

## Supplementary Material

**Appendix S1.** Complete sequence of *Batrachochytrium dendrobatidis* gBlock gene fragment prepared by IDT Asia in Singapore. Synthetic gBlock was made from references to partial ITS sequence of Bd strain JEL 197 as described by Boyle et al. (2004).

Sequence retrieved from <https://www.ncbi.nlm.nih.gov/nuccore/AY598034.1?report=fasta>

>AY598034.1 *Batrachochytrium dendrobatidis* internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence.

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tgcgatgctacgtgcgtcacatgcagtacCCTTGATATAATACAGTGTGCCATATGTCACGAGTCGAACAAAATTTATTTATTTTTTCGACAAATTAATTGGAAATTGAATAATTTA
ATTGAAAAAATTGAAAATAAATATTAATAAAACAACCTTTTGACAACGGATCTCTTGGCTcactagctcagattcagtagaccgctgttg

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**Green:** ITS1-3 Chytr (forward primer)

**Yellow:** 5.8S Chytr (reverse primer)

**Cyan:** Chytr MGB2 (probe)

**Grey:** adapter sequences

Full gBlock length: 206 bp

**Appendix S2.** Prevalence of *Batrachochytrium dendrobatidis* in three native frog species *Leiopelma hamiltoni*, *L. archeyi*, and *L. hochstetteri* in captive and wild populations across six sites in New Zealand. Confidence intervals (95%) were calculated using the Wilson method.

Frog Species	Location	Number of Individuals	No. of Bd-positive	Total Bd prevalence (%)	95% Confidence Intervals (%)
<b>Wild population</b>					
<i>L. archeyi</i>	Whareorino Forest	197*	19	9.6	6.3–14.6
<i>L. hamiltoni</i> <sup>a</sup>	Maud Island	18	2**	11.1	3.1–32.8
<i>L. archeyi</i>	Pukeokahu	17*	0	0.0	0.0–18.4
<b>Captive indoor population</b>					
<i>L. archeyi</i>	Auckland Zoo	13	0	0.0	0.0–22.8
<i>L. hamiltoni</i> <sup>a</sup>	University of Otago	23	3	13.0	4.5–32.1
<i>L. hochstetteri</i>	University of Otago	14	0	0.0	0.0–21.5
<b>Captive outdoor population</b>					
<i>L. archeyi</i>	Auckland Zoo	11	0	0.0	0.0–25.9
<i>L. hamiltoni</i> <sup>a</sup>	Zealandia Wildlife Sanctuary	31	0	0.0	0.0–11.0

<sup>a</sup>formerly classified as *L. pakeka*

\*Frog swabs were collected and provided by the Department of Conservation (For Whareorino Forest,  $n = 158$ )

