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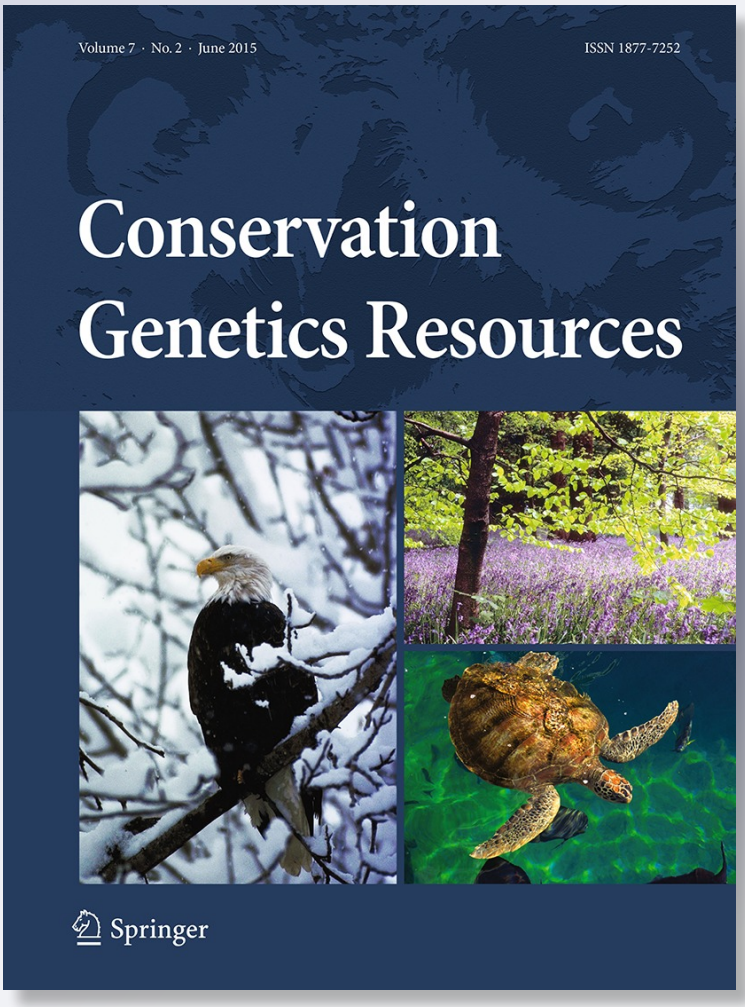

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MICROSATELLITE LETTERS

Microsatellites for two Neotropical dominant ant species, *Camponotus renggeri* and *C. rufipes* (Hymenoptera: Formicidae)

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Abstract *Camponotus renggeri* and *C. rufipes* are two important ants of the Brazilian Cerrado savanna, a threatened Biome. Thirty-one microsatellites were characterized and cross amplified for both species. 27 loci were polymorphic for *C. renggeri* ($H_E = 0.575$ and $H_O = 0.575$) and 24 for *C. rufipes* ($H_E = 0.567$ and $H_O = 0.564$). The average number of alleles per locus was 6.9 for *C. renggeri* and 5.9 for *C. rufipes*. The high levels of genetic diversity of these novel markers make them useful tools for genetic and evolutionary studies involving these ecologically important ants, and for the conservation of the Cerrado.

Keywords Carpenter ants · Formicinae · *Myrmothrix* · Cerrado savanna · Simple sequence repeats

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Ants are among the most common and dominant groups of insects on earth, playing important ecological roles across terrestrial ecosystems. They promote nutrient cycling, seed and pollen dispersal, animal community control and may be considered as biological indicators of anthropogenic environmental impacts (Del Toro et al. 2012). Ants are especially abundant in the Brazilian Cerrado savanna, which is one of the world's most biodiverse and threatened Biomes (Oliveira and Marquis 2002). Two species in the subfamily Formicinae, *Camponotus renggeri* (Emery) and *C. rufipes* (Fabricius), are especially relevant for participating in numerous interspecific interactions across the Cerrado landscape (Oliveira and Freitas 2004). The ecological dominance of these ants makes them interesting biological systems for conservation-focused studies. Here we describe new microsatellites markers that will enable the study of the genetic diversity of these species.

