCAAGCGTCA R: CGATGCCGAGGACATGGCATATC	63	1.0	179–216	1–2	disomic	15	32	0.69	0.92
ZB-P1D4B	EU135993	$(CT)_{33}$	F: TCTGCGAATGGCAAGGAGAATTAC R: GTGTGGGCAGGAATGCATGTG	51	1.0	238–286	1–2	disomic	17	32	0.91	0.93
ZB-P1D7B*	EU135994	$(TC)_{27}$	F: GTTTCACTTGAAATAATTTTGATT R: TTCTCCCACGAGAATAAATCT	54	3.0	95–118	1–2	disomic	6	32	0.44	0.71
ZB-P1E12	EU135995	$(TG)_{13}$	F: GTTTCTATACCAATCCCATTTCGTT R: CACACCCACTCTTTCACACTAACC	52	2.0	86–122	1–2	disomic	10	32	0.63	0.73
ZB-P1E3*	EU135996	$(GA)_{31}$	F: CTAGATTAGCAATATTAGCGTTCTTCA R: ATCTATTCATTTGTCCATCTGTTTATCT	50	3.0	134–379	1–2	disomic	9	31	0.32	0.45
ZB-P1E4*	EU135997	$(CT)_{17}$	F: CGGGCTGCTCAAATCCCAACT R: CCAAACTCTGATAATACAAGGCTCTGAGA	63	2.5	254–356	1–2	disomic	12	32	0.50	0.89
ZB-P1F6	EU135998	$(TC)_{11}(TAG)_{24}$	F: CCTGCCCCTTTCAGAATTCCTAGAC R: GTTGGAAGATGTCAAAACATCAAATCTCA	54	2.0	121–225	1–2	disomic	28	32	0.84	0.97
ZB-P1G8	EU135999	$(CT)_{26}$	F: CCAATGTTGGGGAGTTCAAGGTTAA R: TGTGATACAAGGCAGTGACATTGTTCTAA	53	1.0	231–276	1–2	disomic	20	32	0.75	0.95
ZB-P1H10	EU136000	$(TC)_{40}$	F: CTTGTCCTTCGGTTTCCTTCGTTTTAGTA R: GGCCAGCCTTACCATGTTCAGTCTC	57	1.0	250–292	1–2	disomic	11	30	0.70	0.86
ZB-P2C12	EU136001	$(CAG)_6(CAA)_2CAG(CAA)_9$	F: CAATAATAACAACAACAAAAACAATAGC R: ATTTTTGACCGTGATGTAAGCATTAC	54	2.5	99–143	1–2	disomic	8	32	0.59	0.75
ZB-P2H12	EU136002	$(GA)_{14}$ ... $(AAG)_{20}(AAC)_{12}$	F: AAAAATGAAATGCTGAATCACATGAGTA R: CAGGGGCCATTGGCACAA	53	2.5	168–285	1–2	disomic	27	32	0.84	0.96
ZB-P3A7	EU136003	$(TTC)_7TTT(TTC)_5$	F: CTTGGCTGGAAGGAACTGGAAAGAAG R: ATCTGCGCATGCGCACTTTGTAGTTG	57	1.0	426–430	1–2	disomic	2	32	0.03	0.03
ZB-P3A8*	EU136004	$(TCC)_6$	F: CTCAGTAACCCTTCCACTCCCACCA R: GGATTTAGGGGCATAGAGAAGGTTCG	61	2.0	116–240	1–2	disomic	6	32	0.09	0.26
ZB-P3F11	EU136005	$(AC)_{25}(AT)_5(ACAT)_{14}$	F: AGACCTAAATGAATTGCTTTATGGATGAG R: GTTCCCGCAAGCGTATTCATTC	58	4.0	134–264	1–2	disomic	28	31	0.90	0.96
ZB-P3G3*	EU136006	$(CAG)_3(CAA)_6CAG$ $(CAA)_5CAG(CAA)_8$	F: AGAGCCAATTACGCTTCATATCAGGTG R: CACTCGCACATACAAAGTCGTCATGG	57	2.5	231–287	1–2	disomic	10	30	0.07	0.84
ZB-P5A8	EU136007	$(TAC)_{16}$	F: AATTCCCAAACCGTTTACGGGTTAAGTA R: ACGGCTTGGCTGTCTGGCTAGG	58	1.0	182–234	1–2	disomic	17	32	0.84	0.94
ZB-P5C10	EU136008	$(GTT)_{24}$	F: AGCGTGCATTCTCACAGCCTTTCAG R: TTCCCGTAAGCTGCCACTGTTGAGA	57	1.5	387–569	1–2	disomic	20	27	0.93	0.92
ZB-P6A2B*	EU136009	$(GTCT)_3(GTCC)_2$	F: ATGTGTAAATAGTTGGCCAAGGATGAAGTGA R: CTGTATCCCTTATAGGCGCCTCATGTAAAA	58	2.5	177–197	1–2	disomic	2	32	1.00	0.51
ZB-P6B2*	EU136010	$(AGAT)_3(AGAG)_3$ $(ATAC)_2ATGC(ATAC)_{10}$	F: CGGGATTCCGAAACGGTAAAAG R: CAATCGTCGCTCTGTCTATCTATCCA	61	2.0	221–355	1–2	disomic	15	32	0.63	0.87
ZB-P6B8	EU136011	$(GCTT)_{10}$ ... $(ACTT)_{13}$	F: TTATTCATCTGTTCATTCATTCACTTAC	54	2.0	371–457	1–2	disomic	13	32	0.66	0.81

