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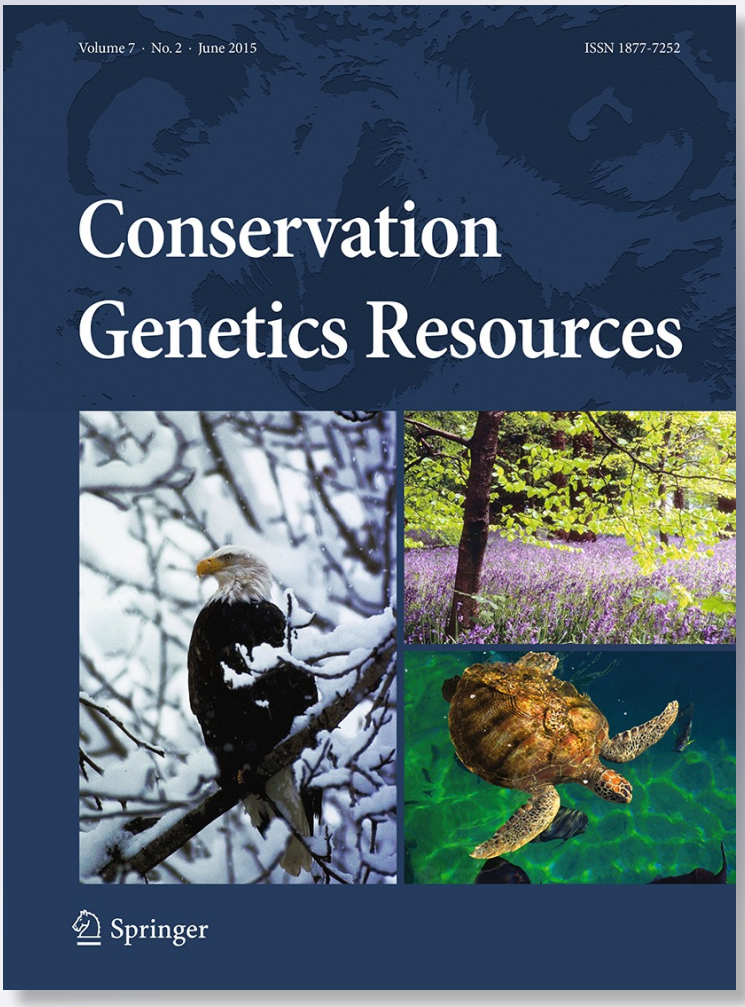

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MICROSATELLITE LETTERS

Development and characterization of microsatellite markers from the humivorous termite *Cavitermes tuberosus* (Isoptera: Termitinae) using pyrosequencing technology

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Abstract Eighteen microsatellite markers from the humivorous termite *Cavitermes tuberosus* (Isoptera: Termitidae: Termitinae) were developed using a procedure of microsatellite-enriched libraries pyrosequencing. They were optimized in four multiplex PCR sets, and tested on 38 individuals collected in French Guiana. The number of alleles per locus ranged from 3 to 13. The expected and observed heterozygosities varied from 0.279 to 0.867 and from 0.237 to 0.789, respectively. Cross-species amplifications of these loci were performed in eight other neotropical species of Termitidae. These new microsatellite markers will constitute useful tools to study population genetics, reproductive strategies and dispersal patterns of *C. tuberosus*, which is a common representative of the humivorous guild, fulfilling an essential function in soil nutrient recycling.

Keywords *Cavitermes tuberosus* · Termite · Microsatellite · Pyrosequencing · Population genetics

Termites represent a considerable fraction of the animal biomass in tropical ecosystems, where they play a major ecological role (Bignell and Eggleton 2000). These eusocial insects show a great diversity of life history traits and, more particularly, a challenging complexity of breeding systems (Bignell et al. 2011). Most studies investigating population genetic structure and breeding systems focused on species causing serious economical damage, such as subterranean termites (Bignell et al. 2011). Despite their ecological importance, soil-feeding termites have been very scarcely studied in these respects. We describe here the development and characterization of 18 polymorphic microsatellite loci for the humivorous termite *Cavitermes tuberosus*, a common arboreal-nesting species in French Guiana rainforests.

Samples were collected from seven localities in French Guiana. Total DNA was isolated from 50 individuals and mixed in a single tube for about 1,000 ng of genomic DNA using the kit NucleoSpin Tissue XS (Macherey–Nagel). Construction and pyrosequencing of microsatellite-enriched DNA libraries were carried out by Genoscreen, France (http://www.genoscreen.fr/). 24,090 sequences were sorted and primer pairs were designed for a total of 238 (over 6,345 sequences showing microsatellite motifs) sequences longer than 100 base pairs that contained at least 5-repeat microsatellite loci and tandem-repetition-free flanking regions.