Two microsatellite-enriched libraries (one for each species) were built according to Billote et al. (1999), using six and five workers from the same nest of C. *rufipes* and *C. renggeri*, respectively, from Itirapina, state of São Paulo, Brazil. Genomic DNA was extracted from entire workers following a modified CTAB protocol and digested with *Afa*I. DNA fragments were ligated to the double-strand adapters 5′-CTCTTGCTTACGCGTGGACTA-3′ and 5′-TAGTCCACGCGTAAGCAAGAGCACA-3′. The enrichment was performed using a hybridization-based capture with $(CT)_8$ and $(GT)_8$ biotin-linked probes and streptavidin magnetic-coated beads (Promega). Selected fragments were then cloned into a pGEM-T easy vector (Promega) and inserted into *Escherichia coli* XL1-Blue competent cells. From each library, we sequenced 96 positive clones, in which 79 repeat motifs were identified in 52 clones for *C. renggeri* and 82 in 58 clones for *C. rufipes* using SSRIT (http://archive.gramene.org/db/markers/ssrtool). A total of

Table 1 Characteristics of 31 microsatellite markers for *C. renggeri* and *C. rufipes*

Locus	Primer sequence (5′–3′)	Repeat motif	TD (°C)	*Camponotus renggeri*				*Camponotus rufipes*				GenBank accession
				SR	A	H_E	H_O	SR	A	H_E	H_O	
Cgg01	F: CATAAATAATTGACGGAGAA R: AATGTGGCTTTAATCTGTCT	$(ca)_9$	57–52	189–199	6	0.545	0.567	191–241	10	0.838	0.933	KP101484
Cgg02	F: TAGCAATAATGGAAGGAATAGC R: CGAAGCAGACAAAATCAAATC	$(agt)_4$	57–52	239	1	–	–	239–245	3	0.385	0.333	KP101485
Cgg03	F: TGCAAAGTGCGTGCTTCATA R: GCCTGCGTAACGGTAAAAAC	$(gt)_5$	60–55	132	1	–	–	132	1	–	–	KP101486
Cgg04[b]	F: TAGGCCCGATACTTGAGGAA R: AAGAAGAGGTCCGCTCTCG	$(aaac)_4(ac)_{21}(at)_5$	57–52	250–278	10	0.452	0.433	234–270	8	0.479	0.400	KP101487
Cgg05	F: CGTGAATCCCGCTAGACAAT R: TCGAAACGGGATCTTACCAC	$(tg)_4ta(tg)_4$	57–52	251	1	–	–	251	1	–	–	KP101488
Cgg06[b]	F: GTGCATTCGCGTTGTAACAT R: CGGATAACAGGAATCGGAAA	$(ca)_7at(ca)_4...(ca)_4$	57–52	222–242	6	0.599	0.767	218–232	4	0.367	0.367	KP101489
Cgg07[b]	F: GAGCGAAGGAAAGAGATGGACA R: ATTGCCAACGCCTTCTGACAC	$(ag)_4...(ag_7...(ag)_7$ $...(ag)_{10}...(ag)_9$	57–52	280–310	6	0.564	0.533	274–296	9	0.806	0.700	KP101490
Cgg08	F: GAGCATTAGCATTACCCAAAAAG R: GCGACGATGATCAAAGCAG	$(tg)_6$	57–52	204–208	3	0.543	0.533	204	1	–	–	KP101491
Cgg09	F: TCGATTAAATTAGCACCGTTC R: TGAAAATCACAGTCCCAAATGTA	$(tg)_{10}$	57–52	150–152	2	0.206	0.233	134	1	–	–	KP101492
Cgg10	F: ATAAATAACCAGCCGCCACA R: CATTCGTGCCGACTATGCT	$(tg)_4ta(tg)_4...(tg)_4$	57–52	229–231	2	0.491	0.600	229–231	2	0.391	0.533	KP101493
Cgg11	F: CGGGCCGACTCTTCAAAC R: TATCCCATGTTTCACGAATGAG	$(tg)_9...(gt)_4$	57–52	264–266	2	0.278	0.333	256–276	7	0.808	0.8	KP101494
Cgg12	F: ATCGGTTCTAGAAAAATCCAAG R: AGGGTGCACTTTAAAGAATGA	$(ca)_6$	60–55	253–259	4	0.157	0.167	255–271	6	0.494	0.333	KP101495
Cgg13	F: TACGTGCGAGCAAATAAGATAAT R: AGAAGGCGAGGGTAAACGAC	$(ac)_8...(ca)_4$	57–52	218–228	4	0.698	0.567	206–222	5	0.652	0.600	KP101496
Cgg14	F: CAATCTAAGAACTCCAGCAAA R: GATTAATCTATGGAAAGCACATC	$(tg)_{10}cg(gt)_7$	57–52	236–268	16	0.891	0.800	234	1	–	–	KP101497
Cru01	F: AGGCAAAATCGATGGTGTA R: TGTTTTCATGCTTATTCAAGG	$(gt)_9...(ta)_4(tg)_4$	60–55	257–297	14	0.842	0.800	241–289	10	0.821	0.833	KP101498
Cru02	F: GTATCGGGGACGTCACCTTTTG R: ACGCGTTGCCGGAAGAGAA	$(ac)_5$	57–52	166–172	5	0.617	0.633	168–174	3	0.383	0.500	KP101499
Cru03	F: CAGCGAAGGAGGAAGGGAAA R: CCTCGCGTGCCATTCAGAT	$(gt)_9$	60–55	216–218	2	0.033	0.033	216–222	4	0.317	0.367	KP101500