\*\*Number of Bd-weakly-positive individuals

**Appendix S3.** Frog individuals collected in different sampling locations that were declared with Bd-positive, Bd-negative, and Bd-weakly-positive status. Samples that showed amplification on two or more out of three replicates were designated as positive. Weakly-positive status was given to swab samples where only one out of three replicates showed amplification.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies per swab	Bd Status
LP2	<i>Leiopelma hamiltoni</i> **	Maud Island	1	40.1	1.00	22.84	Weak-positive
LP4	<i>Leiopelma hamiltoni</i> **	Maud Island	1	40.0	1.05	24.13	Weak-positive
A221	<i>Leiopelma archeyi</i>	Whareorino*	3	39.30	5.12	117.09	Positive
LA22	<i>Leiopelma archeyi</i>	Whareorino	2	39.0	0.62	14.23	Positive
D74	<i>Leiopelma archeyi</i>	Whareorino*	3	38.6	2.51	57.53	Positive
C837	<i>Leiopelma archeyi</i>	Whareorino*	2	38.6	3.29	75.26	Positive
B456	<i>Leiopelma archeyi</i>	Whareorino*	3	38.2	3.94	90.21	Positive
D1092	<i>Leiopelma archeyi</i>	Whareorino*	2	38.1	3.64	83.18	Positive
B465	<i>Leiopelma archeyi</i>	Whareorino*	3	37.99	12.81	293.14	Positive
B495	<i>Leiopelma archeyi</i>	Whareorino*	3	37.28	7.34	168.02	Positive
A223	<i>Leiopelma archeyi</i>	Whareorino*	3	36.76	12.48	285.47	Positive
A229	<i>Leiopelma archeyi</i>	Whareorino*	3	36.71	68.88	1575.97	Positive
LP76	<i>Leiopelma hamiltoni</i> **	University of Otago	3	36.65	6.07	138.91	Positive
C927	<i>Leiopelma archeyi</i>	Whareorino*	2	36.42	11.28	258.13	Positive
B412	<i>Leiopelma archeyi</i>	Whareorino*	2	36.36	41.06	939.45	Positive
C10	<i>Leiopelma hamiltoni</i> **	University of Otago	3	36.36	7.15	163.61	Positive
LP87	<i>Leiopelma hamiltoni</i> **	University of Otago	3	35.73	5.11	116.93	Positive
LA24	<i>Leiopelma archeyi</i>	Whareorino	3	35.52	10.24	234.25	Positive
B539	<i>Leiopelma archeyi</i>	Whareorino*	3	35.50	62.28	1425.06	Positive
A225	<i>Leiopelma archeyi</i>	Whareorino*	2	34.16	402.24	9203.20	Positive
C905	<i>Leiopelma archeyi</i>	Whareorino*	3	33.60	133.61	3057.06	Positive
A233	<i>Leiopelma archeyi</i>	Whareorino*	2	33.20	655.00	14986.39	Positive
B540	<i>Leiopelma archeyi</i>	Whareorino*	3	30.31	1698.61	38864.28	Positive
A232	<i>Leiopelma archeyi</i>	Whareorino*	3	27.92	20040.19	458519.46	Positive
A60164	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B30290	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
A60152	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
A60154	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B30292	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B50213	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B50214	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B50215	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B50216	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B50223	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60124	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60332	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60333	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60334	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative

## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
B60335	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60336	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60337	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60338	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60339	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60340	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60341	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60342	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60346	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
B60347	<i>Leiopelma archeyi</i>	Auckland Zoo	0	0	0	0	Negative
LP12	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP17	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP1	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP11	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP13	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP14	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP16	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP3	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP5	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP6	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP8	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP9	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
LP7	<i>Leiopelma hamiltoni</i> **	Maud Island	0	0	0	0	Negative
P403	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P449	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P433	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P475	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P290	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P374	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P248	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P239	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P476	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P262	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P447	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P130	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P345	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P278	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P155	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P71	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative
P460	<i>Leiopelma archeyi</i>	Pukeokahu	0	0	0	0	Negative

## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
LP30	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
Mi3	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP55	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
P3	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
H1	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H10	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H12	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H16	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H17	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H18	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H19	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H2	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H20	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H21	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H4	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H7	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H8	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
H9	<i>Leiopelma hochstetteri</i>	University of Otago	0	0	0	0	Negative
C1	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C11	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C3	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C5	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C6	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C7	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
C8	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP51	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP62	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP64	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP79	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP81	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LP84	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
Mi1	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
P2	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
P4	<i>Leiopelma hamiltoni</i> **	University of Otago	0	0	0	0	Negative
LA13	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA1	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA10	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA11	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA12	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA14	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative

## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
LA15	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA16	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA17	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA18	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA19	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA2	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA20	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA21	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA23	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA25	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA26	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA27	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA28	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA3	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA30	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA31	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA32	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA33	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA34	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA35	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA36	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA37	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA38	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA39	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA4	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA40	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA5	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA6	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA7	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA8	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
LA9	<i>Leiopelma archeyi</i>	Whareorino	0	0	0	0	Negative
D121	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B592	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B537	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B565	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D561	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D956	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D18	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
873	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
883	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative

## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
62	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
200	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
872	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
884	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
885	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
802	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
886	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
81	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
265	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
421	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
130	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
631	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
258	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
74	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
76	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
120	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
696	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
887	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D158	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D941	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D940	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D3	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D947	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D696	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D784	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D956	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D987	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D979	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D977	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D998	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D615	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D3	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D696	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D999	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D802	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D136	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D692	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D784	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D998	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D265	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative



## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies per swab	Bd Status
D990	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1071	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1072	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D593	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D859	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D359	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D911	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1014	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1015	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1031	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1063	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1022	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D613	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1158	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1159	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1105	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D3	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
D1160	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B419	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B58	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B448	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B449	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B424	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B289	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B494	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B538	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B511	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B521	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B541	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B524	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B504	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B568	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B591	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B548	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B606	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B607	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B608	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B429	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
B458	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A192	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative

## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
A195	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A184	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A197	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A186	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A195	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A184	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A192	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A184	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A97	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A119	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A222	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A218	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A230	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A231	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
106	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
219	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
108	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
104	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
892	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A197	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
A249	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C458	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C807	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C879	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C63	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C694	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C953	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C954	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C856	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C955	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C928	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C814	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C956	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C925	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C914	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C930	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C991	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C11	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C399	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1076	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative



## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies per swab	Bd Status
C1063	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1077	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1078	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1079	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1128	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C892	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1093	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C467	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C414	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C840	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C108	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C839	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C104	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C103	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1055	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1129	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1130	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C531	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C990	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C789	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
C1117	<i>Leiopelma archeyi</i>	Whareorino*	0	0	0	0	Negative
LP20	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP21	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP22	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP23	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP24	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP25	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP26	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP27	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP28	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP29	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP30	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP31	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP32	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP33	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP34	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP35	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP36	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP37	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP38	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative

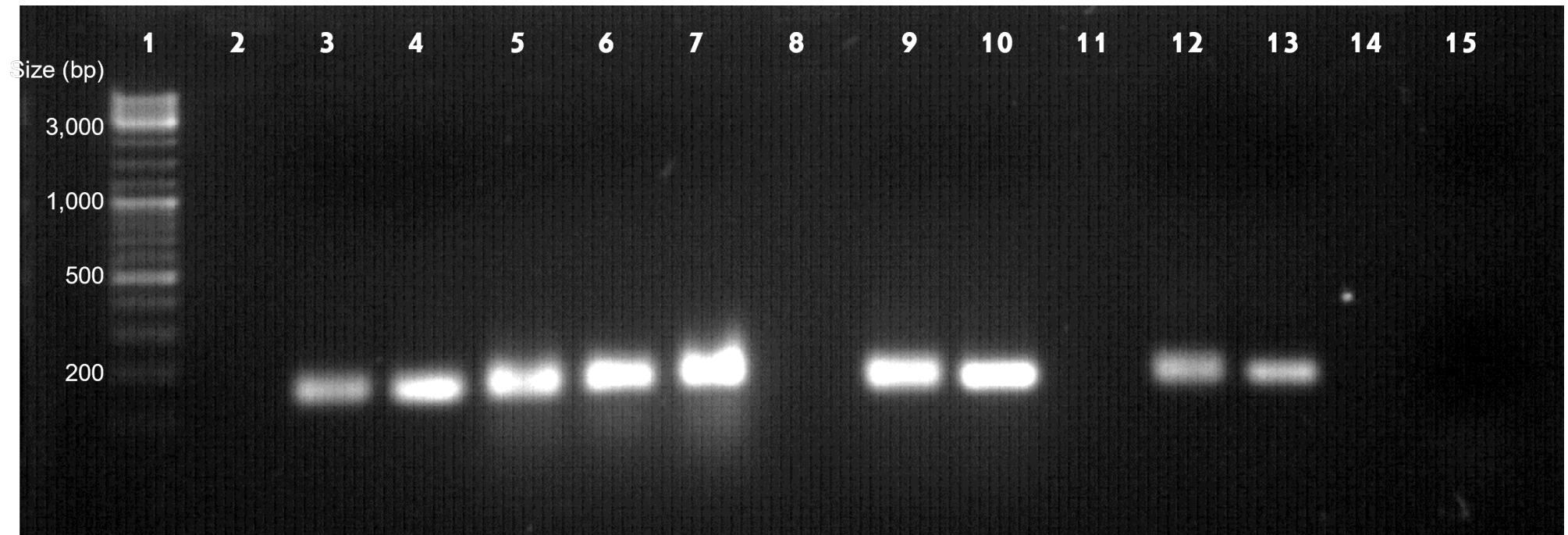
## Appendix S3. Continued.

