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A recent specimen of a Tasmanian Boobook *Ninox leucopsis* recovered on Lord Howe Island

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Abstract. On 11 July 2019, during the Rodent Eradication Program on Lord Howe Island using aerial and ground rodent-baiting, the recovery of dead non-target birds included a recently dead boobook *Ninox* sp. found on a resident's property. Two *Tyto* species were also recovered. Despite automated sound-recording equipment stationed within the forests of the Island, no records of *Ninox* vocalisations were made before discovery of the boobook specimen; however, two instances of *Ninox* owl calls were reported anecdotally within The Settlement. There was speculation from some Island residents that the recovered boobook could have been an individual of the extinct endemic subspecies, the Lord Howe Boobook *N. novaeseelandiae albaria*. The boobook was forwarded to the Australian Museum for further visual scrutiny, collection of morphometric data, DNA analysis, and preparation for the Australian Museum collection. There was overlap in plumage and morphological measurements between both the Tasmanian Boobook (*N. leucopsis*) and the nominate Tasman Morepork from New Zealand (*N. n. novaeseelandiae)*, but the specimen was distinct from the larger Australian mainland *N. boobook*. DNA analysis provided conclusive evidence that the bird was a male *N. leucopsis*, exhibiting an overall clean-white spotted pattern and darker brown coloration. The occurrence of a *Ninox* species on Lord Howe Island is the first record in more than 50 years and should prompt further exploration of the dispersal and possible migration of boobooks from Tasmania.

Introduction

The Lord Howe Island Group (31°30′S, 159°05′E) is dominated by an island of volcanic origin, largely forested and of subtropical climate, located 580 km east of Australia's New South Wales coast in the South Pacific Ocean. The main Lord Howe Island (LHI) (see Figure 1), of 1455 ha, is bounded to the south by volcanic plugs of height 777 m and 875 m and to the north by part of the former caldera, forming hills reaching 209 m. The central lowland areas have been partially cleared for agriculture within The Settlement (Figure 1) and are dissected by a network of narrow roads.

An early account of the avifauna of LHI was published by Ramsay (1882), who listed 29 bird species occurring on the Island. Following major publications by Mathews (1928) and then Hindwood (1940), the number of species known increased to 85. Hutton (1991) provided a thorough contemporary account of all species and subspecies on the Island, as well as discussing extinct species. Later reviews by McAllan *et al.* (2004) and Frith (2013) provided further revisions of all known species, subspecies and vagrants. Based on the comprehensive account by McAllan *et al.* (2004), there are 182 species (including 11 extinct taxa) recorded for the Island. One of the extinct subspecies is the endemic Lord Howe Boobook *Ninox novaeseelandiae albaria.*

The taxonomy of the Southern Boobook group has been subject to several revisions and remains unresolved. Schodde & Mason (1997) treated the Southern Boobook *N. boobook* and the New Zealand (or Tasman) Morepork *N. novaeseelandiae* as separate species. However, Christidis & Boles (2008) considered *N. novaeseelandiae* and *N. boobook* a single species. Using bioacoustics and mitochondrial DNA, Gwee *et al.* (2017) studied *Ninox* in Wallacea and included representatives of several subspecies from the Australian mainland, Tasmania and New Zealand. Reconstructions of phylogenetic trees placed Tasmanian *N. leucopsis* closer to the New Zealand Morepork *N. novaeseelandiae* than to the mainland Australian species. Further, bioacoustics analyses also revealed a distinct separation of the mainland Australian group from Tasmanian and New Zealand representatives (Gwee *et al.* 2017).

Based on Dickinson *et al.* (2013) and del Hoyo *et al.* (2014), the mainland Southern Boobook is currently listed by BirdLife Australia (2019) as *N. boobook* (with three subspecies). The Tasmanian Boobook is recognised as a separate full species, *N. leucopsis*, and the New Zealand Morepork *N. novaeseelandiae* is treated as a distinct species with three subspecies (nominate in New Zealand, extinct *albaria* on LHI, and the Norfolk Island Morepork survives as *undulata* × introduced *novaeseelandiae* progeny). The last pure *undulata* on Norfolk Island was a female that bred with one of two introduced *N. n. novaeseelandiae* in 1989 and 1990 (Olsen 1996), with the pure *undulata* declared extinct in 2000 (Garnett *et al.* 2011).

During the recent Rodent Eradication Program (REP) on LHI (DoEE 2017), the recovery of owl remains was part of the LHI Board's mitigation management of non-target species. For the REP, a cereal-based bait containing the rodenticide brodifacoum was aerially distributed over 75% (the forested area) of the Island. In The Settlement, bait was distributed in >19,000 ground baiting stations, placed 10 m apart (Harper *et al*. 2020). The bait was dyed green to reduce its attractiveness to birds, but it was expected that consumption of poisoned rodents would affect,

