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PERMANENT GENETIC RESOURCES NOTE

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Abstract

This article documents the addition of 238 microsatellite marker loci to the Molecular Ecology Resources Database. Loci were developed for the following species: *Alytes dickhilleni*, *Arapaima gigas*, *Austropotamobius italicus*, *Blumeria graminis* f. sp. *tritici*, *Cobitis lutheri*, *Dendroctonus ponderosae*, *Glossina morsitans morsitans*, *Haplophilus subterraneus*, *Kirengeshoma palmata*, *Lysimachia japonica*, *Macrolophus pygmaeus*, *Microtus cabrerae*, *Mytilus galloprovincialis*, *Pallisentis* (*Neosentis*) *celatus*, *Pulmonaria officinalis*, *Salminus franciscanus*, *Thais chocolata* and *Zootoca vivipara*. These loci were cross-tested on the following species: *Acanthina monodon*, *Alytes cisternasii*, *Alytes maurus*, *Alytes muletensis*, *Alytes obstetricans almogavarii*, *Alytes obstetricans boscai*, *Alytes obstetricans obstetricans*, *Alytes obstetricans pertinax*, *Cambarellus montezumae*, *Cambarellus zempoalensis*, *Chorus giganteus*, *Cobitis tetralineata*, *Glossina fuscipes fuscipes*, *Glossina pallidipes*, *Lysimachia japonica* var. *japonica*, *Lysimachia japonica* var. *minutissima*, *Orconectes virilis*, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Salminus brasiliensis* and *Salminus hilarii*.

This article documents the addition of 238 microsatellite marker loci to the Molecular Ecology Resources Database. Table 1 contains information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column. A full description of the development protocol for the loci presented here can be found on the Molecular Ecology Resources Database (http://tomato.biol.trinity.edu/).

Table 1 Information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Alytes dickhilleni	13	*A. obstetricans pertinax*, *A. obstetricans obstetricans*, *A. obstetricans boscai*, *A. obstetricans almogavarii*, *A. muletensis*, *A. maurus*, *A. misternasii*	45209–45221	HQ693828–HQ693840	Carvalho, B. M.; Lopes, S.; Van de Vliet, M. S.; Dias, G.; Benítez, M.; Beltrán, J. F.; Tejedo, M.; Ferrand, N.; Gonçalves, H.

Table 1 Continued

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Arapaima gigas	10	n/a	45253–45262	HM013750–HM013759	Santos, Carlos Henrique dos Anjos; Climaco, Gisele Torres; Sousa, Carolina Fernandes da Silva; Paula-Silva, Maria Nazaré; Sousa, Adna Cristina Barbosa; Farias, Izeni Pires; Campos, Tatiana; Almeida-Val, Vera Maria Fonseca
Austropotamobius italicus	12	*Pacifastacus leniusculus*, *Cambarellus zempoalensis*, *C. montezumae*, *Orconectes virilis*, *Procambarus clarkii*	45393–45404	HQ593123–HQ593134	Pedraza-Lara, C.; Gonzalez, E. G.; Bloor, P.; Doadrio, I.
Blumeria graminis f. sp. *tritici*	9	n/a	45222, 45223, 45225–45231 (see also 45224)	HQ631364, HQ631366–HQ631373	Parks, Ryan; Booth, Warren; Cowger, Christina
Cobitis lutheri	11	*C. tetralineata*	45281–45291	HQ158597–HQ158607	Kwan, Ye-Seul; Lee, Wan-Ok; Won, Yong-Jin
Dendroctonus ponderosae	50	n/a	45343–45392	GO486077, GT317345, GT320845, GT322895, GT324623, GT324841, GT325939, GT328703, GT331212, GT339861, GT344705, GT345241, GT350467, GT350767, GT356832, GT357891, GT363660, GT369500, GT373329, GT381367, GT383057, GT393905, GT401041, GT403944, GT404280, GT408450, GT413070, GT413201, GT415941, GT416554, GT419741, GT421807, GT429515, GT430043, GT433817, GT436798, GT451465, GT457678, GT458184, GT461671, GT464982, GT465588, GT473994, GT474165, GT485805, GT486724, GT489170, GT490424, GT490498, GT490735, GT491361	Samarasekera, N Gayathri; Keeling, Christopher I.; Bohlmann, Jörg; Murray, Brent W.
Glossina morsitans morsitans	14	*G. fuscipes fuscipes*, *G. pallidipes*	45232–45252	See paper for details	Hyseni, Chaz; Beadell, Jon S.; Gomez Ocampo, Zaneli; Ouma, Johnson O.; Okedi, Loyce M.; Gaunt, Michael W.; Caccone, Adalgisa
Haplophilus subterraneus	11	n/a	45198–45208	HQ670723–HQ670733	Congiu, L.; Boscari, E.; Bino, G.; Barbisan, F.; Leśniewska, M.; Fusco, G.

