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A GLOBAL MOLECULAR PHYLOGENY OF THE SMALL *PUFFINUS* SHEARWATERS AND IMPLICATIONS FOR SYSTEMATICS OF THE LITTLE–AUDUBON'S SHEARWATER COMPLEX

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Abstract.—A molecular phylogeny based on 917 base pairs (bp) of the mitochondrial (mt) DNA cytochrome-*b* gene was used to test and reassess the systematics and conflicting taxonomic treatments of the small, black-and-white *Puffinus* shearwaters, including the *P. assimilis–lherminieri* species complex. Three geographically discrete clades were identified in the North Atlantic, Southern (Australasia) and tropical Pacific and Indian oceans that contain most of the *P. assimilis–lherminieri* taxa. Together with four other lineages (*P. puffinus*, *P. opisthomelas*, *P. mauretanicus–P. yelkouan*, *P. newelli–P. myrtae*), they form an unresolved polytomy. *Puffinus huttoni–P. gavia*, *P. nativitatis*, and *P. subalaris* are basal to this. The phylogenetic positions of *P. myrtae* and *P. subalaris* are unexpected and warrant further investigation. None of the competing taxonomic treatments of the *P. assimilis–lherminieri* complex are supported. Instead, our phylogeny suggests that 14 taxa should be recognized, whereas five others (*loyemilleri*, *colstoni*, *nicolae*, *polynesiae*, and *atrodorsalis*) are phylogenetically undifferentiated from more widespread species (*lherminieri*, *dichrous*, and *bailloni*) and are probably not valid. Similarities in plumage and external morphological characters between unrelated species and differences between closely related species suggest that those traditional taxonomic characters are poor indicators of phylogenetic relatedness. *Received 15 August 2003, accepted 28 March 2004.*

Resumen.—Utilizamos una filogenia molecular basada en 917 pares de bases (pb) del gen de ADN mitocondrial citocromo-*b* para analizar y reconsiderar la sistemática y taxonomía conflictiva de las fardelas del genero *Puffinus,* incluyendo el complejo de especies *P. assimilis–lherminieri.* Se identificaron tres clados geográficamente discretos en el Atlántico Norte, sur de Oceanía-Australasia, y en los mares tropicales del Pacífico y Océano Índico, los cuales contuvieron la mayoría de los taxa *P. assimilis–lherminieri.* Junto con otros cuatro linajes (*P. puffinus*, *P. opisthomelas*, *P. mauretanicus–P. yelkouan*, *P. newelli–P. myrtae*), éstos forman una politomía no resuelta. *Puffinus huttoni–P. gavia*, *P. nativitatis* y *P. subalaris* son basales a ésta. Las posiciones filogenéticas de *P. myrtae* y *P. subalaris* son inesperadas y requieren de más investigación. Ninguno de los tratamientos taxonómicos del complejo *P. assimilis–lherminieri* es robusto. En cambio, nuestra filogenia sugiere que se deberían reconocer 14 taxa, mientras que otros cinco (*loyemilleri*, *colstoni*, *nicolae*, *polynesiae*, y *atrodorsalis*) no se diferenciaron filogenéticamente de otras especies de distribución amplia (*lherminieri*, *dichrous*, y *bailloni*) y probablemente no son válidos. Las similitudes en el plumaje y en los caracteres morfológicos externos entre especies no relacionadas y las diferencias entre especies cercanamente relacionadas sugieren que estos caracteres taxonómicos tradicionalmente usados son malos indicadores de las relaciones filogenéticas.

The order Procellariiformes remains one of the most controversial groups in avian systematics (Cracraft 1981, Warham 1990). Dispute occurs at every phylogenetic level: (1) relationships with other avian orders; (2) numbers of families and genera; and (3) relationships among taxa at the family, genus, and species levels (Alexander et al. 1965, Imber 1985, Sibley and Ahlquist 1990, Warham 1990). Species limits are perhaps the most controversial aspect (Imber 1985, Bretagnolle et al. 1990, Bretagnolle 1995, Austin 1996), partly because most petrels are remarkably similar in morphology and are not distinctively colored. Strong philopatry has probably resulted in minor

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phenotypic variation between populations, causing additional taxonomic confusion. More recently, DNA–DNA hybridization (Sibley et al. 1988) and DNA sequences (Austin 1996, Bretagnolle et al. 1998, Heidrich et al. 1998, Nunn and Stanley 1998) have been used to assess species limits and phylogeny; the results have sometimes conflicted with traditional taxonomies.

Difficulties in procellariiform taxonomy are particularly evident in the Little–Audubon's Shearwater complex (*Puffinus assimilis–lherminieri*). Members of the complex are widespread, small-sized, black-and-white shearwaters from tropical, subtropical, and sub-Antarctic seas, breeding from 38°N to 50°S (Harrison 1987, Mougin et al. 1992; Fig. 1). Together with other small- to medium-sized, dark and light shearwaters, they form a morphologically (Jouanin and Mougin 1979, Wragg 1985) and genetically (Austin 1996, Nunn and Stanley 1998) well-defined subgroup within the genus. Major reviews of the complex have been published by Murphy (1927) and Jouanin and Mougin (1979). Here, we summarize the various taxonomic treatments; for clarity, we refer to two species groups "lherminieri" and "assimilis" and italicize subspecies names. The traditional view retains two species, with ~12 subspecies in the lherminieri group and ~8 in the assimilis group, though up to 40 taxa have been described for the complex in the past (Jouanin and Mougin 1979, Warham 1990, Mougin et al. 1992; Fig. 1 and Table 1). Jouanin and Mougin (1979) recognized that three taxa (all belonging to the lherminieri group) might be separate species: *heinrothi* (see also Harrison 1983), *bannermani* (as suggested by Vaurie 1965), and *persicus* (Table 1). That traditional view has been repeatedly challenged, with respect to number of species and assignment of particular populations to one or the other species. Since the check-list of Jouanin and Mougin (1979), two new subspecies of the lherminieri group—*temptator* from the Comores (Louette and Heremans 1985) and *colstoni* from Aldabra (Shirihai and Christie 1996)—have been described, and a new species, *atrodorsalis*, has been proposed (Shirihai et al. 1995). That proposal has been very controversial (Bourne 1995, Bretagnolle and Attié 1996, Bretagnolle et al. 2000); *atrodorsalis* is now regarded as closely

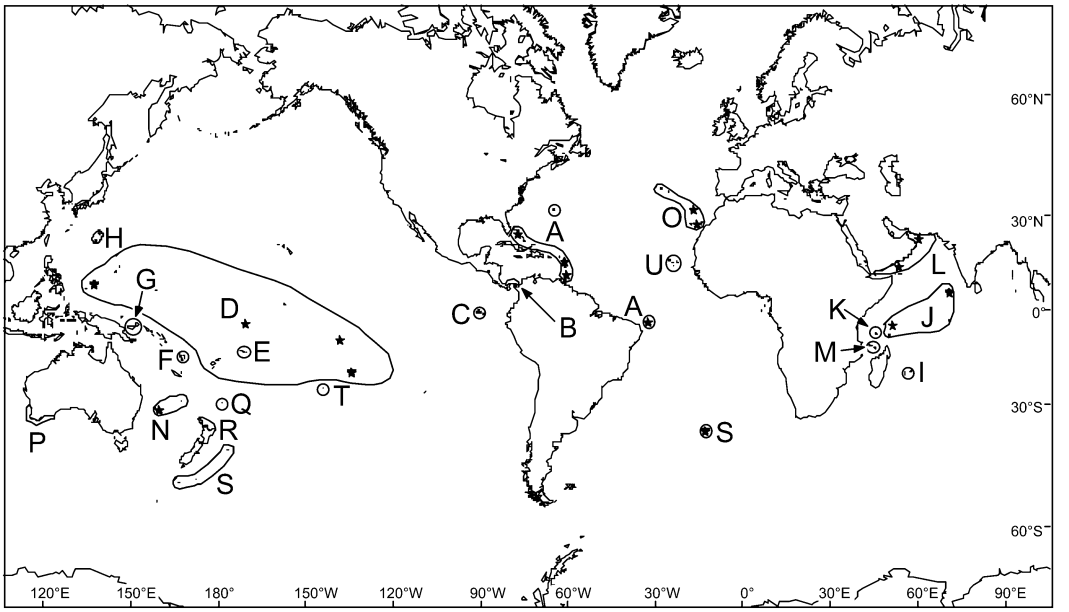

FIG. 1. Distribution of the *assimilis–lherminieri* complex. (A) *lherminieri*, (B) *loyemilleri*, (C) *subalaris*, (D) *dichrous*, (E) *polynesiae*, (F) *gunax*, (G) *heinrothi*, (H) *bannermani*, (I) *bailloni*, (J) *nicolae*, (K) *colstoni*, (L) *persicus*, (M) *temptator*, (N) *assimilis*, (O) *baroli*, (P) *tunneyi*, (Q) *kermadecensis*, (R) *haurakiensis*, (S) *elegans*, (T) *myrtae*, (U) *boydi*. Stars indicate sampling localities for widely dispersed taxa.

