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# ISOLATION AND CHARACTERISATION OF EIGHT MICROSATELLITE MARKERS OF THE THORN-TAILED RAYADITO *APHRASTURA SPINICAUDA*

# AISLAMIENTO Y CARACTERIZACIÓN DE OCHO MARCADORES MICROSATÉLITES DEL RAYADITO *APHRASTURA SPINICAUDA*

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SUMMARY.—Eight novel microsatellites were isolated and characterised in the thorn-tailed rayadito *Aphrastura spinicauda* in order to evaluate genetic diversity in three contrasting populations, two of them at the boundaries of the geographic range of the species. All loci were found to be polymorphic and 3-15 alleles were found per locus. Expected and observed heterozygosity ranged from 0.26 to 0.89 and from 0.27 to 0.87, respectively. Global and pairwise Weir and Cockerham's $F_{st}$ showed that genetic differentiation was greater between the northern population and the ones in central and southern Chile. The eight markers developed will be useful to study the genetic diversity of the thorn-tailed rayadito across its distribution.

*Key words*: Chile, Furnariidae, habitat specialist, population genetics.

RESUMEN.—Para evaluar la diversidad genética en tres poblaciones del rayadito *Aphrastura spinicauda* que habitan zonas de características contrastantes (dos de ellas en los límites de su distribución), desarrollamos ocho marcadores moleculares (microsatélites). Todos los loci fueron polimórficos, encontrando de 3 a 15 alelos por locus. La heterocigosidad esperada y observada varió entre 0,26 a 0,89 y de 0,27 a 0,87 respectivamente. Los valores de $F_{st}$ de Weir y Cockerham indicaron que la mayor diferenciación genética ocurre entre las poblaciones del norte y del centro y entre las poblaciones del norte y el sur. Los ocho microsatélites desarrollados para este estudio pueden ser útiles para estudiar la diversidad genética del rayadito a todo lo largo de su distribución.

*Palabras clave*: Chile, especialista de hábitat, Furnariidae, genética poblacional.

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The family Furnariidae, with more than 240 recognised biological species, comprises a large group of passerines found in Mexico, Central and South America. They have been described as one of the most morphologically, behaviorally and ecologically diverse passerine families (Ridgely and Tudor, 1994). Anthropogenic habitat destruction (deforestation, burning, grazing) is the main threat to this family because it reduces and fragments their habitats (Remsen, 2003). Currently the IUCN lists five species of furnariids as "Critically Endangered", seven as "Endangered", 16 as "Vulnerable" and 23 as "Near Threatened", so that, in total, 21% of species are under some degree of threat (IUCN, 2014).

Despite the size of this family and the number of threatened species, relatively few studies have investigated genetic diversity, and these have principally used mtDNA and fingerprinting (ISSR) markers (e.g., Gonzalez and Wink, 2010; Fernandes *et al*., 2013). In some cases microsatellites developed from other passerine families have been used but, as has been observed in other groups, such cross-species VNTR markers were not very useful. For example Cardoni *et al*. (2013) tested 49 passerine microsatellite loci in an Argentinian saltmarsh furnariid species and only three of them were polymorphic. Thus it is worthwhile to isolate and characterise specific microsatellite markers in the Furnariidae in order to analyse genetic diversity.

The thorn-tailed rayadito *Aphrastura spinicauda* is an insectivorous and forest specialist passerine (Remsen, 2003) distributed in Argentina and Chile. In Chile it inhabits forests, ranging from a natural fragmented and highly isolated forest in the north (Fray Jorge National Park, 30° S) to a pristine and continuous forest in the south (Navarino Island 55° S). One of the most useful features of thorn-tailed rayaditos is that they build their nests in cavities, making the capture process easier by using artificial nest boxes (Moreno *et al*., 2005; Quilodrán *et al*., 2012; Quirici *et al*., 2014). The wide geographical range of the thorn-tailed rayadito allowed us to study three different populations characterised by contrasting parameters at ecological, geographical, geological and human intervention levels. In order to test our microsatellites, we compared populations located at both distributional margins (northern and southern populations) and another situated very near the city of Santiago (central population, 32º S).