We chose 96 sequences that we tested in separate PCRs on seven individuals. PCR amplification of DNA was performed in 25 μL reactions containing 2 μL (1 U) FastStart Taq DNA Polymerase (Roche Diagnostics), 2.5 μL 10× PCR buffer, 1.5 μL 25 mM $MgCl_2$, 0.6 μL 10 mM dNTP mix, 1 pmol of each forward and reverse primer, 2 μL (~20 ng) DNA and PCR-grade water (q.s.). The following cycling conditions were used: an initial denaturing step at 95 °C for 10 min; 40 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s; with a final extension step at 72 °C for 10 min. Visualization of the amplicons was

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Table 1 Microsatellite loci developed for the termite *Cavitermes tuberosus*

Locus	Genbank Accession Numbers	Repeat motif	Primer sequences (5′–3′)	5′ dye	Quantity (pmol)	Allele size range (bp)	N_a	H_o	H_e
PCR multiplex set 1									
Ctub-21F	KJ922353	(AG)10	F: AGCACATGCGAAGTCATCAG	VIC	12	165–197	6	0.737	0.728
			R: TCCAGCACAAACATCTTTCA		12				
Ctub-42F	KJ922354	(AC)13	F: AGAAGGTGTCATTCAATCATTTG	NED	8	239–255	6	0.526*	0.662
			R: GATTCATGACTGCTGATGATTTT		8				
Ctub-90F	KJ922367	(AC)18	F: GGACACGCTGTAGGATTTGT	6FAM	10	101–123	3	0.474	0.548
			R: GAATTATAATAGCATGGAATGGAAA		10				
Ctub-94F	KJ922369	(TCT)20	F: AATGTTTGCTTATCACGTTTTGA	NED	14	100–154	5	0.500	0.629
			R: CTTAAGGCTGAGGTGCTTCC		14				
Ctub-95F	KJ922370	(TCT)21	F: GAATCTGAACACAAGTACCCTGC	PET	4	130–151	5	0.447	0.446
			R: TGGTTGAGAAGGCCAAAACT		4				
PCR multiplex set 2									
Ctub-43F	KJ922355	(GT)13	F: ACCCCGATTATGTGAAATGG	VIC	14	132–148	5	0.684	0.710
			R: TGAAATTTCTGTACGTGGACCTT		14				
Ctub-60F	KJ922357	(CTT)11	F: TGAGACAATTTCGCATCAGC	NED	4	239–248	4	0.553	0.460
			R: AAACCACCAAGGGTGTAGCA		4				
Ctub-86F	KJ922366	(AC)15	F: TGGCCTTACCGTTTATCACC	PET	6	104–114	4	0.579	0.638
			R: TGGCATAGATGTCACAAGAAACA		6				
Ctub-91F	KJ922368	(CTT)18	F: TTGGTTGGTTTTATCCCGTC	6FAM	2	148–217	13	0.789	0.867
			R: AGTCAGCGTGAAAATACGGC		2				
PCR multiplex set 3									
Ctub-74F	KJ922360	(CTT)13	F: CTGCCTATATTCCACCTTTTTCTT	VIC	6	108–132	7	0.605	0.637
			R: ACACGTCGGCGTAAATATCC		6				
Ctub-77F	KJ922361	(CA)14	F: GCGCTACAATTCATATCGGG	6FAM	6	148–168	3	0.395	0.459
			R: GTTGTGTAGGTTGTCGGCG		6				
Ctub-84F	KJ922364	(CA)15	F: GCAAAGAGTAAGAATTATGTCGTTT	PET	1.25	236–274	7	0.632	0.656
			R: TGTCTGAAATCACGGAGATGA		1.25				
Ctub-85F	KJ922365	(AC)15	F: GTTAAGGGGTTATCAGCGCC	NED	10	157–167	5	0.237	0.279
			R: CACCCATAGTGTTGACGCAG		10				
PCR multiplex set 4									
Ctub-45F	KJ922356	(TCT)18	F: GTTTCACACCTGTGTTGTTAAAAT	NED	4	157–187	11	0.737	0.839
			R: GCTTAAGCAGACGGACCCTA		4				
Ctub-70F	KJ922358	(TTC)12	F: AGTGGGACCGGCGAATAC	6FAM	5	115–133	4	0.237†	0.347
			R: GCAAGATAGAAGAAGGTGGGG		5				
Ctub-72F	KJ922359	(TG)12	F: TGCACTAGTAAGAATATGCACGG	VIC	10	144–150	4	0.553	0.596
			R: CGACATCACGTTCATAGCAAG		10				
Ctub-78F	KJ922362	(AC)14	F: TGGTAGAGCTAGACAGGCCA	PET	6	148–150	3	0.632	0.594
			R: TTCCACTTTCACTTGGGTCC		6				
Ctub-80F	KJ922363	(AC)15	F: TCTTCGCGATGACAGACACT	NED	1.25	276–294	4	0.579	0.606
			R: AAACGTTAGTTATGCGGCGA		1.25				

The observed size range (in base pairs), the number of alleles (N_a), and the estimates of observed (H_o) and expected (H_e) heterozygosities are based on 38 individuals. * significant deviation from Hardy–Weinberg equilibrium after correction for multiple tests ($p < 0.01$); † significant presence of null alleles

conducted on an ABI3730XL sequencer (Applied Biosystems). Genotypes were obtained using GENEMAPPER version 2.7 software (Applied Biosystems).