TABLE 1. Recent taxonomic treatments of the *assimilis–lherminieri* complex.

Authority	Number of species	Species	Number of subspecies
Jouanin and Mougin (1979)	2	*lherminieri*	11
		assimilis	7
Bourne (1986)	1	*assimilis* [a]	19
Sibley and Monroe (1990) [b]	5	*lherminieri*	
		assimilis	
		heinrothi	
		bannermani	
		persicus	
Warham (1990)	2	*lherminieri*	12
		assimilis	7
Carboneras (1992)	3	*lherminieri*	10
		assimilis	8
		heinrothi	
Shirihai et al. (1995)	8	*lherminieri*	5
		assimilis	8
		nicolae	2
		gunax	
		bannermani	
		heinrothi	
		persicus	
		atrodorsalis	

[a] Owing to anteriority; *assimilis* was described in 1838, whereas *lherminieri* was described in 1839.
[b] No subspecies were proposed.

related to *bailloni* (Shirihai 2000) or identical to it (Bretagnolle and Attié 1996, Bretagnolle et al. 2000). Number of proposed species has varied from one to eight (Table 1). Sibley and Monroe (1990) recognized five species in their checklist, including the three identified by Jouanin and Mougin (1979). Bourne (1986), following Vaurie (1965), suggested that only one species should be considered, on the basis of the difficult taxonomic treatment of *boydi* (Cape Verde Island), sometimes treated as belonging to the lherminieri group (Murphy 1927, Jouanin and Mougin 1979), sometimes as belonging to the assimilis group (Cramp and Simmons 1977, Carboneras 1992), or more recently as a separate species (Hazevoet 1995). Partly from *boydi* again, Austin (1996) reached a different, biogeographic conclusion and proposed that the complex comprises three ocean-based clades. Lastly, Shirihai et al. (1995) hesitated between lumping all taxa into one species or splitting the series into eight different species.

Difficulties in this species complex arise mainly through the taxonomic treatment of some distinctive populations. Conflict originates from the criteria used to distinguish the two traditional species. Basically, the assimilis group has darker upper parts than the lherminieri group, blue instead of pink legs, white instead of brown undertail coverts, shorter wing and tail than the lherminieri group, and white inner webs of the primaries (gray in the lherminieri group). However, *boydi* has blue legs but gray inner webs; some *lherminieri* from Guadeloupe have blue legs and brown undertail; and *bailloni* has white undertail but gray inner webs, and pink or blue legs (Bretagnolle and Attié 1996). Juvenile *bailloni* are black-backed, whereas adults are brown-backed (Bretagnolle and Attié 1996). Color of undertail coverts was also found to vary extensively within populations (Lee 1988); back color with molt stage and age (Lee 1988); and leg color from blue to pink or pale, according to individuals, at least in *lherminieri*, *polynesiae*, *dichrous*, and *bailloni* (Bretagnolle 1996, V. Bretagnolle pers. obs.).

The aim here is twofold: (1) to discern which competing taxonomic treatment, if any, is supported by a molecular phylogeny, especially with regard to number of distinct species; and (2) to reconstruct the phylogeny of the whole complex, investigating whether taxa within a proposed species are monophyletic or not. We used DNA sequences from the mitochondrial (mt) cytochrome-*b* gene, which has proved valuable for phylogenetic analysis at the

species level in birds (see review in Moore and DeFilippis 1997). Austin (1996), Heidrich et al. (1998), and Nunn and Stanley (1998) have previously used cytochrome-*b* gene sequences from 19, 8, and 12 shearwater taxa, respectively, to investigate phylogenetic relationships within the genus. However, Austin (1996) used partial sequences (307 base pairs [bp]) and sequenced five taxa of the complex (*baroli*, *boydi*, *lherminieri*, *assimilis*, and *haurakiensis*). Heidrich et al. (1998) sequenced the complete gene but included only one taxon (*baroli*); Nunn and Stanley (1998) also used complete gene sequences and included only *lherminieri* and *baroli*. Here, we present a more thorough analysis based on 917 bp from the mtDNA cytochrome-*b* gene, including 19 taxa of the *assimilis–lherminieri* complex.

METHODS

Samples.—We collected tissue, blood, and feather samples from 68 *Puffinus* shearwaters, 40 of which came from registered museum specimens or vouchered tissue collections (Appendix). Tissue samples from museum skins consisted of small pieces of skin cut from the webbing of the foot. Twelve museum specimens failed to yield DNA (Appendix), three of which represented the only specimens available for *P. auricularis*, *gunax*, and *bannermani*. We were unable to obtain any samples for *heinrothi*. Thirty-four cytochrome-*b* sequences were obtained from GenBank (see Heidrich et al. 1998, Nunn and Stanley 1998); GenBank accession numbers and locality data (for *lherminieri* and *baroli* only) are in parentheses: *Calonectris leucomelas* (AF076045), *C. diomedea* (U74356), *P. pacificus* (AF076088), *P. bulleri* (AF076081), *P. creatopus* (AF076083), *P. carneipes* (AF076082), *P. gravis* (U74354), *P. griseus* (U74353), *P. tenuirostris* (U74352), *P. nativitatus* (AF076086), *P. huttoni* (AF076084), *P. puffinus* (U74355, AJ004213–4215), *P. yelkouan* (AJ004216–4224), *P. mauretanicus*, (AJ004208–4212), *P. opisthomelas* (AF076087), *P. lherminieri* (AF076085: at sea, offshore North Carolina, USA), and *P. baroli* (AF076080 and AJ004206–4207: Madeira, Portugal). Sequences from *P. pacificus*, *P. bulleri*, *P. carneipes*, *P. creatopus*, *P. griseus*, *P. gravis*, *P. tenuirostris*, *C. leucomelas*, and *C. diomedea* were included for outgroup comparison. On the basis of the phylogeny of Nunn and Stanley (1998), those species are clades that are sister to all of the smaller species of *Puffinus*, including the *assimilis–lherminieri* complex.

Laboratory procedures.—To minimize risk of contamination, especially of museum material, we carried out pre- and post-polymerase chain reaction (PCR) procedures in a separate area, using dedicated equipment and supplies and following general precautions for work on ancient DNA (Austin et al. 1997). We always performed DNA extractions on museum material independently of modern samples. Where the 917-bp sequence was obtained from two or three smaller overlapping fragments (see below), those were PCR amplified and sequenced independently, reducing the chance of contamination affecting the entire sequence.

We extracted DNA from small (2–3 mm^3) pieces of finely minced tissue (muscle, kidney, or skin); 5 μL of blood; or single feather tips, using proteinase-K digestion, phenol–chloroform, and centrifugal dialysis (Cooper 1994). We obtained a 917-bp segment of the mtDNA cytochrome-*b* gene, via PCR amplification, as a single fragment or two or three shorter overlapping fragments, using various combinations of the following primers: CYTB1 (14990), 5′-ATC CAA CAT CTC AGC ATG ATG AAA-3′ (Kocher et al. 1989), CYTB1B (14994), 5′-AAC ATC TCA GCA TGA TGA AAY TTY-3′ (R. H. Thomas pers. comm.), CYTB2A (15295), 5′-AAT GAT ATT TGT CCT CAS GG-3′ (R. H. Thomas pers. comm.), CYTB2 (15298), 5′-CCC TCA GAA TGA TAT TTG TCC TCA-3′ (Kocher et al. 1989), H15312, 5′-GAT AGC TGA GAA TAG GTT GGT GAT G-3′ (present study), L15287, 5′-CAT AGC AAC TGC CTT CGT AGG ATA-3′ (present study), CB3H (15706), 5′-GGC AAA TAG GAA RTA TCA TTC-3′, (Palumbi 1996), L15598, 5′-GAC ATT CTA GGC TTT ATA CTC T-3′ (present study), L15656, 5′-AAC CTA CTA GGA GAC CCA GA-3′ (Helm-Bychowski and Cracraft 1993), H15914, 5′-GGT TGT TCT ACT GGT TGG C-3′ (Helm-Bychowski and Cracraft 1993), H16065, 5′-AAC TGC AGT CAT CTC CGG TTT ACA AGA C-3′ (Helm-Bychowski and Cracraft 1993). Numbers refer to the 3′ position of the primer in the chicken (*Gallus gallus*) mitochondrial genome sequence (Desjardins and Morais 1990). We carried out PCR amplifications in 25-μL volumes containing 1×PCR Buffer B (Promega, Madison, Wisconsin), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μm of each primer, 0.2 mg mL^{-1} BSA, 0.5 U of *Taq* DNA polymerase (Promega), and 2 μL of genomic DNA, using the following cycling parameters: 2 min at 94°C, 35 cycles of 30 s at 94°C, 30–40 s at 50–55°C, and 45–90 s at 72°C, with a final extension of 5 min at 72°C. For all museum-skin samples and some modern samples, we performed a secondary PCR amplification, using the same reaction conditions (with the exclusion of BSA) and cycling parameters, adding 1 μL of the primary amplification as template. Amplified products were agarose-gel purified (Qiaex II Gel Extraction Kit, Qiagen, Valencia, California); both strands were sequenced directly, using the same primers as for PCR and automated cycle sequencing chemistry (Big Dye, Applied Biosystems [ABI], Foster City, California) with an ABI 373 or 377 DNA sequencer. All sequences are deposited in GenBank under accession numbers AY219925–AY219980.

