Usefulness of heterologous microsatellites obtained from *Genypterus blacodes* (Schneider 1801) in species *Genypterus* off the Southeast Pacific

Utilidad de microsatélites heterólogos de *Genypterus blacodes* (Schneider 1801) en especies de *Genypterus* del Pacífico suroriental

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RESUMEN

Analizamos la utilidad de cinco loci de microsatélites aislados en *Genypterus blacodes* de Australia en especies de *Genypterus* presentes en Chile (*G. chilensis*, *G. maculatus* y *G. blacodes*). Los resultados muestran que los loci microsatélites amplifican positivamente en cada especie y podrían ser utilizados en futuros estudios poblacionales.

Microsatellites are currently one of the most popular and most used genetic markers in molecular ecology (Barbará *et al.* 2007). These markers are especially useful for evaluating patterns of populations genetic variability, and the microevolutionary mechanisms behind these patterns (e.g. Mundy *et al.* 1997). One strategy used to obtain microsatellite loci is the construction of a genomic library. Although cloning microsatellite loci for any taxon is relatively simple (Zane *et al.* 2002), the development of microsatellites still requires deep knowledge experts on molecular biology and a considerable amount of time and resources. An alternative strategy is to test primers described for microsatellite loci in closely related species (that is, cross-priming strategy or heterologous microsatellites). This cross-species amplification is only effective if the flanking regions of the primers are conserved among species. For example, highly conserved flanking regions have been reported in cetaceans (Schlötterer *et al.* 1991), turtles (FitzSimmons *et al.* 1995), and fish (Angers & Bernatchez 1996, Rico *et al.* 1996). A potentially useful application of cross-species amplification of microsatellites could be its use with species of congeneric commercial importance fishes.

Within the order Ophidiiformes, *Genypterus* is the genus with the largest number of species of economic importance (Nielsen *et al.* 1999). In the southeast Pacific Ocean three species of the genus can be found: *G. chilensis*, *G. maculatus*, and *G. blacodes*; and despite their importance, there are few genetic studies on *Genypterus* species.

There are no existing genetic studies regarding *G. chilensis*, and only one in relation to *G. maculatus* through allozymes

(Alay *et al.* 1993). There are a few studies involving *G. blacodes* (although not from the southeast Pacific), two of these studies used allozymes (Smith 1979, Smith & Francis 1982), and two other studies use microsatellite loci (Ward & Reilly 2001, Ward *et al.* 2001). The lack of genetic studies regarding these species could result in inadequate measures of fishery management policies.

The availability of microsatellite loci isolated from *G. blacodes* from Australia could contribute to population studies regarding species of the genus off the southeast Pacific. Therefore, the objective of the present study is to determine the usefulness of these markers in *G. chilensis*, *G. maculatus* and *G. blacodes* from the southeast Pacific.

We tested five pairs of microsatellite primers previously isolated from *G. blacodes* in Australia (Ward & Reilly 2001: cmrGb4.2A, cmrGb4.2B, cmrGb4.11B, cmrGb5.2B, and cmrGb5.9) on six *G. blacodes* (from the southeast Pacific), five *G. chilensis* and seven *G. maculatus* individuals. DNA was extracted from muscle tissue samples according to the protocol described by Grijalva-Chon *et al.* (1994). The quantity and quality DNA was measured by an Eppendorf® BioPhotometer and the DNA was diluted to 20 ng for amplifications. The PCR conditions for each locus were applied as described by Ward *et al.* (2001). The PCR products were separated by means of electrophoresis in 6% polyacrylamide gel and were visualized with silver staining (Acrylamide:Bis-Acrylamide Kit; Winkler®). A 10 pb marker (Invitrogen, Life Technologies, USA) was used as a standard weight marker to evaluate the size of the amplified products.

The genotypes were identified using the LabWork™ v4.6.00.0 software (UVP®, Inc.), which estimates the size of alleles based on electrophoretic mobility in relation to the mobility of the weight marker. Deviations from Hardy-Weinberg equilibrium between markers were tested using Arlequin v3.0 (Excoffier *et al.* 2005). The Genetix v4.05 program (Belkhir *et al.* 2000) was used to conduct a Factorial Correspondence Analysis (FCA). With this approach, we evaluated the usefulness of these markers in species differentiation. Additionally, a dendrogram was generated based on the Nei's standard genetic distance (Ds), using the UPGMA clustering method in the TFPGA v1.3 program (Miller 1997).