Frog ID	Frog species	Location	Number of replicates with amplification	Mean CT value	Mean qPCR-generated DNA	Overall mean number of Bd DNA copies copy count per swab	Bd Status
LP39	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP40	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP41	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP42	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP43	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP44	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP45	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP46	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP47	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP48	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP49	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
LP50	<i>Leiopelma hamiltoni</i> **	Zealandia	0	0	0	0	Negative
After_rinse	After_rinse	Control	0	-	0		
Blank_PrepMan	Blank_PrepMan	Control	0	-	0		
Blank_Swab	Blank_Swab	Control	0	-	0		
Blank_PrepMan	Blank_PrepMan	Control	0	-	0		
Clean_Rinse	Clean_Rinse	Control	0	-	0		
NTC	NTC	Control	0	-	0		
Blank_PrepMan	Blank_PrepMan	Control	0	-	0		

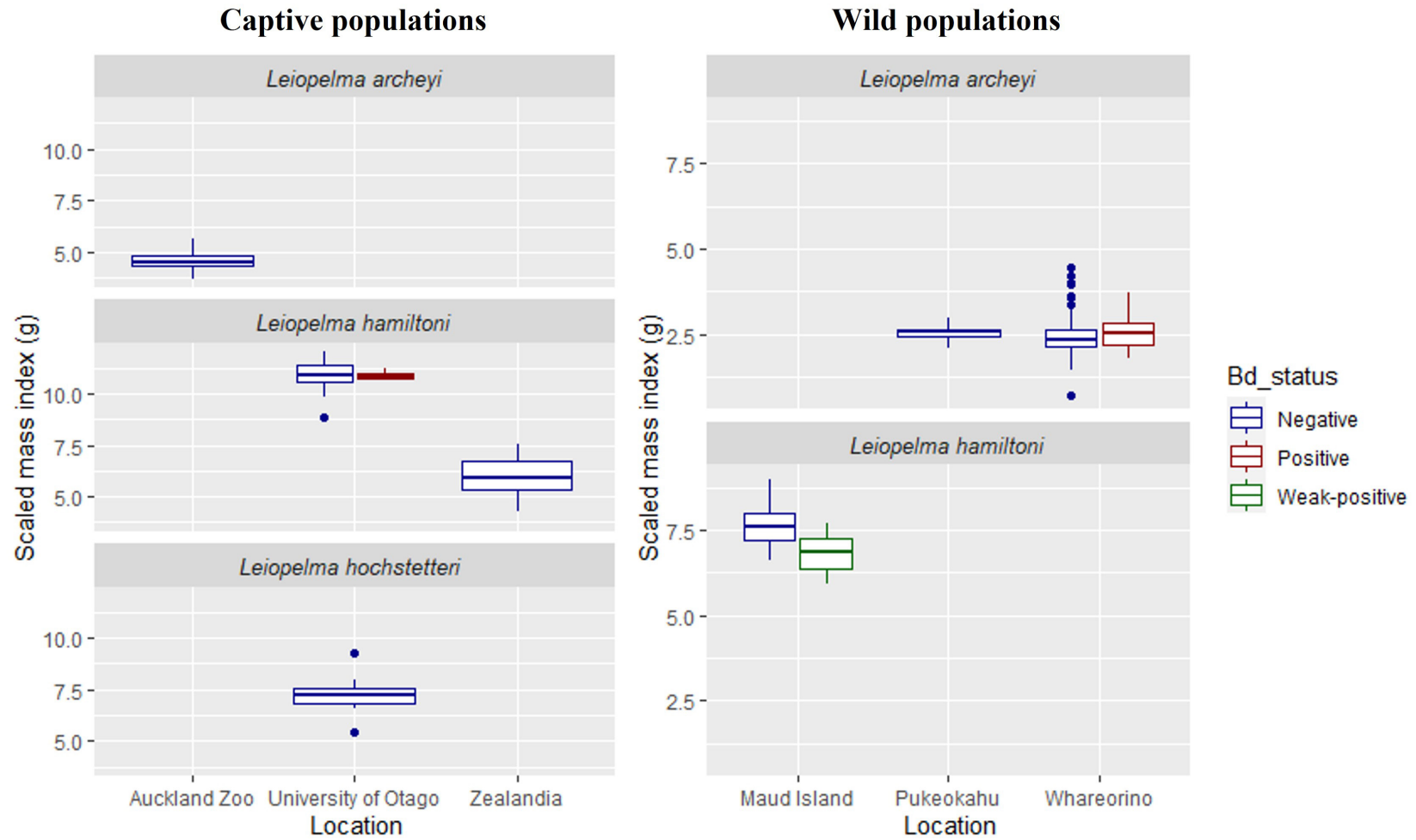
\*Frog swabs collected and provided by the Department of Conservation

