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 & Davies, 1990. 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 extinct 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. (2003)) 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 ±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
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 fossils, 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. fullagarri 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. fullagarri 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
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* *Lord Howe* *Norfolk* *Kermadecs* *C* *A* *B* *RVST6* *RVST1* *RVST5*)

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.

<table>
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<th>this study</th>
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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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Shaughnessy, P. D. 1993 Commentary on a statistical oversight used to describe a masked booby subspecies. Mar. Ornithol. 21, 69–70.


