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Supplementary Material

A southern range extension for *Sminthopsis macroura* in Western Australia, at Eucla

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Supplementary Material for Umbrello et al. 2024 A southern range extension for *Sminthopsis macroura* in Western Australia, at Eucla.

Molecular laboratory methods

Total genomic DNA was obtained from WAM M65257 liver tissue at the Western Australian Museum. Approximately 15 mg of tissue was used to extract DNA in a Qiagen DNeasy tissue and blood tube kit according to manufactures' instructions, and DNA was eluted into 100 µL low-EDTA tris buffer. Both loci were amplified via polymerase chain reaction (PCR) in 25 µL reaction volumes containing 1 µL of template DNA, 1x PCR buffer containing 1.5 mM MgCl2 (Applied Biosystems, Branchburg, NJ, USA), 0.3 µM of each primer (Integrated DNA Technologies) and 1 unit of MyHSTAQ DNA polymerase (Applied Biosystems). Primers used for CR, and 12S are listed in Table S1.

PCR cycling conditions follow Krajewski *et al.* (1997) for 12S and are as follows: 95 °C for 5 min, then 9 low stringency cycles of 95 °C for 45 s, annealing at 49 °C for 50 s, and extension at 72 °C for 55 s, followed by 25 high-stringency cycles of 95 °C for 45 s, 55 °C for 50 s and extension at 72 °C for 1 min followed by a final extension at 72 °C for 5 min. For CR, PCR reactions were performed at the following conditions; 95°C for 5 min, then 35 cycles of denaturation of 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 60 s, and followed by a final extension at 72°C for 10 min. as detailed in Umbrello *et al.* (2017) PCR products were visualised on the E-Gel® Electrophoresis system (Life Technologies, Melbourne, Australia) on pre-cast 2% agarose gels with ethidium bromide.

DNA purification and bi-directional sequencing was carried out at the Australian Genome Research Facility, Perth, WA. Assembly, quality control and alignment of sequences was performed in Geneious Prime (Kearse *et al.* 2012), including checking for stop codons and trimming primer sequences.

Table S1: Primers used for PCR amplification and sequencing

Name	Direction	Sequence	Reference
		Control region (left domain)	
L15999M	FWD	5'-ACCATCAACACCCAAAGCTGA-3'	Fumagalli *et al.* (1997)
H16498M	REV	5'-CCTGAAGTAGCAACCAGTAG-3'	Fumagalli *et al.* (1997)
		12S RNA	
L12C	FWD	5'-AAAGCAAAACACTGAAAATG-3'	Springer *et al*. (1995)
H12GG	REV	5'-TRGGTGTARGCTRRRTGCTTT-3'	Springer *et al*. (1995)

References

Fumagalli L, Pope LC, Taberlet P, Moritz C (1997). Versatile primers for the amplification of the mitochondrial DNA control region in marsupials. *Molecular Ecology* **6**, 1199–1201.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* **28**, 1647–9. doi:10.1093/bioinformatics/bts199

Krajewski C, Blacket M, Buckley L, Westerman M (1997). A multigene assessment of phylogenetic relationships within the dasyurid marsupial subfamily Sminthopsinae. *Molecular Phylogenetics and Evolution* **8**, 236–248. doi:10.1006/mpev.1997.0421

Springer MS, Hollar LJ Burke A (1995). Compensatory substitutions and the evolution of the mitochondrial 12S rRNA gene in mammals. *Molecular Biology and Evolution* **12**, 1138–1150.

Umbrello LS, Woolley PA, Westerman M (2017). Species relationships in the dasyurid marsupial genus *Pseudantechinus* (Marsupialia: Dasyuridae): a re-examination of the taxonomic status of *Pseudantechinus roryi*. *Australian Journal of Zoology* **65**, 240–247. doi:10.1071/ZO17059