Table 1 Continued

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Kirengeshoma palmata	8	n/a	45176–45183	AB571675–AB571678, AB571681, AB571682, AB598398, AB598399	Yamashiro, T.; Yamashiro, A.
Lysimachia japonica	10	*L. japonica* var. *japonica*, *L. japonica* var. *minutissima*	45162, 45163, 45331–45342	AB591815–AB591824	Shinohara, Wataru; Kakezawa, Akihiro; Kawaguchi, Eri; Agata, Kiyokazu
Macrolophus pygmaeus	10	n/a	45264–45273	HM208591–HM208599, HQ853699	Sanchez, Juan A.; La Spina, Michelangelo; Perera, Omaththage P.
Microtus cabrerae	12	n/a	45292–45303	AF268902, AF268903, EF666983, EF666984, EF666987, EF666990, EF666991, EU101013, EU101014, EU101016, EU101021, FR820649	Alasaad, Samer; Soriguer, Ramón C.; Wandeler, Peter; Jowers, Michael J.; Sánchez, Antonio
Mytilus galloprovincialis	15	n/a	45132–45146	AJ625605, AJ626093, AJ624322, AJ938131, AJ938131, EH663192, EH663098, EH663076, EH662757, FL498494.1, FL500528.1, FL498564.1, FL495095.1, FL501296.1, BV725482	Pardo, B.G.; Vera, M.; Pino-Querido, A.; Álvarez-Dios, J.A.; Martínez, P.
Pallisentis (*Neosentis*) *celatus*	11	n/a	45324–45336	HQ588802–HQ588812	Li, Yang: Liu, Xiaolin: Liu, Haixia; Wang, Weimin; Zhu, Changcheng
Pulmonaria officinalis	8	n/a	45304–45311	HQ452963–HQ452970	Meeus, S.; Honnay, O.; Vandepitte, K.; Jacquemyn, H.
Salminus franciscanus	10	*S. brasiliensis*, *S. hilarii*	45184–45197 (includes monomorphic loci)	HQ317313–HQ137316, HQ137320–HQ137326	Rossini, Bruno C.; Nunes, Aline G.; Freitas, Patrícia D.; Galetti Jr, Pedro M.
Thais chocolata	12	*Acanthina monodon*, *Chorus giganteus*	45312–45323	HQ700360–HQ700371	Gallardo-Escárate, CristianValenzuela-Bustamante, Myriam; Haye Pilar
Zootoca vivipara	12	n/a	45164–45175	HQ337631–HQ337642	Stevens, Virginie M.; Richard, Murielle; Bleay, Colin; Clobert, Jean

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Microsatellite isolation in a population of the geophilomorph centipede *Haplophilus subterraneus* with high frequency of morphological anomalies

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Abstract

This study reports the first isolation and characterization of microsatellites from the major arthropod clade Chilopoda. The species of interest is the geophilomorph centipede *Haplophilus subterraneus*, for which a high incidence of morphological anomalies has been recorded in several European populations. With the aim of investigating the causes of malformations in the context of the phylogeographic history of the species, we set up a panel of microsatellites to be used in population genetics studies.