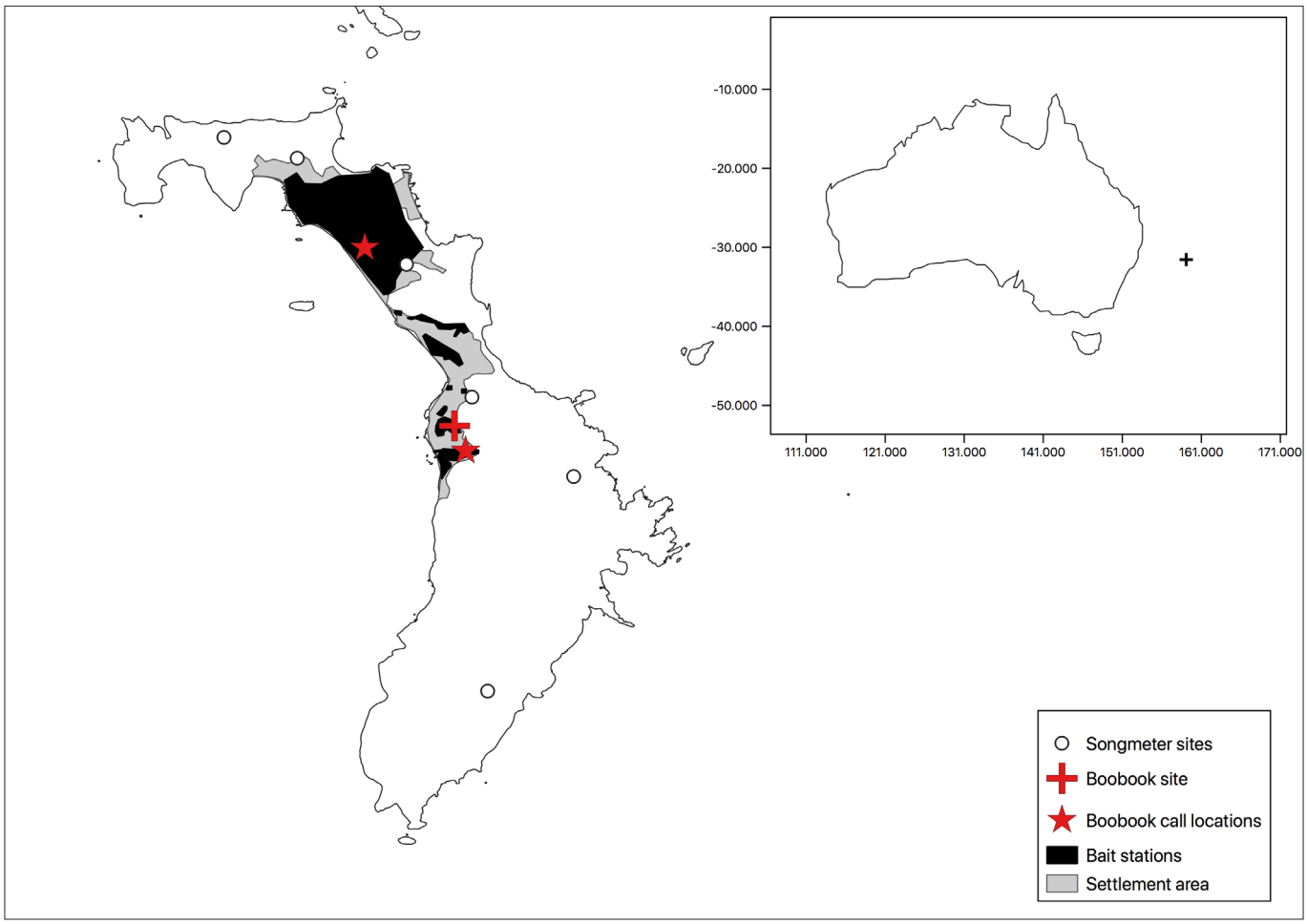

Figure 1. Locations of songmeters (circles), reports of calling boobook (stars) and the location where the carcass was found (cross). The light shaded area shows the area baited with ground bait stations and the dark shaded area shows the extent of The Settlement. The inset shows the location of LHI in relation to mainland Australia and Tasmania.

through secondary poisoning, avian species that consume vertebrates (LHIB 2009). The only resident owl species on the Island, the introduced population of the Masked Owl *Tyto novaehollandiae*, was expected to be affected (Walsh *et al.* 2019). Remains of this species were recovered, and also the skeletal remains of a Barn Owl *T. alba* (a likely vagrant; see Discussion). The REP was covered by two federal permits issued by the Department of Environment and Energy under the *Environment Protection and Biodiversity Conservation Act 1999* (DoEE 2017) and the Australian Pest and Veterinarian Medicines Authority (APVMA 2018). As part of these permits, a mitigation management program was initiated to attempt to monitor and, if possible, ameliorate the impacts of rodent poison in the LHI environment. Servicing of each individual ground baiting station occurred approximately every 10 days between 21 May and 31 October 2019.

The Lord Howe Woodhen *Hypotaenidia sylvestris* and Lord Howe Pied Currawong *Strepera graculina crissalis* were taken into captivity during the REP (LHIB 2009) but the other avian species were either considered not at risk or not deliberately targeted for poisoning. The Island supports a hybrid Masked Owl population which resulted from subsequent breeding between introduced Tasmanian Masked Owls *Tyto novaehollandiae castanops* and south-eastern Australian mainland Masked Owls *T. n. novaehollandiae* (Hindwood 1940), as recently confirmed by Hogan *et al.* (2013). It was expected that the Masked Owl would undergo a significant population reduction on the Island. The removal of the Masked Owl was planned for during the REP via secondary poisoning to eliminate an introduced predator of native fauna. This effect was to be followed up with a protracted shooting program (Milledge *et al.* 2018). Based on casual observations and the direct sampling of songmeter recordings used to provide an index of the numbers of Masked Owls and potential roosting localities across the main Island, it was determined that there were no other owl species resident on the Island before the REP was implemented.

On 11 July 2019, the intact remains of a *Ninox* owl were recovered from an orchard near the southern end of The Settlement by REP baiting staff member Josh Adams (Figure 1). Although the liver was removed by staff of the Science Division of the Department of Planning and Environment (DPE) for potential analysis of poisoning, the bird was left mostly intact for shipment to the Australian Museum. News of the *Ninox* recovery within the LHI community led some people within that community to place blame with the REP for inadvertently killing the last known specimen of the endemic *N. n. albaria*. Initial measurements by Science Division staff of the DPE suggested that the *Ninox* specimen might have been of either New Zealand or Tasmanian origin, but this information did not deter some members in the community from contacting the media to express their concern (Benns 2019).

Here we report on the identification of this *Ninox* specimen via three different methods: (1) vocalisation, (2) morphometric and (3) DNA analyses.

Methods

Analysis of vocalisations and bird monitoring

Analyses of attempts to sample owl vocalisations across the forested areas of LHI leading up to and during the REP provided an opportunity to determine the potential timing of the arrival and presence of the *Ninox* owl on LHI. These recordings were supplemented with casual observations of when *Ninox* owls were heard vocalising within The Settlement. As part of the gathering of evidence on the spread and frequency of occurrence of the hybrid Masked Owl within the forested areas of the main LHI before and during the REP, six acoustic recorders (Wildlife Acoustics ®, Songmeter SM4®) were established in areas previously used in triangulation of owl surveys (Milledge *et al.* 2018; and see Figure 1). Songmeters were set to record for 1.5 hours each side of dawn and dusk. Recordings from January to June 2019 were checked using Kaleidoscope Pro Analysis 5.0 (Wildlife Acoustics ®) with vocalisations of Tasmanian Boobooks (Fred van Gessel; Professional Wildlife Sounds) as classifiers to run against the LHI recordings. Additionally, instances of vocalisations of a boobook-type owl *Ninox* sp. were collected from reports of staff associated with the REP from April to June 2019.

Any bird specimens found during the servicing of the ground baiting stations were returned to the mitigation management team run by DPE staff. Recovered bird remains were dissected to determine if brodifacoum poisoning was the likely source of death, principally through staining of the intestinal system and, if still fresh, significant impacts on organs and internal bleeding (Murray 2018). Of note is the recovery of the skeletal remains of a Barn Owl during the REP (see Discussion).

Morphometric analysis

Morphometric data were collected from museum study skins representing boobooks from the eastern Australian mainland (*N. boobook*), Tasmania (*N. leucopsis*) and New Zealand (*N. n. novaeseelandiae*) within the Australian Museum and American Museum of Natural History collections (Appendix 1). Measurements of lengths of wing chord, bill and tarsus were taken by LRT, and compared with the LHI specimen. Given the similarities in plumage between Tasmanian and New Zealand species, only specimens with confirmed locality data (data inscriptions on specimen tags) were included in this study.

