



Phylogenetic relationships of the extinct St Helena petrel, *Pterodroma rupinarum* Olson, 1975 (Procellariiformes: Procellariidae), based on ancient DNA

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Palaeontological studies show that three endemic procellariid seabird species became extinct on the remote island of St Helena in the South Atlantic Ocean. At least one of these, *Pterodroma rupinarum* Olson, 1975, is likely to have survived until human colonization of the island, although it is known only from subfossil bones. Several species of *Pterodroma* are distributed across the Atlantic, but the skull and bill of *Pt. rupinarum* were judged to be more similar to the Indo-Pacific *Pterodroma rostrata* group, which was recently split into the separate genus *Pseudobulweria*. We used ancient DNA techniques to sequence the mitochondrial cytochrome *b* gene of the extinct *Pt. rupinarum*, and conducted phylogenetic analyses to investigate the placement of this enigmatic taxon. In trees constructed using maximum likelihood and Bayesian inference, *Pt. rupinarum* did not group with *Pseudobulweria*, but instead fell within a strongly supported clade of Atlantic *Pterodroma*, including the endangered Black-capped [*Pterodroma hasitata* (Kuhl, 1820)] and Bermuda [*Pterodroma cahow* (Nichols & Mowbray, 1916)] petrels, as well as the Macaronesian petrels [*Pterodroma madeira* Mathews, 1934, *Pterodroma feae* (Salvadori, 1899) and *Pterodroma deserta* Mathews, 1934]. *Pterodroma rupinarum* shared a particularly close relationship with *Pt. feae* of the Cape Verde Islands, which is also the geographically closest species within the clade. Considering the osteological distinctiveness of *Pt. rupinarum* it was probably a separate species, or at least a highly diverged population that was isolated for a substantial period of time prior to its extinction.

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INTRODUCTION

Seabirds are highly vagile, and may forage widely over the open oceans, travelling hundreds to

thousands of kilometers on a single foraging trip during the breeding season (Brooke, 2004). Their ability to disperse widely over expansive pelagic ecosystems makes them a useful indicator of marine conditions (Piatt, Sydeman & Wiese, 2007; Wiley *et al.*, 2013), and as top marine predators they play ecologically important roles (Sánchez-Piñero & Polis, 2000; Croll *et al.*, 2005). Unfortunately, seabirds are one of the most endangered groups of birds in the

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world (Croxall *et al.*, 2012). The family Procellariidae contains approximately 80 species of petrels and shearwaters, although the taxonomy of this group is challenging and often disputed (Brooke, 2004; Penhallurick & Wink, 2004; Rheindt & Austin, 2005). The Procellariidae ranks as the third most endangered seabird family (Croxall *et al.*, 2012), and many of these species face substantial threats both at the breeding grounds as well as at sea (Weimerskirch, Brothers & Jouventin, 1997; Lewison & Crowder, 2003; Wanless *et al.*, 2007; Fontaine, Gimenez & Bried, 2011; Barbraud *et al.*, 2012). Despite this, investigation of the fossil record indicates that in the recent past seabird extinction has been relatively rare, especially when compared with the extinction rate of terrestrial species on oceanic islands (Steadman, 1995; Steadman, 2006).

Fossils from Saint Helena, a remote island in the South Atlantic Ocean (Fig. 1), showed that three apparently endemic species of Procellariidae became extinct there, and populations of three other procellariids, along with several additional species of seabirds, were extirpated (Olson, 1975). Of the three extinct species, only one, *Puffinus pacificoides* Olson, 1975, is likely to have disappeared prior to human arrival, probably during the Pleistocene. Another species, *Bulweria bifax* Olson, 1975, was much more abundant in the Pleistocene than in recent deposits, and may have been decreasing in number towards modern times. The third species, *Pterodroma rupinarum* Olson, 1975, was fairly common in the most recent deposits, and may have become extinct after the discovery of the island in 1502 by Portuguese sailors (Olson, 1975). Little is known about the



Figure 1. Approximate recent breeding locations of *Pterodroma* petrels in the Atlantic Ocean.

avifauna of St Helena at the time of human colonization, as there appear to be no written accounts from that period; however, after permanent settlement in 1513 there are numerous accounts of large numbers of feral invasive species, including goats, pigs, rats, mice, and cats (Gosse, 1938), which would presumably have caused severe environmental perturbation and led to greatly increased predation on burrow-nesting seabirds.