Phylogenetic analyses.—We aligned sequences by eye, without the need for indels. To reduce computer-intensive analysis times, we removed identical sequences using the Filter Taxa option in MACCLADE, version 3.07 (Maddison and Maddison 1997). We chose a subset of those sequences for final analyses by eliminating multiple representatives of non-*assimilis–lherminieri* taxa (i.e. *P. puffinus*, *P. yelkouan*, *P. mauretanicus*, and *P. opisthomelas*). Preliminary analyses showed that multiple sequences for those four taxa were monophyletic, with little intraspecific diversity relative to interspecific divergence (see Table 2).

We did phylogenetic analyses using maximum-likelihood (ML), maximum-parsimony (MP), and neighbor-joining (NJ) methods, implemented in PAUP, version 4.0b10 (Swofford 2000). In ML analyses, we used the Tamura-Nei model of evolution (Tamura and Nei 1993), incorporating empirically determined base frequencies, proportion of invariable sites, and a discrete gamma-distributed rate heterogeneity for variable sites. Hierarchical likelihood-ratio tests (Huelsenbeck and Crandall 1997), implemented in the program MODELTEST, version 3.06 (Posada and Crandall 1998), showed that this parameter-rich model fitted the data significantly better than simpler, alternative models of sequence evolution. We estimated model parameters for the ML analyses from data on an initial NJ tree generated using LOGDET (Lockhart et al. 1994) distances. We conducted 10 random addition heuristic searches to find the tree of maximum likelihood. We based NJ analyses on ML-corrected distances, using the same model parameters as the ML analysis. We estimated support for branches on the NJ tree using 1,000 bootstrap replicates.

Maximum-parsimony analyses used heuristic searches with equal weighting to all sites and substitution types, tree-bisection-reconnection (TBR) branch swapping, and 100 random additions of taxa. Differential weighting of codon positions and transitions–transversion substitutions were not implemented because sequence divergence was low and saturation effects were not evident in the data set (see below). We examined support for branches on the MP trees using 1,000 bootstrap replicates.

Results

We obtained 917 bp of mtDNA cytochrome-*b* gene sequence from 90 individual *Puffinus* and *Calonectris* shearwaters, including 1–10 individuals for 19 taxa in or associated with the *assimilis–lherminieri* complex listed in Table 1, and 15 of the remaining 16 species of *Puffinus* shearwaters. Sixty-six different sequences were found; 54 sequences were unique to single individuals; and 12 sequences were shared between two, three, or four individuals.

Nucleotide variation.—Sequences showed typical mtDNA codon-position and transition biases with majority of substitutions involving synonymous, third-codon transitions: 81% and 86% of the 264 variable sites were at third-codon positions and involved only transitions, respectively. Sequences appeared to be largely unaffected by multiple substitutions because sequence divergence between all taxa was below the threshold at which saturation effects have been detected in procellariiform birds (Austin 1996, Nunn et al. 1996) and plots of observed transition and transversion differences against ML corrected distances showed a linear relationship for all comparisons (data not shown).

Within-taxon genetic diversity was low (Table 2). Mean pairwise corrected sequence divergence ranged from 0% within *P. nativitatis* (two individuals), *P. huttoni* (two individuals), *P. newelli* (two individuals), and *subalaris* (three individuals) to 0.5% within *P. puffinus* (five individuals). Two distinct patterns of intertaxon sequence divergence were observed. In the first sequence divergence within three groups of taxa (*loyemilleri–lherminieri*, *dichrous–nicolae–colstoni–polynesiae*, and *atrodorsalis–bailloni*) was extremely low (0–0.7%, 0–0.7%, and 0–0.1%, respectively) and clearly within the range of within-taxon diversity. Some individuals from different taxa shared identical sequences (i.e. *loyemilleri* [Panama] and *lherminieri* [Martinique, one sample; Brazil, two samples], *colstoni* [Aldabra Atoll, two samples] and *nicolae* [Seychelles archipelago, two samples], and *atrodorsalis* and *bailloni* [Reunion Island]). In contrast, pairwise sequence divergence between all other taxa was greater than within-taxon diversity. Excluding outgroup comparisons, sequence divergence ranged from 0.7% between *assimilis* and *tunneyi* to 8.1% between *nativitatis* and *baroli*.

Phylogenetic relationships.—Maximum-parsimony searches found 12 most parsimonious trees (length 603 steps). The ML and NJ analyses using the Tamura-Nei model (transversion rates: A–G = 32.78, C–T = 34.69, proportion of invariable sites = 0.64, gamma-shaped parameter = 1.96) found trees very similar in topology to those. The ML tree ($-\ln L = 4248.65$) is shown in Figure 2. Bootstrap support (MP and NJ) for branches within the phylogeny were generally concordant, and nodes recovered in one analysis and not another received low support. A summary cladogram—

