

Appendix A

Table S1. Specimens data and GenBank accession numbers for the *Nothophryne* sequences generated in the present study. Mozambique (MZ); Malawi (MW); Mount (Mt). Collections: (HF) Universidade Lúrio; (WC and PEM) Port Elisabeth Museum; (QQ) South African Institute of Biodiversity, (NA) not available.

Nº/ Molecular Accession	Collection Nº / Field Nº	Locality	Latitude	Longitude	12S	16S	RAG1	RHOD
AC769	NA	Mt Inago, MZ	-15.045000	37.396111	KU761268	KU761276	KU761284	KU761292
AC770	NA	Mt Namuli dome, MZ	-15.361540	37.072110	KU761269	KU761277	KU761285	KU761293
AC776	NA	Mt Namuli dome, MZ	-15.361540	37.072110	KU761270	KU761278	KU761286	KU761294
T5638	HF 21	Taratibu, MZ	-12.821783	39.686578	KU761271	KU761279	KU761287	KU761295
T5786	PEM A11343	Pese dome, Mt Namuli, MZ	-15.387418	37.031650	KU761272	KU761280	KU761288	KU761296
T5791	PEM A11320	Mt Namuli dome, MZ	-15.361540	37.072110	KU761273	KU761281	KU761289	KU761297
T5795	PEM A11369	Mt Ribáuè, MZ	-14.911860	38.316230	KU761274	KU761282	KU761290	KU761298
T6469	QQ 0710	Mt Mulanje, MW	-15.892307	35.614891	KU761275	KU761283	KU761291	KU761299

Table S2. Primers used in this study.

Gene	Primer	Study/Source
12S	12S L1091: AAAAAGCTTCAAACCTGGGATTAGATACCCCACTAT	Kosher et al., 1989
	12S H1478: TGA CTGCAGAGGGTGACGGGCGGTGTGT	Kosher et al., 1989
16S	16S H3062: CCGGTTTGA ACTCAGATCA	16sbr-H modified Palumbi et al., 1991
	16SB FROG: CGCCTGTTACCAAAAACAT	16sar-L modified Palumbi et al., 1991
RAG1	RAG1.Mart.FL1: AGCTGCAGYCARTAYCAYAARATGTA	Pramuk et al., 2008
	RAG1.AMP.R1: AACTCAGCTGCATTKCCAATRTCA	Pramuk et al., 2008
RHOD	Rhod-1A: ACCATGAACGGAACAGAAGGYCC	Bossuyt and Milinkovitch, 2000
	Rhod-1D: GTAGCGAAGAARCCTTCAAMGTA	Bossuyt and Milinkovitch, 2000

Table S3. PCR protocols.

Gene	Annealing temperature	Time	Cycles
12S	51°C	1 min	35
16S	51°C	1 min	35
RAG1	50°C	25 s	45
RHOD	51°C	1 min	35

References:

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- Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. 1991. *The simple fool's guide to PCR*. Department of Zoology and Kewalo Marine Laboratory, Hawai, 47 pp.
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