Initial work by Bourne (1956) on the taxonomic identification of a limited number of *Pterodroma* bones from St Helena ruled out affinity with *Pterodroma arminjoniana* (Giglioli & Salvadori, 1869), the only other tropical South Atlantic petrel (Fig. 1). Instead, he suggested that the fossils might belong to Macaronesian species of *Pterodroma* breeding on Madeira and the Cape Verde Islands in the North Atlantic. Recent genetic work on the Macaronesian petrels suggests the existence of three recently diverged species: *Pterodroma feae* (Salvadori, 1899) in the Cape Verde archipelago; *Pterodroma deserta* Mathews, 1934 on Bugio Island (first suggested as a distinct species by Bourne in 1955); and *Pterodroma madeira* Mathews, 1934 on the island of Madeira in the Madeira Archipelago (Fig. 1), although the species status of *Pt. deserta* is still somewhat controversial (Zino, Brown & Biscoito, 2008; Jesus *et al.*, 2009; Gangloff *et al.*, 2013). Bourne (1965) later suggested that the fossil *Pterodroma* from St Helena might belong with the *Pterodroma rostrata* group instead. Examination of greatly expanded material collected by Olson (1975) resulted in the naming of the fossils as a new species, *Pt. rupinarum*, some characters of which, particularly the skull and the angle of the bill, were considered to be more similar to the *Pterodroma rostrata* group than to other *Pterodroma* petrels. Genetic work (Bretagnolle, Attié & Pasquet, 1998) has since shown that the *Pterodroma rostrata* group actually represents a distinct genus, *Pseudobulweria*, first proposed by Mathews in 1936 [type species *Thalassidroma (Bulweria) macgillivrayi* G.R. Gray, 1859]. The Indo-Pacific genus *Pseudobulweria* contains four extant species: *Pseudobulweria rostrata* (Peale, 1848), *Pseudobulweria becki* (Murphy 1928), *Pseudobulweria aterrima* (Bonaparte, 1857), and *Pseudobulweria macgillivrayi* (Gray, 1860). These are among the most endangered of procellariiforms, with three out of four of the species considered critically endangered, being known only from a handful of museum specimens, and often having been feared extinct for many years (Attié, Stahl & Bretagnolle, 1997; Shirihai, 2008; Shirihai *et al.*, 2009; Gangloff *et al.*, 2012).

Here, we report on ancient DNA sequences from the extinct *Pt. rupinarum* and assess the placement of

this species in a phylogeny including nearly all *Pterodroma* and *Pseudobulweria* petrels.

MATERIAL AND METHODS

SAMPLES AND MOLECULAR TECHNIQUES

Of the thousands of available bones of *Pt. rupinarum* collected by Olson in 1971, ten were selected from three sites on St Helena: eight from Prosperous Bay (USNM 176607, 176608, 176610, 176613, 176615, 176623, 176627, 176641), one from Sandy Bay (USNM 176679), and one from Sugarloaf Hill, site 2 (USNM 176702). Bones that were clearly identifiable as *Pt. rupinarum* were selected based on size, thickness of bone walls, and un-weathered appearance. A mixture of complete and partial elements was selected to minimize the effects of destructive sampling. The age of the samples is not certainly known, but all of the deposits are thought to be late Holocene. A small piece of bone (approximately 4 mm × 6 mm) was cut from each specimen using a rotary saw with disposable blades. Each sample was ground into powder using a mortar and pestle and DNA was extracted using a phenol/chloroform procedure with centrifugal dialysis (Fleischer *et al.*, 2000). All laboratory work was conducted in a physically isolated facility dedicated for ancient DNA work, and strict protocols were followed to prevent contamination from modern sources. Ancient DNA was extracted in batches of six samples, each of which included an extraction control.

Most phylogenetic work on procellariiform seabirds has focused on the mitochondrial cytochrome *b* gene (Bretagnolle *et al.*, 1998; Nunn & Stanley, 1998; Austin, Bretagnolle & Pasquet, 2004; Gangloff *et al.*, 2012), so we targeted that gene for our study. Polymerase chain reaction (PCR) was carried out as described in Welch *et al.* (2012). Briefly, PCR was conducted in 25- μ L reaction volumes with 1 unit Amplitaq Gold DNA polymerase (Applied Biosystems), 2–3 μ L of DNA extract, and with a total of 45 cycles. We used a series of short (120–200 bp), overlapping PCR primers designed specifically to amplify in *Pterodroma* (Welch *et al.*, 2012), in *Pseudobulweria* and *Bulweria* (Table 1), and in all Procellariiformes (Pyle, Welch & Fleischer, 2011). Negative controls were used to detect potential contamination. All amplifications were conducted a minimum of two times for each primer–sample combination. PCR products were cleaned using ExoSAP-IT (USB), cycle-sequenced in both directions with Big Dye Terminator 3.1 (Applied Biosystems), then purified using Sephadex G-50 fine columns (GE Healthcare Bio-Sciences), and electrophoresed in an ABI 3130 xL Genetic Analyzer (Applied Biosystems).

