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# Isolation and characterisation of 15 microsatellite loci from *Lethrus apterus* (Coleoptera: Geotrupidae)

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Fifteen new microsatellite markers for the beetle *Lethrus apterus* were developed and tested in 45 specimens from the North Hungarian Mountains. Fourteen of the markers developed were polymorphic, and the number of alleles per locus ranged from two to nine. The observed and expected heterozygosities of the polymorphic markers ranged from 0.178 to 0.578, and 0.201 to 0.698, respectively. One locus showed significant deviation from the Hardy-Weinberg equilibrium, probably due to null alleles. The primers were tested on four other *Lethrus* species (*L. bituberculatus*, *L. scoparius*, *L. strymonensis* and *L. perun*) and six other coleopteran species (*Copris hispanus*, *Geotrupes stercorarius*, *Melolontha melolontha*, *Onthophagus taurus*, *Oryctes nasicornis* and *Protaetia affinis*). Thirteen loci showed cross-amplification in the *Lethrus* species and only three loci could be amplified in some of the six other coleopteran species. The developed markers will be valuable in investigating the population genetic structure, behaviour and reproductive biology of *L. apterus*.

## Introduction

The subfamily Lethrinae within the scarabaeoid family Geotrupidae is represented by a single genus, *Lethrus*, which comprises about 120 species (Hillert 2004, Král & Nikolajev 2006). The genus is considered monophyletic based on morphological characters (Nikolajev 2003, Scholz & Grebenikov 2005), with a wide distribution in the Palearctic, however, most of the species are

known from Central Asia (Nikolajev 2003, Král & Nikolajev 2006, Král & Hillert 2013). The beetle *Lethrus apterus* is distributed in eastern Europe and Anatolia, and the western edge of its distribution is in Hungary (Merkl & Vig 2009) where it is protected by law. Until recently, *L. apterus* was common in Hungary, and it was considered a pest. However, most of its habitats have been destroyed due to intense agriculture and now it is confined to fragmented areas (Merkl & Vig 2009). The species is well-known for its highly developed parental care (Wilson 1971).

*Lethrus apterus* is a biparental species, the sexes are dimorphic and — according to literature sources — there is a division of parental roles between the sexes (Emich 1884, Schreiner 1906, von Lengerken 1939, Wilson 1971, Clutton-Brock 1991): males are responsible for leaf collection and defend the nest burrow from intruders, while females prepare food balls for the offspring. Recent observations suggest a change in division of labour between the parents in northern Hungary: leaf collecting activity is highly female biased (A. Kosztolányi unpubl. data). One of the several possible explanations for this shift is that the area of this species has recently been fragmented, and because of this fragmentation the density of breeding individuals may have locally increased. High male density may increase the frequency of extra-pair matings, leading to a reduced incentive to care by males (Kokko & Jennions 2008). The observed change in parental duties may provide a unique opportunity to shed light on the evolutionary origin of biparental care and on how the social environment influences this cooperative behaviour.

Microsatellites are considered highly variable and co-dominant DNA markers, thus they are suited for investigations of genetic structure, reconstruction of pedigrees and estimation of parentage (Harris *et al*. 1991). Until now, there have been no published microsatellite markers available for any of the approximately 120 species in the genus *Lethrus*. Here, we present 15 microsatellite loci developed for *L. apterus*.

## Material and methods

Genomic DNA was isolated by homogenizing muscle of the thorax in 800 $\mu$l extraction buffer proposed by Gilbert *et al*. (2007). The samples were incubated for 24 h at 56 °C with gentle agitation and then centrifuged at 14 000 rpm for 1 min. The supernatant was washed with an equal volume of chloroform–isoamyl alcohol (24:1) to remove proteins. DNA was precipitated using 80 $\mu$l ammonium acetate (7.5 M) and an equal volume of ice-cold isopropanol stored at –20 °C for 4 h. DNA was pelleted by centrifugation at 14 000 rpm for 10 min at 4 °C. After centrifugation, the supernatant was discarded and the DNA pellet was washed twice with 70% ice-cold ethanol. The pellet was air-dried for 1 h and dissolved in 50 $\mu$l elution buffer (10 mM Tris HCl, pH 8.0 and 0.5 mM EDTA, pH 9.0).

