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SHORT COMMUNICATION

Microsatellites for the Neotropical ant, *Camponotus leydigi* (Hymenoptera: Formicidae)

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Abstract

Ants (Hymenoptera: Formicidae) are dominant social insects that play important ecological roles in terrestrial ecosystems. *Camponotus leydigi* (Forel) is widely distributed in the Neotropical region and is frequently found in the Brazilian cerrado savanna interacting with plants and other insects. Field observations indicate that *C. leydigi* has a polydomous nesting habit, but little is known about the genetic relationship among workers. In this study, we identify the first nine microsatellite loci for *C. leydigi* that will allow further investigation on its genetic diversity. We used a microsatellite-enriched library method. According to this method, repetitive sequences are captured with $(CT)_8$ and $(GT)_8$ biotin-linked probes, with subsequent recovery by streptavidin magnetic-coated beads. We observed that eight loci were polymorphic. The mean (± standard error) observed and expected heterozygosities were 0.55 ± 0.23 and 0.73 ± 0.28, respectively. The rarified allelic richness ranged from 1 to 5.32. The polymorphism contents were similar to diversity estimates found in markers previously developed for other *Camponotus* ants. These markers will be useful for future studies on population genetics and ecology of *Camponotus* ants in cerrado, including nesting ecology, colony structure, dispersal and conservation.

Key words: *Camponotus leydigi*, cerrado savanna, formicinae, molecular markers, neotropics, simple sequence repeat, social insects.

Ants are distributed worldwide and outnumber all other terrestrial animals (Wheeler 1910). In tropical rainforests, ants account for over 80% of the arthropod biomass and up to nearly 90% of the arthropod individuals inhabiting the canopy environment (Majer 1990; Tobin 1995). Ants are abundant and occur in large numbers of species throughout the Brazilian cerrado savanna (Vasconcelos *et al.* 2008), where they feed on sweet secretions of extrafloral nectaries and insect trophobionts, scavenge for animal matter, hunt for arthropod prey, and collect fleshy seeds and fruits (Oliveira & Freitas 2004; Christianini & Oliveira 2010; Kaminski *et al.* 2010; Lange *et al.* 2019). Carpenter ants (genus *Camponotus*) are widely distributed in cerrado savanna (Vasconcelos *et al.* 2008). The ground-nesting species *Camponotus leydigi* (Forel) (Fig. 1) is frequently seen on the leaflitter hunting for insect prey, and on leaves collecting extrafloral nectar and insect honeydew (Costa *et al.* 1992; Schoereder *et al.* 2010; Bächtold *et al.* 2012; Soares 2018). Behavioral and spatial data support the existence of polydomy (i.e. physically separated but socially connected nests; Debout *et al.* 2007) in *C. leydigi* colonies in the cerrado (Soares 2018). However, little is known about the genetic relationship among workers from different nest units. Genetic polymorphism influences the species ability to respond to environmental changes, with implications for their conservation in nature (Romiguier *et al.* 2014; Ellegren & Galtier 2016). In ants, due to the haplodiploid sex determination and eusocial organization (with few

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Figure 1 Worker of *Camponotus leydigi* (Photo by Sebastián Sendoya).

reproductive individuals), genetic diversity is potentially low and make ants vulnerable to climate change, demographic fluctuations, and extinction (Hedrick & Parker 1997; Chapman & Bourke 2001). Therefore, elucidating patterns and processes underlying genetic variation is important to preserve ant populations and maintain their ecological functions and services (Del-Toro *et al.* 2012).

Microsatellites are molecular tools commonly employed to investigate species genetic diversity (Sunnucks 2001). They consist of tandem repetitive sequences of one to six nucleotides, which are frequent and randomly distributed in the genomes of eukaryotes (Selkoe & Toonen 2006). These regions are highly polymorphic and have codominant inheritance, being considered as neutral markers (Goldstein & Schlötterer 1999). Microsatellites are of interest to ecologists due to their applicability in understanding ecological and evolutionary patterns and processes at fine scales (Selkoe & Toonen 2006; Katada *et al.* 2007). For ants in particular, microsatellites are useful tools to investigate colony genetic structure (Bolton *et al.* 2006; Qian *et al.* 2012), breeding systems (e.g. number of queens and queen mating frequency in colonies; Goodisman & Hahn 2005; Azevedo-Silva *et al.* 2020), kinship between individuals, population and colony delimitation (e.g. identification of polydomy; Elias *et al.* 2005; Ellis *et al.* 2017). Here, we identify and characterize microsatellite markers for the ant species *Camponotus leydigi*. We provide nine new microsatellite loci that will allow further investigation on the behavioral ecology and genetic structure of *C. leydigi* colonies, and which can also be tested as potential molecular tools in other *Camponotus* species.