Table 1. Polymerase chain reaction (PCR) primers developed from *Pseudobulweria* and *Bulweria* sequences that were used in this study, in addition to primers developed from sequences of other procellariiform taxa (Pyle *et al.*, 2011; Welch *et al.*, 2012)

Primer	Sequence (5'→3')
aJugAF	GGATCTCTCCTAGGYATCTGCCTAAT
aJugAR	TCGGATTAGTCAGCCGTAYTG
aJugBF	CAACCCTAGCCTTTTCATCC
aJugBR	CCGTAATAGAATCCTCGTCCA
aJugCF	CAAACGGAGCCTCATTYTTTC
aJugCR	ATCCTACGAAGGCRGTTGCT
aJugDF	CCTGAGGGGGATTCTCAGTA
aJugDR	ACCTAGGGGGTTGTTTGAGC
aJugEF	CGCAGGACTCASMCTAATCCA
aJugER	TGGGAGGAATATAATTGTGAARCC
aJugFF	TCCACCCCTACTTCACCCTA
aJugFR	GGTTTGATATGRGGRRGGTGT
aJugGF	AACTTCACCCCAGCAAACC
aJugGR	GGAGGAATGGGGTTAGGAAT

Sequences were visually inspected and edited in SEQUENCHER 4.9.

DATA ANALYSIS

In addition to the data we obtained for *Pt. rupinarum*, we downloaded representative DNA sequences from the GenBank database for procellariid taxa (Table 2), selecting sequences from all species of *Pseudobulweria* and nearly all species of *Pterodroma* (see Results). Here, we consider the three Macaronesian taxa of *Pterodroma* from the North Atlantic as distinct species. Only partial sequences were available for the enigmatic *Ps. aterrima*, *Ps. becki*, and *Ps. macgillivrayi*, so for those species multiple sequences were included. We also included multiple sequences for taxa of interest that breed in several different locations (e.g. *Ps. rostrata* and *Pt. arminjoniana*). Diving petrels of the genus *Pelecanoides* were used to root the tree (Nunn & Stanley, 1998). The cytochrome *b* gene is relatively well conserved in Procellariiformes, and sequences were aligned in SEQUENCHER and then manually adjusted by eye. To detect the presence of nuclear copies of mitochondrial DNA in our data set (NUMTs; Sorenson & Fleischer, 1996), the sequences were characterized and translated in MacClade 4.08 (Maddison & Maddison, 2008). The program jModelTest 0.1.1 (Posada, 2008) and the Akaike information criterion (AIC) were used to select the best-fitting substitution model. The GTR + I + G model was selected for the full data set as well as for data sets composed of the nucleotides in first and third codon positions, and

the GTR + I model was selected for the data set composed of nucleotides in the second codon position. The transition/transversion bias was estimated using maximum likelihood in MEGA 5 (Nei & Kumar, 2000; Tamura *et al.*, 2011). Calculations of pairwise sequence divergence using the best-fit substitution model were conducted in PAUP* (Swofford, 2002) and MEGA 5.

We constructed phylogenetic trees using maximum likelihood and Bayesian inference. For both methods we first constructed trees using the full data set and then performed the analysis again, partitioning the data by codon position. Maximum-likelihood analyses were conducted with the RAxML BlackBox webserver (Stamatakis, Hoover & Rougemont, 2008), and branch support was estimated through 1000 bootstrap replicates. Bayesian inference was conducted using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003; Altekar *et al.*, 2004). For the codon-partitioned analyses, some parameters of the GTR substitution matrix in the partition for the first codon position had particularly low effective sample size (ESS) compared with the others (e.g. some types of transversions were rare in the data set), leading to poor mixing overall; therefore, the HKY + I + G model was implemented instead. Default priors were used and all parameters except for branch lengths and topology were unlinked. We used the default heating scheme with one cold and three heated chains. Runs were conducted for 10 million generations, with the first 10% discarded as burn-in. We conducted multiple independent analyses with different, randomly generated starting seeds. We examined plots of $-\ln L$ across generations and assessed the ESS for each parameter (> 500 in all cases) in TRACER 1.5 (Drummond *et al.*, 2012). To test whether the codon-partitioned model fit the data better than the unpartitioned model, we conducted a likelihood ratio test (LRT) for maximum-likelihood analyses, and estimated Bayes factors (BF) in TRACER for Bayesian analyses (Suchard, Weiss & Sinsheimer, 2001).

We investigated the divergence time of *Pt. rupinarum* using a strict molecular clock gene tree analysis in BEAST 1.7.4 (Drummond *et al.*, 2012). The analysis was performed using the unpartitioned data set with the GTR + I + G substitution model, empirical base frequencies, and a random starting tree. We used the Yule tree prior with other priors set at their default values. The substitution rate for the cytochrome *b* gene was set to 1.89% per million years, as estimated for procellariiform seabirds using fossil and biogeographic calibrations (Weir & Schluter, 2008). Multiple independent analyses with randomly generated starting seeds were conducted for 50 million generations, with the first 10% discarded as burn-in. As above, plots of $-\ln L$ across generations and ESS were examined in TRACER 1.5.