Isolation and characterisation of microsatellites was performed by ATGgenetics (www.atggenetics.com). Genomic DNA of the thorn-tailed rayadito was used for cloning. Libraries of Hae III, Alu I and Rsa I digested genomic DNA were modified by linker adaptation using T4 DNA ligase and the primers:

M28 5' CTCTTGCTT**GAATTC**GGACTA
M29p 5' pTAGTCC**GAATTC**AAGCAAGAGCACA.

Screening began with 95 random clones each from the di mix library and tetra mix libraries. Amplified plasmid inserts were screened by dot blot hybridisation with biotin-labelled oligos. Hybridising clones (~ 25%) were identified by chemiluminescence (Phototope) and then treated with exonuclease I and alkaline phosphatase to prepare sequencing templates.

Primers were designed manually or with Primer 3.0. In order to assess the novel primers, Genomic DNA was amplified in 12 µl polymerase chain reactions (PCRs) as follows: 1 µL diluted genomic DNA, 1X UltraTherm buffer (Bocascientific Inc), 0.5 µM each primer pair, 200 µM each dGTP, dTTP, dCTP, dATP, 0.05 units Ultratherm DNApol (Bocascientific Inc) and 0.01 units Vent DNApol (New England Biolabs). Our thermal profile began with an initial denaturing step at 95ºC for 3 min, followed by 35 cycles at 55ºC (annealing temperature) and extension to 72ºC for five minutes.

TABLE 1

Polymorphic microsatellite loci from *Aphrastura spinicauda*.
*[Loci polimórficos de los microsatélites de* Aphrastura spinicauda.*]*

| Locus | Repeat | Primers | Size-Range (bp) | Pop | $N_A$ | $H_{obs}$ | $H_{exp}$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| As1 | $(AC)_{19}$ | F:TTTCCAGTTGTATCTCTCAGCA | 143-203 | North | 7 | 0.35 | 0.61 | 0.00 |
| | | R:GAAGAATGGGATCTAAGAAGTC | | Central | 5 | 0.33 | 0.72 | 0.00 |
| | | | | South | 10 | 0.39 | 0.73 | 0.00 |
| As25-10 | $(CA)_8A(AC)_{20}$ | F:GGAGTTATACCAGTTATAAAGG | 143-201 | North | 9 | 0.72 | 0.73 | 0.70 |
| | | R:TGCTGTTGTCTGGCTAGCA | | Central | 13 | 0.71 | 0.82 | 0.05 |
| | | | | South | 14 | 0.86 | 0.82 | 0.51 |
| As25-5 | $(TG)_{16}$ | F:TGGGTTCAGTATCCTGGAAGA | 177-211 | North | 9 | 0.72 | 0.74 | 0.29 |
| | | R:GAGTTGCTCTTCTCTCCCTCA | | Central | 12 | 0.84 | 0.88 | 0.43 |
| | | | | South | 15 | 0.86 | 0.89 | 0.04 |
| As25-1 | $(CA)_{18}$ | F:GGAGGGTATTTGGCAAGGTT | 177-211 | North | 7 | 0.82 | 0.78 | 0.41 |
| | | R:AGGATGGCTTGCTAGCTGTG | | Central | 4 | 0.27 | 0.29 | 0.17 |
| | | | | South | 7 | 0.64 | 0.71 | 0.04 |
| As25-8 | $(CA)_9$ | F:AAGAAGCTCACCCGCTACCT | 220-234 | North | 4 | 0.69 | 0.67 | 0.74 |
| | | R:TGTTGTCGTGCCTGAAGAAG | | Central | 5 | 0.64 | 0.67 | 0.13 |
| | | | | South | 4 | 0.59 | 0.67 | 0.00 |
| As25-14 | $(GATA)_{13}$ | F:TTTCTGCTGCTGGAAAGGTT | 179-225 | North | 9 | 0.68 | 0.82 | 0.03 |
| | | R:GTTCATCCAGGGAGAGTCCA | | Central | 8 | 0.71 | 0.83 | 0.05 |
| | | | | South | 13 | 0.65 | 0.82 | 0.00 |
| As18 | $(GT)_9$ | F:GGAAGCCATCTTAGGCTGTG | 214-222 | North | 4 | 0.54 | 0.50 | 0.42 |
| | | R:GGGCATAGATGGTTGCTGAT | | Central | 3 | 0.33 | 0.31 | 1.00 |
| | | | | South | 5 | 0.43 | 0.44 | 0.16 |
| As7 | $(GT)_{13}$ | F:GCTGGGCTTGCATATTCTTC | 217-251 | North | 5 | 0.27 | 0.26 | 0.14 |
| | | R:TCTTGTTTTGAAGGGAAGTGGA | | Central | 12 | 0.62 | 0.86 | 0.00 |
| | | | | South | 15 | 0.84 | 0.82 | 0.22 |