High throughput sequencing was performed on an Illumina HiScan-SQ platform by a commercial service provider (UD-GenoMed Medical Genomic Technologies Ltd., Debrecen, Hungary). Genomic DNA libraries of two individually tagged specimens were prepared according to the Illumina DNA library preparation method. The average insert size was 250–300 bp. TruSeq DNA Sample Preparation Kit was used (Illumina, San Diego, CA) and paired-end 100 bp sequencing was carried out. The paired-end sequenced reads were de-multiplexed by individuals and assembled using the ngopt ver. 20130326 software (ngopt, NextGenOptimator, http://sourceforge.net/projects/ngopt/), with default settings. A total of 202.1 K and 214.8 K contigs (total length of the contigs: 240.6 and 257.7 Mb, N50 values: 2311 and 2837, respectively) were obtained from 57.7 M and 86.9 M aligned reads of the two individuals. After assembling, we searched in the assembled contigs for the motifs $(AAT)_n$, $(GT)_n$, $(CT)_n$ fulfilling three conditions: (i) $n \geq 5$, (ii) the length of flanking regions had to be at least 100 bp at both ends, and (iii) there had to be a size difference in repeat length between sequences of the two individuals. This process resulted in 18 potential loci. Primers were designed by manually inspecting potential priming regions, and the potential primers were tested and further modified to meet optimal priming criteria using the Primer Stats program of the Sequence Manipulation Suite ver. 2 (Stothard 2000).

Microsatellite polymorphism was tested on 45 specimens collected from four locations in

the North Hungarian Mountains: Cserháthaláp (47°59´2´´N, 19°22´30´´E, 4 individuals), Dorogháza (47°59´29´´N, 19°53´36´´E, 26 individuals), Kisterenye (48°0´41´´N, 19°50´44´´E, 10 individuals) and Nemti (48°0´31´´N, 19°53´20´´E, 5 individuals). The latter three locations are within a 2.5-km radius, whereas Cserháthaláp is located about 50 km to the east from the other three locations. DNA was extracted by homogenizing the middle leg in 800 $\mu$l extraction buffer proposed by Gilbert *et al*. (2007), and using the protocol described above. DNA aliquots were stored at 4 °C. DNA amplification from 1 $\mu$l of DNA extracts was carried out in 10 $\mu$l final reaction volumes containing 10 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 units/$\mu$l of Taq DNA polymerase (Dream Taq Green, Fermentas) and 0.5 $\mu$M of each fluorescent dye-labeled primer (Table 1). The following cycling conditions were used on ABI Veriti Thermal Cycler: initial denaturation 2 min at 95 °C; 40 cycles of 15 s at 95 °C, 30 s at the locus specific annealing temperature of 60 °C, 1 min at 72 °C; final elongation of 14 min at 72 °C. PCR amplicons were run on 2% agarose gels. Three primer pairs did not amplify the target sequences consistently, therefore these were excluded from further analyses. After amplification, microsatellite products were multiplexed and fragment analysis was carried out on an ABI 3130 Genetic Analyzer in the Molecular Taxonomy Laboratory of the Hungarian Natural History Museum (Budapest, Hungary). Allele sizes were estimated using the Peak Scanner software (Applied Biosystems). All allele sizes were double checked to assure reproducibility and correct readings. Micro-Checker 2.2.3 (van Oosterhout *et al*. 2004) was used for calculating null allele frequency by Monte Carlo simulation of expected homozygote frequencies and heterozygote allele size differences. Parameters of polymorphism, including the number of alleles per locus ($N_a$), observed heterozygosity ($H_o$) and expected heterozygosity ($H_e$) were calculated by GENALEX 6.4 (Peakall & Smouse 2006). Linkage disequilibrium test and deviation from Hardy-Weinberg equilibrium at each locus were performed by GENEPOP 4.2 (Raymond & Rousset 1995, Rousset 2008). Because of small and unequal numbers of individuals per sampling location, we treated the samples as one dataset and calculated the polymorphism measures uniquely.