Table 2. Taxa included in phylogenetic analyses and the corresponding accession number for the mitochondrial cytochrome *b* sequence obtained from GenBank

Taxon	GenBank accession no.
<i>Bulweria bulwerii</i>	U74351
<i>Calonectris diomedea</i>	AY139626
<i>Calonectris leucomelas</i>	AF076045
<i>Daption capense</i>	AF076046
<i>Fulmarus glacialis</i>	U74348
<i>Fulmarus glacialoides</i>	AF076055
<i>Halobaena caerulea</i>	AF076057
<i>Aphrodroma brevirostris</i>	NC_007174
<i>Macronectes giganteus</i>	U48941
<i>Macronectes halli</i>	AF076061
<i>Pachyptila desolata</i>	AF076068
<i>Pachyptila salvini</i>	AF076069
<i>Pachyptila turtur</i>	AF076070
<i>Pachyptila vittata</i>	U74349
<i>Pagodroma nivea</i>	AF076071
<i>Pelecanoides garnoti</i>	AF076073
<i>Pelecanoides georgicus</i>	AF076074
<i>Pelecanoides magellanicus</i>	AF076075
<i>Pelecanoides urinatrix</i>	AF076076
<i>Procellaria aequinoctialis</i>	U74350
<i>Procellaria cinerea</i>	U48940
<i>Procellaria parkinsoni</i>	AF076077
<i>Procellaria westlandica</i>	AF076078
<i>Pseudobulweria aterrima</i>	JF522101
<i>Pseudobulweria aterrima</i>	U70483
<i>Pseudobulweria becki</i>	JF522102
<i>Pseudobulweria macgillivrayi</i>	JF522104
<i>Pseudobulweria macgillivrayi</i>	JF522105
<i>Pseudobulweria rostrata rostrata</i> – Fiji (F)	JF522103
<i>Pseudobulweria rostrata rostrata</i> – Marquesas (M)	JF522106
<i>Pseudobulweria rostrata rostrata</i> – Gambier (G)	U70482
<i>Pseudobulweria rostrata trouessarti</i> – New Caledonia (N)	U70493
<i>Pterodroma arminjoniana</i> – Round (R)	GQ328970
<i>Pterodroma arminjoniana</i> – Trindade (T)	GQ328969
<i>Pterodroma axillaris</i>	U74342
<i>Pterodroma barau</i>	U70489
<i>Pterodroma cahow</i>	U74331
<i>Pterodroma cookii</i>	U74345
<i>Pterodroma deserta</i>	U74333
<i>Pterodroma externa</i>	U74339
<i>Pterodroma feae</i>	FJ196356
<i>Pterodroma hasitata</i>	EU167017
<i>Pterodroma heraldica</i>	GQ328988
<i>Pterodroma hypoleuca</i>	AF076079
<i>Pterodroma incerta</i>	U74335
<i>Pterodroma inexpectata</i>	U74346
<i>Pterodroma lessonii</i>	U74337
<i>Pterodroma longirostris</i>	U74344
<i>Pterodroma macroptera</i>	EU979357
<i>Pterodroma madeira</i>	FJ196362
<i>Pterodroma magentae</i>	U74338
<i>Pterodroma mollis dubia</i>	U74655
<i>Pterodroma mollis mollis</i>	U74334

Table 2. *Continued*

Taxon	GenBank accession no.
<i>Pterodroma neglecta</i>	U74341
<i>Pterodroma nigripennis</i>	U74343
<i>Pterodroma phaeopygia</i>	HQ420347
<i>Pterodroma rupinarum</i>	KF010837
<i>Pterodroma sandwichensis</i>	HQ420351
<i>Pterodroma solandri</i>	U74347
<i>Pterodroma ultima</i>	HQ420379
<i>Puffinus assimilis</i>	AF076080
<i>Puffinus bryani</i>	HQ589355
<i>Puffinus bulleri</i>	AF076081
<i>Puffinus carneipes</i>	AF076082
<i>Puffinus creatopus</i>	AF076083
<i>Puffinus gravis</i>	U74354
<i>Puffinus griseus</i>	U74353
<i>Puffinus huttoni</i>	AF076084
<i>Puffinus lherminieri</i>	AF076085
<i>Puffinus nativitatis</i>	AF076086
<i>Puffinus newelli</i>	HQ589356
<i>Puffinus opisthomelas</i>	AF076087
<i>Puffinus pacificus</i>	AF076088
<i>Puffinus puffinus</i>	U74355
<i>Puffinus tenuirostris</i>	U74352
<i>Thalassoica antarctica</i>	AF076095

RESULTS

DNA sequences were obtained from two samples: 194 bp from one sample from Prosperous Bay (USNM 176607) and 786 contiguous base pairs from the sample from Sandy Bay (USNM 176679). The sequence from Prosperous Bay fully overlapped with the sequence from Sandy Bay and was identical. After alignment, the total sequence length for *Pt. rupinarum* was 949 bp. This sequence has been deposited in the GenBank database under accession number KF010837. The full alignment of comparative sequences was 1143 bp and contained 76 taxa, with an average sequence length of 1045 bp. No gaps or premature stop codons were detected. There were 478 variable sites with 21, 6, and 73% occurring in the first, second, and third codon positions, respectively. After translation, 14% of the substitutions resulted in non-synonymous changes. The nucleotide frequencies were A = 28.15%, T = 26.34%, C = 32.91%, and G = 12.60%, and the transition/transversion bias was estimated to be 9.42. This pattern of variation suggests that these sequences are likely to be of mitochondrial, and not nuclear, origin. Additionally, the independent amplification of multiple overlapping fragments for *Pt. rupinarum* did not uncover any ambiguous sequences, which provides additional evidence for the mitochondrial origin of those sequences.