We sampled 10 nests from a polydomous colony of *C. leydigi* in the cerrado reserve in Itirapina (22°15′10”S, 47°49′22” W), state of São Paulo, southeast Brazil. The whole foraging area of the colony covered nearly 1700 m^2, with nest units at least 10 m apart from one another (Soares 2018). The total genomic DNA was extracted from entire workers, following the protocol by Saghai-Maroof *et al.* (1984). The method consisted of individual maceration in a 2% CTAB solution (200 mM Tris–HCl pH 8.0; 50 mM EDTA pH 8.0; 700 mM NaCl) followed by 10–30 min of incubation at 65°C. DNA was purified with chloroform/isoamyl alcohol (24:1) and precipitated with isopropanol. A microsatellite-enriched library was built based on Billotte *et al.* (1999), using six workers of *C. leydigi* from the same nest. Repetitive sequences were selected using $(CT)_8$ and $(GT)_8$ biotin-linked probes and recovered with streptavidin magnetic coated beads (Promega, Madison, WI, USA). The recovered fragments were cloned into pGEM-T vectors (Promega). The plasmids were inserted into *Escherichia coli* XL1-Blue, and recombinant colonies containing inserts were identified by colorimetric detection. Forty-eight positive clones were sequenced (forward and reverse) using the 3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA). The electropherograms were analyzed and edited with the program CLC Genomics Workbench v 4.9 (CLC bio, Arhus, Denmark). Any vector sequences and enzyme restriction sites were identified and removed from the sequences using the software Seqman (DNAStarInc, Madison, WI, USA). We used Blastn (Altschul *et al.* 1990) to compare the edited sequences with public database (NCBI) and to eliminate possible contamination. Microsatellites were identified in the sequences using the web-based program SSRIT (Temnykh *et al.* 2001). For the primer design, we used the programs Primer Select (DNAStar Inc.) and Primer3Plus (Untergasser *et al.* 2007), with the following criteria: (i) total fragment sizes between 100 bp and 300 bp; (ii) primers size between 18 and 22 bp; (iii) hybridization temperature (Tm) between 45°C and 65°C; (iv) maximum difference of 3°C between the Tm of each primer in the pair; (v) GC content above 35%; and (vi) absence of complementarity between the primer pair. At the 5′ end of each forward primer of the pair, a M13 tail (5′-CACGACGTTGTAAAACGAC - 3′; Schuelke 2000) was added, enabling genotyping in the sequencer 3500 Genetic Analyzer (Applied Biosystems). Four fluorescents (6-FAM, VIC, NED and PET; Applied Biosystems) were used to optimize the

Table 1 Characteristics of 9 microsatellite markers for *Camponotus leydigi*

Locus	Primer sequences (5′–3′)	Motif	TD (°C)	SR	A	H_E	H_O	PIC	Null	GenBank accession
Cl05	F:CACGACGTTGTAAAACGAC CGATTAGAATTATTAACGGTTG R:CGAGAAAATTACCCTCTGAG	$(GT)_{25}$	57–52	130–176	5.32	0.84	0.75	0.80	0.075	MT674622
Cl10	F:CACGACGTTGTAAAACGA CCTTCATAGTAGGACTGTTGTG R:AAAGTAGACGGATTGTAGCG	$(AC)_7$… $(CA)_{22}$… $(AT)_3$	57–52	266–380	4.67	0.76	0.80	0.73	0	MT674623
Cl17	F:CACGACGTTGTAAAACGAC GCCGAGTGAACTGTGATT R:GTGTCTACGAAAGCAAATGTA	$(AT)_3$… $(AG)_3$… $(AT)_3$… $(TA)_3$… $(TG)_{16}$… $(TGTA)_3$	57–52	238–256	2.27	0.52	0.83	0.41	0.001	MT674624
Cl22	F:CACGACGTTGTAAAACG ACGGCCGCGACTGTGTCTCA R:CGCGAACAAAAAACGAAAAA	$(GT)_7$… $(TG)_7$	57–52	185–277	1.44	0.10	0.069	0.097	0.00005	MT674625
Cl26	F:CACGACGTTGTAAAAC GACTTCGTTACGTATATGCTGGAA R:CGGGAGATTACTTCTTTATGTG	$(TAA)_3$	57–52	96–102	2.12	0.46	0.66	0.36	0	MT674626
Cl36	F:CACGACGTTGTAAAAC GACTTCATGAAAGATGCGATACTC R:TTTGCCTAGCGACTAAGTTC	$(TC)_5$… $(CG)_3$… $(CT)_{25}$	60–55	346–364	3.79	0.70	0.83	0.64	0.0164	MT674627
Cl39	F:CACGACGTTGTAAAAC GACAATGATTAATATACTTCGTGAA R:CACAACTTTGATTTCTGAA	$(TTTA)_3$	57–52	142	1	0	0	0	-	MT674628
Cl42	F:CACGACGTTGTAAAACG ACAGGCAGCTATTGAACACTCTAA R:GCCGAACAGAAGGAGAAA	$(TC)_4$	57–52	124–144	2.31	0.54	0.93	0.42	0	MT674629
Cl49	F:CACGACGTTGTAAAAC GACGGCAGCGAATCCCTTAG R:CGCTTCATTTTGTATGTGTATG	$(CA)_4$… $(AC)_4$… $(CA)_3$… $(CA)_3$… $(CG)_3$	57–52	213–223	1.99	0.50	1	0.37	0	MT674630
Mean					2.77	0.55	0.73	0.48	0.01	