Genetic analysis

Duplicate tissue samples (muscle; designated A and B) were taken from the unknown LHI specimen for processing. For comparison, tissue samples from boobooks from across their Australian range were obtained from the Australian Museum, Sydney, New South Wales (n = 8) and the Queen Victoria Museum and Art Gallery, Launceston, Tasmania (QVMAG) (n = 1) ornithology collections (remaining tissue and DNA from the QVMAG sample were subsequently accessioned into the Australian Museum Collection with permission) (see Appendix 2 for sample details). Total genomic DNA was extracted from the frozen or ethanol-preserved tissue samples using the Bioline Isolate II Genomic DNA Kit following the manufacturers' instructions; extraction blanks were included in all extractions.

Two mitochondrial DNA genes, cytochrome b (Cyt b) and cytochrome oxidase 1 (CO1), were amplified for the purpose of identification of species. This was done via Polymerase Chain Reaction (PCR) using the primers Cyt b - L14841 (F) and H15149 (R) (Kocher *et al.* 1989) and CO1 - BAK1490 (F) and BAK2198 (R) (Neaves *et al.* 2018). PCRs were conducted in 25 µl reactions using 100–500 ng of genomic DNA, 1 x Reaction Buffer (Bioline MyTaq Red Reagent Buffer; Bioline, Australia), 2 pmol primers and Bioline MyTaq Red DNA polymerase (0.5 unit). Negative controls were included in each PCR. Thermocycling was performed on an Eppendorf Mastercycler Pro S (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 94°C (3 min.), 38 cycles of denaturation at 94°C (20 sec.), annealing at 55°C (40 sec.) and extension at 72°C (40 sec.) with a final extension step of 72°C for 5 minutes. PCR products were cleaned using ExoSap-IT (Thermo Fisher Scientific). Sequencing was resolved on an AB 3730xl Sequencer at the Australian Genome Research Facility, Sydney.

The sex of the LHI specimen was ascertained by amplification of the sex-linked chromo-helicase-DNA binding protein using the primers 2550F and 2718R (Fridolfsson & Ellegren 1999). PCR reactions and thermocycling conditions were as above except that annealing was at 50°C (40 sec.). Sex was determined by visualising the PCR products on a pre-cast 2% agarose gel (ThermoFisher Scientific) with reference to *Ninox boobook* individuals that had been sexed previously via morphological examination: O.72065 (female), O.70987 (female), O.73976 (male) and O.69004 (male).

Sequences were visually checked with reference to chromatograms using SEQUENCHER VERSION 5.2.4 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequence alignments were carried out in Mega version 6 (Nei & Kumar 2000; Tamura *et al.* 2013). CO1 and Cyt b sequences available from GenBank for Australian and New Zealand *Ninox* species were used for comparison and as outgroups. Phylogenetic relationships were estimated using both Bayesian inference (BI) and Maximum Likelihood (ML). Mega version 6 (Nei & Kumar 2000; Tamura *et al.* 2013) was used to determine an appropriate model of evolution (HKY + G) based on the Bayesian Information Criterion (BIC scores) and Akaike Information Criterion, corrected (AIC scores). All phylogenetic analyses were carried out using this model. Bayesian Inference (BI) analysis was conducted in MrBayes version 3.2 (Ronquist *et al.* 2012). Metropolis-Coupled Markov Chain Monte Carlo sampling was used to calculate posterior probabilities. The analyses were run using default settings for priors. Chains were run for one million generations and sampled every 100 generations to obtain 10,000 sampled trees. Maximum Likelihood was estimated using Mega version 6 (Tamura *et al.* 2013) with 1000 bootstrap replicates. Tracer version 1.7.1 (Rambaut *et al.* 2018) was used to check for chain convergence and adequate effective sample size (>200). Posterior probabilities (decimals) bootstrap values (percentages) were used to assess the level of branch support.

Table 1. Morphometric measurement data of museum study skins [showing mean, range and standard deviation (SD)] and from the Lord Howe Island (LHI) specimen. Measurements are rounded to the nearest 0.1 mm where applicable.

Taxon	*Wing chord (mm)*			*Bill (to skull) (mm)*			*Tarsus (mm)*		
	Mean	*Range*	*SD*	*Mean*	*Range*	*SD*	*Mean*	*Range*	*SD*
N. boobook (*n* = 86)	243.2	211–260	9.7	26.6	22.4–30.9	1.5	38	30.7–45.3	2.3
N. n. novaeseelandiae (*n* = 19)	192.1	179–205	8.6	23.4	21.5–25.7	1.4	24.9	21.2–29.4	2.6
N. leucopsis (*n* = 9)	200.4	192–216	8.5	25.9	24.3–28.1	1.3	30.6	23.3–33.9	4.0
LHI specimen (O.80000)	202			24.5			36.4		

Results

Vocalisation analysis

Analysis of acoustic recordings totaling 543 h, spanning 181 days, did not provide any positive *Ninox* sp. detection, indicating that the owl did not frequent the forested areas of the Island. During the same period, calls of the hybrid Masked Owl were detected on 79 days across the six recorders. Two records of vocalisations from a *Ninox* owl in the same period were reported. HB noted a call at dusk in late April or early May (precise date uncertain) from within the central area of The Settlement, and the REP baiting team leader (Peter Carr) also noted a call at dusk in early May from the southern section of The Settlement (Figure 1).

Morphometric analysis

Morphometric data were collected from museum study skins representing boobooks from the eastern Australian mainland (*N. boobook*, *n* = 86), Tasmania (*N. leucopsis*, *n* = 9) and New Zealand (*N. n. novaeseelandiae*, *n* = 19). Specimen measurements (rounded to the nearest 0.1 mm where applicable) of wing chord, bill and tarsus are shown in Table 1 and Figures 2a–c.