Maximum likelihood and Bayesian inference produced congruent topologies, with *Pt. rupinarum* placed within a clade of *Pterodroma* petrels from the Atlantic with high support. For both methods of analysis there was strong support for the codon-partitioned model over the unpartitioned model (LRT = 1254, $P < 0.001$; $\log_{10}BF = 238$), although both models also produced congruent topologies. Overall, the topology recovered here is generally in agreement with other molecular analyses of Procellariidae (Fig. 2; Nunn & Stanley, 1998; Austin *et al.*, 2004; Penhallurick & Wink, 2004; Jesus *et al.*, 2009; Gangloff *et al.*, 2012). In general the cytochrome *b* gene provides poor resolution for deeper nodes beyond the level of genus. The branch leading to *Pseudobulweria* received strong support (posterior probability, PP 1.0; bootstrap support, BS 99). Within *Pseudobulweria*, relationships were largely unresolved, and further analyses are particularly needed to determine subspecific relationships and taxonomic status within the Tahiti petrel (*Ps. rostrata*; Gangloff *et al.*, 2012). Support was also strong for the branch leading to *Pterodroma* (PP 1.0; BS 100). Within *Pterodroma*, deeper relationships were unresolved, but some strongly supported clades were also detected.

In the phylogenies, *Pt. rupinarum* did not group with *Pseudobulweria*, but instead fell within a