Talbe 1 *Continued*

Locus	GenBank Accession No.	Repeat motif	Primer sequence (5′–3′)	T_a (C)	$MgCl_2$ (mM)	Product size range (bp)	No. of alleles per individual	Presumptive inheritance	No. of alleles per locus	N	H_O	H_E
			R: GTAACTTCGCTTTATCCCACTGA									
ZB-P6D3*	EU136012	$(GTTT)_5$ ... $(GTTT)_5$	F: TTTGGTTTTATGATGACCTTTTTCTATT R: AATAAACAATTCGGTGAATAAATAAATGA	57	3.0	232–236	1–2	disomic	2	26	0.04	0.11
ZB-P6E12	EU136014	$(AG)_{38}$	F: GGTGCATAACAAGTTAATAGGAGAATGTCA R: CTGTAGCCTTTGGAAAAGCAAAGAAAC	58	1.5	210–240	1–2	disomic	15	32	0.72	0.92
ZB-P6G11	EU136015	$(GAT)_6$ ... $(TAG)_{13}$	F: ATGAAAGTTAGAAGAATAATGGTATTATGA R: TTATATTACTGCTGCTGCCGTTAC	57	3.5	111 - 151	1–2	disomic	16	32	0.91	0.93
ZB-P7C7	EU136016	$(TC)_3CT(TC)_3$	F: GGCGGGTCGTGATGCTTGTATAGTG R: AGACCGACAGAAAGACAGATAGACAGACAAAAAG	55	1.0	399–431	1–2	disomic	3	32	0.16	0.20
ZB-P7E10*	EU136017	$(TG)_3TC(TG)_4(TC)_2$	F: CGTGGATTTTTCCCTGGGTTTCTAC R: CCGGCGTATCTCGGCCACTA	64	2.5	206–270	1–2	disomic	2	32	0.03	0.20
8 LOCI WITH PRESUMED POLYSOMIC INHERITANCE												
ZB-P1D6B	EU136018	$(TC)_{36}$	F: CCCCCATCACAACTTCCTTCTCCA R: ACGGAAATACAGACGGAGCAAAAATCA	53	1.0	161–285	1–4	tetrasomic	33	32	—	—
ZB-P1F7	EU136019	$(GTAT)_{27}$	F: GAGACAATCCTGAGGGGGCTTTACAA R: GCTGGCTTGGAATGATCAGCATTTC	58	2.5	228–335	1–4	tetrasomic	13	32	—	—
ZB-P2E9	EU136020	$(GTT)_8GAT\ (GTT)_5(GAT)_3$	F: CTGCCATATTCATTCCCACCCATTC R: CATCGCCTACTGAGTCGTTTTACTGGTATC	58	1.0	315–453	1–5	> tetrasomic	19	32	—	—
ZB-P4D2	EU136021	$(ACA)_2ACT\ (ACA)_4(GCA)_5$	F: CAGCAAAAGAATAAATTTCAACAACAACC R: GATATTTCTTAGCGGGATTTGCACTTC	57	3.0	301–373	1–4	tetrasomic	13	32	—	—
ZB-P4F4B	EU136022	$(TCTG)_3TATG(TCTG)_4\ (TG)_5$ $(TCTG)_9TACG\ (TCTG)_{11}AC(TC)_{37}$	F: TTCCACTTACAAATGCCGCTGATGC R: GAATGTACTTCAGTGCATTTTTCACGTGAGAC	63	1.0	271–463	1–4	tetrasomic	34	30	—	—
ZB-P5E2	EU136023	$(AG)_{52}$	F: TCGACATTGGGGCAAATAGAGGTTTG R: TCCTCGGATGAACTCTTTGGTCTTTGAG	58	1.5	250–455	1–4†	tetrasomic	32	32	—	—
ZB-P7D11	EU136024	$(GTCT)_{17}$	F: CTCGTCGGCATTATCGTTGTTCA R: GCGTTTTTGCGTAATCCTGCTAA	61	1.5	151–356	1–4	tetrasomic	24	32	—	—
ZB-P7E12	EU136025	$(CTT)_4$ ... $(CTT)_5$ ... $(CTT)_3$ ... $(CTT)_3GT(CTT)_{51}$	F: AATCGATTGCGTTTCTTCTTTAAGGATACG R: CTGTTTCGGCCCGTAGGTGAGC	60	2.0	252–478	1–4	tetrasomic	45	32	—	—