Plumage characteristics of the recovered LHI specimen most closely resembled the Tasmanian/New Zealand group, exhibiting an overall clean-white spotted pattern (Figures 3a–c; see also Appendix 3), including finer clean-white spots on the cap, nape and upper mantle, whereas *N. boobook* is described as having either streaks or streaks and spots (Higgins 1999). The LHI specimen showed much darker plumage ventrally and dorsally than mainland birds (see Figures 3a, c). *N. leucopsis* individuals have darker 'colder' brown coloration (Higgins 1999) on the upperwing-coverts (see Appendix 3), a feature that was also present in the recovered LHI specimen (Figure 3b). Overall, *N. leucopsis* is much smaller than the Australian mainland *N. boobook* and also larger than the New Zealand *N. n. novaeseelandiae* (Higgins 1999). The measured features of the LHI specimen reflect these differences when comparing the LHI specimen (AM O.80000) with the average measurements of the other species. The measurements of the LHI specimen overlap with both *N. leucopsis* and *N. n. novaeseelandiae* in wing chord and bill measurements (see range data in Table 1). These two measured features of the LHI specimen (wing = 202 mm; bill = 24.5 mm) align closest with the average measurements of *N. leucopsis*. There was a slight difference in tarsus measurements between the unprepared LHI specimen compared with prepared study skins of *N. leucopsis*. This could be explained by a smaller sample size for *N. leucopsis* capturing less variation in tarsus length, and by specimen shrinkage in museum study skins as part of the drying process after preparation of study skins (Winker 1993; Wilson & McCracken 2008; Williams 2017). Despite this, the tarsus measurement of the LHI specimen (36.4 mm) is closest to the official average tarsus measurement of a male *N. leucopsis* (36.8 mm) reported in Higgins (1999). Further support for the LHI specimen being a biological male is provided via molecular analysis (see genetic analysis below). In *N. leucopsis*, males are smaller than females, and this reversed sexual dimorphism also occurs in *N. novaeseelandiae* and *N. boobook* (Higgins 1999).

Genetic analysis

A total of 622 base pairs (bp) of sequence data was obtained for CO1 and 249 bp for Cyt b. For the LHI specimen, sequences from both A and B samples were identical and showed no sequence divergence (sd) (0%) from samples of *N. leucopsis* from Tasmania and consistently grouped with them under phylogenetic analysis (Figure 4; see also Appendix 2). Tasmanian *N. leucopsis* were divergent from Australian mainland *N. boobook* [3.4% (CO1), 2.5% (Cyt b), average sd] and from *N. n. novaeseelandiae* from New Zealand [1.7% (CO1), 2.8% (Cyt b), average sd). There was 3.1% (CO1), 3.9% (Cyt b) and average sd between New Zealand and mainland Australian boobooks. Little divergence was detected within eastern Australian mainland *N. boobook* [0.11% (CO1), 0% (Cyt b), average sd] despite sampling being spread from southern New South Wales (O.73976, Goulburn) to north-western Queensland (O.65798, Musselbrook) and north-eastern Queensland (O.69004, Lake Eacham) (over ~1900 km) (Figure 4; see also Appendix 2). Molecular sexing determined the LHI specimen to be male.

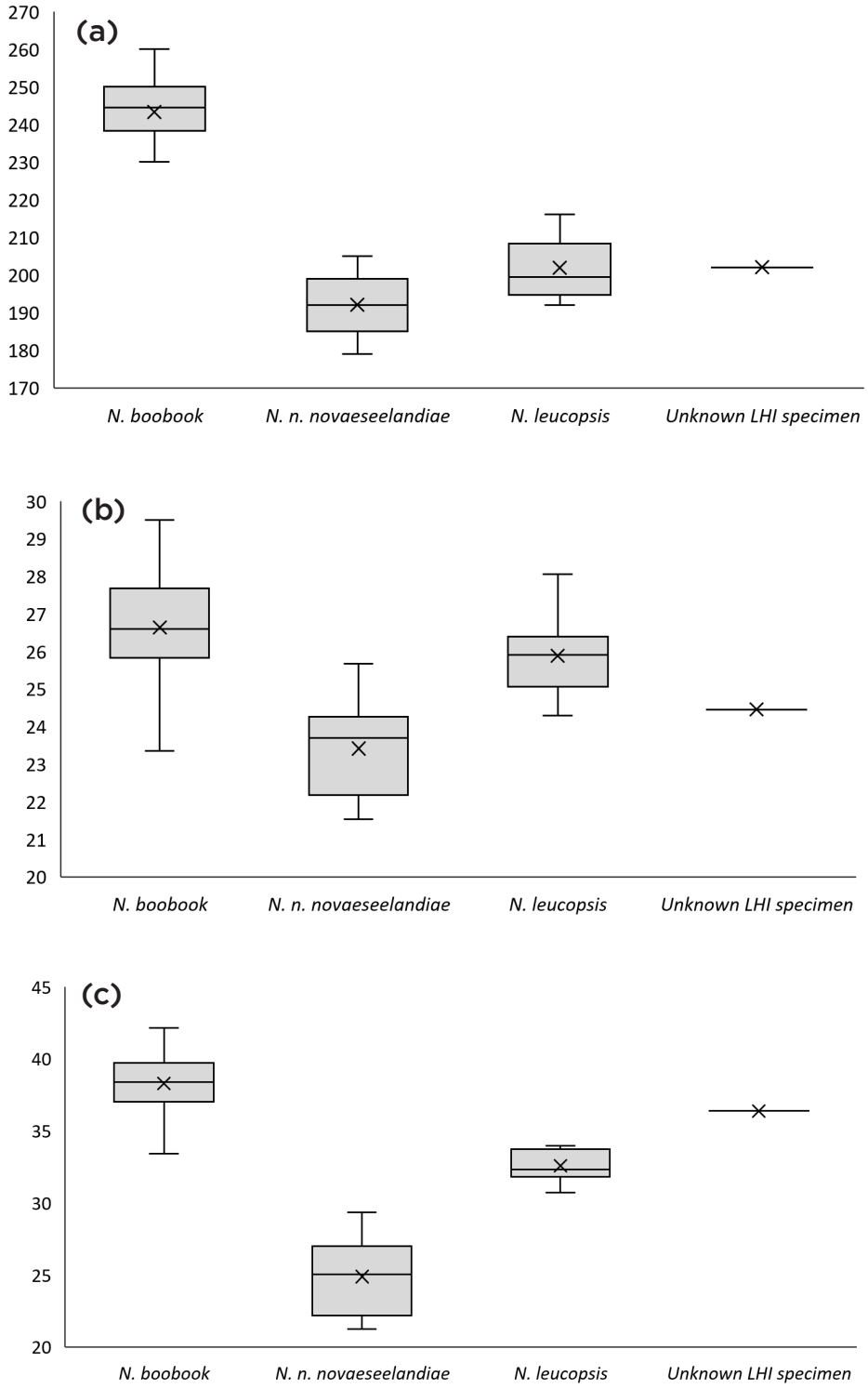

Figure 2. Box and whisker plots (range, standard deviation) showing morphometric measurements (mm on y-axis) of museum study skins, and the recovered LHI specimen: (a) wing chord, (b) bill (to skull) and (c) tarsus.