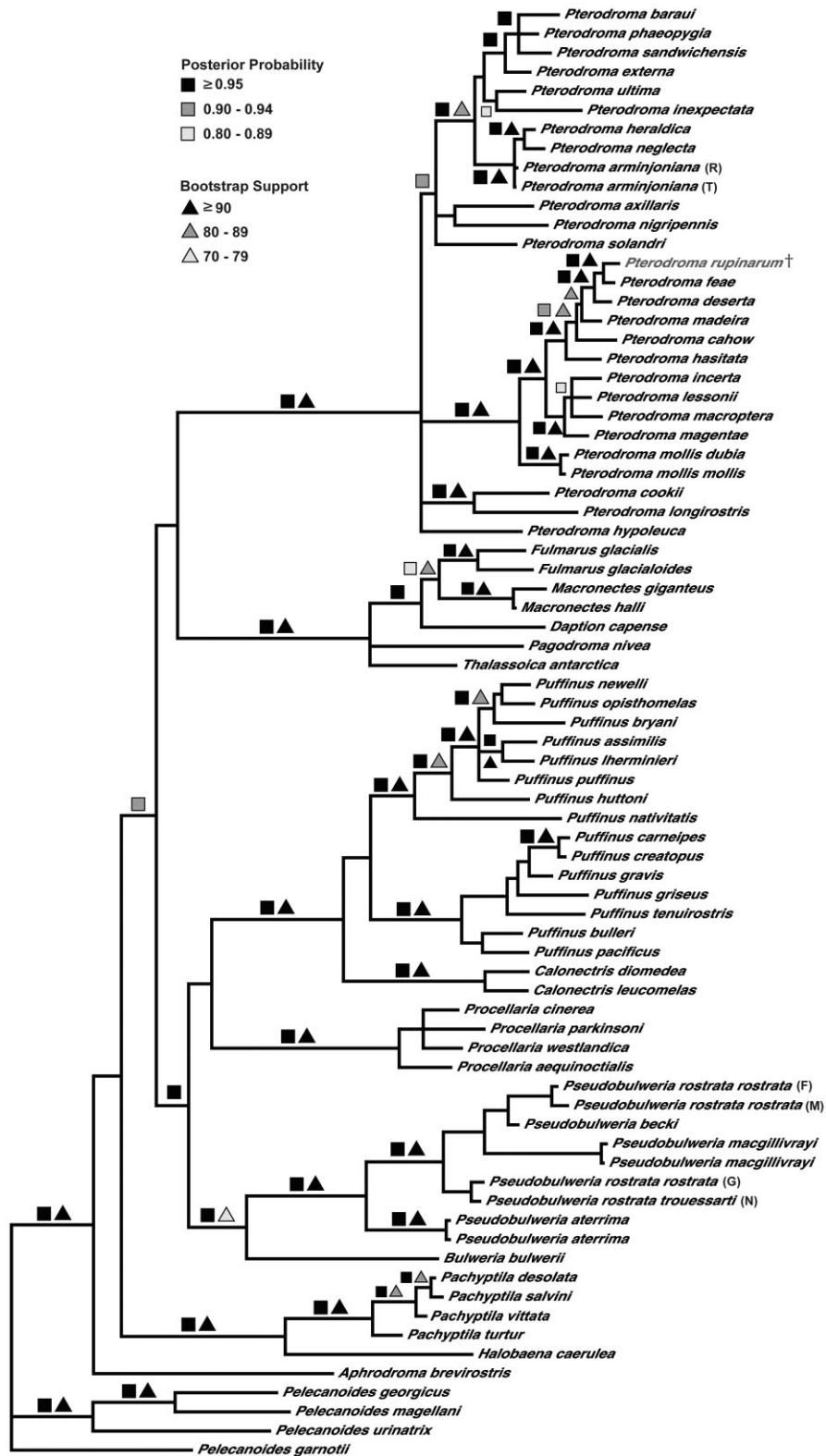


Figure 2. Bayesian consensus tree of cytochrome *b* sequences from 72 procellariid taxa and four *Pelecanoides* out-groups. Maximum-likelihood analyses yielded a congruent topology. Bayesian posterior probability and maximum-likelihood bootstrap support are shown along the branches. Sampling locations for *Pterodroma arminjoniana* and *Pseudobulweria rostrata* are as listed in Table 2.

strongly supported Atlantic clade of *Pterodroma* (Fig. 2) that includes *Pterodroma hasitata* (Kuhl, 1820), *Pterodroma cahow* (Nichols & Mowbray, 1916), and the Macaronesian petrels, *Pt. madeira*, *Pt. deserta*, and *Pt. feae* (PP 1.0; BS 98). Within this clade, *Pt. rupinarum* was most closely related to *Pt. feae* (PP 0.99; BS 91), which breeds in the Cape Verde Islands, the most southerly of the Macaronesian islands and geographically closest to St Helena. Divergence time estimates from the strict molecular clock gene tree analysis suggest that *Pt. rupinarum* diverged from *Pt. feae* approximately 260 000 years ago (95% highest posterior density 126 000–418 000 years ago). The pairwise genetic distance (Table 3) between *Pt. rupinarum* and *Pt. feae* was 1.10%, and between *Pt. rupinarum* and the next closest taxon, *Pt. deserta*, was 1.83%, whereas distances to species of *Pseudobulweria* were much larger, ranging from 12.2 to 14.6%, and averaging approximately 13%. The calculation of genetic distances can be influenced by missing data (Fregin *et al.*, 2012), and therefore these distances may be slightly revised if longer sequences from *Pt. rupinarum* or the critically endangered *Pseudobulweria* petrels become available.

DISCUSSION

PHYLOGENETIC PLACEMENT

Phylogenetic analyses of the cytochrome *b* sequence of the extinct *Pt. rupinarum* strongly support its placement in the genus *Pterodroma*. It groups within a clade of petrels distributed in the North Atlantic, and is most closely related to the Macaronesian petrels, in particular *Pt. feae* of the Cape Verde islands. It is unlikely that the placement of *Pt. rupinarum* could result from contamination, postmortem DNA damage, or poor taxonomic sampling. Negative DNA extraction and PCR controls did not display evidence of contamination. Additionally, sequences of multiple, overlapping fragments from the same sample were identical, as were sequences obtained from two different individuals collected at different sites under different conditions of local preservation, suggesting minimal contamination or errors from miscoding regions in the DNA. PCR amplification success is higher for shorter fragments, typical of ancient DNA. Taxonomic sampling is also not likely to be an issue. Using sequences available in the GenBank database all extant *Pseudobulweria* petrels were included in the phylogeny. One additional extinct taxon potentially belonging to that genus remains to be described (Gangloff *et al.*, 2012). Cytochrome *b* sequences > 600 bp were not available for seven Pacific taxa: *Pterodroma alba* (Gmelin, 1789), *Pterodroma atrata* (Mathews,

Table 3. Pairwise genetic distances (%) between *Pterodroma rupinarum* and *Pterodroma* and *Pseudobulweria* petrels, and other selected taxa of Procellariidae