TD, range of temperature for touchdown PCR amplification; SR, size range after addition of M13 tail; A, rarified allelic richness; H_E and H_O, expected and observed heterozygosities; PIC, polymorphism content; Null, estimate of null allele frequency; and GenBank accession number. Mean values of A, H_E, H_O, PIC and Null are shown.

genotyping process. The loci were amplified using two touchdown PCR protocols (Don *et al.* 1991), with the following steps: (i) 94°C for 4 min; (ii) 10 cycles of [94°C for 45 s, 60° or 57°C (– 0.5°C / cycle) for 1 min and 72°C for 1 min and 15 s]; (iii) 25 cycles of [94°C for 45 s, 50°C for 1 min and 72°C for 1 min and 15 s], and (iv) 72°C for 10 min. Amplifications were evaluated with polyacrylamide gel in the sequencer 3500 Genetic Analyzer (Applied Biosystems), using the program Geneious prime 2019.2 (Biomatters Limited, New Zealand). Loci that amplified according with expected sizes, and without nonspecificity, were chosen for further characterization. For this purpose, three workers per nest, totalling 30 workers were used. Observed and expected heterozygosity (H_O and H_E, respectively) and polymorphism content (PIC) (Botstein *et al.* 1980) were calculated in the Excel based program Microsatellites Toolkit (Park 2008). Rarefied allelic richness was estimated with the software HP-Rare (Kalinowski 2005). Linkage disequilibrium (LD) between each pair of markers was evaluated using the program FSTAT 2.9.4 (Goudet 1995). For LD estimates, the significance value (0.05) was corrected for multiple comparisons using Bonferroni correction. Microsatellite loci were evaluated for the occurrence of stuttering and reduced amplification of large fragments using the Micro-Checker program (Oosterhout *et al.* 2004). The frequency of null alleles was estimated with the software FreeNA (statistical significance not provided; see Chapuis & Estoup 2007).

From the initial 48 clones, 44 presented more than one microsatellite sequence. We were able to design primer pairs for 13 microsatellite loci. We successfully amplified nine of these markers, eight of which were polymorphic. Average H_E (mean ± SE) was 0.55 ± 0.23, with the loci Cl5 (0.84), Cl10 (0.76) and Cl36 (0.70) presenting the highest values (Table 1) whereas H_O (mean ± SE) was 0.73 ± 0.28, whereas PIC was 0.48 ± 0.23 (Table 1). The rarified allelic richness ranged from 1 to 5.32 alleles per locus (Table 1). We did not find any pair of loci under linkage disequilibrium. Additionally, there was no evidence of allele stuttering, or reduced amplification of large fragments. The frequency of null alleles is close to zero for most of the markers (Table 1).

The microsatellites we developed showed a high level of polymorphism, with diversity estimates (Table 1) similar to markers previously developed for other *Camponotus* ants. Booth *et al.* (2009), analyzing microsatellite markers of *C. femoratus* (Fabricius) found a variation in the observed heterozygosity ranging from 0.28 to 0.71. Macaranas *et al.* (2011) obtained values from 0.17 to 0.54 for *C. ephippium* (F. Smith). The allelic richness in our markers are also in agreement with other markers developed for other tropical *Camponotus*. For instance, Azevedo-Silva *et al.* (2015) also using 30 individuals found 1 to 19 alleles per locus for *C. renggeri* Emery and 1 to 15 for *C. rufipes* (Fabricius).

Ecological evidence indicates that *C. leyidigi* has a polydomous colony (Soares 2018). Ants with polydomous nesting habits are often successful due to diversification of the diet and increased rate of resource exploitation (through expansion of the foraging area and/or increase in foraging efficiency; Debout *et al.* 2007). Identifying polydomy is therefore essential to understand the life history and evolutionary success of particular ant species.

These are the first molecular markers developed for *C. leydigi*, and could be used as a tool to better explore the nesting ecology and colony structure in this ant species. Our microsatellite data may hopefully be useful for future research on the preservation of *C. leydigi* and other *Camponotus* species, and of their numerous interspecific interactions in tropical cerrado savanna.

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