Discussion

This study has confirmed that the *Ninox* specimen recovered on LHI in July 2019 was a male Tasmanian Boobook *N. leucopsis*. This is the first record of any *Ninox* species on LHI in well over 50 years. There has not been a confirmed sighting of a boobook species there since the extinction of the endemic *Ninox* subspecies, the Lord Howe Boobook *N. n. albaria*, which is thought to have occurred during the 1960s (McAllan *et al.* 2004; Frith 2013). The morphometric data indicate the possibility that the LHI bird was a Tasmanian Boobook, and genetic analysis confirms that this is indeed the case.

On 11 May 2019, just before *Ninox* calls were detected near The Settlement in early May, a significant weather system, with sustained west to south-westerly winds of 49 knots emanating from a vigorous cold front associated with a low-pressure system, with multiple centres (BOM unpubl. data), passed over LHI. This system could have caused the accidental dispersal of the *Ninox* owl to the Island.

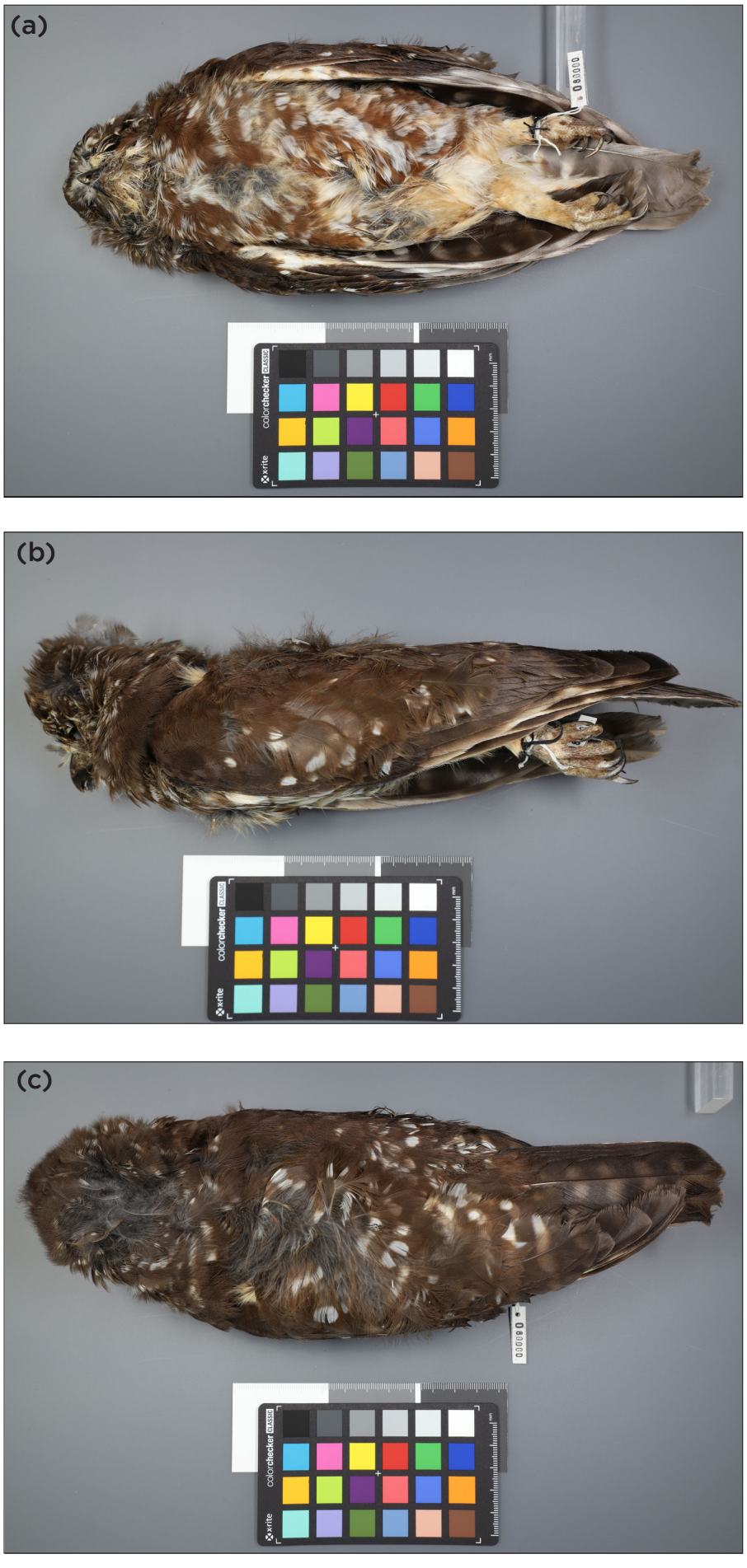

Figure 3. Unprepared recovered LHI specimen (registration AM O.80000) showing (a) ventral, (b) lateral and (c) dorsal views. Photos: R. Lovatt