* Loci where CERVUS detected null alleles at $P > 0.15$. † One individual may have had seven bands.

Table 2 Cross-species amplification of 33 polymorphic *Zulzalpheus brooksi* microsatellite markers in four other *Zulzalpheus* species and in a population of *Z. brooksi* from Belize. *Zulzalpheus chacei*, *Z. elizabethae* and *Z. regalis* were collected in Belize, whereas *Z. pectiniger* were collected in the Florida Keys, USA. All loci were tested on two individuals of each species (all apparent males, except for one ovigerous female *Z. pectiniger*) and visualized on a 2% agarose gel with a positive control sample of *Z. brooksi* from the Florida population. Markers that amplified and did not amplify genomic DNA are indicated by + and –, respectively

Locus	*Z. chacei*	*Z. elizabethae*	*Z. pectiniger*	*Z. regalis*	*Z. brooksi* (Belize)
25 LOCI WITH PRESUMED DISOMIC INHERITANCE					
ZB-P1A12	–	–	–	–	+
ZB-P1D4B	+	+	+	+	+
ZB-P1D7B	+	+	+	+	+
ZB-P1E12	+	+	+	+	+
ZB-P1E3	–	–	+	+	+
ZB-P1E4	–	–	+	–	+
ZB-P1F6	–	–	–	–	+
ZB-P1G8	–	–	–	–	+
ZB-P1H10	–	–	–	–	–
ZB-P2C12	+	+	–	+	+
ZB-P2H12	–	+	+	–	+
ZB-P3A7	–	–	–	–	+
ZB-P3A8	+	–	–	–	+
ZB-P3F11	+	–	–	–	+
ZB-P3G3	–	–	–	–	–
ZB-P5A8	–	–	–	–	+
ZB-P5C10	–	–	–*	–	+
ZB-P6A2B	+	+	+	–	+
ZB-P6B2	+	–	–	–	+
ZB-P6B8	+	–	+	–	+
ZB-P6D3	–	–	+	–	–
ZB-P6E12	–	–	–	–	+
ZB-P6G11	–	–	–	–	–
ZB-P7C7	+	–	+	+	+
ZB-P7E10	–	–	–	–	+
8 LOCI WITH PRESUMED POLYSOMIC INHERITANCE					
ZB-P1D6B	–	+	–	–	+
ZB-P1F7	+	–	–	–	–
ZB-P2E9	–	–	–	–	+
ZB-P4D2	+	–	–	–	+
ZB-P4F4B	–	–	–	–	–
ZB-P5E2	–	–	–	–	+
ZB-P7D11	–	–	–	–	+
ZB-P7E12	–	–	–	–	+