Taxon	<i>Pterodroma rupinarum</i>
<i>Pterodroma feae</i>	1.10
<i>Pterodroma deserta</i>	1.83
<i>Pterodroma madeira</i>	2.39
<i>Pterodroma mollis dubia</i>	2.53
<i>Pterodroma hasitata</i>	3.25
<i>Pterodroma cahow</i>	3.32
<i>Pterodroma mollis mollis</i>	3.38
<i>Pterodroma magentae</i>	3.42
<i>Pterodroma lessonii</i>	4.11
<i>Pterodroma incerta</i>	4.25
<i>Pterodroma macroptera</i>	4.55
<i>Pterodroma axillaris</i>	7.65
<i>Pterodroma nigripennis</i>	7.67
<i>Pterodroma solandri</i>	8.01
<i>Pterodroma externa</i>	8.16
<i>Pterodroma neglecta</i>	8.94
<i>Pterodroma barau</i>	9.09
<i>Pterodroma longirostris</i>	9.16
<i>Pterodroma ultima</i>	9.42
<i>Pterodroma arminjoniana</i> – Trindade	9.46
<i>Pterodroma heraldica</i>	9.54
<i>Pterodroma sandwichensis</i>	9.56
<i>Pterodroma hypoleuca</i>	9.58
<i>Pterodroma phaeopygia</i>	9.73
<i>Pterodroma cookii</i>	9.98
<i>Pterodroma inexpectata</i>	10.32
<i>Pseudobulweria rostrata rostrata</i> – Marquesas	12.15
<i>Pseudobulweria rostrata rostrata</i> – Fiji	12.28
<i>Pseudobulweria aterrima</i>	12.36
<i>Pseudobulweria rostrata trouessarti</i> – New Caledonia	12.71
<i>Pseudobulweria macgillivrayi</i>	13.11
<i>Pseudobulweria rostrata rostrata</i> – Gambier	13.56
<i>Pseudobulweria becki</i>	14.64
<i>Bulweria bulwerii</i>	12.72
<i>Fulmarus glacialisoides</i>	14.86
<i>Pachyptila desolata</i>	13.93
<i>Pelecanoides magellanicus</i>	15.45
<i>Procellaria parkinsoni</i>	13.26
<i>Puffinus puffinus</i>	14.18

1912), *Pterodroma brevipes* (Peale, 1848), *Pterodroma cervicalis* (Salvin, 1891), *Pterodroma defilippiana* (Giglioli & Salvadori, 1869), *Pterodroma leucoptera* (Gould, 1844), and *Pterodroma pycrofti* Falla, 1933, none of which are thought to belong in the same clade as the Atlantic taxa that grouped with *Pt. rupinarum*. No sequences were available for the extinct Jamaica

petrel, *Pterodroma caribbaea* Carte, 1866, which has sometimes been considered a subspecies of *Pt. hasitata* (see below).

Analyses relying strictly on mitochondrial DNA can be biased in some circumstances. In cases of hybridization, phylogenetic analysis of mitochondrial DNA will group hybrids with the maternal species. It is possible that the individuals of *Pt. rupinarum* we sequenced were the product of a *Pterodroma* × *Pseudobulweria* hybridization event, although this seems unlikely. There is no evidence for the existence of another species of *Pterodroma* on St Helena, and no species of *Pseudobulweria* are known from the Atlantic. Furthermore, continental landmasses as well as non-physical barriers may reduce the frequency of dispersal of *Pseudobulweria* into the Atlantic (Steeves, Anderson & Friesen, 2005; Friesen, Burg & McCoy, 2007). Also, hybridization is thought to be rare in procellariid seabirds in general, with only a few reported cases in the family (Hunter, 1982; Brown *et al.*, 2010). Finally, it is unclear whether a hybridization event between such divergent species would produce viable offspring. Positive selection may also act on the mitochondrial genome (Bazin, Glémin & Galtier, 2006), which could potentially influence the results of phylogenetic analyses. Additional data from the nuclear genome would help to verify the findings presented here, but the results are consistent with the geographical distributions of other species of *Pterodroma* in the Atlantic. Therefore, biases related to hybridization and positive selection do not seem likely.

The present analysis indicates, with high support, that *Pt. rupinarum* belongs within a clade of *Pterodroma* petrels that are distributed around the North Atlantic. This group includes the endangered black-capped petrel (*Pt. hasitata*) of Hispaniola, Cuba, and Lesser Antilles, the vanished Jamaica petrel (*Pt. caribbaea*), which was once considered a subspecies of *Pt. hasitata* (Brooke, 2004), and was last observed in the mid 19th century, the endangered Bermuda petrel (*Pt. cahow*), as well as the three species of Macaronesian petrels breeding in the eastern North Atlantic. The closest taxon to *Pt. rupinarum* appears to be *Pt. feae* of the Cape Verde Islands, with *Pt. deserta* of Bugio Island in the Madeira Archipelago being slightly more distant. The species status of *Pt. deserta* has been debated. There appears to be little or no gene flow with *Pt. feae*, but the two taxa have diverged only very recently, within the last 40 000 years (Gangloff *et al.*, 2013), and so the divergence process may not be complete. *Pseudobulweria* petrels breed in the Indian and Pacific oceans, and given this wide distribution it may not have been surprising to find a member of the genus in the Atlantic; however, the phylogenetic

placement of *Pt. rupinarum* within *Pterodroma* is consistent with a biogeographic scenario in which a closely-knit clade of petrel species radiated throughout the Atlantic and Caribbean, reaching as far south as St Helena (Fig. 1).