## Results and discussion

Fourteen of the developed markers were polymorphic. The number of alleles per locus ranged from two to nine (Table 1). Observed ($H_o$) and expected ($H_e$) heterozygosities ranged from 0.178 to 0.578, and from 0.201 to 0.698, respectively (Table 1). Two loci (Lethrus11 and Lethrus13) showed significant deviation from the Hardy-Weinberg equilibrium and significant linkage disequilibrium was observed in 16.2% of all possible comparisons before correcting for multiple comparisons. The Micro-Checker analysis detected evidence for null alleles at the Lethrus11 locus by general excess of homozygotes for most allele size classes. After Bonferroni correction (Rice 1989), only locus Lethrus11 displayed deviation from the Hardy-Weinberg equilibrium (at $p < 0.0033$) probably due to null alleles, and only one significant linkage disequilibrium was observed (at $p < 0.00048$), affecting loci Lethrus01 and Lethrus05.

The primers were also tested on the DNA of 2–5 individuals of four closely related species: *Lethrus bituberculatus*, *L. scoparius*, *L. strymonensis* and *L. perun* and on the DNA of six other coleopteran species: *Copris hispanus*, *Geotrupes stercorarius*, *Melolontha melolontha*, *Onthophagus taurus*, *Oryctes nasicornis* and *Protaetia affinis* in order to investigate the primer pairs' effectiveness in other taxa. Out of the 15 loci, 13 showed cross-amplification based on gel electrophoresis, and amplifications were successful predominantly in *Lethrus* species (Table 2). Our results show that most of our markers are specific for *Lethrus* species, two of them for *L. apterus* in particular.

This newly developed microsatellite marker set will allow to study the relationship of environmental factors, behaviour, and reproductive biology of *Lethrus apterus* with its genetic structure in a new light. In particular, the newly developed markers make it possible to investigate parentage and population differentiation in this species with an enigmatic parental care

**Table 1.** Characteristics of 15 microsatellite loci in *Lethrus apterus*. Values are based on the analyses of 45 individuals. Dye = fluorescent dye label, Mix = the number of multiplexed microsatellite sets, $T_a$ = optimal annealing temperature (°C), $N$ = number of individuals from the 45 in which the locus amplified, $N_a$ = number of alleles per locus, $H_o$ = observed heterozygosity, $H_e$ = expected heterozygosity, HWE = exact $p$ value for the Hardy-Weinberg equilibrium test (the asterisk indicates a significant deviation from the Hardy-Weinberg equilibrium, $p < 0.0033$ after Bonferroni correction).