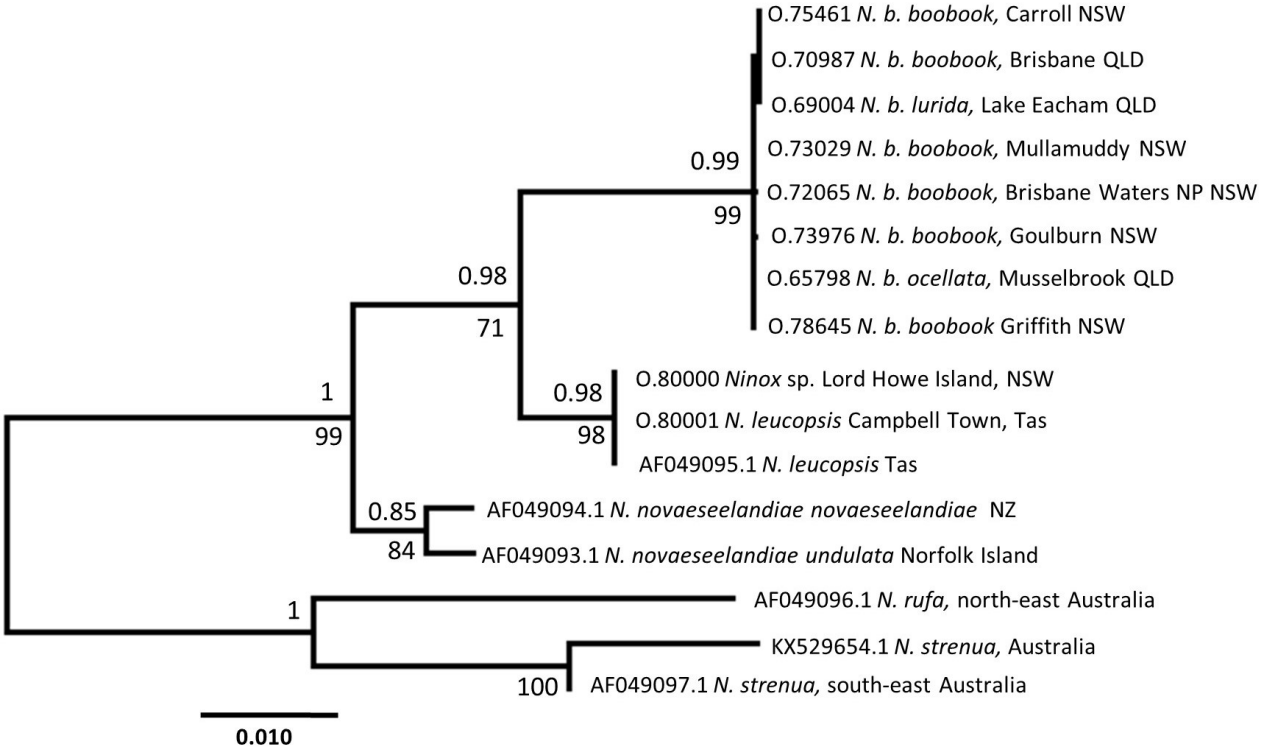

Figure 4. Cyt b phylogenetic tree reconstruction of *Ninox* samples used for DNA comparisons. Reconstruction inferred from 249 bp of Cyt b mitochondrial DNA sequence data. Posterior probabilities are indicated above the node and Bootstrap values are indicated below the node. Both Maximum Likelihood and Bayesian Inference inferred the same tree topology.

As the songmeters were situated within the forested areas of LHI, it is unlikely that the calls from a foraging *Ninox* owl, centred more on the open pastures, would have been obtained. Although rodents are an important dietary item for boobooks (McNabb 2002; Trost *et al.* 2008), and are a secondary poisoning pathway, House Mice *Mus musculus* were common only within The Settlement. This was confirmed from monitoring ground baiting sites during the REP where interactions with bait stations by mice (which typically removed bait to just outside the stations, rather than the actions of Black Rats *Rattus rattus*, which fully removed baits for caching) occurred predominantly in open grass or grassland-dominated areas, rather than forested habitats (HB unpubl. data). If the boobook had been targeting mice as a primary food source, it would have frequented these more open sites and consumed mice that had fed on baits. Although no subsequent analysis of the liver removed from the boobook was made, death by secondary poisoning is assumed.

Three species of owls were introduced to LHI in the 1920s to control rodents: the Barn Owl (mainland Australian birds as well as individuals from North America), Masked Owl [from Tasmania (Hindwood 1940) and the Australian mainland (Hogan *et al.* 2013)], and mainland Southern Boobooks (Hutton 1991; Higgins 1999). A fresh Barn Owl skeleton was recovered on the Island in 1971 (Hutton in Higgins 1999). Although the Barn Owl had not been recorded on the Island over a 20-year period (McAllan *et al.* 2004), earlier records might have been occasional arrivals of individuals. Photographic records also exist of a Barn Owl present on the Island in 2014 (J. Shick *in litt.*). Evidence of Barn Owls colonising New Zealand has been reported by Hyde *et al.* (2009), who described the first documented breeding event and related behaviours of a single pair, observed over a 24-month period (2008–2010). The skeletal remains of a Barn Owl recovered during the REP is another example of this occasional immigration.

The possibility of Tasmanian Boobooks migrating to the mainland has been subject to some discussion, most recently by Olsen & Debus (2013) and Mooney (2013). The long-range dispersal would be expected to occur between March and September (Mees 1964), and there have been reports of boobooks being sighted in Bass Strait by boat crews during that period (Mooney 2013). Although LHI is a considerable distance from mainland Australia, the possibility that a *N. leucopsis* from Tasmania dispersed to the Island cannot be ruled out. The prevailing weather system, and the LHI specimen being heard in April or May, are consistent with a north-bound autumn migrant or disperser from Tasmania being blown off course. The DNA and morphological evidence leave no doubt that the bird found dead in May 2019 was a Tasmanian Boobook *N. leucopsis*.

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Appendix 1. List of *Ninox* study skins measured for morphometric analysis by LRT. Institution abbreviations: AM = Australian Museum; AMNH = American Museum of Natural History.

Taxon	*Specimen registration no.*	*Institution*
N. boobook boobook	A.11172	AM
N. boobook boobook	A.11940	AM
N. boobook boobook	A.18989	AM
N. boobook boobook	O.10925	AM
N. boobook boobook	O.12127	AM
N. boobook boobook	O.1267	AM
N. boobook boobook	O.15599	AM
N. boobook boobook	O.16054	AM
N. boobook boobook	O.16372	AM
N. boobook boobook	O.16531	AM
N. boobook boobook	O.18285	AM
N. boobook	O.18286	AM
N. boobook boobook	O.20559	AM
N. boobook boobook	O.21212	AM
N. boobook boobook	O.21214	AM
N. boobook boobook	O.22724	AM
N. boobook	O.23638	AM
N. boobook boobook	O.23641	AM
N. boobook boobook	O.27331	AM
N. boobook boobook	O.27906	AM
N. boobook boobook	O.32253	AM
N. boobook boobook	O.33033	AM
N. boobook boobook	O.33169	AM
N. boobook boobook	O.33170	AM
N. boobook boobook	O.33391	AM
N. boobook boobook	O.3701	AM
N. boobook boobook	O.3702	AM
N. boobook boobook	O.37041	AM
N. boobook lurida	O.37647	AM
N. boobook boobook	O.37726	AM
N. boobook boobook	O.37766	AM
N. boobook boobook	O.39644	AM
N. boobook boobook	O.4051	AM
N. boobook boobook	O.40747	AM
N. boobook boobook	O.44204	AM
N. boobook boobook	O.43124	AM
N. boobook boobook	O.43342	AM
N. boobook boobook	O.44407	AM
N. boobook boobook	O.44491	AM
N. boobook boobook	O.45135	AM
N. boobook boobook	O.45334	AM
N. boobook boobook	O.45381	AM
N. boobook boobook	O.45471	AM
N. boobook boobook	O.46925	AM
N. boobook boobook	O.46979	AM
N. boobook	O.47156	AM
N. boobook boobook	O.47539	AM