The phylogenetic placement of *Pt. rupinarum* may seem contradictory to earlier osteological analyses. The skull of this taxon was described as being similar to petrels of the genus *Pseudobulweria*, which have a marked downward slope of the bill as compared with *Pterodroma* petrels, where the bill attaches to the skull in a horizontal manner (Olson, 1975). When these analyses were conducted nearly 40 years ago no skeletons of *Pseudobulweria* were available, and therefore comparisons were made between the three-dimensional fossils and X-radiographs taken of museum study skins. Similarly, there were very few skeletons of *Pterodroma* species in museum collections, and none of the Macaronesian species. This is slowly beginning to change. Now there are at least a good series of skeletons of several different populations of *Ps. rostrata*, and although very scarce and scattered in small and remote museums, there are also a few skeletons now available for the Macaronesian species of *Pterodroma*. Until proper comparisons of this new material can be made with the fossils of *Pt. rupinarum*, it remains unclear whether the apparently distinctive features of the skull and mandible are truly similar to, and presumably convergent with, *Pseudobulweria*, or whether such features may also occur in some or all of the Macaronesian species of *Pterodroma*. Likewise, we are unable at this point to say how much the osteology of *Pt. rupinarum* is similar to or differs from its apparent closest relative, *Pt. feae*, of the Cape Verde Islands.

SPECIES STATUS

Pterodroma rupinarum shows a close relationship to *Pt. feae* and *Pt. deserta*, with an uncorrected pairwise sequence divergence of 1.1–1.8%. Further work is needed to investigate genetic variation within these taxa, which may lead to a revision of the estimated genetic distance in the future; however, a distance of 1.1% is within the normal range of pairwise cytochrome *b* sequence divergences for acknowledged full species of petrels (Austin *et al.*, 2004; Techow, Ryan & O’Ryan, 2009). The lowest pairwise divergence between species in the data set analysed here was 0.7%, which occurred between *Pachyptila desolata* (Gmelin, 1789) and *Pachyptila salvini* (Mathews, 1912), and between *Puffinus creatopus* Coues, 1984 and *Puffinus carneipes* Gould, 1844. Overall, a pairwise sequence divergence of 1.1%, in addition to osteological differences, suggests that

Pt. rupinarum is a distinct species from *Pt. feae*. At the very least it may represent a substantially differentiated population of *Pt. feae* that was isolated for a long period of time prior to its extinction, perhaps about a quarter of a million years, assuming an evolutionary rate of 1.89% per million years for the cytochrome *b* gene (Weir & Schluter, 2008).

EXTINCTION AND CONSERVATION OF PETRELS

Several species of procellariid seabirds have become extinct or extirpated since humans colonized the islands where they breed, including *Puffinus spelaeus* Holdaway & Worthy, 1994 and *Puffinus olsoni* McMinn, Jaume & Alcover, 1990 (Rando & Alcover, 2008) in the Canary Islands, and *Puffinus parvus* Shufeldt, 1916 (= *P. boydi*) (Olson, 2004) on Bermuda, as well as other species in the Pacific. In addition, many bones have been found for which the taxonomic identity remains unclear, and these may or may not represent undescribed species (Steadman, 2006). Furthermore, many populations of extant species have been extirpated as well, and these could have represented distinct but unrecognized evolutionary units. It is interesting that two extinct species occur in the same clade of *Pterodroma*: *Pt. rupinarum* on St Helena and *Pt. caribbaea* on Jamaica (although the extinction of the Jamaica petrel has not been confirmed). Which factors are consistently related to seabird extinctions, if any, remains unclear. Anthropogenic influences on these islands are similar to those of other oceanic islands where procellariid species have persisted. Gangloff *et al.* (2012) suggest that petrels that breed on gently sloping islands, as opposed to islands with steep, inaccessible cliffs, may be at increased risk for extinction. This may have been the case for *Pt. rupinarum* (Olson, 1975), but *Pt. caribbaea* was known to breed on steep cliffs (Brooke, 2004). Another factor may be that *Pt. rupinarum* and *Pt. caribbaea* are both single-island endemics, which have an increased risk of extinction because of restricted ranges, small population sizes, and lower genetic diversity (Frankham, 1997). Additionally, the characteristic philopatry of petrel species may mean that birds continually return to the same high-mortality breeding sites year after year (Igual *et al.*, 2007). Although at least one, and potentially two, instances of extinction have occurred in this clade, there is also at least one case of successful conservation restoration. *Pterodroma cahow* was thought to be extinct for over 200 years until a small population was rediscovered in the early 1900s. Through intensive management, population sizes have been steadily increasing and translocated birds have begun breeding in their new colonies (Carlile *et al.*, 2003; Gehrman, 2012; Birdlife International,

2013); therefore, although some petrel extinctions have already occurred, it may still be possible to prevent additional extinctions in the future.

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