Table 1 continued

Locus	Primer sequence (5′–3′)	Repeat motif	TD (°C)	*Camponotus renggeri*				*Camponotus rufipes*				GenBank accession
				SR	A	H_E	H_O	SR	A	H_E	H_O	
Cru04[b]	F: ATTATTCGCGCCCTTTCC	$(at)_5(ac)_{12}$	57–52	234–256	9	0.627	0.667	236–246	6	0.763	0.800	KP101501
	R: AGATGTGGTGCAACTTCTTCAA											
Cru05[a]	F: CACAATGTATTTGTTACGGTGT	$(ga)_{16}$	60–55	258–292	11	0.846	0.733	240–282	11	0.763	0.867	KP101502
	R: TCATAAACGACGAATCGGT											
Cru06	F: CCGTCTTATTTAGGAACACA	$(ca)_8$	57–52	196–198	2	0.124	0.133	200–202	2	0.095	0.100	KP101503
	R: CTGTCAAGAAGTGGATAGTGTC											
Cru07[b]	F: GCTCGATGTGCTAAGAATAAG	$(tg)_7$	57–52	170–176	5	0.713	0.633	172–186	6	0.395	0.400	KP101504
	R: CAACAATAGGATCATACGTGTC											
Cru08	F: CCTATTTTCCTTTCCCCTGCTA	$(ca)_{13}$	57–52	215–229	4	0.575	0.700	213–225	6	0.672	0.600	KP101505
	R: CGGTTTTATCCCATCGGTTAC											
Cru09[b]	F: CCAACAGAATAGGCAACA	$(at)_{5...}(at)_4(tg)_7ta$	57–52	204–248	15	0.861	0.767	224–284	16	0.892	0.933	KP101506
	R: AATGAAGATGAGTTTAGAAGG	$(tg)_{12}(ta)_{4...}(at)_4$										
Cru10	F: GCGTGACGTCAGATAAACAG	$(tg)_8$	57–52	135–169	11	0.866	0.867	135–137	2	0.124	0.067	KP101507
	R: CGGACTGATAATCGTAGAGGA											
Cru11	F: GCGGTTACAGCAAACAAATA	$(tc)_6tt(tc)_{11}a(ct)_6$	57–52	195–271	18	0.910	0.900	191–223	11	0.753	0.600	KP101508
	R: CATTCCCTTCCCCCATAA											
Cru12	F: GCATCCTGACCTGAAAAT	$(ct)_8$	57–52	228	1	–	–	228	1	–	–	KP101509
	R: GAAGAGAAGAGATGTTACGC											
Cru13	F: GCGCGAGTTGGAAGTGAC	$(ag)_9ac(ag)_5$	57–52	226–270	16	0.873	0.967	228–252	9	0.650	0.833	KP101510
	R: GAGGCGATCGAGTGGACA											
Cru14	F: CCCTTTCACCCTCCATTTCTG	$(tc)_{9...}(ct)_{5...}(ct)_9$	60–55	186–196	5	0.626	0.467	176–182	4	0.446	0.400	KP101511
	R: CTCAAGCATGGTGCCTGTTCTA											
Cru15[b]	F: TTCCTAAATCTTCACGACAG	$(tc)_{17}$	60–55	206–224	6	0.744	0.767	210–226	5	0.556	0.400	KP101512
	R: GAACCACCAAATATCACTCA											
Cru16[b]	F: TGAAAATCCACAAATCTCCTT	$(ac)_7$	57–52	251–255	2	0.206	0.233	253–259	4	0.599	0.733	KP101513
	R: GTGAATATAAAAGAATCGGCAA											
Cru17	F: AGTGCGAGGAATCACGGA	$(ag)_{12}$	57–52	233–247	7	0.633	0.700	229–265	8	0.721	0.667	KP101514
	R: TGGAAGAAGAAGGGTAAAGGTG											