NA represents number alleles observed; Pop. represents the three populations in this study; Hobs. and Hexp. are observed and expected heterozygosities, respectively; P value is the probability of significant deviations between observed and expected heterozygosities.

Sixteen possible primers were received and tested in our laboratory to assess polymorphism and reproducibility. Each primer pair was tested using a bank of eight genomic DNAs and a positive control before sending them to be labelled by Applied Biosystems Inc. Finally, eight primer pairs showing strong polymorphic products and reproducibility were selected (table 1).

Size-fragment analysis was conducted by Applied Biosystems 3730xl automated sequencers with 50cm capillary arrays. Chromatograms were scored using GeneMarker 2.2.0 Demo (Softgenetics LLCR). Presence of null alleles, large allele dropout and stutter were assessed using MicroChecker (Van Oosterhout *et al*., 2004). Allelic richness and deviations from Hardy-Weinberg equilibrium were assessed for each population using ARLEQUIN v3.5 (Excoffier, 2010). Genetix v4.05 (Belkhir *et al*., 2000) was used to calculate values of global and pairwise Weir and Cockerham's $F_{ST}$ (Weir and Cockerham, 1984) and to test their significance through permutation tests (10,000 permutations).

Results from eight loci scored on 176 individuals (north = 68 individuals, central = 45 individuals, south = 63 individuals) are presented in table 1. The number of alleles ranged from three to 15 (north = 6.89, central = 8.22, south = 10.44), and genetic diversity from only one locus showed significant deviation from the Hardy-Weinberg Equilibrium in the three populations analysed (Locus As1), probably as a consequence of the presence of null alleles as detected by the MicroChecker analysis. Scoring errors due to stuttering or large allele dropout were not detected. Expected heterozygosity (H), although not statistically significant (paired comparisons > 0.05) was highest in the southernmost population (0.74 ± 0.40), followed by the central population (0.67 ± 0.36) and finally, the lowest value corresponded to the northernmost population (0.64 ± 0.34). The same trend was observed in allelic richness (A); it was highest in the southernmost population (10.44 ± 4.25), followed by the central population (8.22 ± 4.06) and lowest in the northernmost population (6.89 ± 2.09); the southernmost population was statistically different from the northernmost population (permutation test, P = 0.04). Regarding population genetic structuring, there was a highly significant degree of differentiation at a global scale with the eight loci analysed ($F_{ST}$ = 0.108, P < 0.001). The pairwise $F_{ST}$ indices showed that there was greater differentiation between the northernmost population and central and southernmost population than between the central and southernmost population. All values were highly significant, as shown in table 2.

Our study makes available the first specific microsatellite markers for a representative of the Furnariidae and provides very preliminary results on the geographic distribution of genetic diversity of the thorn-tailed rayadito across its geographical range. It suggests the existence of genetic differentiation between distant populations, as well as the existence of a latitudinal gradient in genetic diversity, decreasing from south (higher latitude) to north (lower latitude). The wide geographical range of the thorn-tailed rayadito makes it

TABLE 2

Pairwise $F_{ST}$ between sampling localities of *Aphrastura spinicauda*. *P < 0.001
*[Valores de $F_{ST}$ apareados entre las localidades muestreadas de* Aphrastura spinicauda. *$P < 0.001$.]*

| $F_{ST}$ | Central | South |
|---|---|---|
| North | 0.145* | 0.125* |
| Central | — | 0.042* |

an adequate model species to test the effects of different environmental and historical factors on its genetic diversity, such as the existence of sharp vegetation transition, habitat fragmentation caused by anthropogenic activities and the effect of the last glaciation on the southernmost populations.

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