

Supplementary material

Monitoring post-release survival of the northern corroboree frog, *Pseudophryne pengilleyi*, using environmental DNA

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Table S1. Sequence data used to design primer and hydrolysis probe assay targeting *P. pengilleyi* and *P. corroboree*. *ANWC – Australian National Wildlife Collection. MV – Museum Victoria herpetology collection.

Species	Accession number/ID	Collection/Source	Sequence data (ND4 mtDNA)
<i>Crinia signifera</i>	A02601	ANWC	GAAGCCAAATTATTCTCCCATTAGCGGCAGCCTGATGACTCATTTGTTACTAT ACTTAATATAGCTCTCCCCCATCTACCAACTTCATGGGAGAACTACTTATC CTTCTCTCTACGTTCAACTGGGCCCCAACATCTATACTCTTGGCAGCCCTC
<i>Crinia signifera</i>	A02762	ANWC	GGAGCCAAATTATTCTTCCCTTGGCAGCAGCCTGATGACTTATTTGTTACTAT ACTTAATATAGCCCTCCCCCCTCCACCAATTTTCATGGGCGAACTACTTATC CTTCTCTCCACATTCAACTGGGCCCCAACATCTATAATACTGGCAGCCCTC
<i>Limnodynastes tasmaniensis</i>	A02058	ANWC	GCAGTCAAGTTATACTACCTCTTGCAGCAGCCTGATGATTACTTTCAACTAT ATTAACATAGCACTTCCTCCCTCAACTAACTTTATAGGCGAACTTTTAATT TTAACATCAGTATTTAAGTGATCCACTTACTCTATTACCCTCGCCGGCCTA
<i>Limnodynastes tasmaniensis</i>	A02785	ANWC	GCAGTCAAGTTATTCTACCTCTTGCAGCAGCCTGATGATTACTTTCAACTAT ATTAACATAGCACTTCCTCCCTCAACTAACTTTATAGGCGAACTTTTAATT TTAACATCAGTATTTAAGTGATCCACTTACTCTATCACCTCGCCGGCCTA
<i>Pseudophryne bibronii</i>	A01926	ANWC	GAAGTCAAATTATCCTCCCCTTAGCCGCAGCATGATGACTAATCTGCACAA TACTTAACATAGCTCTACCACCATCAACCAACTTCATGGGGGAACTCTTAA TTCTTCTCTCCACTTTCCACTGGTCCCCAATCTCACTTACTTTAGCAGCCCTA
<i>Pseudophryne bibronii</i>	A02300	ANWC	GAAGTCAAATTATCCTCCCCTTAGCCGCAGCATGATGACTAATCTGCACAA TACTTAACATAGCTCTACCACCATCAACCAACTTCATGGGGGAACTCTTAA TTCTTCTCTCCACTTTCCACTGGTCCCCAATCTCACTTACCTTAGCAGCCCTA
<i>Pseudophryne bibronii</i>	A02301	ANWC	GAAGTCAAATTATCCTTCCCTTAGCCGCAGCATGATGACTAATCTGCACAA TACTTAACATAGCTCTACCACCATCAACCAACTTCATGGGGGAACTCTTAA TTCTTCTCTCCACTTTCCACTGGTCCCCAATCTCACTTACTTTAGCAGCCCTA
<i>Pseudophryne dendyi</i>	A034570	MV	GAAGTCAAATTATCCTCCCCTTAGCCGCAGCATGATGACTAATCTGCACAA TACTTAACATAGCTCTACCACCATCAACCAACTTCATAGGAGAACTCTTAA TTCTTCTCTCCACTTTCCACTGGTCCCCAATCTCACTTIGCTTTAGCAGCCCTA
<i>Pseudophryne dendyi</i>	A034571	MV	GAAGTCAAATTATCCTCCCCTTAGCCGCAGCATGATGACTAATCTGCACAA TACTTAACATAGCTCTACCACCATCAACCAACTTCATGGGAGAACTCTTAA TTCTTCTCTCCACTTTCCACTGGTCCCCAATCTCACTTIGCTTTAGCAGCCCTA