Cgg and *Cru* markers names refer to microsatellites developed for *C. renggeri* and *C. rufipes*, respectively

TD range of temperature for touchdown PCR amplification, *SR* size range including M13 tail, *N* number of alleles, H_E expected heterozygosity, H_O observed heterozygosity

[a,b] Significant departures from Hardy–Weinberg equilibrium for *C. renggeri* and *C. rufipes*, respectively

23 and 22 primer pairs were designed to *C. renggeri* and *C. rufipes*, respectively, using PrimerSelect (DNASTAR) software. To each forward primer we added a M13 tail (5′-CACGACGTTGTAAAACGAC-3′) at its 5′ end, which enabled the fragments to be scored on 6.5 % polyacrylamide gels on 4300 DNA Analysis System (Li-Cor Biosciences).

For each species, polymorphism levels for microsatellite markers were quantified using 30 workers from 30 different nests from Reserva Biológica de Mogi-Guaçu, state of São Paulo, Brazil. Polymerase chain reactions (PCR) were carried out in a final volume of 10 μL containing 1.5 ng of template DNA, 1× PCR buffer, 3 mM magnesium chloride, 0.2 μM of each dNTP, 0.1 μM of each primer, 0.1 μM of 700 or 800 nm infrared dyes (Li-Cor Biosciences) and 1 U *Taq* DNA polymerase. All loci were amplified using touchdown PCR, according to the following thermocycling conditions: 94 °C for 4 min; 10× [94 °C for 45 s, 60 or 57 °C (−0,5 °C/cycle) for 1 min and 72 °C for 1 min 15 s]; 25× [94 °C for 45 s, 50 °C for 1 min and 72 °C for 1 min 15 s]; and 72 °C for 10 min.

Of the initial 45 loci tested, 31 loci successfully cross amplified for both species and 27 were polymorphic for *C. renggeri* and 25 for *C. rufipes* (Table 1). The number of alleles, expected (H_E) and observed (H_O) heterozygosities and loci adherence to Hardy–Weinberg equilibrium (HWE) were calculated using the PopGenReport in R (Adamack and Gruber 2014). For *C. renggeri* and *C. rufipes*, the mean number of alleles was 6.9 and 5.9, the H_E ranged from 0.033 to 0.91 and 0.095 to 0.892 and the H_O ranged from 0.033 to 0.967 and 0.067 to 0.933, respectively. Significant departure from HWE was found for 1 and 8 loci in *C. renggeri* and *C. rufipes* samples, respectively (Table 1).

The genetic variation of *C. renggeri* and *C. rufipes* revealed by these novel molecular tools should provide valuable information to support Cerrado conservation strategies and also promote further investigation on phylogeography, population and landscape genetics.

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