Appendix 1 continued

Taxon	*Specimen registration no.*	*Institution*
N. boobook boobook	O.47714	AM
N. boobook boobook	O.47715	AM
N. boobook boobook	O.58170	AM
N. boobook boobook	O.58171	AM
N. boobook boobook	O.58750	AM
N. boobook boobook	O.59416	AM
N. boobook boobook	O.60154	AM
N. boobook boobook	O.60155	AM
N. boobook boobook	O.60771	AM
N. boobook boobook	O.62089	AM
N. boobook ocellata	O.65798	AM
N. boobook boobook	O.66174	AM
N. boobook boobook	O.66404	AM
N. boobook boobook	O.66517	AM
N. boobook lurida	O.69004	AM
N. boobook boobook	O.69028	AM
N. boobook boobook	O.69132	AM
N. boobook lurida	O.69142	AM
N. boobook lurida	O.69143	AM
N. boobook boobook	O.70987	AM
N. boobook boobook	O.71475	AM
N. boobook boobook	O.72065	AM
N. boobook boobook	O.72671	AM
N. boobook boobook	O.73168	AM
N. boobook boobook	O.75098	AM
N. boobook boobook	O.76316	AM
N. boobook boobook	O.78645	AM
N. boobook boobook	O.78646	AM
N. boobook boobook	O.78647	AM
N. boobook boobook	O.78648	AM
N. boobook boobook	O.796	AM
N. boobook boobook	O.8242	AM
N. boobook boobook	O.8313	AM
N. boobook boobook	O.8431	AM
N. boobook boobook	O.8459	AM
N. boobook boobook	O.8857	AM
N. boobook boobook	O.8965	AM
N. boobook boobook	O.9444	AM
N. boobook	O.9498	AM
N. boobook boobook	A.11172	AM
N. leucopsis	630526	AMNH
N. leucopsis	630527	AMNH
N. leucopsis	630528	AMNH
N. leucopsis	630529	AMNH
N. leucopsis	630530	AMNH
N. leucopsis	630531	AMNH
N. leucopsis	630532	AMNH
N. leucopsis	O.23795	AM

Appendix 1 continued

Taxon	*Specimen registration no.*	*Institution*
N. leucopsis	O.29982	AM
N. novaeseelandiae novaeseelandiae	202982	AMNH
N. novaeseelandiae novaeseelandiae	630360	AMNH
N. novaeseelandiae novaeseelandiae	630361	AMNH
N. novaeseelandiae novaeseelandiae	630362	AMNH
N. novaeseelandiae novaeseelandiae	630363	AMNH
N. novaeseelandiae novaeseelandiae	630364	AMNH
N. novaeseelandiae novaeseelandiae	630365	AMNH
N. novaeseelandiae novaeseelandiae	630366	AMNH
N. novaeseelandiae novaeseelandiae	630370	AMNH
N. novaeseelandiae novaeseelandiae	630372	AMNH
N. novaeseelandiae novaeseelandiae	A.1944	AM
N. novaeseelandiae novaeseelandiae	O.30411	AM
N. novaeseelandiae novaeseelandiae	O.30412	AM
N. novaeseelandiae novaeseelandiae	O.3081	AM
N. novaeseelandiae novaeseelandiae	O.37296	AM
N. novaeseelandiae novaeseelandiae	O.37297	AM
N. novaeseelandiae novaeseelandiae	O.37298	AM
N. novaeseelandiae novaeseelandiae	O.37299	AM
N. novaeseelandiae novaeseelandiae	O.4073	AM

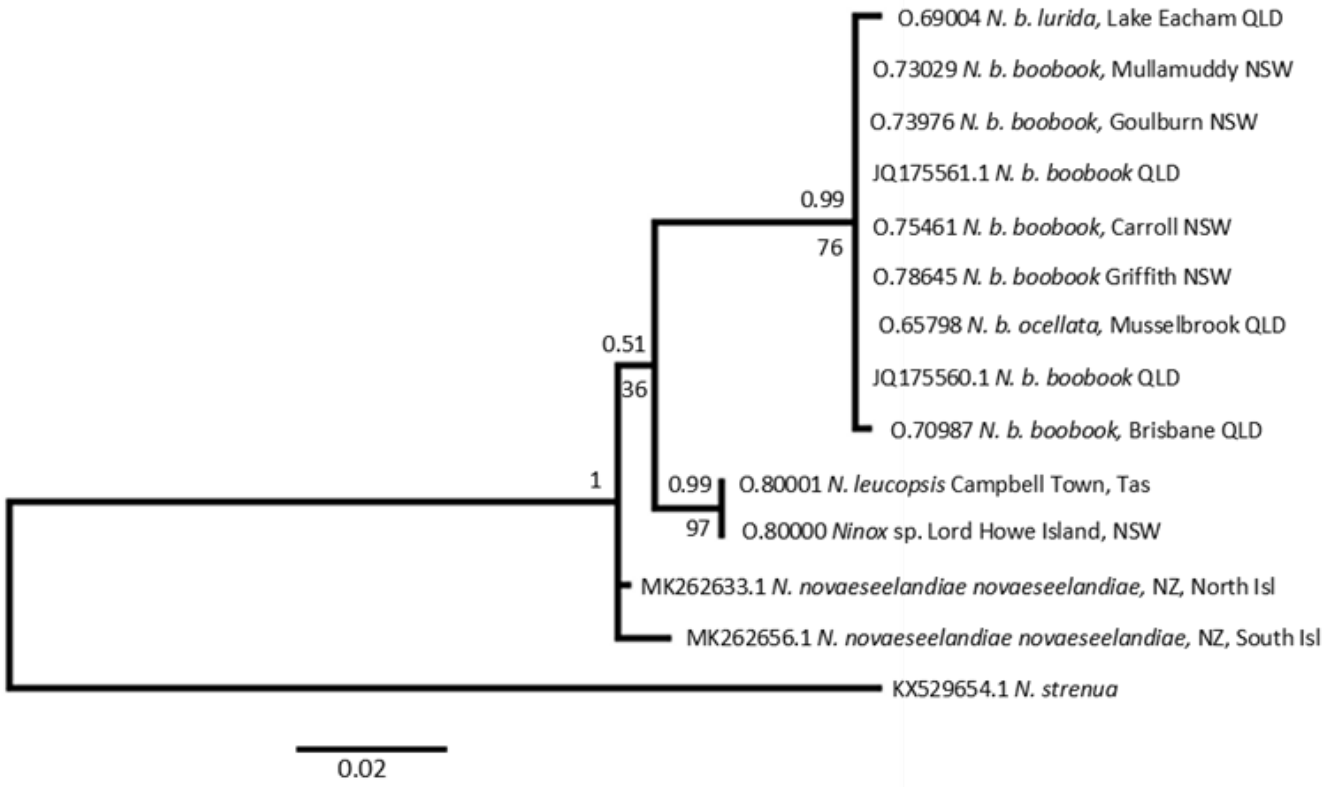
Appendix 2. (a) *Ninox* specimens and GenBank numbers used for DNA comparisons and (b) CO1 phylogenetic tree reconstruction of these *Ninox* samples. (a) Details of *Ninox* specimens (grouped by species, then ordered by AM registration number in descending order, where applicable) and associated GenBank numbers used for DNA comparisons. AM = Australian Museum, USNM = Smithsonian National Museum of Natural History, LB = Auckland Museum. Locality: LHI = Lord Howe Island, NE = north-eastern, NP = National Park, NSW = New South Wales, NZ = New Zealand, Qld = Queensland, SE = south-eastern, and Tas. = Tasmania. N/A = not applicable.