Seventy-eight sequences yielded an unambiguous allelic pattern and 40 were polymorphic. Four multiplex PCRs of eighteen microsatellites were optimized using Multiplex

Table 2 Cross-species PCR tests for 18 *Cavitermes tuberosus* microsatellite loci in eight termite species

	Spinitermes trispinosus	*Inquilinitermes inquilinus*	*Termes* sp.	*Orthognathotermes aduncus*	*Planicapritermes planiceps*	*Neocapritermes taracua*	*Embiratermes neotenicus*	*Anoplotermes banksi*
Ctub-21	–	–	–	–	–	2 (181–193)	2 (187–193)	1 (203)
Ctub-42	2 (249–259)	1 (241)	1 (263)	2 (249–251)	2 (247–252)	1 (249)	–	1 (249)
Ctub-43	2	1 (128)	2 (148–152)	–	3 (132–152)	4 (128–152)	5 (126–150)	2 (142–152)
Ctub-45	–	–	–	–	–	3 (148–157)	–	–
Ctub-60	–	1 (245)	1 (245)	–	–	2 (245–257)	–	–
Ctub-70	–	–	–	–	–	–	1 (133)	2 (133–139)
Ctub-72	4 (138–150)	1 (144)	2 (138–148)	–	1 (148)	3 (140–156)	–	2 (150–152)
Ctub-74	1 (105)	2 (123–126)	1 (129)	–	–	–	4 (123–129)	2 (102–129)
Ctub-77	4 (142–154)	–	1 (152)	–	–	2 (148–150)	1 (154)	1 (152)
Ctub-78	–	–	–	–	–	–	–	–
Ctub-80	–	–	–	–	–	3 (286–304)	–	–
Ctub-84	–	–	–	–	–	–	–	–
Ctub-85	–	–	–	–	–	–	–	–
Ctub-86	–	–	–	–	–	–	–	–
Ctub-90	–	–	–	–	–	–	1 (135)	2 (135–139)
Ctub-91	4 (139–148)	1 (142)	2 (139–148)	–	1 (148)	3 (142–154)	–	2 (148–151)
Ctub-94	3 (112–118)	2 (142–151)	2 (148–154)	1 (145)	–	1 (145)	–	1 (145)
Ctub-95	2 (136–145)	3 (142–148)	2 (148–151)	1 (145)	2 (142–145)	1 (145)	2 (142–145)	1 (145)

Amplification failure is indicated by a dash

Manager 1.1 (Holleley and Geerts 2009) and were tested on 38 individuals. We extracted DNA from the head and the thorax using a Chelex method. PCR amplifications were carried out as above, except for the primers concentration (see Table 1).

GenAlEx version 6.5 (Peakall and Smouse 2012) and GenePop on the Web (http://genepop.curtin.edu.au/index.html) were used to estimate the number of alleles, expected and observed heterozygosities (H_e and H_o), and to test Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD). A sequential Bonferroni correction for multiple tests was applied when appropriate. Presence of null alleles was tested using Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004).

The number of alleles ranged from 3 to 13 per locus (mean ± standard error = 5.50 ± 0.63). Observed and expected heterozygosities varied from 0.237 to 0.789 and 0.279 to 0.867, respectively (Table 1). Locus *Ctub-42* deviated significantly from HWE. No LD was found over the combinations of 153 pairs of loci. Tests for presence of null alleles were significant at locus *Ctub-70*.

Cross-species amplifications were carried out as described above on three individuals from eight other neotropical species (Table 2). Loci *Ctub-78, Ctub-84, Ctub-85 and Ctub-86* did not amplify with any of the species sampled. Otherwise, all loci were polymorphic in at least one species (Table 2).

The microsatellite loci characterized here will provide suitable tools for investigating population genetic structure, breeding systems and dispersal patterns of the humivorous termite *C. tuberosus* and of related species.

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References

Bignell DE, Eggleton P (2000) Termites in ecosystems. In: Abe T, Bignell DE, Higashi M (eds) Termites: evolution, sociality, symbiosis, ecology. Kluwer Academic Publishers, Dordrecht, pp 363–387

Bignell DE, Roisin Y, Lo N (eds) (2011) Biology of termites: a modern synthesis. Springer, Dordrecht

Holleley CE, Geerts PG (2009) Multiplex Manager 1.0: a crossplatform computer program that plans and optimizes multiplex. PCR BioTech 46:511–517. doi:10.2144/000113156

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539. doi:10.1093/bioinformatics/bts460

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x