The results showed positive amplification of microsatellite loci in the three species. The percentage of amplification, mean number of alleles and size range of each locus varied between species (Table 1). Three loci showed significant deviation from Hardy-Weinberg equilibrium (Table 1). The percentage of variation in the FCA (Fig. 1) was explained by the first two axes (Axis1: 51.45; Axis2: 48.55). With these results, *G. blacodes* can be differentiated from other species. The dendrogram constructed by means of UPGMA corroborates these results (Fig. 2). The most closely related species are *G. chilensis* and *G. maculatus*, and *G. blacodes* appears as sister group of both species.

Our results confirm that flanking regions of microsatellite loci have been conserved in closely related species of the genus *Genypterus*, as observed in other studies with congeneric species or other taxonomic groups (Schlötterer *et al.* 1991, FitzSimmons *et al.* 1995, Angers & Bernatchez 1996, Rico *et al.* 1996). The percentage of amplification (Table 1) does not indicate a great loss of diversity among species (83.3% for *G. blacodes*, 88% for *G. chilensis*, and 97.1% for *G. maculatus* positive amplification), considering that a percentage of 57.2% and 57.3% of polymorphism and amplification, respectively, has been described for fish species (Barbará *et al.* 2007). However, given the low number of analyzed samples, these results should be taken with precaution. Our observations differ from other studies, in which a decrease in genetic diversity between species has been observed when using heterologous microsatellites (Primmer & Merilä 2002). The presence of null alleles could reduce genetic variability with increasing phylogenetic distance from a focal species (*e.g.* in the oyster *Crassostrea*, Li *et al.* 2003). Particularly among fish, the decrease of genetic variability is greater between genera of the same family, than between species of the same genus (Barbará *et al.* 2007), in conformity with the results obtained here.

TABLE 1: Loci microsatellite used to evaluate interspecific amplification. N: number of individuals; Na: number of alleles. Values in bold show significant deviation from Hardy-Weinberg equilibrium ($P \leq 0.05$).

TABLA 1: Marcadores microsatélites usados para evaluar amplificación interespecífica. N: número de individuos; Na: número de alelos. Los valores en negrita muestran desviación significativa de equilibrio de Hardy-Weinberg ($P \leq 0,05$).

	N	Parameter	cmrGb4.2A	cmrGb4.2B	cmrGb4.11B	cmrGb5.2B	cmrGb5.9	Mean
<i>G. blacodes</i>	6	% Amplification	66.6	100	66.6	83.3	100	83.3
		Na	2	8	6	6	7	5.8
		Range	174-206	161-183	199-246	114-154	146-168	-
		H&W (p-value)	0.671	1.000	0.809	0.683	1.000	
<i>G. chilensis</i>	5	% Amplification	60	80	100	100	100	88
		Na	5	7	4	6	5	5.4
		Range	202-246	147-190	210-216	126-166	130-156	-
		H&W (p-value)	1.000	0.003	0.162	0.282	0.197	
<i>G. maculatus</i>	7	% Amplification	85.7	100	100	100	100	97.1
		Na	7	8	8	7	6	7.2
		Range	186-238	155-185	202-234	126-154	136-162	-
		H&W (p-value)	0.003	0.864	0.415	0.045	0.163	

The positive amplification of heterologous microsatellites in species of *Genypterus* from the southeast Pacific indicates that these could be used to test polymorphism in other species of *Genypterus* spp., such as *G. brasiliensis* (southeast Atlantic), *G. capensis* (south of Africa) and *G. tigerinus* (Australia). Finally, five of the microsatellite loci isolated from *G. blacodes* from Australia (Ward & Reilly 2001) proved to be useful for genetic studies of congeneric species.

FIGURE 1: Factorial Correspondence Analysis (FCA) between species of the genus *Genypterus* of the southeast Pacific.

FIGURA 1: Análisis Factorial de Correspondencia (AFC) entre las especies del género *Genypterus* del Pacífico sureste.

FIGURE 2: Dendrogram based on Nei's standard genetic distance using UPGMA. Values above branches depict the bootstrap support values.

FIGURA 2: Dendrograma basado en la distancia genética estándar de Nei usando UPGMA. Los valores sobre las ramas representan los valores de soporte de bootstrap.

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