<i>Pseudophryne corroboree</i>	A01858	ANWC	GGAGTCAAATCATCCTCCCACTAGCCGCAGCATGATGACTAATCTGCACAA TACTCAACATAGCTCTACCCCATCAACCAACTTCATAGGGGAACTCTTAA TCCTTCTTTCTACCTTTCCTGATCCCCAATCTCACTTGTCTTAGCAGCTTTA
<i>Pseudophryne corroboree</i>	A01860	ANWC	GGAGTCAAATCATCCTCCCACTAGCCGCAGCATGATGACTAATCTGCACAA TACTCAATATAGCTCTACCCCATCAACCAACTTCATAGGGGAACTCTTAA CCTTCTTTCTACCTTTCCTGATCCCCAATCTCACTTGTCTTAGCAGCTTTA
<i>Pseudophryne corroboree</i>	A01872	ANWC	GGAGTCAAATCATCCTCCCACTAGCCGCAGCATGATGACTAATCTGCACAA TACTCAATATAGCTCTACCCCATCAACCAACTTCATAGGGGAACTCTTAA CCTTCTTTCTGCCTTTCCTGATCCCCAATCTCACTTGTCTTAGCAGCTTTA
<i>Pseudophryne corroboree</i>	AA70256	Unknown	GGAGTCAAATCATCCTCCCACTAGCCTCAGCATGATGACTAATCTGCACAA TACTCAATATAGCTCTACCCCATCAACCAACTTCATAGGGGAACTCTTAA CCTTCTTTCTACCTTTCCTGATCCCCAATCTCACTTGTCTTAGCAGCTTTA
<i>Pseudophryne pengilleyi</i>	AA70219	Tidbinbilla breeding program	GGAGTCAAATCATCCTCCCACTAGCCGCAGCATGATGACTAATCTGCACAA TACTCAATATAGCTCTACCCCATCAACCAACTTCATAGGGGAACTCTTAA CCTTCTTTCTACCTTTCCTGATCCCCAATCTCACTTGTCTTAGCAGCTTTA

Table S2. Nucleotide mismatches in hydrolysis probe assay binding regions between target and non-target species. Note that the mismatches indicated for the non-target species correspond to minimum values.

Species	Left primer	Right primer	Probe	Total
<i>Pseudophryne</i> <i>corroboree</i>	0	0	0	0
<i>Pseudophryne</i> <i>pengilleyi</i>	0	0	0	0
<i>Crinia signifera</i>	5	10	1	16
<i>Limnodynastes</i> <i>tasmaniensis</i>	6	11	5	22
<i>Pseudophryne bibronii</i>	4	4	1	9
<i>Pseudophryne dendyi</i>	4	4	1	9

Table S3. *P.cor* ND4 primer and probe sequences showing mismatches with closely related and co-occurring species.

Details of <i>P.cor</i> _ND4 primer and hydrolysis probe assay		Forward primer sequence (5' – 3')	Reverse primer sequence (5'- 3)	Probe sequence (5' – 3)
		GGAGTCAAATCATCCTCCCAC	TCTCACTTGTCTTAGCAGCTTTA	ACCCCCATCAACCAACTTCA
Species incorporated into assay design				
Common name	Scientific name			
Northern corroboree frog	<i>Pseudophryne pengilleyi</i>
Southern corroboree frog	<i>Pseudophryne corroboree</i>
Common eastern froglet	<i>Crinia signifera</i>	.A..C.....T..T.....T	CA..TA.AC.....G.....CC.C	C.....T.....
Common eastern froglet	<i>Crinia signifera</i>C.....T..T..T..CT	CA..TA.AA.AC.G.....CC.C	C.....C..C.....T..T.
Spotted marsh frog	<i>Limnodynastes tasmaniensis</i>	.C.....G.T..A..A..T.	A...TA..AC.C.C..C.GCC..	T..T..C.....T.....T.
Spotted marsh frog	<i>Limnodynastes tasmaniensis</i>	.C.....G.T..T..A..T.	A...TA.CAC.C.C..C.GCC..	T..T..C.....T.....T.
Bibron's toadlet	<i>Pseudophryne bibronii</i>	.A.....T.....CTACT.....CC..	...A.....
Bibron's toadlet	<i>Pseudophryne bibronii</i>	.A.....T.....T..CTACT.....CC..	...A.....
Bibron's toadlet	<i>Pseudophryne bibronii</i>	.A.....T.....CTAC.....CC..	...A.....
Dendy's toadlet	<i>Pseudophryne dendyi</i>	.A.....T.....CTCT.....CC..	...A.....

Table S4. *Pseudophryne pengilleyi* assay sensitivity for amplifying low concentrations of DNA from synthetic oligonucleotides.

DNA concentration (copies/ μ L)	DNA amplification success
1×10^6	11/11
1×10^5	11/11
1×10^4	11/11
1×10^3	11/11
1×10^2	11/11
80	7/11
60	8/11
40	6/11
20	5/11
10	4/11
1	0/11
1×10^{-1}	0/11
1×10^{-2}	0/11