TABLE 2. Tamura-Nei corrected cytochrome-*b* gene pairwise genetic distances between *Calonectris* and *Puffinus* shearwaters (below diagonal) and within species (on diagonal). Numbers in parentheses next to species names are number of individuals sampled (>1).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *C. leucomelas*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. *C. diomedea*	0.035	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3. *P. bulleri*	0.093	0.084	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4. *P. pacificus*	0.094	0.085	0.036	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5. *P. tenuirostris*	0.088	0.088	0.052	0.061	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6. *P. griseus*	0.105	0.103	0.058	0.059	0.055	–	–	–	–	–	–	–	–	–	–	–	–	–
7. *P. gravis*	0.091	0.086	0.044	0.049	0.041	0.043	–	–	–	–	–	–	–	–	–	–	–	–
8. *P. carneipes*	0.097	0.091	0.052	0.056	0.048	0.040	0.028	–	–	–	–	–	–	–	–	–	–	–
9. *P. creatopus*	0.097	0.093	0.052	0.056	0.050	0.038	0.026	0.009	–	–	–	–	–	–	–	–	–	–
10. *P. nativitatis* (2)	0.101	0.093	0.086	0.088	0.093	0.110	0.089	0.092	0.092	0	–	–	–	–	–	–	–	–
11. *P. huttoni* (2)	0.096	0.089	0.085	0.085	0.100	0.094	0.084	0.095	0.088	0.080	0	–	–	–	–	–	–	–
12. *P. gavia*	0.091	0.080	0.077	0.080	0.086	0.093	0.077	0.089	0.089	0.072	0.031	–	–	–	–	–	–	–
13. *P. puffinus* (5)	0.088	0.077	0.076	0.073	0.079	0.086	0.077	0.082	0.078	0.075	0.056	0.048	0.005	–	–	–	–	–
14. *P. yelkouan* (10)	0.089	0.085	0.087	0.085	0.086	0.090	0.085	0.090	0.085	0.080	0.055	0.054	0.036	0.002	–	–	–	–
15. *P. mauretanicus* (6)	0.092	0.085	0.085	0.085	0.093	0.095	0.087	0.093	0.090	0.076	0.055	0.054	0.038	0.022	0.005	–	–	–
16. *P. opisthomelas* (2)	0.088	0.074	0.081	0.076	0.088	0.095	0.080	0.085	0.085	0.075	0.059	0.050	0.030	0.044	0.044	0.002	–	–
17. *P. newelli* (2)	0.088	0.074	0.079	0.079	0.088	0.089	0.080	0.083	0.081	0.076	0.058	0.050	0.032	0.039	0.039	0.025	0	–
18. *P. subalaris* (3)	0.098	0.081	0.101	0.097	0.097	0.107	0.097	0.095	0.097	0.075	0.072	0.067	0.067	0.076	0.075	0.068	0.069	0
19. *P. elegans* (2)	0.091	0.081	0.087	0.087	0.093	0.097	0.091	0.087	0.085	0.070	0.066	0.061	0.040	0.047	0.050	0.037	0.035	0.076
20. *P. haurakiensis* (2)	0.090	0.080	0.085	0.080	0.088	0.097	0.085	0.089	0.087	0.069	0.062	0.055	0.039	0.041	0.040	0.038	0.037	0.071
21. *P. kermadecensis* (3)	0.088	0.077	0.082	0.084	0.090	0.099	0.085	0.091	0.091	0.067	0.061	0.052	0.037	0.042	0.042	0.033	0.034	0.068
22. *P. tunneyi*	0.093	0.084	0.092	0.092	0.099	0.105	0.091	0.098	0.092	0.075	0.067	0.064	0.040	0.050	0.052	0.045	0.044	0.077
23. *P. assimilis*	0.091	0.084	0.094	0.094	0.099	0.105	0.093	0.098	0.092	0.077	0.064	0.064	0.040	0.046	0.051	0.047	0.046	0.080
24. *P. boydi*	0.087	0.073	0.083	0.079	0.086	0.095	0.081	0.086	0.084	0.080	0.061	0.053	0.036	0.046	0.046	0.035	0.036	0.072
25. *P. baroli* (6)	0.085	0.070	0.083	0.077	0.085	0.092	0.081	0.084	0.081	0.081	0.059	0.052	0.035	0.042	0.043	0.036	0.036	0.072
26. *P. lherminieri* (9)	0.084	0.072	0.086	0.076	0.091	0.093	0.085	0.087	0.083	0.075	0.060	0.053	0.036	0.041	0.046	0.034	0.031	0.068
27. *P. loyemilleri*	0.085	0.073	0.087	0.076	0.092	0.091	0.083	0.086	0.082	0.077	0.059	0.053	0.036	0.040	0.046	0.033	0.032	0.068
28. *P. myrtae* (2)	0.090	0.078	0.087	0.082	0.091	0.095	0.086	0.091	0.087	0.074	0.057	0.052	0.030	0.036	0.038	0.027	0.013	0.068
29. *P. temptator*	0.081	0.077	0.076	0.080	0.094	0.099	0.087	0.093	0.091	0.076	0.057	0.048	0.031	0.033	0.037	0.028	0.026	0.070
30. *P. persicus* (2)	0.084	0.080	0.083	0.086	0.094	0.098	0.086	0.092	0.088	0.076	0.058	0.050	0.033	0.038	0.041	0.033	0.027	0.075
31. *P. bailloni* (2)	0.080	0.077	0.075	0.081	0.088	0.092	0.081	0.086	0.083	0.079	0.057	0.050	0.036	0.032	0.036	0.031	0.026	0.073
32. *P. atrodorsalis*	0.079	0.075	0.075	0.081	0.087	0.091	0.081	0.084	0.082	0.078	0.056	0.048	0.036	0.031	0.035	0.031	0.025	0.071
33. *P. dichrous* (6)	0.080	0.077	0.074	0.077	0.091	0.093	0.082	0.087	0.086	0.073	0.054	0.044	0.032	0.033	0.038	0.029	0.024	0.070
34. *P. polynesiae*	0.081	0.077	0.074	0.075	0.092	0.093	0.081	0.086	0.084	0.073	0.056	0.046	0.032	0.034	0.039	0.027	0.024	0.072
35. *P. colstoni* (4)	0.082	0.076	0.075	0.076	0.093	0.094	0.082	0.087	0.085	0.074	0.057	0.047	0.033	0.036	0.040	0.029	0.026	0.073
36. *P. nicolae* (3)	0.083	0.077	0.076	0.077	0.093	0.095	0.083	0.088	0.086	0.075	0.057	0.048	0.033	0.036	0.041	0.029	0.026	0.073

TABLE 2. Continued.

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
19. *P. elegans* (2)	0.001	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20. *P. haurakiensis* (2)	0.032	0.001	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
21. *P. kermadecensis* (3)	0.027	0.020	0.002	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22. *P. tunneyi*	0.033	0.028	0.016	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23. *P. assimilis*	0.033	0.030	0.018	0.007	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24. *P. boydi*	0.041	0.034	0.033	0.043	0.045	–	–	–	–	–	–	–	–	–	–	–	–	–
25. *P. baroli* (6)	0.040	0.035	0.034	0.042	0.045	0.012	0.001	–	–	–	–	–	–	–	–	–	–	–
26. *P. lherminieri* (9)	0.039	0.038	0.037	0.044	0.047	0.025	0.022	0.004	–	–	–	–	–	–	–	–	–	–
27. *P. loyemilleri*	0.039	0.039	0.037	0.045	0.047	0.024	0.022	0.003	–	–	–	–	–	–	–	–	–	–
28. *P. myrtae* (2)	0.035	0.034	0.034	0.041	0.041	0.035	0.035	0.029	0.031	0.001	–	–	–	–	–	–	–	–
29. *P. temptator*	0.041	0.035	0.035	0.045	0.047	0.039	0.037	0.034	0.035	0.027	–	–	–	–	–	–	–	–
30. *P. persicus* (2)	0.044	0.039	0.038	0.043	0.048	0.045	0.044	0.040	0.040	0.026	0.015	0.003	–	–	–	–	–	–
31. *P. bailloni* (2)	0.041	0.036	0.036	0.045	0.047	0.042	0.040	0.037	0.038	0.027	0.014	0.017	0.001	–	–	–	–	–
32. *P. atrodorsalis*	0.041	0.035	0.035	0.045	0.047	0.041	0.039	0.037	0.037	0.027	0.013	0.016	0.001	–	–	–	–	–
33. *P. dichrous* (6)	0.040	0.033	0.034	0.043	0.046	0.038	0.038	0.033	0.034	0.023	0.012	0.015	0.010	0.010	0.003	–	–	–
34. *P. polynesiae*	0.040	0.033	0.034	0.044	0.046	0.038	0.038	0.032	0.032	0.023	0.012	0.015	0.010	0.010	0.003	–	–	–
35. *P. colstoni* (4)	0.041	0.035	0.036	0.045	0.047	0.040	0.040	0.033	0.033	0.025	0.014	0.016	0.012	0.011	0.005	0.002	0.001	–
36. *P. nicolae* (3)	0.042	0.035	0.036	0.045	0.048	0.040	0.040	0.034	0.033	0.025	0.014	0.017	0.012	0.012	0.005	0.002	0.001	0.001

derived as a strict consensus of the ML, NJ, and 12 MP trees—is shown in Figure 3.

In all analyses, assimilis taxa, lherminieri taxa, and the *assimilis–lherminieri* complex as a whole are not resolved as monophyletic clades. Instead, they are split among five separate and well-supported lineages that, in some cases, include taxa from outside the complex. (1) A North Atlantic and Caribbean clade is geographically, but not taxonomically, well defined with two strongly supported sister-groups, *lherminieri–loyemilleri* in the west and *baroli–boydi* in the east. (2) A southern hemisphere and subtropical–subantarctic clade contains all the Australasian and Southern Ocean assimilis taxa: *assimilis*, *tunneyi*, *kermadecensis*, *haurakiensis*, and *elegans*. The pattern of cladogenesis within that group has a strong east-to-west orientation—*elegans* from the South Atlantic is basal, and western taxa are progressively more derived. (3) An Indian and Pacific clade contains populations of the lherminieri group from the tropical Indian and Pacific oceans: *nicolae*, *colstoni*, *polynesiae*, *dichrous*, *bailloni*, *atrodorsalis*, *persicus*, and *temptator.* Within that clade, *bailloni*, *persicus*, and *temptator* form three separate lineages. For the remainder, there is almost no divergence between *nicolae* and *colstoni*, genetic divergence between those and *polynesiae* and *dichrous* is low, and *dichrous* is paraphyletic. Bootstrap support values for nodes separating the latter four taxa are low. Relationships between those and *bailloni*, *persicus*, and *temptator* are poorly resolved. A haplotype network for sequences from that clade confirmed the phylogeography for taxa from that group (Fig. 4). All sequences from the Pacific Ocean taxa *dichrous* and *polynesiae* are phylogenetically intermediate between those of the Indian Ocean taxa *bailloni*, *persicus*, *temptator*, *nicolae*, and *colstoni*. (4) A fourth clade comprises *myrtae* and *P. newelli* from the central Pacific; (5) the fifth lineage is *subalaris* from the Galápagos. Four additional lineages are well supported: *puffinus*, *mauretanicus–yelkouan*, *opisthomelas*, and *gavia–huttoni*. Phylogenetic relationships among those nine lineages are generally poorly resolved, as indicated by the short internal branches in the ML tree (Fig. 2) and NJ tree (not shown); conflicting

