Merging ancient and modern DNA: extinct seabird taxon rediscovered in the North Tasman Sea

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Ancient DNA has revolutionized the way in which evolutionary biologists research both extinct and extant taxa, from the inference of evolutionary history to the resolution of taxonomy. Here, we present, to our knowledge, the first study to report the rediscovery of an ‘extinct’ avian taxon, the Tasman booby (*Sula tasmani*), using classical palaeontological data combined with ancient and modern DNA data. Contrary to earlier work, we show an overlap in size between fossil and modern birds in the North Tasman Sea (classified currently as *S. tasmani* and *Sula dactylatra fullagari*, respectively). In addition, we show that Holocene fossil birds have mitochondrial control region sequences that are identical to those found in modern birds. These results indicate that the Tasman booby is not an extinct taxon: *S. dactylatra fullagari* O’Brien & Davies, 1990 is therefore a junior synonym of *Sula tasmani* van Tets, Meredith, Fullagar & Davidson, 1988 and all North Tasman Sea boobies should be known as *S. d. tasmani*. In addition to reporting the rediscovery of an extinct avian taxon, our study highlights the need for researchers to be cognizant of multidisciplinary approaches to understanding taxonomy and past biodiversity.

Keywords: *Sula tasmani*; *Sula dactylatra fullagari*; masked booby; ancient DNA; mitochondrial DNA; phylogeography

1. INTRODUCTION

Once the realm of palaeontologists, interpretation of the extensive Quaternary avian fossil record has become a multidisciplinary pursuit. Classical palaeontological data augmented by ancient DNA are often used to assess the taxonomic status of extinct taxa (e.g. moa;

Bunce *et al.* 2003; Huynen *et al.* 2003). When these data are further augmented by modern DNA, they may also be used to describe previously unrecognized extinct lineages (e.g. geese; Paxinos *et al.* 2002) or even extinct species (e.g. penguins; Boessenkool *et al.* 2009). Here, we combine classical palaeontological data with ancient and modern DNA data to assess the taxonomic status of two seabird taxa, one of which was thought to have become extinct during the late eighteenth century (van Tets *et al.* 1988).

Masked boobies (*Sula dactylatra*) are large colonial seabirds that breed on oceanic islands throughout the tropics and subtropics, and exhibit extensive morphological variation. As many as six subspecies have been proposed based largely on geographical variation in bare-part coloration (Nelson 1978; O’Brien & Davies 1990). The southernmost populations, currently referred to as *Sula dactylatra fullagari*, breed on the Lord Howe, Norfolk and Kermadec Island groups in the North Tasman Sea, and comprise fewer than 1500 breeding pairs (Garnett & Crowley 2000; Taylor 2000; Priddel *et al.* 2005). In addition to having longer wings than masked boobies elsewhere, the North Tasman Sea birds have sepia, not yellow, irides (O’Brien & Davies 1990; but see Shaughnessy 1993; figure 1a). An extinct congeneric sulid species, the Tasman booby (*Sula tasmani*), has also been described from Holocene fossil deposits on Norfolk and Lord Howe Islands (van Tets *et al.* 1988). Whereas the extinction of the Tasman booby on Norfolk Island was attributed to Polynesian colonization before AD 1200, the ultimate demise of the species was attributed to hungry European sailors on Lord Howe Island during the late eighteenth century (van Tets *et al.* 1988). However, preliminary analysis of additional fossil material collected in the 1980s and in 1995 has suggested the fossils of ‘extinct’ *S. tasmani*, described by van Tets *et al.* (1988), might instead be from individuals of the upper size range of extant *S. dactylatra* (Holdaway & Anderson 2001).

To clarify the taxonomic status of North Tasman Sea boobies, we used a multidisciplinary, two-stage approach: (i) we compared standard morphometric measurements of new fossil material collected from Norfolk Island to new modern specimens collected in the North Tasman Sea, and (ii) we used ancient and modern DNA methods to compare mitochondrial control region sequences from the Norfolk Island fossils to those in a global sample of modern birds.