| Locus | Primer sequence (5´–3´) | Repeat motif | Dye | Mix | $T_a$ | $N$ | Size (bp) | $N_a$ | $H_o$ | $H_e$ | HWE | GenBank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lethrus01 | F: GCACAAAGACGTTATTACGAG<br>R: ATTTTCGTCCATTGTTTGCG | $(GT)_8$ | FAM | 2 | 60 | 45 | 148–150 | 2 | 0.289 | 0.401 | 0.066 | KJ934622 |
| Lethrus02 | F: GTAACGTTTGATTTTCCACACG<br>R: GTRGTGATGGATAAGAACAGAGC | $(AAT)_5$ | VIC | 2 | 60 | 44 | 98–101 | 2 | 0.386 | 0.407 | 0.718 | KJ934623 |
| Lethrus03 | F: TTCAAATGGGTCATTGATGAAA<br>R: ATGTATAATGGACACACTTATCTG | $(AAT)_5$ | PET | 1 | 60 | 45 | 150–153 | 2 | 0.489 | 0.500 | 1.000 | KJ934624 |
| Lethrus04 | F: CGTTTTGACAATAAAACCTGC<br>R: GATTGTGTTGCTATCCATGA | $(CT)_9$ | NED | 2 | 60 | 45 | 155–171 | 5 | 0.578 | 0.698 | 0.095 | KJ934625 |
| Lethrus05 | F: CGCACAAAGACGTTATTACG<br>R: TTTTCGTCCATTGTTTGCG | $(GT)_8$ | VIC | 1 | 60 | 45 | 149–151 | 2 | 0.289 | 0.401 | 0.069 | KJ934626 |
| Lethrus06 | F: TGACCGTATCACCTCCAA<br>R: ACTTGCTGTTTCTAAGTAGCG | $(GT)_8$ | FAM | 1 | 60 | 45 | 189–195 | 2 | 0.444 | 0.480 | 0.756 | KJ934627 |
| Lethrus07 | F: GGTTAAATATGGACGAACG<br>R: CCGTAAATCATAACAAGCG | $(GT)_8$ | NED | 1 | 60 | 45 | 165–169 | 3 | 0.289 | 0.363 | 0.370 | KJ934628 |
| Lethrus10 | F: GTTTATTAACAATACGCAAACC<br>R: GTTCCTGTTCCTTATAGTTGG | $(CT)_{17}$ | FAM | 2 | 60 | 44 | 185–197 | 4 | 0.455 | 0.504 | 0.798 | KJ934629 |
| Lethrus11 | F: TCCCGTTGTTACTACTTTCG<br>R: ATGAGGCTGGGAATGGTC | $(CT)_{10}$–TT–$(CT)_{10}$ | NED | 1 | 60 | 45 | 230–238 | 4 | 0.511 | 0.663 | 0.000* | KJ934630 |
| Lethrus13 | F: AAGATCGCAAATCAATGTCG<br>R: AGGTTTGCGACTTCTTGG | $(AAT)_8$ | NED | 2 | 60 | 42 | 258–261 | 3 | 0.262 | 0.368 | 0.033 | KJ934631 |
| Lethrus14 | F: CGAGATGACAAAAATTGTTCC<br>R: TACAAACCAAGAGCCAATCC | $(GT)_7$ | FAM | 1 | 60 | 45 | 366 | 1 | | monomorphic | | KJ934632 |
| Lethrus15 | F: AGTTGAATGTACCGATGACG<br>R: GTAACTATGTGTGTTGCAAGC | $(GT)_{11}$–A–$(GT)_2$–CA–GT | FAM | 1 | 60 | 45 | 259–265 | 3 | 0.178 | 0.201 | 0.432 | KJ934633 |
| Lethrus16 | F: GTTCTCATTTATTCTAGTGAGC<br>R: TACACGCACAAATCACACG | $(GT)_2$–TT–$(GT)_{18}$ | PET | 1 | 60 | 45 | 324–352 | 9 | 0.422 | 0.446 | 0.186 | KJ934634 |
| Lethrus17 | F: CGTGTAAATGACGTGAGC<br>R: CCGACTTCCTTATAGACAGG | $(GT)_8$ | VIC | 2 | 60 | 45 | 187–191 | 2 | 0.511 | 0.475 | 0.758 | KJ934635 |
| Lethrus19 | F: GATTATGTACTAAGGTCAGC<br>R: GCATAGTTCGTTTAGATACG | AAT–A–$(AAT)_7$ | PET | 2 | 60 | 41 | 343–346 | 2 | 0.293 | 0.369 | 0.205 | KJ934636 |

**Table 2.** Cross-amplification of *Lethrus apterus* microsatellite loci in four species of the genus *Lethrus* and six other Coleopteran species. *N* = number of individuals tested, L1 … L19 are the abbreviations of the loci (Lethrus01 … Lethrus19, respectively). '–' = the locus was not amplified.

| Species | *N* | Number of individuals in which the locus was amplified | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L10 | L11 | L13 | L14 | L15 | L16 | L17 | L19 |
| *Lethrus scoparius* | 4 | 1 | – | 4 | – | 3 | – | – | – | – | 4 | – | 4 | – | – | – |
| *Lethrus strymonensis* | 5 | 5 | 4 | 5 | 5 | 5 | 5 | – | 5 | 2 | 5 | 3 | 5 | – | 4 | 3 |
| *Lethrus bituberculatus* | 5 | 3 | – | 3 | – | 3 | – | – | – | 1 | 3 | – | 5 | – | 4 | – |
| *Lethrus perun* | 5 | 5 | 5 | 5 | 5 | 5 | 5 | – | 4 | 5 | 5 | 5 | 5 | – | 5 | 4 |
| *Copris hispanus* | 4 | – | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – |
| *Geotrupes stercorarius* | 2 | – | – | – | – | – | – | – | – | – | – | – | 2 | – | – | – |
| *Oryctes nasicornis* | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Protaetia affinis* | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *Melolontha melolontha* | 5 | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | – |
| *Onthophagus taurus* | 5 | – | – | – | – | – | – | – | – | – | – | – | 1 | – | – | – |

system and of great conservation concern in Europe.