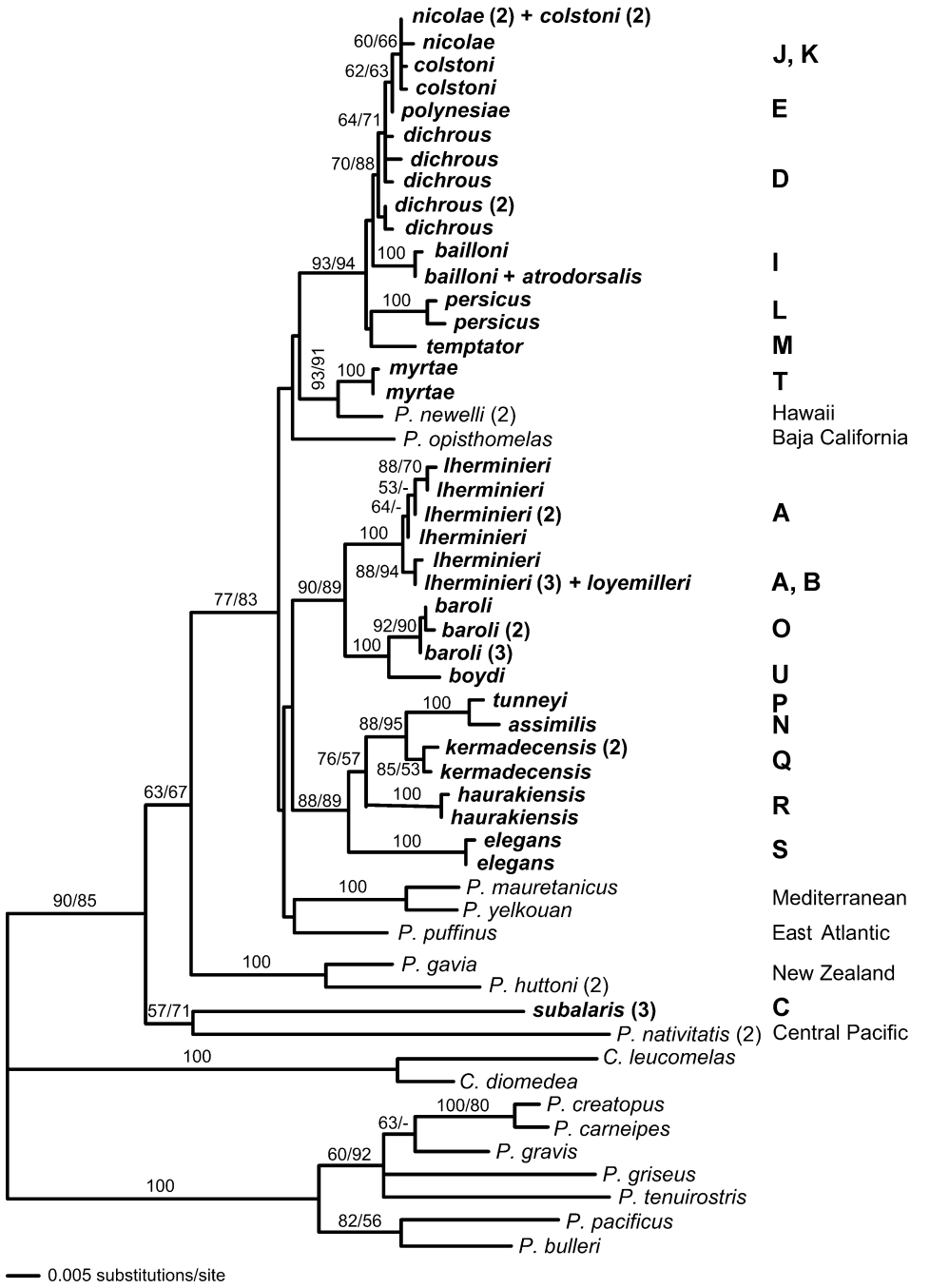

FIG. 2. Maximum-likelihood phylogenetic tree for *Puffinus* shearwaters based on 917 bp of mtDNA cytochrome-*b* gene sequence (–ln *L* = 4248.65). Numbers above and adjacent to branches are maximum-parsimony (MP) and neighbor-joining (NJ) bootstrap support values. Where only one value is given, MP and NJ support values were identical. Uppercase letters refer to breeding ranges shown in Figure 1.

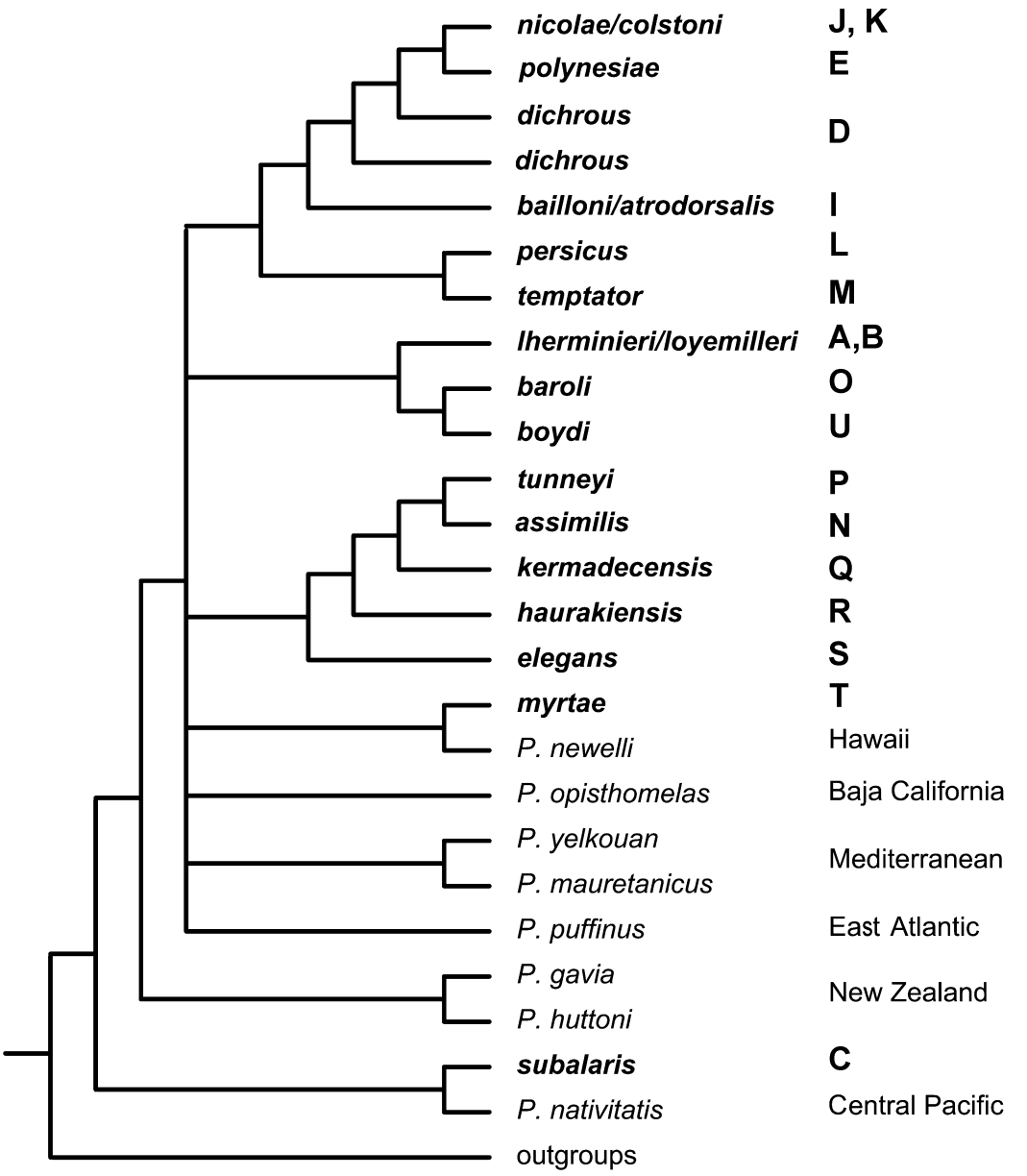

FIG. 3. Summary consensus cladogram for members of the *assimilis–lherminieri* complex and other closely related *Puffinus* shearwaters. The cladogram is a strict consensus of the maximum-likelihood, neighbor-joining, and maximum-parsimony trees. Uppercase letters refer to breeding ranges shown in Figure 1.

topologies between MP, ML, and NJ trees; and low-support values. That basal polytomy is reflected in the consensus cladogram in Figure 3. However, both *subalaris* and *gavia–huttoni* were consistently placed as basal branches on the tree relative to all other small shearwaters.