Main text

An exotic population of the geophilomorph centipede *Haplophilus subterraneus* (also cited as *Stigmatogaster subterranea*), living in a city park of Poznań (West Poland), exhibits a high proportion (26%) of individuals with morphological anomalies of several kinds. The study of these anomalies, in particular those affecting trunk segmentation, provided precious information about normal developmental dynamics in these animals during late embryogenesis, with implications for the process of segmentation in arthropods at large (Leśniewska *et al.* 2009a).

However, the causes of the anomalies are still to be identified. The high incidence and the extreme diversity of the morphological defects would suggest the presence of some environmental physico-chemical factors responsible for the recorded high level of developmental instability. However, further observations allowed to exclude this hypothesis: (a) physico-chemical parameters of the soil, including heavy metal content and radioactivity, are within standard values; (b) no comparable frequency of morphological anomalies of any kind was found in any of the other nine species of centipedes living in the same site, including other four geophilomorph species; (c) preliminary data show that segmental anomalies of the same kind occur with comparable high frequency also in other European populations of *H. subterraneus*, both within and outside its natural range (Leśniewska *et al.* 2009b).

Specifically targeted investigations are thus necessary to identify the causes of the high level of developmental instability in the populations of this species. With this aim, we have planned a study for evaluating the genetic structure of the European populations of *H. subterraneus* in its relation to the incidence of anomalies, and for reconstructing the phylogeography of the same population, as a basis for the comparative analysis of developmental instability.

The absence of microsatellite markers for the species made necessary the isolation here reported, which also represent the first microsatellite isolation of the whole clade Chilopoda.

Genomic DNA was extracted from 4-5 trunk segments using a salting-out protocol (Patwary *et al.* 1994). As for sample conservation, only animals stored in 100% ethanol immediately after collection yielded DNA of acceptable quality. Lower concentrations of ethanol resulted in highly degraded DNA.

A partial genomic library was constructed after enrichment with a pool of biotin-labelled probes ($(AC)_{17}$ $(AG)_{17}$ $(CAG)_{11}$ $(AAC)_{12}$ $(AAT)_{12}$ $(GATA)_9$ and $(CACC)_8$), following the FIASCO procedure (Zane *et al.* 2002a) adapted as in Zane *et al.* (2002b). About 211 recombinant colonies were screened by PCR with universal M13 primers and those with an insert longer than approximately 400bp were selected for sequencing analyses. All the PCRs were performed on GeneAmp PCR System 9700 thermal-cyclers (Applied Biosystems). Out of 108 sequenced clones, 75 contained a microsatellite motif, and 19 also had adequate flanking regions for primer designing that was performed with the software Oligoexplorer version 1.2 (www.genelink.com). A standard amplification with unlabelled primer was performed directly from genomic DNA of 3 individuals in 20 µl of reaction mixture containing: *Taq* buffer 1X (Resnova), $MgCl_2$ 1.5 mM, 0.6 µM of each primer, 200 µM dNTP's, 1 units of *Taq*, and about 50 ng of genomic DNA. Thermal cycler was set as follows: 94°C 30 sec, 50°C 30 sec, 72°C 30 sec, 30 cycles with an initial denaturing step of 5 min at 94°C and a final elongation of 5 min at 72°C. Amplification products were ran on 1.8% agarose gel to check amplification. A further selection was made on the basis of the efficiency of PCR amplification from genomic DNA. Finally, 12 loci (Tab. 1) were labelled with the appropriate dyes for fragment analysis with ABI PRISM 3100 or 3700 automated sequencer all yielding reliable and reproducible profiles (external service, BMR Genomics).

