Genus	Supplement 14: 59-62	Wrocław, 15 XII 2007

Cross-species amplification of microsatellite loci in *Orchesella flavescens* (Apterygota: Collembola)*

Robert Rutkowski[1*], Izabella Olejniczak[2], Paweł Boniecki[2], Anna Tereba[1], Magdalena Cybulska[3] & Anna Parys[4]

[1]Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland
[2]Center for Ecological Research, Polish Academy of Sciences, M. Konopnickiej 1, 05-092 Łomianki, Poland
[3]Warsaw Agricultural Academy, Ciszewskiego 8, 02-786 Warsaw, Poland
[4]Institute of Zoology, Department of Ecology, Warsaw University, Banacha 2, 02-097 Warsaw, Poland
*Corresponding author: E-mail: robertrut@miiz.waw.pl

Abstract. Cross-species amplification of microsatellite loci, described for *Orchesella cincta* and *O. villosa*, was performed in *O. flavescens*. Among 14 tested markers, 11 were amplified successfully and 5 of them were polymorphic, with 2 to 5 alleles, depending on the locus. Generally, success of amplification was higher for loci described for *O. cincta*, which might suggest close genetic relation between this species and *O. flavescens*.

Key words: Collembola, *Orchesella*, microsatellite markers, cross-species amplification.

INTRODUCTION

Microsatellites are defined as tandem repeats of a short (from two to six nucleotides) DNA motif, forming more or less uniform tracts up to 100 nucleotides long (Chambers et Macavoy 2000). High level of polymorphisms, together with the power that they provide to solve biological problems, as well as the possibility of analysis using fast and effective technique of PCR, makes the microsatellites a useful genetic marker for wide range of genetic investigation, ranging from identification of individuals to studies on population level (e.g. Sloane et al. 2000; Girman et al. 2001; Lee et al. 2001; Roeder et al. 2001). One of the factors limiting even broader use of microsatellites is the fact that the initial identification of the marker is expensive and labour-consuming, and also

*Proceedings of the 8th Conference of the Polish Taxonomical Society, Wiechlice 18-20 V 2007

requires cloning and sequencing. To overcome these disadvantages, researchers often adapt information about microsatellite markers originally developed for one species (source) for use in other, usually closely related species (the target). This strategy, based on using PCR primers described for one species to amplify homologous microsatellite in others, was called cross-species microsatellite amplification and was widely applied, frequently with success (Moore et al. 1991; Gemmell et al. 1997; Primmer et Ellegren 1998; Gibbs et al. 2000). However, application of cross-species microsatellite amplification has been shown to have many limitations. Firstly, the strategy works preferably for species belonging to the same genus or to recently separated genera (Scribner et Pearce 2000). Secondly, in many cases a given microsatellite may fail to amplify or may be less or even non-polymorphic in target species (Rubinsztein et al. 1995; Morin et al. 1998). Thus, application of cross-species strategy requires a pilot study which would assess the amplification success of particular markers and their level of polymorphisms in target species, as only polymorphic microsatellites can be successfully used in population genetic studies (Frankham et al. 2003).

In this paper, we describe results of cross-species amplification of microsatellite loci in *Orchesella flavescens* using PCR primers described for two other species of *Orchesella*: *O. villosa* and *O. cincta*.

MATERIAL AND METHODS

We collected 41 individuals of *O. flavescens* from two sampling sites: one local population from Kampinoski National Park (Poland) ($n = 25$) and one local population from southern Finland ($n = 16$).

Genomie DNA was isolated using DNeasy® Tissue Kit (Qiagen). Microsatellite loci were amplified using PCR with primers and conditions described by Van Der Wurff et. al. (2001) for *O. cincta* and by Spinsanti et al. (2006) for *O. villosa*. Results of amplification were assessed using capillary electrophoresis in CEQ 8000 automatic sequencer (Beckman Coulter).

For each successfully amplified locus we described the size of PCR products and the number of alleles. Using GenAlEx version 5.04 (Peakall et Smouse 2001) we estimated observed heterozygosity for each locus.

RESULTS AND DISCUSSION

Among 14 tested markers 11 (79%) were successfully amplified (Table 1), showing characteristic microsatellite's structure of a peak after analysis of PCR product in sequencing machine. One locus (OvMic47), though amplified, was impossible to interpret due to multiple peaks on the chromatograme, despite applying wide range of annealing temperatures. Monomorphic locus F2a4f was amplified exclusively in specimens from Poland. Five loci (45% of all successfully amplified) presented polymorphism with from 2 to 5 alleles. In one locus (Rt11b4) we found two alleles but no heterozygotes — allele 116 bp was found exclusively in population from Poland, whereas allele 118 bp only in sample from southern Finland.

Generally, success of amplification in *O. flavescens* was higher in the case of primers described for *O. cincta* (100%) and size of alleles in these two species appeared to be very similar. This might suggest close genetic relation between O. *flavescens* and O. *cincta*, as PRIMMER et al. (2005) showed that the success of amplification of a locus in cross-species strategy was higher when the genetic distance between source and target species was small.

The success of amplification and the ratio of polymorphic loci among those successfully amplified (79% and 45% accordingly) are both similar to values reported by other authors applying the cross-species strategy (e.g., PRIMMER et al. 1996; GALBUSERA et al. 2000).

We have identified 5 polymorphic microsatellites in *Orchesella flavescens*. Whilst it is postulated that at least 10 polymorphic loci are necessary to avoid erroneous inference in genetic population studies (KOSKINEN et al. 2004), studies based on four to six microsatellite markers are not rare (e.g. RICHARD et THORPE 2001). Moreover, as obtaining large samples of Collembola is rather easy, it could be stated that we described a useful molecular tool for "small scale" as well as "wider" population genetic analysis of *O. flavescens*.

ACKNOWLEDGEMENTS

The study was supported by Polish State Committee for Scientific Research (grant nr.: P04F 04025).

Table 1. Results of cross-species amplification of microsatellite loci described for *Orchesella cincta* (WURFF et. al. 2001) and *O. villosa* (SPINSANTI et al. 2006) in *O. flavescens*. * — cited data ** — range of observed heterozygosities found in different sampling localities, size/range — size of PCR products in base pairs and (in the case of polymorphic loci) size range of alleles; A — number of alleles in locus ; H_O — observed heterozygosity; *n* — sample size

	*O. cincta** (*n* = 30)			*O. flavescens* (*n* = 41)		
Locus	Size/range	A	H_O**	Size/range	A	H_O
Ft29b3	208–214	4	0.0–0.2	256–268	5	0.6
Rt17f2	204–206	2	0.1–0.5	202	1	0.0
Rt23d11	307–310	2	0.2	301–307	3	0.4
Rt11b4	114–116	2	0.1–0.5	116–118	2	0.0
Rt10d9	183–193	5	0.6–0.9	176	1	0.0
Rt18d7	230–234	3	0.4–0.8	236	1	0.0
F2a4f	190–196	2	0.0–0.2	216	1	0.0
	*O. villosa** (*n* = 75)					
OvMic3	109–125	11	0.33–0.66	143–145	2	0.2
OvMic7	132–146	10	0.46–0.73	132	1	0.0
OvMic11	105–109	3	0.13–0.53	–	–	–
OvMic16	115–147	18	0.13–0.40	–	–	–
OvMic32	131–149	9	0.40–0.80	73	1	0.0
OvMic38	107–131	12	0.26–0.73	131–137	4	0.3
OvMic47	153–167	10	0.80–0.86	–	–	–

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