DISCUSSION

The present study is the first comprehensive phylogenetic assessment of the *Puffinus assimilis–lherminieri* complex, incorporating almost all described subspecies, multiple populations,

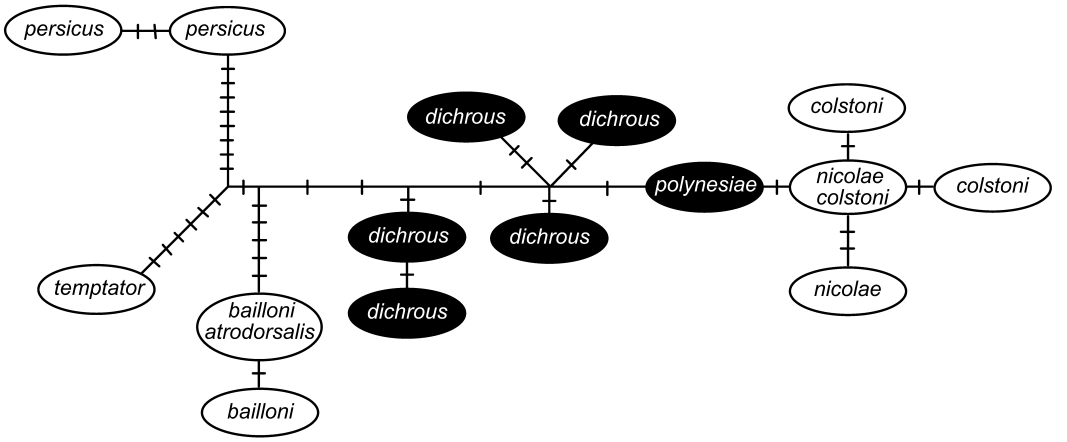

FIG. 4. Haplotype network for sequences obtained from taxa within the Indian and Pacific oceans clade derived using TCS, version 1.3 (Clement et al. 2000). Haplotypes are labeled with the taxa in which they were found, haplotypes are filled black (Pacific Ocean) or white (Indian Ocean), and haplotypes are joined by lines showing number of nucleotide substitutions.

and *Puffinus* congeners for comparison. Significantly, most subspecies of the complex are well resolved as genetically differentiated, independent lineages. There is strong support for three biogeographically defined groups: (1) North Atlantic, (2) Australasia and the Southern Ocean, and (3) tropical Indian and Pacific oceans. However, that phylogeny is not congruent with any previous classifications of the *assimilis–lherminieri* complex on the basis of phenetic similarity (Table 1). In addition, the lack of sequence divergence, paraphyly of sequences, or both between (1) *loyemilleri* and *lherminieri*; (2) *colstoni*, *nicolae*, *polynesiae*, and *dichrous*; and (3) *atrodorsalis* and *bailloni* was unexpected, as were the basal position of *subalaris* and the close relationship between *myrtae* and *P. newelli*.

Authenticity of cytochrome-b *gene sequences.*—The incongruence between molecular phylogeny and traditional taxonomy may indicate errors or artifacts in our data. Several lines of evidence support the identity of all samples in the study and the accuracy, authenticity, and mitochondrial origin of the cytochrome-*b* sequences reported here. All samples have good locality provenance and were collected on islands or in areas where only that taxon is known to breed. None of the sequences have unusual nucleotide compositions, patterns of nucleotide substitution, amino-acid translations, stop-codons, insertions or deletions indicative of methodological errors (Hackett et al. 1995, Helbig and Seibold 1996), contamination (Edwards and Arctander 1996), or presence of a nuclear copy of the gene (Arctander 1995, Zhang and Hewitt 1996, Sorenson and Quinn 1998). Sequences obtained from multiple samples for 18 taxa, either by us or independently by Nunn and Stanley (1998) and Heidrich et al. (1998) using different tissue sources and PCR primers all show high sequence-identity, which suggests sequence accuracy (Helbig and Seibold 1996) and reproducibility (Austin et al. 1997). Phylogenies derived separately from the three overlapping cytochrome-*b* subfragments amplified from most museum specimens were congruent with the topology derived from the complete 917 bp (data not shown), indicating that the sequences are not chimeric. That is strong evidence for authenticity, because it is unlikely that a sample mix-up, contamination, or nuclear pseudogenes would affect all three fragments independently (Hackett et al. 1995, Sorenson and Quinn 1998, J. J. Austin pers. obs.). In the three cases where identical sequences were obtained from different taxa, we conducted DNA extraction, PCR amplification, and DNA sequencing of all samples completely independently of each other.

Phylogeny and evolutionary history of the assimilis–lherminieri *complex.*—Austin (1996) previously discussed the general phylogenetic history of *Puffinus* shearwaters on the basis of

short cytochrome-*b* gene sequences. The larger data set confirms and refines earlier findings, in particular the monophyly of all the small- to medium-sized *Puffinus* shearwaters and a largely unresolved, basal radiation within the clade. The phylogeny indicates that the majority of the *assimilis–lherminieri* complex has arisen via radiations in three geographically separate regions, and that the ancestors of those three lineages were part of a rapid radiation involving most of the small- to medium-sized shearwaters 2.5–5.2 mya (based on 2.3–4.7% divergence and an evolutionary rate for Procellariidae cytochrome-*b* of 0.9% Ma^{-1}; Nunn and Stanley 1998)

The three primary clades in the North Atlantic, Southern, and tropical Indian and Pacific oceans are biogeographically well defined. North Atlantic populations have been isolated from the Pacific since the formation of the Panama landbridge ~3 mya (Keigwin 1978, Coates et al. 1992), a date that is concordant with the split between *lherminieri* and taxa in the central and eastern Pacific (3.2–3.8 mya). The tropical equatorial waters of the central Atlantic may be a barrier to southern dispersal, because *baroli* and *boydi* have never been recorded in the southern hemisphere (Sinclair et al. 1982, Berruti 1990). The split between the Australasian and Southern oceans clade and the tropical Indian and Pacific oceans clade corresponds with the boundary between subtropical and tropical oceanic zones (Ashmole 1971). Changes in sea-surface temperature and food availability across that boundary may act as an ecological barrier to dispersal between the two clades. There is evidence that warm nutrient-poor waters may act as ecological barriers to dispersal even in wide-ranging oceanic birds, for example, albatrosses, gannets, and some larids (e.g. Nelson 1978, Nunn et al. 1996). Dispersal and allopatric speciation have occurred longitudinally within each of the three lineages and would have been reinforced by the generally nonmigratory habits and natal philopatry of most procellariform populations (Ovenden et al. 1991, Warham 1996) and shearwaters in particular (Wooller et al. 1990, Rabouam et al. 1998). There is a strong geographic component to the pattern of cladogenesis. In the North Atlantic, taxa are split between eastern and western clades. In the southern hemisphere, topology suggests sequential colonization and speciation in a westerly direction from the Atlantic to Australia. In the tropical Indian and Pacific oceans, the pattern is more difficult to interpret given the phylogeography of the clade (Fig. 4). The presence of three genetically divergent but geographically restricted taxa—*bailloni*, *persicus*, and *temptator*—in the Indian Ocean suggests an origin there. However, the limited genetic differentiation among the remaining four taxa, separated by >16,000 km, is surprising; it contrasts sharply with other assimilis and lherminieri taxa that show divergence over much smaller geographic scales (Fig. 2). In addition, the phylogeographically intermediate position of the Pacific Ocean taxa *dichrous* and *polynesiae* suggests dispersal from the Indian Ocean to the Pacific and then back again. That scenario conflicts with patterns of differentiation and speciation in all other *Puffinus* shearwaters and clearly requires more detailed examination. Regardless, the limited genetic differentiation among populations from across the Indian and Pacific oceans suggests a recent long-distance expansion, 0.1–0.5 mya (based on an evolutionary rate for procellariid cytochrome-*b* of 0.9% Ma^{-1}; Nunn and Stanley 1998).

In conclusion, phylogeny (Figs. 2 and 3) indicates that the *assimilis–lherminieri* complex has an evolutionary history involving (1) radiations in three separate ocean regions, (2) allopatric speciation on individual islands or island archipelagoes, and (3) at least one case of a recent long-distance expansion across two ocean basins.

More or less similar phylogeographic schemes have already been suggested for other procellariiforms. A phylogeny of the gadfly petrels (genus *Pterodroma*) suggests that there is a clade of Atlantic species (*P. feae–madeira*, *P. hasitata*, and *P. cahow*), a clade of southern species (*P. incerta*, *P. macroptera*, and *P. lessoni*), and a clade of tropical species (*P. neglecta*, *P. externa*, and *P. phaeopygia*; though the latter also includes *P. inexpectata* from New Zealand) (Nunn and Stanley 1998). However, a new and more complete phylogeny (using a super-tree approach) provided slightly different results, lumping the Atlantic and southern clades but raising a larger tropical clade with the inclusion of Indian Ocean species within the Pacific Ocean ones (Kennedy and Page 2002). Similarly, for albatrosses, the three species breeding in the

northern hemisphere and *Phoebastria irrorata* from the Galápagos form a monophyletic clade (Nunn et al. 1996, Kennedy and Page 2002). Therefore, speciation within oceanic water masses via allopatry seems a common process in several groups of Procellariiformes, at different phylogenetic levels.