PCR amplifications from genomic DNA were performed in 20 individuals from the Poznań population, and 11 out of the 12 tested loci resulted to be polymorphic, with a number of alleles ranging from 2 to 7. For all loci amplification conditions were the same as previously reported for unlabelled primers with the only exception that annealing temperature was optimized according to Table 1. Scoring was performed using Peak Scanner 1.0 software (Applied Biosystem). Genepop

3.4 (Raymond & Rouset 1995) was used to test for deviation from Hardy-Weimberg equilibrium (HWE) and linkage disequilibrium (LD) with a statistical confidence interval (CI) of 99%; Sequential Bonferroni correction for multiple comparison (Rice 1989) was applied. No locus pairs were in significant LD and only locus H.sub13 was found to be significantly out of HWE. MICROCHECKER (Van Oosterhout *et al.* 2004) analyses indicated the possible presence of null alleles for locus H.sub13 (CI: 99%).

The relatively low number of alleles recorded in the Poznań population of *H. subterraneus* might be the result of a founder effect, as the species has been probably accidentally introduced with garden soil (Leśniewska *et al.* 2009b), but further comparative studies among populations inside and outside the natural range of the species will be necessary to test this hypothesis.

Table 1. Characteristics of microsatellites loci in *Haplophilus subterraneus*. * = Significant deviation from HW equilibrium (P < 0.01) after sequential Bonferroni correction. Fluorescent dyes used are reported for each labelled forward primer.

Locus	Primer sequences (5'-3')	Repeat motif	Annealing	GenBank accession number	No. alleles	Size range (bp)	Ho/He
H.sub16	$\mathbf{F}_{(NED)}$ GATAAATTGAGCATCAGCGAGTTT **R** CGTACGGGTTTACATCTTGTTC	$(CAA)_6$	54 °C	HQ670723	3	284-293	0.35/0.38
H.sub1	$\mathbf{F}_{(FAM)}$ GGATCTGCACTCAGATTTTCA **R** AACCCTTCATTCTTCCCTTTC	$(TG)_{12}$	50 °C	HQ670724	3	159-181	0.25/0.46
H.sub47	$\mathbf{F}_{(FAM)}$ TTATGATGGACAGAACTGTA **R** ACGTGAAGTATAGAAGTGTA	$(GA)_{11}$	54 °C	HQ670725	3	322-338	0.45/0.59
H.sub48	$\mathbf{F}_{(VIC)}$ CGTGGTGACGTGAGAATTT **R** GTTGACAACTTCGATCGGT	$(GA)_{16}$	53 °C	HQ670726	7	188-218	0.65/0.78
H.sub56	$\mathbf{F}_{(NED)}$ CGTCATTGTGGGAAGATCTAATTC **R** GTGGAATAACAGGGATGCTAT	$(CT)_9$	54 °C	HQ670727	3	253-259	0.55/0.55
H.sub13	$\mathbf{F}_{(NED)}$ CTCGGGAAATCCAGACC **R** GGCTGTCGGCTGATTTT	$(CA)_3CC(CA)_5$	58 °C	HQ670728	2	139-147	0.00/0.26*
H.sub36	$\mathbf{F}_{(FAM)}$ TAACACATCACAATTTTATCTACG **R** AAGGTTGTTTTGTGATACAA	$(CT)_8$	54 °C	HQ670729	3	88-108	0.25/0.56
H.sub85	$\mathbf{F}_{(FAM)}$ AGGCAGCTGTAATAATCCAA **R** TAGTATACACATCTCGCAGC	$(CA)_7$	54 °C	HQ670730	3	226-230	0.40/0.46
H.sub61	$\mathbf{F}_{(VIC)}$ AACCCTTTTCCTTGTATATAATC **R** ATTCATTGAAAATTACACCGAGC	$(TC)_6$	54 °C	HQ670731	2	173-175	0.3/0.38
H.sub45	$\mathbf{F}_{(VIC)}$ AGTTGTTATTGACTCCCGTT **R** CAACAGCTCAACGATCTTAC	$(GA)_9$	50 °C	HQ670732	5	242-254	0.5/0.6
H.sub43	$\mathbf{F}_{(VIC)}$ TGAGTGAGCATATTTGCGAG **R** CAATCGCCGCTTTATTCTCT	$(TG)_7$	50 °C	HQ670733	1	325	

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