*Loci that amplified bands of unexpected size.

using iCycler thermal cyclers (Bio-Rad Laboratories). For the remaining loci, variability was assessed using a universal fluorescent labeling protocol (Boutin-Ganache *et al*. 2001; Waldbieser *et al*. 2003) that included the forward primer with an additional 20-bp tag (CGAGTTTTCCCAGTCACGAC) on the 5′ end, a fluorescently labelled tag (6-FAM) of the same 20-bp sequence, and the reverse primer. The concentrations of all reagents remained the same, except for the forward primer, which was reduced from 10 M to 1.0 M. PCRs consisted of an initial step of 94 C for 4 min, followed by 30 cycles of 94 C for 50 s, T_a for 1 min, 72 C for 1 min, an additional 10 cycles of 94 C for 50 s, 50 C for 1 min, 72 C for 1 min, and a final extension of 72 C for 30 min. The 'pigtail' sequence GTTTCT was added to the 5′ end of the reverse primer of all loci (Brownstein *et al*. 1996).

Of the 66 *Z. brooksi* loci screened for variability, 33 were polymorphic and had between two and 45 alleles (mean = 16.0) (Table 1). However, eight of 33 polymorphic loci showed evidence of > 2 bands per individual (in both sexes); the number of bands per individual ranged from one to four for seven loci, but at one locus, two of 32 individuals had five bands (Table 1). These results suggest that *Z. brooksi* may be polyploid, as is common in crustaceans (Lecher *et al*. 1995; Otto & Whitton 2000;

Gregory & Mable 2005), including some Alpheid shrimp (Bachmann & Rheinsmith 1973). Alternatively, this could be evidence of gene duplication, which has been observed in insects (Zhang 2004). However, the mixture of disomic and presumably tetrasomic loci, which is also common in invertebrates and some vertebrates (Dowling & Secor 1997; Rodzen & May 2002; Gregory & Mable 2005), is consistent with the hypothesis of polyploidy via recent gene duplication followed by rediploidization (Marsden *et al*. 1987). Finally, we tested each locus for amplification in four other *Zuzalpheus* species and a population of *Z. brooksi* from Belize (Table 2).

Observed heterozygosities of the 25 disomic loci ranged from 0.03 to 1.00 (Table 1). We did not conduct tests of Hardy–Weinberg equilibrium because individuals were sampled from multiple colonies (i.e. sponges) with a high likelihood of strong genetic subdivision among colonies (Duffy 1996b). Although none of the disomic loci showed strong evidence of linkage disequilibrium (all $P > 0.05$) (GENEPOP version 3.4; Raymond & Rousset 1995), nine of 25 loci showed possible evidence of null alleles ($P > 0.15$) (CERVUS version 3.0; Kalinowski *et al*. 2007), presumably because of high population structure among sponges. Because the mean expected heterozygosity of these 25 disomic loci was 0.71 and they had a combined parental exclusionary power > 0.99999 (CERVUS version 3.0; Kalinowski *et al*. 2007), they will be useful for examining familial relationships and parentage in *Zuzalpheus* shrimp.

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