Taxonomic implications.—Previous taxonomic groupings of the *assimilis–lherminieri* complex do not reflect phylogenetic relationships of the taxa involved. Given that levels of genetic divergence among members of the complex are similar to those among the larger *Puffinus* shearwaters and that most of the described subspecies (identified on morphological and other grounds) are also phylogenetically independent lineages, there appears to be little justification for maintaining the current taxonomy. Our molecular data suggest (1) that five major clades should be regarded as "higher-level" taxa, (2) that at least 13 currently recognized subspecies (*bailloni*, *persicus*, *temptator*, *dichrous*, *myrtae*, *lherminieri*, *baroli*, *boydi*, *assimilis*, *tunneyi*, *kermadecensis*, *haurakiensis*, and *elegans*) should be regarded as "lower-level" taxa, and (3) that five taxa (*loyemilleri*, *polynesiae*, *nicolae*, *atrodorsalis*, and *colstoni*) should be synonymized with the previous ones (Table 3).

Taxa from the Southern Hemisphere and Australasia are a single clade (higher-level taxon: *P. assimilis*, Little Shearwater), consisting of five lineages (lower-level taxa: *assimilis*, *tunneyi*, *kermadecensis*, *haurakiensis*, and *elegans*; Table 3). Taxa from the tropical Indian and Pacific oceans are a clade (higher-level taxon: *P. bailloni*, Tropical Shearwater), consisting of four lineages (lower-level taxa). Taxa *temptator*, *persicus*, and *bailloni* are divergent from each other and the rest of the clade (1.1–1.7% corrected sequence divergence); whereas *dichrous*, *polynesiae*, *colstoni*, and *nicolae*—though forming a clade—are not genetically differentiated from each other (0–0.7% corrected sequence divergence); and the sequence from *atrodorsalis* is identical to that from *bailloni*. The status of *colstoni* and *atrodorsalis* has been disputed on the basis of coloration and morphology (Bretagnolle and Attié 1996, Bretagnolle et al. 2000). The lack of genetic divergence between *nicolae* on Aldabra and *colstoni* in the nearby Seychelles reflects the known history of Aldabra. Aldabra was completely submerged at least twice during Pleistocene sea-level changes, with the most recent submergence 125,000 years ago (Taylor et al. 1979). Colonization of the present fauna must have occurred since then. Molecular data

TABLE 3. Proposed taxonomy for the *Puffinus assimilis–lherminieri* complex. "Lower-level" taxa represent monophyletic and genetically differentiated terminal clades. "Higher-level" taxa correspond to deeper branches in the molecular phylogeny (see text) and represent ocean-based taxa (*bailloni*, *lherminieri*, and *assimilis*) or taxa that were misclassified (*subalaris* and *myrtae*).

Original description	Proposed "lower-level" taxon	Proposed "higher-level" taxon and common name
Puffinus lherminieri nicolae Jouanin, 1971	*dichrous*	*P. bailloni*, Tropical Shearwater
Puffinus lherminieri colstoni Shirihai and Christie, 1996	*dichrous*	*P. bailloni*, Tropical Shearwater
Puffinus lherminieri polynesiae Murphy, 1927	*dichrous*	*P. bailloni*, Tropical Shearwater
Puffinus dichrous Finsch and Hartlaub, 1867	*dichrous*	*P. bailloni*, Tropical Shearwater
Procellaria (nugax) bailloni Bonaparte, 1857	*bailloni*	*P. bailloni*, Tropical Shearwater
Puffinus persicus Hume, 1872	*persicus*	*P. bailloni*, Tropical Shearwater
Puffinus lherminieri temptator Louette and Herremans, 1985	*temptator*	*P. bailloni*, Tropical Shearwater
Puffinus assimilis myrtae Bourne, 1959	*myrtae*	*P. newelli*, Newell's Shearwater
Pufflnus (sic) *lherminieri* Lesson, 1839	*lherminieri*	*P. lherminieri*, Audubon's Shearwater
Puffinus lherminieri loyemilleri Wetmore, 1959	*lherminieri*	*P. lherminieri*, Audubon's Shearwater
Procellaria baroli Bonaparte, 1857	*baroli*	*P. lherminieri*, Audubon's Shearwater
Puffinus lherminieri boydi Mathews, 1912	*boydi*	*P. lherminieri*, Audubon's Shearwater
Puffinus assimilis Gould, 1838	*assimilis*	*P. assimilis*, Little Shearwater
Puffinus assimilis tunneyi Mathews, 1912	*tunneyi*	*P. assimilis*, Little Shearwater
Puffinus assimilis kermadecensis Murphy, 1927	*kermadecensis*	*P. assimilis*, Little Shearwater
Puffinus assimilis haurakiensis Fleming and Serventy, 1943	*haurakiensis*	*P. assimilis*, Little Shearwater
Puffinus elegans Giglioli and Salvadori, 1869	*elegans*	*P. assimilis*, Little Shearwater
Puffinus subalaris Ridgway, 1897	*subalaris*	*P. subalaris*, Galapagos Shearwater

support a recent origin for *colstoni* but cannot reject ongoing gene flow between populations of *colstoni* and those of *nicolae*. Jouanin and Mougin (1979) synonymize *dichrous* and *polynesiae*, a strategy that should be extended here to include *nicolae* and *colstoni*. The *atrodorsalis* sequence is identical to one haplotype of *bailloni*, which suggests that the *atrodorsalis* specimen is conspecific with or recently derived from *bailloni*. Additional information (e.g. using vocalizations, as was successfully done in petrel systematics; see Bretagnolle and Attié 1996) is required to fully assess the appropriate taxonomic level of those taxa, but current data suggest that only four terminal taxa are involved—*dichrous*, *bailloni*, *persicus*, and *temptator* (Table 3).

Clearly, *baroli* and *boydi* are closely related to each other and are closer to *lherminieri* than to *assimilis*, in contrast to previous taxonomic treatments. They belong to a clade (higher-level taxon: *P. lherminieri*, Audubon's Shearwater) that is endemic to the North Atlantic, with three distinct lineages (lower-level taxa: *baroli*, *boydi*, and *lherminieri*; Table 3). The taxon *loyemilleri* is probably not valid, given the wide geographic range of *lherminieri* in the West Indies and western Atlantic, so *loyemilleri* should be synonymized with *lherminieri*. Again, the taxonomic decision with regard to species versus subspecies status will depend on other characters.

Bourne (1959) noticed the atypical structure (long tail) of *myrtae* as compared with the assimilis group. Unexpectedly, we found a close relationship between *myrtae* and *P. newelli* from Hawaii. Interestingly, *P. newelli* in flight has white flank patches (see Harrison 1987 for photos), a character also found in *myrtae* (R. Seitre and A. Guillemont pers. comm.). This unusual relationship may be true but could also indicate introgression, via hybridization, of mtDNA from *P. newelli* to *myrtae*. Such a hybridization event would be old (~1.4 Ma for 1.3% divergence at 0.9% divergence Ma^{-1}; Nunn and Stanley 1998) and would require maintenance of the introgressed mtDNA at relatively high frequency, at least in the population on Rapa Island sampled here. Alternatively, *myrtae* may be a species of hybrid origin, involving *P. newelli* and a species from the tropical Pacific clade (e.g. *dichrous*) or Southern Ocean clade (e.g. *elegans*). W. R. P. Bourne (pers. comm.) has suggested that *myrtae* may be a hybrid species, because the Austral Islands are in an area where tropical and subtropical birds overlap. Here, we recognize *myrtae* as a distinct taxon within the *P. newelli* (Newell's Shearwater) but recommend that a thorough analysis based on measurements, calls, and additional molecular markers be done.

The phylogenetic position of *subalaris* is a striking result. That taxon definitely does not belong to the *assimilis–lherminieri* complex but is a species on its own (Galapagos Shearwater [*P. subalaris*]), very well differentiated from all other black-and-white shearwaters. Murphy (1927) reported that "a curious feature…appears to distinguish *subalaris* from every other form of either *lherminieri* or *assimilis*…, the nasal tubes of the Galápagos race are exceptionally firm and corneous, showing almost no trace of shrinkage in dried specimens." Calls from Galápagos populations made available by the British Library of Wildlife Sounds in 1989 also confirm that *subalaris* is distinct from other lherminieri (V. Bretagnolle unpubl. data).

