



Isolation and characterization of novel microsatellite loci in the genome of the dragonhead sap beetle *Thymogethes norvegicus* (Coleoptera: Nitidulidae)

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Abstract. Here we describe 16 novel microsatellite loci in the genome of the dragonhead sap beetle *Thymogethes norvegicus*, which were identified using a next-generation sequencing approach. In 40 dragonhead sap beetles from a population in southern Norway, we found 2–4 alleles per locus. Expected heterozygosity ranged from 0.18–0.65. Three of the loci showed significant deviation from Hardy-Weinberg equilibrium. After correcting for multiple tests, one pairwise locus combination displayed significant linkage disequilibrium. These novel microsatellite loci will be useful for future population structure, genetic diversity and conservation genetic studies of the dragonhead sap beetle.

INTRODUCTION

The dragonhead sap beetle *Thymogethes norvegicus* (Easton 1959), belonging to the Nitidulidae, was until recently assigned to the genus *Meligethes* Stephens, 1830 (Audisio et al. 2009). Easton (1959) described this species based on a series of individuals collected in southern Norway on the northern dragonhead *Dracocephalum ruyschiana*, which later was identified as its host plant (Stabbetorp & Endrestøl, 2011). Up to the year 2000, the global distribution of the dragonhead sap beetle was restricted to coastal areas in the Bærum municipality in Norway. In recent years, however, the species has been found in neighbouring municipalities (Stabbetorp & Endrestøl, 2011). Despite searching for it in other countries where it may occur (A. Endrestøl, unpubl. data), the dragonhead sap beetle is currently considered endemic to Norway (Stabbetorp & Endrestøl, 2011) and listed as endangered (EN) on the Norwegian Red List (Henriksen & Hilmo, 2015). In order to estimate the connectivity and genetic structure among populations of the dragonhead sap beetle we used high-throughput sequencing to identify novel microsatellite loci in the genome of this species.

MATERIAL AND METHODS

Seven dragonhead sap beetles collected in June 2015 in southern Norway (Latitude: 59.89947843, Longitude: 10.60829173) was sent to Ecogenics GmbH (Balgach, Switzerland) to produce an enriched microsatellite library and test for marker polymorphism. In brief, DNA was extracted from the beetles using a manual commercial kit (DNeasy Blood and Tissue Kit; Qiagen, Hilden, Germany) following the manufacturers protocol. Size-selected fragments of genomic DNA were enriched for simple

sequence repeats (SSRs) by using streptavidin-coated magnetic beads and biotin-labelled GTAT and GATA repeat oligonucleotides. The SSR-enriched library was analyzed on an Illumina MiSeq (Illumina, San Diego, CA, USA) platform at Microsynth AG (Balgach, Switzerland) using the Nano 2 × 250 v2 format. The resulting 335001 sequences were stitched, assembled and candidate microsatellites were searched for in the assembled 21390 contigs at Ecogenics using an in-house script. After assembly, 770 contigs or singlets contained a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least ten repeat units. Primer3 (Rozen & Skaletsky, 2000) was used to design PCR primers for 439 microsatellite candidates. Of the 439 microsatellite candidates, 36 were tested for polymorphism. Then, a selection based on genetic variation, single polymerase-chain reaction (PCR) performance and peak interpretation was performed.

Microsatellite loci were PCR amplified with fluorescently labelled (6FAM) forward primers. PCRs were performed in a final volume of 8.4 µL and the protocol consisted of 95°C for 15 min, 30 cycles of 95°C for 30 s, 56°C for 90 s, 72°C for 60 s and a final extension step of 60°C for 30 minutes. PCR products (0.8 µL) were mixed with Genescan 500 LIZ (Applied Biosystems, Foster City, CA, USA) size standard (0.14 µL) and Hi-Di formamide (6.16 µL). Alleles were separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer and sizes assigned using Genemapper software (Applied Biosystems).

For genotyping, we used DNA from 40 individuals, all collected in the years 2009–2015 in the Oslofjord area in southern Norway. Genomic DNA was extracted from a leg or whole speci-

Table 1. Characterization of 16 novel microsatellite loci in the genome of *Thymogethes norvegicus*.