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## References

Clutton-Brock, T. H. 1991: *The evolution of parental care*. — Princeton University Press, Princeton, New Jersey.

Emich, G. 1884: Die metamorphose des *Lethrus apterus*. — *Mathematische und Naturwissenschaftliche Berichte aus Ungarn* 2: 184–188.

Gilbert, M. T. P., Moore, W., Melchior, L. & Worobey, M. 2007: DNA extraction from dry museum beetles without conferring external morphological damage. — *PLoS One* 2: e272, doi:10.1371/journal.pone.0000272.

Harris, A. S., Bieger, S., Doyle, R. W. & Wright, J. M. 1991: DNA fingerprinting of tilapia, *Oreochromis niloticus*, and its application to agricultural genetics. — *Aquaculture* 92: 157–163.

Hillert, O. 2004: *Lethrus (Paralethrus) crassus* sp. n. from Uzbekistan (Coleoptera: Geotrupidae). — *Linzer Biologische Beiträge* 36: 823–839.

Kokko, H. & Jennions, M. D. 2008: Parental investment, sexual selection and sex ratios. —*Journal of Evolutionary Biology* 21: 919–948.

Král, D. & Hillert, O. 2013: Three new *Lethrus* species close to *L. raymondi* (Coleoptera: Geotrupidae) from the Balkan Peninsula. — *Acta Entomologica Musei Nationalis Pragae* 53: 219–244.

Král, D. & Nikolajev, G. V. 2006: Geotrupidae: Lethrinae. — In: Löbl, I. & Smetana, A. (eds.), *Catalogue of Palaearctic Coleoptera, Scarabaeoidea–Scirtoidea–Dasciloidea–Buprestoidea–Byrrhoidea*, vol. 3: 93–95. Apollo Books, Stenstrup.

Merkl, O. & Vig, K. 2009: *Beetles in the Pannonian Region*. — Vas Megyei Múzeumok Igazgatósága, B. K. L. Kiadó, Magyar Természettudományi Múzeum, Szombathely.

Nikolajev, G. V. [Николяев, Г. В.] 2003: [*Coleoptera, Scarabaeidae, Geotrupinae, Lethrini: biology, taxonomy, distribution, key*]. — Kazak Universiteti, Almaty. [In Russian].

Peakall, R. O. D. & Smouse, P. E. 2006: Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. — *Molecular Ecology Notes* 6: 288–295.

Raymond, M. & Rousset, F. 1995: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. — *Journal of Heredity* 86: 248–249.

Rice, W. R. 1989: Analyzing tables of statistical tests. — *Evolution* 43: 223–225.

Rousset, F. 2008: Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. — *Molecular Ecology Resources* 8: 103–106.

Scholz, C. H. & Grebennikov, V. V. 2005: Scarabaeiformia. — In: Beutel, R. G. & Leschen, R. A. B. (eds.), *Coleoptera beetles*, volume 1: *Morphology and systematics (Archostermata, Adephaga, Myxophaga, Polyphaga partim). Handbuch der Zoologie. Eine Naturgeschichte der Stämme des Tierreichs. Band IV. Arthropoda:*

*Insecta, Teilband 38*: 345–365. Walter de Gruyter, Berlin, New York.

Schreiner, J. 1906: Die Lebensweise und Metamorphose des Rebenschneiders oder grosskopfigen Zwiebelhornkafers (*Lethrus apterus* Laxm.). — *Horae Societatis Entomologicae Rossicae* 37: 197–208.

Stothard, P. 2000: The sequence manipulation suite: JavaScript programs for analysing and formatting protein and DNA sequences. — *Biotechniques* 28: 1102–1104.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. 2004: Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. — *Molecular Ecology Notes* 4: 535–538.

von Lengerken, H. 1939: *Die Brutfürsorge- und Brutpflegeinstinkte der Käfer*. — AkademischeVerlagsgesellschaft M. B. H., Leipzig.

Wilson, E. O. 1971: *The insect societies*. — Belknap Press of Harvard University Press, Cambridge.