Conclusion: Shortcomings of morphology in shearwater systematics and further work.—Morphological similarities and differences are poor indicators of relationships within the small *Puffinus* shearwaters. That is exemplified by the failure of all previous taxonomic treatments to reflect phylogeny, by the conflicting placement of *boydi* in either the assimilis group or the lherminieri group and of *baroli* in the assimilis group when both are sister taxa and closely related to *lherminieri*, and by the sheer number of conflicting taxonomic arrangements that have been proposed. The failure of morphological and plumage characters to accurately reflect phylogeny is likely attributable to two factors. First, many of the morphological and plumage characters used to distinguish taxa are probably ecologically important and, therefore, influenced by selection (Bourne 1998). Second, the overall morphological uniformity of the group, despite moderate phylogenetic diversity, suggests that morphological evolution is constrained. Such stabilizing selection within morphological space could explain the convergent and often conflicting patterns of morphological and plumage characteristics found between unrelated taxa, as well as rapid shifts in morphological and plumage characters between closely related allopatric populations. A recent detailed analysis of biometrics and plumage coloration from populations of

bailloni, *nicolae*, and *colstoni* (Bretagnolle et al. 2000) failed to completely separate *bailloni* from *nicolae*, two taxa that are phylogenetically distinct, whereas *colstoni* was shown to be intermediate between them (Bretagnolle et al. 2000). That demonstrates a shift in morphology between two very closely related forms (*nicolae* and *colstoni*), with convergence toward an unrelated taxon *bailloni*.

Clearly, the mtDNA phylogeny presented here raises a number of unexpected phylogenetic hypotheses with significant taxonomic implications. While calling into question the value of currently available morphological data, our study strongly points to the need for detailed analysis of morphology, life history, and calls of all of the smaller *Puffinus* species toward fully understanding the evolution of the group. In addition, molecular data from unsampled taxa and populations and from multiple nuclear genes (Prychitko and Moore 1997, Lovette and Bermingham 2000, Shapiro and Dumbacher 2001) are required to refine and confirm the current mtDNA-based phylogeny.

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Appendix. *Puffinus* taxa, identifying codes, origins, and tissue sources analyzed in the study. Codes refer to specimen registration numbers for museum skins at the Natural History Museum, England (BMNH); the Muséum National d'Histoire Naturelle, France (MNHN); the Durban Natural Science Museum, South Africa (DNSM); the Museu de Zoologica da Universidade de Sao Paulo, Brazil (MZUSP); the Musee Royal de l'Afrique Centrale, Belgium (RMCA); and tissue collections at the Louisiana State University, Museum of Natural Sciences (LSM). All others are field codes from the authors own collections.

Taxon	Code	Locality	Year	Tissue source
P. nativitatis	Pnat81	Sand Island, Johnston Atoll	1993	Blood
P. puffinus	Pppf86	Tenerife, Canary Islands	1993	Blood
P. mauretanicus	Ppma112	Cabrera Archipelago, Balearic Islands	1993	Blood
P. yelkouan	Ppyk61	Ile de Port-Cros, France	1993	Feather
P. auricularis	BMNH 1959.23.2 [a]	Clarion Island, California	1900	Skin (foot)
P. newelli	Panw106	Kauai Island, Hawaii	1993	Blood
P. newelli	Panw108	Kauai Island, Hawaii	1993	Blood
P. opisthomelas	BMNH 1949.64.56	Santa Catalina Island, California	1922	Skin (foot)
P. gavia	Pgva1	Bream Island, New Zealand	1993	Feather
P. huttoni	Phut10	Seaward Kaikoura Mountains, New Zealand	1992	Feather
P. lherminieri	BMNH 1913.12.26.75	Myra-Provost Island, Bahamas	1891	Skin (foot)
P. lherminieri	LSM B-20918	Near Oregon Inlet, USA	?	Kidney
P. lherminieri	BMNH 1932.4.13.1	Tobago, Caribbean	1932	Skin (foot)
P. lherminieri	Pllh_EP7	Martinique, Lesser Antilles	1996	Blood
P. lherminieri	Pllh_EP8	Martinique, Lesser Antilles	1996	Blood
P. lherminieri	MZUSP 75185	Fernando de Noronha, Brazil	~1998	Skin (body)
P. lherminieri	MZUSP 75186	Fernando de Noronha, Brazil	2000	Muscle
P. lherminieri	MZUSP 75187	Fernando de Noronha, Brazil	2000	Muscle
P. loyemilleri	BMNH 1959.31.1	Bocas del Toro, Panama	1958	Skin (foot)
P. subalaris	BMNH 99.9.1.535	Albemarle Island, Galápagos Islands	1897	Skin (foot)
P. subalaris	MNHN 1970.854	Plaza Island, Galápagos Islands	1962	Skin (foot)
P. subalaris	MNHN 1970.855	Santa Cruz, Galápagos Islands	1962	Skin (foot)
P. subalaris	BMNH 1925.12.22.25 [a]	Galápagos Islands	1924	Skin (foot)
P. dichrous	BMNH 1928.10.27.11	Enderbury Island, Phoenix Island	1924	Skin (foot)
P. dichrous	BMNH 78.10.29.39	Palau	1878	Skin (foot)
P. dichrous	Pldi_EP3	Gambier Island, French Polynesia	1995	Feather
P. dichrous	Pldi_EP9	Ua Pou, Marquisas Islands	1998	Blood
P. dichrous	Pldi_EP10	Ua Pou, Marquisas Islands	1998	Blood
P. dichrous	Pldi_EP11	Ua Pou, Marquisas Islands	1998	Blood
P. polynesiae	BMNH 1948.59.29	Taumanua Island, Samoa	1923	Skin (foot)
P. bailloni	Plba_VB	Reunion	1995	Blood
P. bailloni	Plba_EP2	Reunion	1995	Blood
P. bailloni	BMNH 1969.5.1 [a]	Reunion	1964	Skin (foot)
P. nicolae	BMNH 1946.75.61	Seychelles	1940	Skin (foot)
P. nicolae	Plnc_EP1	Seychelles	1996	Blood
P. nicolae	BMNH 1957.16.2	Maldives	1957	Skin (foot)
P. colstoni	BMNH 1968.43.89	Aldabra	1918	Skin (foot)
P. colstoni	Plco_EP4	Aldabra	1997	Blood
P. colstoni	Plco_EP5	Aldabra	1997	Blood
P. colstoni	Plco_EP6	Aldabra	1997	Blood
P. colstoni	BMNH 1968.43.88 [a]	Aldabra	1968	Skin (foot)
P. temptator	RMCA 83-43-A-756	Comoro Island	1983	Skin (foot)
P. persicus	BMNH 1976.1.27	Masirah Channel, Oman	1974	Skin (foot)
P. persicus	BMNH 1962.9.2	Aden, Yemen	1960	Skin (foot)
P. bannermani	BMNH 1959.23.4 [a]	Bonin Island	1910	Skin (foot)
P. gunax	BMNH 61.6.15.12 [a]	Aneiteum, New Hebrides	1860	Skin (foot)
P. assimilis	Paas162	Lorde Howe Island, Australia	1994	Feather
P. baroli	BMNH 1934.1.1.968	Montana Clara, Canary Islands	1913	Skin (foot)
P. baroli	Pabr91	Tenerife, Canary Islands	1993	Blood

APPENDIX. Continued.

Taxon	Code	Locality	Year	Tissue source
P. baroli	Pabr93	Tenerife, Canary Islands	1993	Blood
P. baroli	BMNH 90.59.42 [a]	Porto Santo, Madeira Islands	1890	Skin (foot)
P. tunneyi	BMNH 1949.64.60	Bunbury, Western Australia	1937	Skin (foot)
P. tunneyi	BMNH 1949.64.61 [a]	Cottesloe Beach, Western Australia	1932	Skin (foot)
*P. kermadecens*is	Pakm1	North Meyer Island, Kermadec Islands	1998	Feather
*P. kermadecens*is	Pakm2	North Meyer Island, Kermadec Islands	1998	Feather
*P. kermadecens*is	Pakm3	North Meyer Island, Kermadec Islands	1998	Feather
*P. kermadecens*is	BMNH 55.10.23.11 [a]	Raoul Island, Kermadec Islands	1854	Skin (foot)
*P. kermadecens*is	BMNH 1905.2.2.17 [a]	Kermadec Islands	?	Skin (foot)
*P. kermadecens*is	BMNH 1928.10.27-140 [a]	Herald Island, Kermadec Islands	1920	Skin (foot)
P. haurakiensis	Paha31	Marotiri Island, New Zealand	1992	Feather
P. haurakiensis	Paha33	Poor Knights Island, New Zealand	1992	Feather
P. elegans	BMNH 1956.36.27	Gough Island	1956	Skin (foot)
P. elegans	BMNH 1956.36.28	Tristan da Cunha Island	1956	Skin (foot)
P. myrtae	MNHN 1975.1788	Rapa, Austral Islands	1975	Skin (foot)
P. myrtae	MNHN 1975.1787	Rapa, Austral Islands	1975	Skin (foot)
P. boydi	BMNH 1936.2.21.87	Rombos Island, Cape Verde Islands	1897	Skin (foot)
P. boydi	BMNH 1911.12.23.223 [a]	Rombos Island, Cape Verde Islands	1897	Skin (foot)
P. atrodorsalis	DNSM 36093	Durban, South Africa	1987	Skin–muscle

[a] Specimens from which DNA sequences could not be obtained.