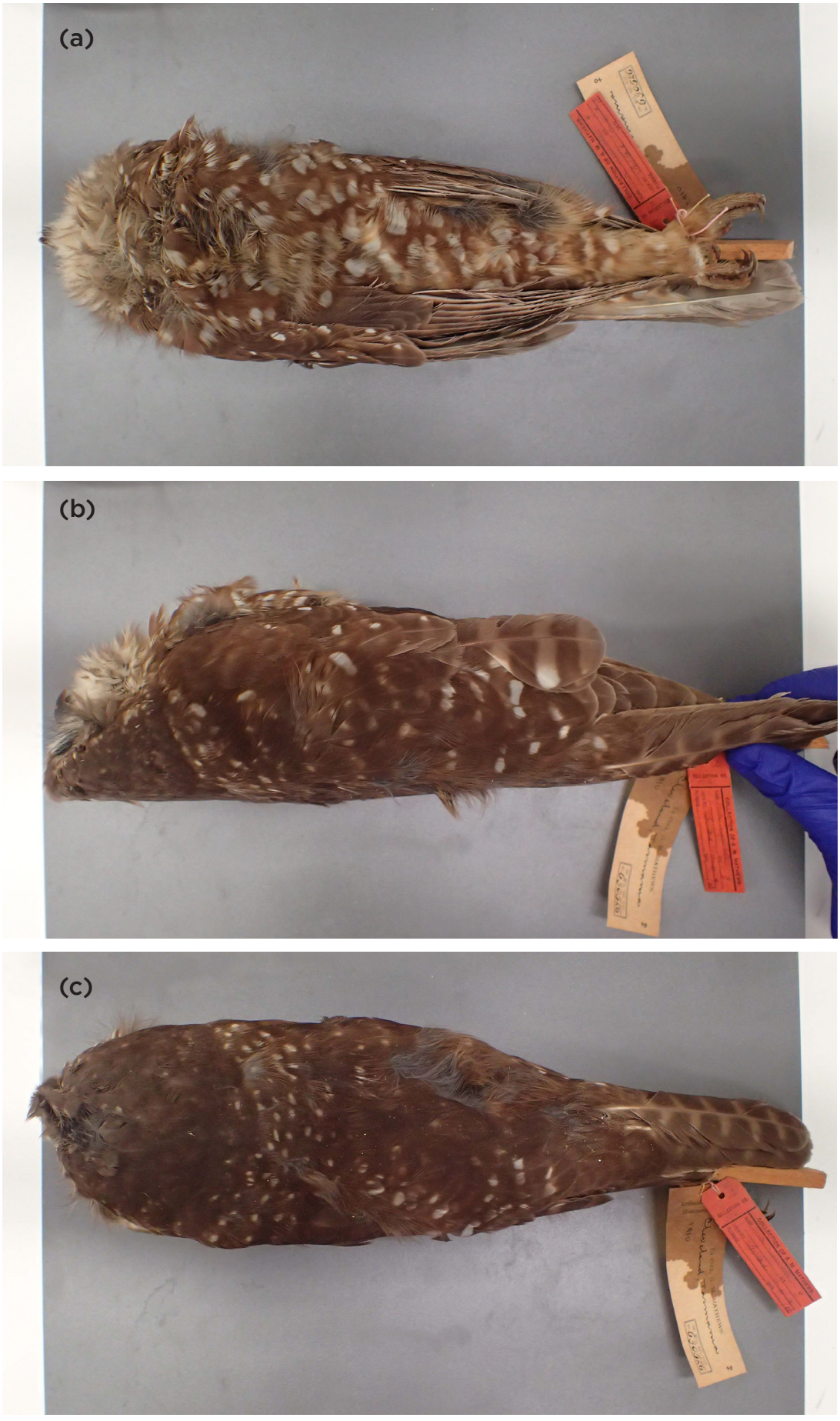
(a) Details of *Ninox* specimens

Taxon	*Museum registration no.*	*Locality*	*GenBank accession no.*		*Reference*
			Cyt b	*CO1*	
Ninox sp.	AM O.80000	LHI, NSW	OL588324	OL583863	This study
N. b. boobook	AM O.78645	Griffith, NSW	OL588333	OL583871	This study
N. b. boobook	AM O.75461	Carroll, NSW	OL588330	OL583867	This study
N. b. boobook	AM O.73976	Goulburn, NSW	OL588326	OL583865	This study
N. b. boobook	AM O.73029	Mullamuddy, NSW	OL588328	OL583866	This study
N. b. boobook	AM O.72065	Brisbane Waters NP, NSW	OL588327	N/A	This study
N. b. boobook	AM O.70987	Brisbane, Qld	OL588331	OL583869	This study
N. b. lurida	AM O.69004	Lake Eacham, Qld	OL588329	OL583868	This study
N. b. ocellata	AM O.65798	Musselbrook, Qld	OL588332	OL583870	This study
N. b. boobook	USNM:Birds:612701	Brisbane, Qld	N/A	JQ175561.1	Schindel *et al*. 2011
N. b. boobook	USNM:Birds:612700	Brisbane, Qld	N/A	JQ175560.1	Schindel *et al*. 2011
N. leucopsis	AM O.80001	Campbell Town, Tas.	OL588325	OL583864	This study
N. leucopsis	N/A	Tas.	AF049095.1	N/A	Norman *et al*. 1998
N. n. novaeseelandiae	N/A	NZ	AF049094.1	N/A	Norman *et al*. 1998
N. n. novaeseelandiae	LB12813	NZ, North Island, Auckland	N/A	MK262633.1	Tizard *et al*. 2019
N. n. novaeseelandiae	N/A	NZ, South Island, Franz Josef	N/A	MK262656.1	Tizard *et al*. 2019
N. n. undulata	N/A	Norfolk Island	AF049093.1	N/A	Norman *et al*. 1998
N. rufa	N/A	NE Australia	AF049096.1	N/A	Norman *et al*. 1998
N. strenua	N/A	SE Australia	AF049097.1	N/A	Norman *et al*. 1998
N. strenua	N/A	Australia	KX529654	KX529654	Sarker *et a*l. 2016

Appendix 2 continued

(b) CO1 phylogenetic tree





Appendix 3. Images of study skin specimen: (a) ventral, (b) lateral and (c) dorsal views of Tasmanian Boobook *N. leucopsis* (AMNH 630526).