SUPPLEMENTARY ELECTRONIC MATERIAL

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SEX RATIO OF ALPINE AND PYRENEAN ROCK PTARMIGANS *LAGOPUS MUTA* ESTIMATED BY A NON-INVASIVE METHOD (FAECAL DNA ANALYSIS) AND FROM HUNTING BAG DATA

LA PROPORCIÓN DE LOS SEXOS DEL LAGÓPODO ALPINO *LAGOPUS MUTA ALPINO* Y PIRENAICO ESTIMADA MEDIANTE UN MÉTODO NO INVASIVO (ANÁLISIS DE ADN EN HECES) Y POR DATOS DE CAZA

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Appendix 1

Description of the polymerase chain reaction (PCR) control.

[Descripción del control por PCR.]

To perform the PCR control, we perform a direct amplification of a fragment of approximately 300 base pairs (bp) of the microsatellite locus Mut12 (Constanzi *et al.*, 2018) using new inner designed primers (LM-Cont-F: 5'-AAAGCCACAGGGGCATGAA-3' and LM-Cont-R: 5'-GTCAGGAACAGGAATCCACGA-3'). The PCR was performed on 13µl of reaction mix containing 5µl of eluted template DNA, 6.5µl of Type-it Microsatellite PCR Kit (Qiagen) and 0.2µM of each primer. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; 40 cycles for 30s at 95°C, 90s at 60°C and 30s at 72°C; and a final extension of 30 minutes at 60°C. PCR amplicons were resolved in 1% agarose gels; the DNA samples with no amplification of the 300 bp fragment were discarded.

PCR for genotyping and sexing the samples:

[PCR para genotipar y sexar las muestras :]

The samples were genotyped using a set of 16 microsatellites described by Costanzi *et al.* (2018) and sexed by the amplification of a fragment of the sex-linked chromodomain-helicase-DNA-binding protein (CHD) gene (using the primers pair PU and P8mod) (Pérez *et al.*, 2011). The PCRs were carried out in four multiplex PCRs, three including primers for four microsatellites (mix 1: Mut01, Mut02, Mut04, Mut17; Mut24; mix 3: Mut03, Mut14, Mut18, Mut22; mix 4: Mut08, Mut12, Mut20, Mut23) and one including primers for four microsatellites and sex determination (mix 2: Mut06, Mut09, Mut16, CHD). Each multiplex was performed on 13µl reaction mix, containing 5µl of eluted template DNA, 6.5µl of Type-it Microsatellite PCR Kit, 0.2µM of each primer. Cycling parameters were as follows: initial denaturation at 95°C for 5 minutes; 30 cycles for 30s at 95°C, 90s at 53°C (mix 2) or at 60°C (mix 1, 3 and 4), and 30s at 72°C; and a final extension of 30 minutes at 60°C. One locus initially used for the genotyping process, Mut 12, which had a pattern that was very difficult to interpret, was discarded from the final microsatellite panel.

REFERENCES

Costanzi, J. M., Bergan, F., Sæbø, M., Jenkins, A. & Steifetten, Ø. (2018). Development and evaluation of 16 new microsatellite loci for the rock ptarmigan (*Lagopus muta*) and cross-species amplification for the willow grouse (*L. lagopus*). *BMC Research Notes*, 11: 1-5.

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