Locus	Clone	Primer sequences (5' – 3')	Primer T _m (°C)	Repeat motif	n	A	H _O	H _E	Expected allele size (bp)	Observed allele size range (bp)	P _{HWE}	F _{NULL}	GenBank no.
Tno01	Melnor_00389s	F: ATGTAGCCCGCTTTGTTTCC R: TCACAGTCACTATCACCACAC	61 54	(TACA) ₈	40	2	0.25	0.32	223	210–222	0.16	0.05	KX831620
Tno02	Melnor_00574s	F: CGAGAAAATTAACCCAATGAGATAGC R: GGAGGACTGGATCAAAACAGC	62 61	(ACAA) ₁₂	40	3	0.40	0.39	188	164–188	0.88	0	KX831621
Tno03	Melnor_00735s	F: GGGTCCCATATCAGCTTCC R: TAAACATGTCCCCGTCTCC	63 62	(TCTA) ₇	40	3	0.33	0.47	165	158–170	0.02	0.10	KX831622
Tno04	Melnor_00857s	F: GCACAATAAACCGGGAGTGG R: TTGGTTCCACGAAAACCTGC	63 63	(ATAC) ₈	40	2	0.48	0.45	211	210–218	1.00	0	KX831623
Tno05	Melnor_01015s	F: GCTACTTTTGAAGTTCCACGC R: AGTGAATAGCGCATAAAACCCG	59 62	(GTAT) ₈	40	3	0.55	0.62	230	230–250	0.58	0.04	KX831624
Tno06	Melnor_01059s	F: TCCATCTACATACCTACTTGACTTG R: TTGCTCTTGTGGCAAACCTGG	57 62	(CAAA) ₇	40	2	0.10	0.18	248	237–245	0.03	0.07	KX831625
Tno07	Melnor_01260s	F: AAATATCCAGAAATCCACCTGC R: GAGTTGCAAGATTCCACCCG	59 63	(TGTA) ₈	40	2	0.53	0.50	142	136–140	0.76	0	KX831626
Tno08	Melnor_01301s	F: GCGGGGCATAACCAACATAG R: ACAAAGTGCCCTACACCAAAATG	62 61	(ATAG) ₁₄	40	4	0.50	0.55	160	159–171	0.25	0.03	KX831627
Tno09	Melnor_02057s	F: TCATGGCTTGAAAATGCAG R: TCCTTTTGTGGATCTTTTGAAC	62 64	(TAGA) ₈	40	2	0.33	0.31	190	184–188	1.00	0	KX831628
Tno10	Melnor_02892s	F: AAAAAGCGGGTGAGCATTAG R: GAAGTAGGTTACGAGTCGGC	60 58	(CATA) ₈	40	2	0.28	0.31	165	159–163	0.60	0.02	KX831629
Tno11	Melnor_03264s	F: CAGCTTCACCTTTTCCCTGC R: GTTTTTGAGCGACCTCTCTGG	62 61	(TAGA) ₁₂	40	3	0.35	0.44	203	191–199	0.02	0.06	KX831630
Tno12	Melnor_05317s	F: CGACCGTTCGATTCTTTCGC R: TGCCTTTTCATTGGGTGCTG	65 64	(CAAA) ₇	40	3	0.38	0.32	244	233–241	0.75	0	KX831631
Tno13	Melnor_07176s	F: GCTCGTGTAACCTGACTTGC R: GAATACAGCATATTCCGCTCG	59 60	(TATC) ₁₅	40	3	0.55	0.59	159	143–159	0.49	0.02	KX831632
Tno14	Melnor_09908s	F: TGGAGGAGAACCTAAAGGCG R: AGAAGTAGTTGAAAAAGCTGTGC	62 57	(TTTG) ₇	40	2	0.45	0.47	245	239–243	0.75	0.01	KX831633
Tno15	Melnor_14604s	F: AGACTAACGCACAGCAATTC R: GCTTTGACCACTTACCTGGG	56 60	(AGAT) ₁₃	40	4	0.68	0.65	97	70–102	1.00	0	KX831634
Tno16	Melnor_20171s	F: GTGATGGGCGCATTGTCTAC R: TGCTATATCCATTAGACCGTC	61 60	(AGAT) ₁₀	40	3	0.28	0.28	161	152–160	0.68	0	KX831635

T_m – melting temperature; n – number of individuals genotyped; A – number of alleles observed; H_O – observed heterozygosity; H_E – expected heterozygosity; P_{HWE} – P-value for test for deviation from Hardy-Weinberg equilibrium; F_{NULL} – estimated frequency of null-alleles according to the Brookfield 2 method implemented in Micro-Checker (van Oosterhout et al., 2004).

mens using a manual commercial kit (Qiagen DNeasy blood and tissue kit) following the manufacturer's protocol.

The mean number of alleles, observed and expected heterozygosities, deviation from Hardy-Weinberg equilibrium and linkage disequilibrium were estimated using Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). A Bonferroni correction for multiple statistical tests (Rice, 1989) was applied to linkage disequilibrium P-values. Null-allele frequencies were estimated using Micro-Checker version 2.2.3 (van Oosterhout et al., 2004).

RESULTS AND DISCUSSION

PCR analysis of seven individuals was done for the 36 microsatellite loci. Of these 36 loci, 16 were polymorphic, twelve were monomorphic, five were difficult to interpret and for three there were more than two alleles per individual. There were 2.7 alleles per locus (range 2 to 4; Table 1) at the 16 polymorphic loci in 40 beetles. The mean expected heterozygosity was 0.43 (range 0.18 to 0.65) and mean observed heterozygosity was 0.40 (range 0.10 to 0.68; Table 1). Evidence for null-alleles was recorded at one locus (Tno03) and three loci (Tno03, Tno06 and Tno11) deviated significantly from Hardy-Weinberg equilibrium (Table 1). After correcting for multiple tests, one locus pair (Tno03 and Tno15) displayed significant linkage disequilibrium. In conclusion, these novel microsatellite loci will be useful for future genetic analyses of the dragonhead sap beetle.

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