2. MATERIAL AND METHODS

(a) Sample collection

Five adult humeri from modern birds, collected at Curtis and Macauley Islands (Kermadec Island Group) and Nepean Island (Norfolk Island Group), were borrowed from Te Papa Tongarewa (accession nos. S24156, S24383, S24384, S27613, S27614). Five adult right humeri and one juvenile right radius were sampled from fossil deposits at Cemetery Bay, Norfolk Island, held by R.N.H. (figure 1b). A total of 55 blood samples (25 new and 30 from Steeves *et al.* (2005b)) were collected from adults or juveniles at three modern colonies in the North Tasman Sea (figure 2).

(b) Morphometric analysis

Modern and fossil humeri were measured to ± 0.1 mm by Vernier calipers or to ± 0.5 mm by steel rule. Sample sizes were too small to warrant comparative statistical analysis.

(c) Genetic analysis

Modern DNA extraction and polymerase chain reaction (PCR) amplification of a 500 bp fragment of the mitochondrial control

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Figure 1. (a) Masked booby roosting at Phillip Island. (b) Caudal view of adult right humeri collected from fossil deposits at Cemetery Bay, Norfolk Island. From left to right: RVST5, RVST4, RVST1, RVST2, RVST3 (juvenile right radius, RVST6, not shown). Reproduced with permission from R.N.H.

region were performed at the University of Canterbury using the protocols outlined in Steeves *et al.* (2005a). Ancient DNA was extracted in a dedicated clean room at Murdoch University using strict ancient DNA guidelines as described in Allentoft *et al.* (2009). Partial or complete amplification of the 500 bp mitochondrial control region fragment described above was achieved using primer pairs that amplified three overlapping fragments, ranging in size from 170 to 179 bp (see table S1 in the electronic supplementary material), using the reaction conditions described in Steeves *et al.* (2005a). PCR products were sequenced in both directions using BigDye v. 3.1 (Applied Biosystems) and visualized using either a 3100 Genetic Analyser or a 3730 DNA Analyser (Applied Biosystems). Sequences were aligned manually using Geneious (Biomatters Limited) and variable sites were confirmed by visual inspection of the chromatograms. Relationships among modern and ancient mitochondrial control region sequences were inferred by constructing statistical parsimony networks using TCS v. 1.21 (Clement *et al.* 2000).

3. RESULTS

(a) Morphometric data

Contrary to van Tets *et al.* (1988), our comparison of new skeletal material revealed a size overlap between modern and fossil specimens for all standard humerus measurements (table 1).

(b) Genetic data

Among the 55 modern samples, we found 14 mitochondrial control region haplotypes defined by 23 variable sites (see table S2 in the electronic

supplementary material). The majority of haplotypes were unique to a single colony in the North Tasman Sea with the following exceptions: three haplotypes were shared among all three colonies and one haplotype was shared between Lord Howe Island and the Norfolk Island Group (figure 2). No haplotypes were shared between the three colonies in the North Tasman Sea and colonies elsewhere in the Indo-Pacific. Two statistical parsimony networks were generated by TCS for the modern control region haplotypes (99% parsimony probability = 4 steps). Both networks contained haplotypes from all three North Tasman Sea colonies (figure 2).

Of the six fossil samples, we achieved complete amplification of the mitochondrial control region for one specimen (500 bp fragment, domain I and II; RVST6) and partial amplification for two additional specimens (170 bp fragment, domain I only; RVST1, RVST5). Subsequent sequence analyses revealed that RVST6 was identical to haplotype Sd_41, and that RVST1 and RVST5 were identical to the 5' end of haplotype Sd_35, both of which were found in modern birds at all three North Tasman Sea colonies (figure 2).

4. DISCUSSION

Despite limited sampling, we describe an overlap in skeletal size between fossil and modern boobies in the North Tasman Sea and show that fossil birds have mitochondrial control region sequences that are identical to those found in modern North Tasman Sea birds. In agreement with previous studies (Steeves *et al.* 2005b), we also show that modern birds from all three colonies in the North Tasman Sea are genetically isolated from colonies elsewhere in the Indo-Pacific. Because Indo-Pacific masked boobies exhibit strong genetic structure (most haplotypes are unique to a single colony; Steeves *et al.* 2005b; this study), haplotype sharing between fossil and modern birds is unlikely to be owing to incomplete lineage sorting. These combined results indicate that extinct *S. tasmani* and extant *S. d. fullagari* are referable to the same taxon. The senior available name for masked boobies in the North Tasman Sea is *Sula tasmani* van Tets, Meredith, Fullagar & Davidson, 1988. We accept this taxon at the subspecific level as *S. d. tasmani* with the same authorship. In addition to reporting the rediscovery of an 'extinct' seabird taxon, our study highlights the need for a multidisciplinary approach when classifying new taxa.