\*\*Formerly classified as *Leiopelma pakeka*

**Appendix S4.** Agarose gel electrophoresis of selected Bd-positive samples was performed to confirm the amplicon observed was the expected size for Bd DNA. The target size for the Bd amplicon was 146 bp. Some amplicons were further purified for sequencing. Bd-positive frog samples with corresponding lane number in parentheses: Sample amplicons: C10 (3), LP76 (4), LP87 (5), LA22 (6), LA24 (7); sandards amplicons: Bd 10 spore  $\mu\text{L}^{-1}$  (12), Bd gBlock 1000 DNA copies  $\mu\text{L}^{-1}$  (13); and no template control (15).



**Appendix S5.** The scaled mass index (g) of the three endemic frog species (*Leiopelma archeyi*, *L. hamiltoni*, and *L. hochstetteri*) in captive and wild populations in relation to their Bd status and location.



**Appendix S6.** Temporal trends in the *Batrachochytrium dendrobatidis* (Bd) prevalence and abundance in *L. archeyi* in Whareorino Forest sampled between 2014–2020. Confidence intervals (95%) were calculated using the Wilson method.

<b>Year of collection</b>	<b>Total number of frogs sampled</b>	<b>Number of frogs swabbed</b>	<b>Number of Bd-positive</b>	<b>Total Bd prevalence (%)</b>	<b>95% Confidence Intervals</b>	<b>Mean Bd DNA copies per frog (<math>\pm</math> sd)</b>
<b>2014</b>	350	35	0	0.0%	0–9.9%	0
<b>2015</b>	382	24	2	8.3%	2.3–25.9%	42 $\pm$ 192
<b>2016</b>	217	12	1	8.3%	1.5–35.4%	8 $\pm$ 26
<b>2017</b>	198	19	4	21.1%	8.5–43.3%	33 $\pm$ 77
<b>2018</b>	368	54	8	14.8%	7.7–26.6%	9504 $\pm$ 62 491
<b>2019</b>	424	56	4	7.1%	2.8–17.0%	273 $\pm$ 2002
<b>2020</b>	164	6	0	0.0%	0–39.0%	0