Despite ample evidence for reversed sexual size dimorphism in modern masked boobies (Nelson 1978), when describing *S. tasmani*, van Tets *et al.* (1988) failed to acknowledge a potential explanation for the minimal size overlap between the fossil and modern birds: the fossil specimens were female and the modern specimens were male. Similarly, although O'Brien & Davies (1990) referenced van Tets *et al.* (1988) when they described *S. d. fullagari* as a new subspecies of masked booby in the North Tasman Sea with longer wings than birds elsewhere in the Indo-Pacific, they did not mention the long wings that characterized the extinct Tasman booby.

O'Brien & Davies (1990) presented convincing morphological and ecological evidence for the taxonomic

Figure 2. (a) Statistical parsimony network of Indo-Pacific masked booby mitochondrial control region haplotypes. (Modified with permission from Steeves *et al.* 2005b.) Circle sizes are proportional to haplotype frequencies. Black circles represent missing haplotypes. Letters A, B and C denote haplotypes Sd_41, Sd_35 and Sd_36, respectively. (b) North Tasman Sea masked booby sampling locations: Lord Howe Island ($n = 30$; Steeves *et al.* 2005b); Norfolk and Phillip Islands, Norfolk Island Group ($n = 11$; this study); North and South Meyer Islands, Kermadec Island Group ($n = 14$; this study). (c) Statistical parsimony network of North Tasman Sea masked booby mitochondrial control region haplotypes. Arrows denote haplotypes found in fossil samples RVST1, RVST5 and RVST6 (see text for details).

Table 1. Adult right humerus measurements of fossil and modern boobies in the North Tasman Sea.

		van Tets <i>et al.</i> (1988)		this study	
humerus (mm)		fossil	modern	fossil	modern
length	mean	209	193	190	192
	range	206–210	186–202	—	182–202
	SD	—	5.2	—	8.2
	n	3	10	1	5
proximal width	mean	30	29	28	28
	range	—	28–30	27–29	26–30
	SD	—	0.9	0.9	1.7
	n	3	9	3	5
shaft width at midpoint	mean	11	8.3	8.8	10
	range	—	7.8–9.1	8.3–9.8	9.5–10.6
	SD	—	0.5	0.9	0.5
	n	3	10	3	5
distal width	mean	23	20	22	21
	range	21–23	19–21	—	20–22
	SD	0.9	0.6	—	0.8
	n	4	10	1	5

distinctiveness of modern masked boobies in the North Tasman Sea (but see Shaughnessy 1993); despite overlapping non-breeding distributions in the Coral Sea, the breeding distribution of the long-winged, sepia-eyed

S. d. tasmani, does not overlap with that of the short-winged, yellow-eyed *S. d. personata*. Although iris colour is rarely used as a sole taxonomic character, recent studies indicate that sepia-eyed (*Thalassarche*

melanophris) and honey-eyed (*Thalassarche impavida*) black-browed albatrosses are indeed genetically distinct (Robertson & Nunn 1998; Burg & Croxall 2001). Similarly, recent genetic evidence for masked boobies in the North Tasman Sea (Steeves et al. 2005b; this study) suggests *S. d. tasmani* may be an incipient species. If future studies indicate that *S. d. tasmani* is indeed on an independent evolutionary trajectory, then elevation of its conservation status from vulnerable to endangered may be warranted (Garnett & Crowley 2000; Taylor 2000). Establishing a robust taxonomic framework for species at risk, such as masked boobies, is critical. This study adds to the growing body of literature (reviewed in Leonard 2008) that has benefited from the integration of ancient DNA data with more conventional approaches to elucidate questions involving taxonomy, demography and conservation.

Sampling was conducted according to permit guidelines issued by the Department of Conservation (permit AK-22658-FAU) and Australian National Parks (permit issued 18 May 2007).

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